ICHTHYOTOXIC EFFECT OF *PHYLLANTUS NIRURI* LEAF

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ABSTRACT

Phyllantus niruri is a versatile African medicinal plant employed for control of several human ailments. It has been reportedly used in control of certain fish infections. It lowers fish blood sugar level, has immunostimulant activities and is a stress reliever in fishes. The present work shows that despites its health benefits, it has some ichthyotoxic effect which is both concentration and time dependent. The affected fishes showed behavioral changes which include weakness, erratic swimming and moribund behavior and finally death. The gills of such fishes showed lamellae inflammation and distortion and serious gill shrinkage and lamellae erosion. They must have died due to poor supply of oxygen. The work therefore suggests that care should be taken when treating fishes with Phyllantus niruri in order not to exceed its safe limit.

Keywords: *Phyllantus niruri, Clarias gariepinus, ichthyotoxicity, gill lamellae inflammation, lamellae erosion*

INTRODUCTION

Phyllantus niruri (Euphorbiaceae) is an annual weed distributed throughout the tropical and subtropical regions. It is a popular African medicinal plant. It has strong antiplasmodial activity (Cimanga *et al*, 2004, Soh *et al*, 2009, Obidike *et al*, 2013), ant-fertility activity in males (Ezeonwu, 2011), antimicrobial activity (Rageshaw, 2008), antiulcer and wound healing activity (Cipriani *et al*, 2008, Okoli *et al*, 2009) nematocidal activity (Shakil *et al*, 2008), antiviral activity (Shead, 1992, Bagaikotkar *et al*, 2010) and anti HIV activity (Qian-catrone *et al*, 1996). *Phyllantus niruri* is also known to have antioxidant and hepatoprotective activity (Harish and Shivanandappa, 2006).

Outbreak of microbial infections in aquaculture is commonly controlled with conventional antibiotics. Many fish species have developed resistance to these synthetic antibiotics. The use of natural products or medicinal plants for control of fish diseases is increasing in popularity mainly because it is cheap and environmentally friendly (Albert and Ransangan, 2013). Hyperglycemia is an indicator of acute stress in fish. Any ingredient that induces reduction of blood

glucose level is considered a stress reliever (Aassoni *et al*, 2013).*Lobec rohita (*Govid *et al,* 2012*)* and gold fish (Ahilan *et al*, 2010) fed with *Phyllantus niruri* feed supplement showed resistance to *Areonmonas hydrophilica* infection. It has also been found that the effective dose of *Phyllantus niruri* extract to modulate stress in Nile tilapia, *Oreochronis niloticus*, is 5% (Ibrahim *et al,* 2015). *Phyllantus niruri* has been reported to have immunostimulant potential (Kumar, 2015).

A good number of plants are known to be toxic to fishes (Morah 1986, 1993). There appears to be no report on ichthyotoxic activity of *Phyllantus niruri.* Since *Phyllantus niruri* is effectively used for treatment of fish infections and control of stress in fishes etc, the present work is focused on its ichthyotoxic effect on *Clarias gariepinus*.

MATERIALS AND METHODS

Phyllantus niruri leaves were harvested from University of Calabar staff quarters. They were carefully rinsed with deionized water, air-dried at 25^0C for four days and finally powered with a blender. The powered leaf (20g) was Soxhlet-extracted with 70% ethanol. The collected ethanol solution of the extract was distilled down *in vacuo* with a rotary evaporator to give 1.5g extract. The extract (1g) was dissolved in $2cm^3$ of dimethyl sulphoxide, DMSO, and made up to $100cm^3$ with deionized water to give a stock solution of $10gdm^{-3}$. This stock solution was used for the preparation of the working solutions containing 50, 100, 150 and $200mgdm^{-3}$ of the extract. The control was made up of water and DMSO without the plant extract. 200 *Clarias gariepinus* fingerlings were purchased from the University of Calabar fish farm. They were left in aquaria to acclimatize to the laboratory condition for seven days.

Each of the aquaria of $10dm^3$ capacity contained $3dm^3$ each of the test solutions with 0, 50, 100, 150 and $200mgdm^{-3}$ of the extract. Each of these aquaria was stocked with 10 fingerlings and there was replicate determinations. The experiment lasted for 96h. The solution in each aquarium was replaced with fresh one of the same concentration every 24h to reduce fluctuation in the extract concentration due to oxidation and degradation of plant products. The behavioral changes and mortality were obtained daily.

Gill of fish sample from each aquarium was extracted with the help of a pair of scissors and forceps after 96h and fixed in 10% buffered formalin for 48h. They were washed with water. These gills were manually dehydrated through graded alcohol (30, 50, 70 90 and 100%) for 2h in each change. The dehydrated gills were impregnated with paraffin wax at 60^0C, blocked out in an embedding mold before cutting it into thin sections (8μ) with a rotary microtome. The sections were mounted on grease free glass slides, de-waxed in xylene and hydrated with water. The sections were stained with haematoxylin and eosine, dehydrated as before with graded alcohol and finally mounted with enthalan for microscope examination.

RESULTS AND DISCUSSION

Table 1. Toxic effect of ethanol extract of *Phyllantus niruri* leaf on *Clarias gariepinus*

Conc./time	Mortality, %				
	24h	48h	72h	96h	Observation
0.0mgdm^{-3}	0	0	0	0	Normal swimming behavior
50mgdm^{-3}	0	0	0	10	Normal swimming behavior
100mgdm^{-3}	0	0	0	10	Weakness and hanging in the water column
150mgdm^{-3}	0	0	0	20	Erratic swimming and weakness
200mgdm^{-3}	10	20	30	50	Moribund swimming and loss of balance

Table 1: shows the effect of different concentrations of the ethanol extract of *Phyllantus niruri* leaf on *Clarias gariepinus* fingerlings. The observed mortality rate of the fingerlings is concentration and time dependent as it is directly proportional to the level of the extract in the aquarium water and the exposure time. After four days (96h), the LC_{50} occurred at 200mgdm^{-3}. The observed behavioral changes include weakness, erratic swimming, moribund behavior, loss of balance and finally death.

Plates 1, 2 and 3 show the effect of *Phyllantus niruri* leaf extract on *Clarias gariepinus* gills. The fishes exposed to the plants extract showed a range of changes from lamellae inflammation and distortion in 100 and 150mgdm^{-3} of the extract as illustrated in plates 2a and 2b respectively. Serious gill shrinkage and lamellae erosion was observed in the highest concentration of 200mgdm^{-3} (plate 3). Gills of fish in the control (0.0mgdm^{-3}) showed normal distribution of gill secondary lamellae as indicated in plates 1a and 1b respectively.

The fish gills are their major organs of respiration but whereby they are exposed to environmental toxicants, the affected fishes could suffer from anoxia and eventual death resulting from lack of oxygen supply for the normal functioning of the cells. It is well known that ichthyotoxic plants lower the dissolved oxygen level in aquatic environments (Morah *et al*, 2015). With the observed ichthyotoxic effect of

Phyllantus niruri, one has to be cautious with its therapeutic application to fishes in order not to exceed its safe level.

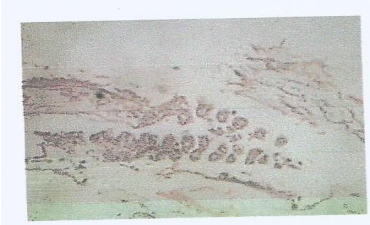

Plate 1a. Normal Distribution of Gill Lamellae (x10)

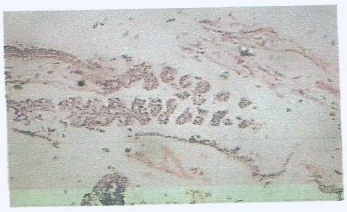

Plate 1b. Normal Gill Lamellae in 5ml/L (x10)

Plate 2a. Inflamed and damaged gill lamellae (x10) 10ml/L

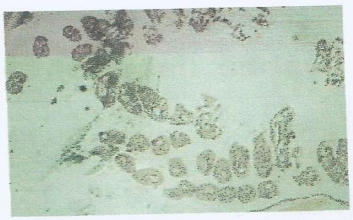

Plate 2b. Highly damaged and distorted gill lamellae (x10) 15ml/L

Plate 3. Shrinkage and erosion of gill lamellae (x10)

REFERENCES

1) Albert, V.and Ransangan, J. (2013). Antimicrobial potential of crude plant extracts against gram negative fish bacterial pathogens. *International Journal of Research in Pharmaceutical and Biosciences 3 (2): 21-27.*
2) Anilan, B., Nithryopiyatharshini, A. and Ravanesharen, K. (2010). Influence of certain herbal additives on the growth, survival and disease resistance of gold fish (*Carassiasaruratus Linnaeus*). *Journal of Veterinary Animal Science 6:5-11.*
3) Bagalkotar, G., Sagineedu, R. S., Soad, M. S. and Stanslac, J. (2010). Phytochemcials from *Phyllantus niruri* Lin and their pharmacological properties review *Journal of Pharmacy and Pharmacology. 58(12):1559-1570.*
4) Cimanga, R. K., Tona, I., Luyndula, N., Mesia, K., Lusakibanza, C. T., Apers, S., De Brugne, T., Merit, S., Hermans, N., Totte, J., Pieters, L. and Vlietinet

A. J. (2004). *In vivio* antiplasmidal activity of callus culture extract and fractions from fresh apical stem of *Phyllantus niruri* part 2 *Journal of Ethnopharmacology.* 95(2-3):399-404.

5) Ciprani, T. R., Mellinger, C. A., de Souze, L. M. and Lacomini, M. (2008). Acidic heteroxylans from medicinal plants and their anti-ulcer activity *Journal of Cancer Policy* 74:274-278.
6) Govid, P., Madhuri, S. and Sahni, Y. P. (2012). Beneficial effect of certain herbal supplements on the health and disease resistance in fish. *Novel Science International Journal of Plant Science* 1: 497-500.
7) Harish, R. and Shivanandappa, T. (2006). Antioxidant activity and hepatoprotective potential of *Phyllantus niruri*. *Food chemistry* 98(2):180-185.
8) Hasain, M. K., Plumenthale, E. J. and Mustapha, A. (2013). Effect of puerain on reduction of glucose and promotion of overall health of acutely stressed Chinock Salmon (*Oncorhynchus tehawytsha*). *Acta Icthyologica et Iscatoria*. 43:85-93.
9) Ibrahim, M., Khan, M., Rinard, J. and Mustapha, A. (2015). Determination of effective dosage of *Phyllantus niruri* to modulate stress in tilapia. *Oreochromis niloticus. Bioengineering and Biosciences.* 3(4): 67-71.
10) Morah, F. N. I. (1986). Naturally occurring fish poisons. *Journal of Science Education* 2(1): 1 -4.
11) Morah, F. N. I. (1993). Toxic constituents of *Adenia* species. *Discovery and Innovations.* 5(2): 111-113.
12) Morah, F. N. I., Ekanem, A. and Ashipu, L. B. (2015). Effect of *Momordica charantia* leaf on water parameters and survival rate of *Clarias gariepinus*. *International Journal of current research in Life Sciences*. 4(11):449-451.
13) Obidike,I., Okhale, S., Aboh, M. and Salawu, O. A. (2013). Isolation, fractionation and evaluation of the antiplasmodial properties of *Phyllantus niruri* resident in its chloroform fraction. *Asian Pacific Journal of Tropical Medicine*. 169- 175.
14) Okoli, C. O., Ezike, A. A., Akah, P. A., Udegbuna S. O., Okoye, T. C, Mbanu, T. P. and Ugwu, E. (2009). Studies on the wound healing and anti-ulcer activities of extract of aerial part of *Phyllantus niruri* L. (Euphorbiaceae). *American Journal of Pharmacology and Toxicology* 4(4) 118-126.
15) Qian-catrone, J., Huang, S., Triuble, J., Li, H., Lin, P., Alan, A., Vlohr, S. E. and Kadow, I. F. (1990). Niruriside, a new HIV REV/RRF binding inhibitor from *Phyllantus niruri*. *Journal of Natural Products* 50(2):196-199.
16) Rajesbwar, Y., Ahmed, R., Shyma, A., Sunda, J., Gupta, D. A. and Mazumder, U. K. (2008). *In vivio* lipid peroxidation inhibitory and antimirocbial activity of *Phyllantus* niruri extract. *International Journal of Pharmacy and Technology*. 7:67-70.

17) Shakil, N.A., Panka, T., Kumar, J., Pandey, R. K. and Sexen, D. B. (2008). Nematocidal activity of mphenylated flavonoids from *Phyllantus niruri*. *Phyotchemistry* 69:559-564.
18) Shead, A., Vickery, K., Pajcos, A., Medhurst, R., Frieman, J., Dixon, R. and Cossart,. (1992). Effect of *Phyllantus* plant extract on duck hepatitis B. *virus in vivio*. *Journal of Anti-viral Research* 18:127-138.
19) Soh, P. N., Banzouzi, J. T., Mangombo, H., Lusakibanz, M., Bulubulu, F. O., Tona, L., Diamuini, A. N., Luyinodulu, S. N. and Benoit –Vical, F. (2009). Antipasmodial activity of various parts of *Phyllantus niruri* according to its geographical distribution. *African Journal of Pharmacy and Pharmacology* 3(12):598-601.