



Toxicological Evaluation of Ethanol Extract of *Adenium obesum* Stem Bark in African Catfish, *Clarias gariepinus*

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ABSTRACT: The toxicity of ethanol extract of *Adenium obesum* stem bark as a tool for aquaculture pond management prior to the stocking of desired fish species was evaluated in *Clarias gariepinus* over a 96-h exposure. The fish were exposed to 6.25 mgL⁻¹, 7.50 mgL⁻¹, 8.20 mgL⁻¹, 8.80 mgL⁻¹ and 10.00 mgL⁻¹ of the extract and a control in an acute static toxicity bioassay after performing a range finding test to determine the median lethal concentration (LC₅₀) of the extract. Exposed fish showed signs of changed behaviours with adaptive responses, respiratory distress and nervous compromise, including mortality in some of the exposed fish. The appearance and intensities of the observed signs were concentration and exposure period-dependent. An LC₅₀ value of 7.35 mgL⁻¹ was established for the extract in the exposed fish where mean mortality was significantly (p<0.05) concentration and exposure period-dependent. The toxic nature of the extract in the exposed hardy fish can be exploited for effective aquaculture pond management against some unwanted predatory and weed aquatic organisms. However, the indiscriminate use of higher extract concentrations could seriously jeopardise the biodiversity of any aquatic environment. @JASEM

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Aquaculture is the fastest growing food sector in the world, accounting for an estimated 43 % of all fish consumed by humans globally (Allsopp *et al.*, 2008). However, the presence of wild and unwanted organisms like frogs, molluscs, insect larvae and fish weeds is a common problem in extensive and semi-extensive aquaculture (Edet and Ikpi, 2008). These organisms greatly decrease aquaculture productivity via predation and competition for available oxygen, food and habitat. Fish farmers tackled this problem with the use of synthetic chemicals, which are non-biodegradable and accumulate in the environment to poses serious toxic threat to both targeted and non-targeted organisms (Randhawa and Kullar, 2011). This is unlike piscicidal plants that are bio-degradable and are more environmentally friendly with little or no residues (Stalin *et al.* 2008).

Adenium obesum is a typical piscicidal plant usually found throughout the Sahel region of Africa into Central Africa and the Arabia where it serves domestic, medicinal and toxicological purposes (Arbonnier, 2004; Oyen 2008). *Clarias gariepinus* is one of the many known predatory aquatic organisms because of its ability to cannibalise fish half its length or 10 % of its own body weight (de Graaf and Janssen, 1996). The fish is also hardy in nature (Hengsawat *et al.* 1997) because of its possession of accessory breathing organs (Safriel and Bruton,

1984). Therefore, piscicidal plants that can kill the fish will inadvertently jeopardise the survival of some other co-inhabitant (predatory and/or weed organisms) of the same aquatic environment. This property can be meaningfully explored for effective aquaculture pond management prior to the stocking of desired fish species. Although *A. obesum* has been used to kill fish globally (Badwen-Davis 2010), information about its median lethal concentration is scanty thereby hampering attempts to establish baseline concentrations of its toxicity viz-a-viz that which results in total fish kill. Therefore, the study aimed to evaluate the toxicity of ethanol extract of *A. obesum* stem bark in African catfish, *Clarias gariepinus*.

MATERIALS AND METHODS

Plant Extraction and Preparation: *Adenium obesum* were collected from the open fields of Rurum town, Rano Local Government Area, Kano State, Nigeria between January – April, 2011 and authenticated at the Herbarium, Department of Biological Sciences, Ahmadu Bello University (A. B. U.), Zaria, Nigeria with Voucher No. 1386 by Mallam Musa Mohammed. The stem bark was removed, sun-dried and pounded into powder where 3.95 kg of it was extracted with 21 L of ethanol (96.0 % vol. Sigma-Aldrich® Inc., St. Louis, MO 63178, USA) over a three-day (72-h) period to obtain a filtrate based on

the maceration methods of Bentley (1977) and Ghani (1990), which was concentrated to dryness as described by Abu-Dahab and Afifi (2007).

Toxicity Bioassay: Adult *Clarias gariepinus* were purchased from a commercial catfish farm (Fannasson Investments Limited, Kano, Nigeria) and authenticated at the Fishery Section, Department of Biological Sciences, A. B. U., Zaria, Nigeria. Fish acclimatization lasted for 21 days under natural day and night photo-periods (12/12-h) with complete changing of pond water once in every three days. Fish were fed to their satisfaction (*ad libitum*) twice daily with 6 mm Coppens® fish feed for aquacultures (Coppens® International bv., 5700 AM Helmond, Holland). Mortality was used as an end point of toxicity and this was determined as described in the OECD guideline No. 203 (1992).

A total of 126 *C. gariepinus* (265.50 ± 4.03 g mean weight and 32.85 ± 0.16 cm mean total length) were exposed to 6.25 mgL⁻¹, 7.50 mgL⁻¹, 8.20 mgL⁻¹, 8.80 mgL⁻¹ and 10.00 mgL⁻¹ of the extract and a control in a static acute toxicity bioassay over a 96-h exposure period (OECD guideline No. 203, 1992) in triplicates. This is after the performance of a range finding test to determine the five extract concentrations as described by Fafioye (2001) and the control with no extract.

Median lethal concentration (LC₅₀) determination: The median lethal concentration (LC₅₀) of the extract over the 96-h exposure period was determined using the Arithmetic method of Karber as adapted by Dede (1992) as follows:

$$LC_{50} = LC_{100} - \frac{\sum \text{Probit}}{\text{No. of fish per extract concentration}}$$

Where LC₁₀₀ = Extract concentration with 100 % fish mortality.

Physicochemical analyses: The temperature, pH, electrical conductivity and the total dissolved solids of the fish culture water were monitored with Hanna "Combo" portable hand instrument (Hi 98129, Hanna Instruments, Mauritius) while their dissolved oxygen content was monitored using the modified method of Winkler-Azide (Lind, 1979; APHA, 1985).

Statistical analyses: Data were expressed as mean (± SEM) and subjected to ANOVA and Tukey's multiple comparison test for statistical significance at p<0.05 using GraphPad software programme (GraphPad Prism, version 4.0, San Diego, California, USA.).

RESULTS AND DISCUSSION

The physicochemical parameters (temperature - 24.65°C; pH - 7.22; dissolved oxygen - 4.56 mgL⁻¹; total dissolved solids - 341.80 ppm and electrical conductivity - 680.30 µScm⁻¹) of the fish culture

water were within acceptable limits for the growth and survival of *C. gariepinus* (Viveen *et al.*, 1985; Peteri *et al.*, 1992). Exposed fish showed signs of toxicity in terms of changed behaviours with adaptive responses, respiratory distress and nervous compromise, which were concentration and exposure period-dependent. Changed behaviours with adaptive responses were characterized by repeated attempts to jump out of fish culture water, erratic and uncoordinated movements, aggression and excessive mucous secretions. These responses were survival strategies by the exposed fish to escape from the toxic aquatic environment as well as coat body surfaces so as to prevent the continuous absorption of the toxicant within their culture water.

The respiratory distress was characterized by frequent opercula movements and air gulping with the continuous exposure of snouts above culture water levels. These were attempts to increase ventilation rates to compensate for low oxygen uptake (Fernandes and Mazon, 2003) by passing large volume of water over gill surfaces at faster rates (Reebs, 2009). This is in addition to the engagement of aerial mode of respiration so as to disengage gill respiration and by implication, prevent continuous gill contact with the toxicant. The respiratory distress might be due to gill epithelia damage or excessive mucous coating of gill epithelia surfaces (Tamse *et al.*, 1995; Abalaka *et al.*, 2010). The nervous compromise was initially characterized by hyperactivity and later, by increasing states of motionlessness, adoption of different postures, sudden darts, swirling/sluggish movements and loss of balance. These might be due to the acetylcholinesterase inhibition property of the plant as similar hyperactivity and uncoordinated movements and inhibited acetylcholinesterase activity was reported in ticks exposed to aqueous extract of *A. obesum* stem bark (Mgbojikwe, 2000).

The toxicity of the extract might have resulted in the mortality observed in some of the exposed fish, which was significantly (p<0.05) concentration and exposure period-dependent as was earlier reported in ticks by Mgbojikwe (2000). The low LC₅₀ value of 7.35 mgL⁻¹ (Table 1) showed that the extract was toxic to the exposed fish as higher LC₅₀ values signify less toxicity (Eisler and Gardener, 1993) and vice versa. The toxic nature of the *A. obesum* might be the reason behind the global use of the plant to poison fish (Oyen, 2008, Badwen-Davis, 2010), which can be exploited for effective aquaculture pond management prior to the stocking of the desired fish species. However, great care should be exercised in the use of the plant for this purpose as it might cause total fauna and flora destruction at concentrations higher and above the established LC₅₀ value thereby interfering with the ecological balance of the aquatic environment.

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Table 1: Determination of the median lethal concentration (LC₅₀) of ethanol extract of *Adenium obesum* stem bark in adult *Clarias gariepinus* over a 96-h exposure period based on the Arithmetic method of Karber (adapted by Dede, 1992).

Extract concentration (mgL ⁻¹)	Concentration differences (A)	Mean mortality	Average mean mortality (B)	Probit (A x B)
0 (Control)	0	0.00	0.000	0.0000
6.25	1.25	1.00	0.500	0.6250
7.50	1.25	3.33	2.165	2.7063
8.20	0.70	5.00	4.165	2.9155
8.80	0.60	5.33	5.165	3.0990
10.00	1.20	10.00	7.665	9.1980
Σ				18.5438

(B): average of the sum of preceding and proceeding mean mortality

$$LC_{50} = 10 - \frac{18.5438}{7}$$

$$= 10 - 2.6491 = 7.3509$$

Therefore, LC₅₀ = 7.35 mgL⁻¹

Conclusion: Ethanol extract of *A. obesum* can be effectively used in aquaculture pond management against some unwanted predatory and weed aquatic organisms as the plant is proven to be toxic to the hardy *C. gariepinus*.

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