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Full Length Research Paper

Acute toxicity of *Clarias gariepi*nus exposed to *Datura innoxia* leaf extract

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Acute toxicity test was carried out with aqueous extract of *Datura innoxia* leaf on *Clarias gariepinus* fingerlings for 96 h under laboratory conditions using static bioassays with continuous aeration. The LC_{50} of the exposed fingerlings was 120.23 mg/L with lower and upper confidence limits of 96.18 and 150.29 mg/L, respectively. The fish exhibited loss of balance, respiratory distress, vertical and erratic movement, accumulation of mucus on the body surface and gill filament and death. Water quality parameters were within recommended acceptable limits.

Key words: *Datura innoxia, Clarias gariepinus*, static bioassays, 96 h LC_{50}.

INTRODUCTION

Different plants assume different importance in different societies. Solanaceae plants occupy a prominent position as source of drugs in medicine, pharmacology but many are toxic when used in excess. Toxin concentrations in a plant can vary tremendously, often by 100 times or more, and can be dramatically affected by environmental stress on the plant (drought, heat/cold, mineral deficiencies, etc) and disease. Different varieties of the same plant species can also have different levels of toxins and nutritional value (D'Mello, 2000). Effect of naturally-occurring plant-made toxins found at low levels in many foods and drugs for humans and animals is based on laboratory tests using toxin concentrations much higher than the concentrations normally found in food and drug.

Datura innoxia, which belongs to the family Solanaceae, has been reported by Djibo and Bouzon (2000) to contain hallucinogenic properties as the flowers and seeds were used for voluntary intoxication in Niger. Dereck (2002) listed *D. innoxia* as one of the poisonous houseplants as well as a sedative. The toxicity of most plant extracts varies depending on the type and animal species involved. This is due in part to the phytochemical composition of the extract and also the very great variation in susceptibility between individual animals.

Ayuba et al. (2011) reported the phytochemical components of the plant, *D. innoxia,* to include atropine scopolamine, saponins, phenols, flavonolds and cardiac glycocides.

Biologically, the African catfish, *Clarias gariepinus*, is undoubtedly an ideal aquaculture species. It is widely distributed, not only in African countries but also in the Netherlands; it thrives in diverse environments, temperate to tropical *(*Hecht et al., 1996). It is hardy and adaptable principally as a consequence of its air breathing ability, feeds on a wide array of natural prey under diverse conditions, is able to withstand adverse environmental conditions, is highly resistant to diseases, and is highly fecund and easily spawned under captive conditions. It has a wide tolerance of relatively poor water quality and possibly the most exciting feature of the species is its potential for highly intensive culture without prerequisite pond aeration or high water exchange rates and its excellent meat quality (Hecht et al., 1996), hence its choice for this study.

MATERIALS AND METHODS

Fresh samples of *D. innoxia* leaves were collected from the University of Agriculture, Makurdi, Nigeria and brought to hydrobiology and Fisheries Research Laboratory, University of Jos Nigeria, where they were washed and air dried to constant weight. The dried samples were pounded using a clean laboratory mortar to a fine powder which was then sieve through 0.25 mm sieve. 500 g of the resultant powder was dissolved in 2 L of water at room

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Table 1. Ingredient and proximate composition of diet fed to *C. gariepinus.*

Ingredient (%)	Diet
Fish meal	30
Soyabean meal	35
Bone meal	10
Rice bran	15
Corn oil	3
*Vitamin and mineral mix	5
Starch	2
Proximate composition (% DM)	
Crude protein	44.5
Crude fat	8.6
Crude fibre	5.1
Ash	16.4
NFE	25.5

Table 2. Mortality rate of *C. gariepinus* fingerlings exposed to acute concentrations of *D. innoxia* leaf extract.

Concentration (mg/L)	Log	Time (h) for 50% mortality	Mean total mortality (%)	Mean probit value
200.00	2.30	36 (0.00)	90 (10.00)	6.28 (3.72)
180.00	2.25	36 (0.00)	80 (0.00)	5.84 (0.00)
160.00	2.20	48 (0.00)	55 (5.05)	5.13 (3.35)
140.00	2.15	54 (6.00)	50 (0.00)	5.00 (0.00)
120.00	2.08	90 (0.00)	50 (10.00)	5.00 (3.72)
100.00	2.00	-	25.(0.05)	4.87 (3.35)
0.00	0.00	-	-	-

temperature (23± 0.5°C) for 24 h. The extract was filtered through Whatman's filter paper (No. 1) with the aid of a vacuum pump. The filtrate was freeze dried and used for the experiment.

Healthy specimens of *C. gariepinus* fingerlings weighing 10.3(± 0. 35) g were collected from Rock water fish farm Jos. They were acclimatized for two weeks under laboratory conditions; during that period, fish were fed 4% of their body weight with laboratory formulated feed (Table 1); their mortality during acclimation period was less than 3%.

After acclimation, the freeze-dried extract was dissolved in distilled water and introduced into 18 glass aquariums each measuring 60 cm × 30 cm × 30 cm containing dechlorinated well aerated municipal tap water at a concentration 200.00, 180.00, 160.00, 140.00, 120.00 and 100.00 mg/L while 0.00 mgL^{-1} served as control. Each concentration had 3 replicates. Fish were randomly selected and stocked at 10 fingerlings per glass aquarium.

Fish were staved for 24 h prior to and throughout the exposure period which lasted 96 h. Static bioassay techniques as described by Reish and Oshida (1987) were used. The following physio-chemical parameters (temperature, dissolve oxygen, pH, total alkalinity, ammonia-nitrogen and Free carbon dioxide) using methods described by APHA et al. (1985) were measured at the beginning and daily thereafter. Aeration was provided prior to exposure and during exposure in all experimental aquaria. The behaviour of the fish was observed before and during exposure period. The tanks were examined for mortality every 6 h until the end of the 96 h exposure period. Fish were considered dead when the opercula and tail movements stopped and there was no response to a gentle prodding. Dead fish were removed immediately from test solutions to avoid fouling the test media.The 96 h LC_{50} was determined as a probit analysis using the arithmetic method of percentage mortality data. The lower and uppers confidence limits of the LC_{50} were determined as described by UNEP (1989). Result obtained were subjected to statistical analysis with Duncan's multiple range F-test to test for significant difference ($P < 0.05$) between the various concentrations of *D. innoxia* and the control.

RESULTS

The results of the mean mortality rates of the fish *C. gariepinus* exposed to *D. innoxia* leaf extract are presented in Table 2. The logarithmic-probability curves of the mean mortality rates are presented in Figure 1. It was observed during the exposure that fish placed in media devoid of the extract survived the 96 h exposure

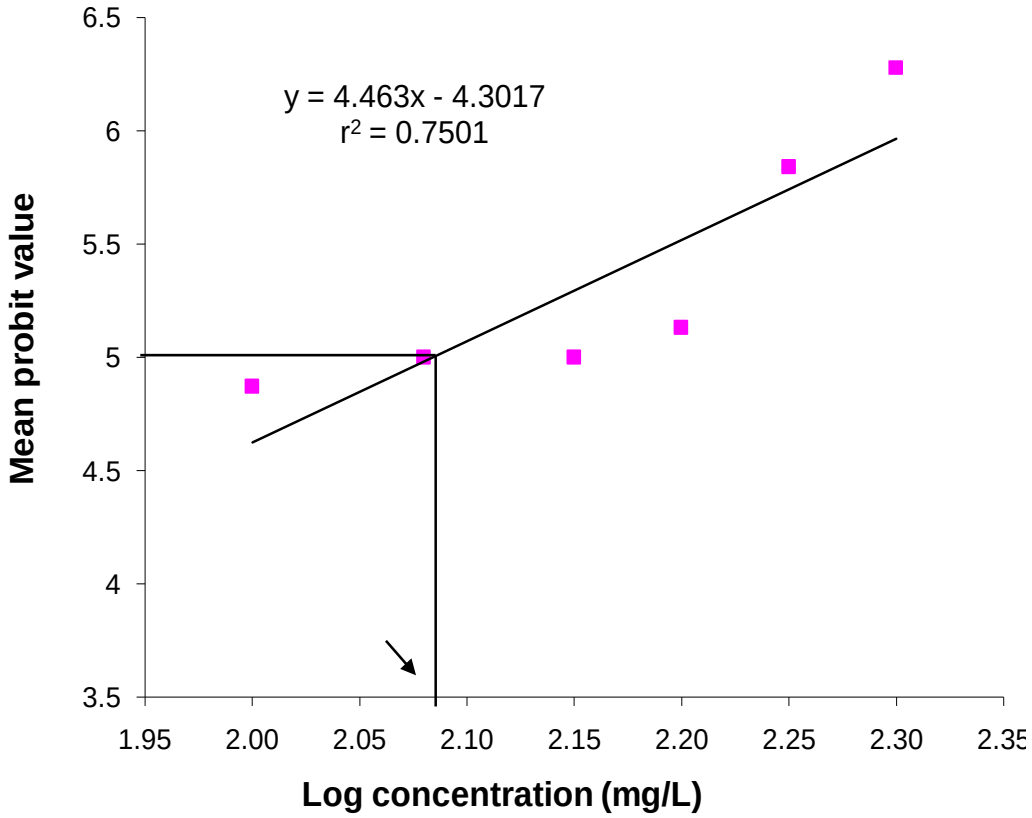

Figure 1. Linear relationship between mean probit mortality and log concentration of *C. gariepinus* exposed to various concentrations of *D. innoxia* leaf extract for 96 h.

period. No mortality was observed in the group exposed to 140 mg/L and below within the first 24 h of exposure. The 96-h LC_{50} of *C. gariepinus* exposed to various concentrations of *D. innoxia* leaf extract was 120.23 mg/L with lower and upper confidence limits of 96.18 and 150.29 mg/L, respectively.

The regression equation of the relationship was calculated to be Probit y = -4.302 + 4.463 log Conc. x and on R square value (r^2) of 0.7501. These expressions, that is, the regression equation r^2 value indicated that mortality rate of the test fish and concentrations of leaf extract are positively correlated. This shows that the mortality rate of the fish increased with increase in the concentrations of *D. innoxia* leaf extract.

During the exposure period, the test fish exhibited various behavioral patterns before death occurred. Restlessness, loss of balance (that is, overturning), air gulping, and convulsion were frequently observed. However, these observations were minimal in the groups of fish exposed to 100 mg/L of the leaf extract. These observations were not noticed in the groups of fish exposed to the media devoid of *D. innoxia* leaf extract.

The opercula ventilation rate and tail beat frequencies per minute are not presented because all the test fish exhibited irregular opercula ventilation rate and tail beat frequency. This may not be unconnected with the presence of assessory organs, which enables it breathe in atmospheric oxygen other than dissolved oxygen.

During the exposure period of the acute toxicity test, it was observed that the mean values of the water quality parameters (Table 3) were not significantly different ($P > 0.05$) for temperature and pH. Even though dissolved oxygen, free carbon dioxide and total alkalinity values differ significantly ($P < 0.05$) compared to the control values, they were still within acceptable limits (Mackereth, 1963).

DISCUSSION

Physical changes observed in *C. gariepinus* exposed to acute toxicity of *D. innoxia* leaf extract included loss of balance, respiratory disorder gulping of air and erratic swimming before death; this is in line with the findings of Omoregie et al. (1998), Ayuba and Ofojekwu (2002), Tawari-Fufeyin et al. (2008); Ololade and Oginni (2010); Okomoda et al. (2010) who made similar observations when they exposed *C. gariepinus* to different toxicants. Behavioural responses of fish to most toxicant and differences in reaction times, according to Bobmanuel et

Table 3. Mean water quality parameters obtained during the exposure of *C. gariepinus* fingerlings to acute concentrations of *D. innoxia* leaf extract for 96 h.

Parameter	Concentrations (mg/L)						
	200.00	180.00	160.00	140.00	120.00	100.00	0.00
Temperature (°C)	22.97(0.65)	22.98(0.6)	22.96(0.65)	22.94(0.66)	22.92(0.6)	22.90(0.6)	22.90(0.6)
Dissolved oxygen (mg/L)	5.24(0.27)	5.62(0.21)	6.24(0.10)	6.26(0.13)	6.30(0.19)	6.34(0.28)	6.94(0.34)
Free carbondioxide (mg/L)	3.80(0.27)	3.58(0.76)	3.32(0.62)	3.18 (0.64)	2.88(0.73)	2.14(0.49)	1.48(0.58)
Total alkalinity (mg/L)	32.60(1.72)	31.80(2.0)	30.50(2.01)	27.00(0.89)	25.80(0.9)	22.80(1.6)	19.88(0.3)
pH	6.94(0.05)	7.00(0.04)	7.00(0.04)	7.06(0.05)	7.06(0.08)	7.02(0.04)	7.00(0.07)
Ammonia (unionized NH_3)	0.23(0.03)	0.22(0.01)	0.22(0.01)	0.22(0.01)	0.22(0.01)	0.22(0.01)	0.22(0.01)

al. (2006), are due to effect of chemicals, their concentrations, species, size and specific environmental conditions Figure 1 which shows the results obtained from this research revealed that the 96 h LC_{50} for the African catfish, *C. gariepinus* exposed to *D. innoxia* leaf extract was 120.23 mg/L with lower and upper confidence limits of 96.18 and 150.29, mg/L, respectively. The 96 h LC_{50} had earlier been reported for *C. gariepinus* by Ayuba and Ofojekwu (2002) to be 204.17 mg/L for *D. innoxia* root extract, while Ayotunde et al. (2010) reported the 96 h LC_{50} of *C. gariepinus* fingerlings exposed to *Carica papaya* seed powder to be 12.9 mg/L; Abalaka and Auta (2010) also recorded 296.14 and 225.48 mg/L for aqueous and ethanol extracts of *Parkia biglobosa* pod respectively for *C. gariepinus*. Fafioye et al. (2010) who worked on the toxicity of aqueous and ethanol extracts of *Parkia biglobosa* and *Raphia vinifera* on *C. gariepinus reported* the 96 h LC_{50} for aqueous and ethanol extracts of *Parkia biglobosa to be* 2.8 and 2.4 ppm respectively. While for *R. vinifera* aqueous and ethanolic extracts, the values were 3.4 and 3.2 ppm, respectively. The difference in the result of the present study and those of these researchers may be due to the difference in toxicants, their concentrations, environmental conditions, age and the size of *C. gariepinus*.

There was no death recorded in the control aquaria, also none in concentrations, 100 and 120 mg/L before 72 and 60 h, respectively. This could be that the fish showed some tolerant to the *D. innoxia* leaf extract at certain level; this agrees with the findings of Datta and Kaviraj (2002), and Fafioye et al. (2004) who reported that *C. gariepinus* is tolerant to pollutants. It is widely known that the toxicity test of pollutant varies with the test organism and chemical quality of the test water. Gaafar et al. (2010) observed that toxicants in the aquatic environment may not necessarily result in the outright mortality of aquatic organisms but can result in several physiological dysfunctions in the fish.

Mucus accumulation was observed on the body surface and gill filament of exposed fish during the present study. This might be as a result of increase in the activity of mucus cells due to subsequent exposure to pollutants. This agrees with the reports of Oti (2002) and Adeyemo (2005) who made similar observations when they exposed *C. gariepinus* fingerlings to cassava mill effluent. The water quality parameters of the test solutions, though fluctuated during the bioassay and were significantly different from those of the control tanks, were within suggested tolerance range (Mackereth, 1963) and so could not have affected the mortality of the test fish. The behavioral alterations that occurred before death in this study may be as a result of nervous impairment due to blockade of nervous transmission among the nervous system and various affector sites, failing organs and retarded physiological processes in fish body functions (Shah, 2002). This may have resulted from enzyme dysfunction and paralysis of respiratory muscle or depression of respiratory centre and disturbances in energy pathways leading to depletion of energy (Gabriel et al., 2010). Since aquatic organisms are in continuous contact with polluted medium, breathing and feeding is impaired due to the damages to respiratory and other organs of the body (Omoregie et al., 1998).

Mortality which is a non response of an organism to the toxic effects of hazardous substances does present a standard form for ranking toxicity and enable the prediction of the effect of potential hazards. Fishes generally produce detoxifying enzymes when exposed to toxicants, but this may be hardly possible in acute exposures which do not allow enough time for the fish to induce detoxifying enzymes as means of increasing immunity against the toxic effects of the chemical. The inability of the fish to detoxify the toxicant and excrete the resultant metabolites, besides direct damage by the toxicant to the epithelial cells of gills, possible destruction of liver and internal asphyxiation may account for the rapid mortality recorded in acute lethal studies of this nature (Farah et al., 2004).

Conclusion

The present study revealed that *D. innoxia* leaf extract causes a regular trend in mortality of *C. gariepinus* fingerlings which increased with increased concentration.

The use of *D. innoxia* leaf in aquatic environment

should be done with caution.

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