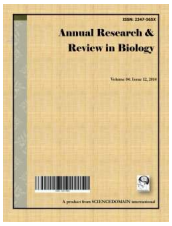

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Acute Toxicity of *Adenia cissampeloides* in Farmed African Catfish (*Clarias gariepinus*)

P. B. Ekpo[1], U. U. Uno[1,2*], C. M. Okolo[3], R. B. Agu[1] and C. F. Onwudike[1]

[1]Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria.
[2]Department of Biology, Cross River State College of Education, Akamkpa, Nigeria.
[3]Department of Biological Science, African Thinkers Community of Inquiry College of Education, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors PBE and UUU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CMO and RBA managed the analyses of the study. Author CFO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study evaluated acute toxicity of *Adenia cissampeloides* leaf extract on early life stages of farmed African catfish. 160 fingerlings were divided into four groups using a completely randomized design in a factorial layout and were exposed to 25, 50 and 100 mg/L of the extract for 24, 48, 72 and 96 hours, respectively while the control animals were kept without any treatment. The percentage mortality rate and acute – lethal toxicity (LC_{50}) were determined for the different durations of exposure. Results obtained indicated a significant dose – dependent increase in mortality in groups of animals treated with the extract of the plant whereas no mortality was recorded in the control group. Moreover, the duration of exposure also affected the mortality rate, with the highest percentage of mortality observed in groups of animals exposed the extract for 96 h. The LC_{50} for the *Adenia cissampeloides* extract to 96 h exposure was 42.38 mg/L with lower and upper confidence limits of 22.20 and 80.88 mg/L. Therefore, the study shows that *Adenia cissampeloides* extract has acute toxic effects on early life stages of farmed African catfish.

**Corresponding author: E-mail: ukamuno@yahoo.com;*

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1. INTRODUCTION

Globally, plants and their derivatives have been used extensively for several purposes including curing and managing of various ailments. Moreover, the ancient practice of poisoning fish as a method of harvesting fish is still practiced today. For example, *Tephrosia vogelii* is still been used for this purpose in Tropical America, Australia, Asia and Tropical Africa [1–3]. Though these plants and their derivatives are believed to be nontoxic compared to their synthetic counterparts, they may contain a number of harmful ingredients on their secondary metabolites which may have deleterious side effects including mutagenic potentials [4]. Hence the need for strict scientific study.

Adenia cissampeloides is a dioecious liana growing up to 30 m long with a stem up to 10 cm in diameter, striped bluish – green and older stem often with whitish powder. The stems have simple tendrils, 10 - 20 cm long. Leaves are alternate, simple, 0.5 – 1 mm long, broadly rounded or triangular and irregularly cleft [5–6]. The genus Adenia consists of about 95 species, about 60 of them occurring in the African continent, 20 in Madagascar and 15 in Asia. They are used mostly in Tropical America, Australia, Asia and Tropical Africa to harvest fish from streams and rivers. The plant contains cacogenic glycoside barterin (tetraphyllin B) and volkenis (epitetraphyllin B) in the leaves, fruits, stem and roots. The leaves also yield gummiferol, a cytotoxic polycetylenic diepoxide with *in vitro* anticancer activity [7].

A. cissampeloides has many uses in traditional medicine throughout tropical Africa. Most frequently recorded are the uses of an infusion or decoction of the root, stem or leaves for the treatment of gastro-intestinal complaints, such as stomach-ache, constipation, diarrhoea and dysentery. Such infusions or decoctions are also taken to treat various inflammatory ailments, commonly oedema and rheumatism, and for pain relief, particularly against headache and back pain. A decoction of the leaves or root is taken to treat fever and malaria, and as a diuretic. Pounded roots, and sometimes other plant parts, are widely used to dress wounds and sores. For leprosy, a decoction of the leaves is applied to the sores, and a root decoction taken orally together with a vapour bath prepared from the leaves. Ashes of the bark or root are mixed with castor oil to treat scabies. In eastern Africa, a root decoction is drunk to treat cholera and taken with milk, to treat anaemia. An extract of the root and stems is administered orally to treat intestinal worms. A leaf decoction is drunk to treat liver ailments. In Tanzania, a paste of the leaves is applied to broken bones and fractures [8–9].

The leaves and bark are also rich in iron and also possess hepatotoxic compounds with *in vitro* liver damaging activity and excessive use of the plant been associated with liver complaints [10]. Fresh leaves of the plant are used as vegetables in Ngbo in Ebonyi state in Nigeria [11]. Therefore, this study sought to evaluate acute toxicity of *Adenia cissampeloides* leaf extract on early life stages of farmed African catfish in Calabar, Cross River State of Nigeria.

2. MATERIALS AND METHODS

2.1 Experimental Fish

One hundred and sixty fingerlings of *C. gariepinus* with average body weight of 2.50 g were obtained from the University of Calabar fish farm. The fish were allowed to acclimatize to laboratory condition in an aquarium containing 100 L of de – chlorinated tap water. The water quality monitoring was carried out prior to, during and after the experiment. The physico – chemical parameters of the water were measured using the APHA [12] method of water quality assessment. All animals were treated in line with national regulations with approval from the ethical committee of the University of Calabar.

2.2 Collection and Preparation of Plant Material

The plant (*A. cissampeloides*) was collected from Idundu in Cross River State of Nigeria. The leaves were washed, air – dried for 48 h and then oven – dried for 6 h at 50℃. The dried leaves were then pulverized using an electric blender to fine powder. The powdered leaves were then subjected to Soxhlet extraction procedure using 70% ethanol as solvent. The extract was separated using rotary evaporator.

2.3 Experimental Design and Procedure

The 160 fingerlings were randomly divided into four groups using a completely randomized

design. The fish were exposed to three different concentrations of the *A. cissampeloides* extract (25, 50 and 100 mg/L) for 24, 48, 72 and 96 hours, respectively. The aquaria was set up for each concentration containing 10 fish. Control animals were kept under similar conditions without any treatment. Mortality was recorded every 24 hours throughout the 96 h exposure period. Fish were considered dead if they failed to respond to vigorous poking with a glass rod [13]. Dead fish were removed from the aquaria as soon as possible in order to prevent their bodies from decomposing.

2.4 Statistical Analysis

Data obtained were analyzed using analysis of variance (ANOVA) test. Significant means were separated using least significant difference (LSD) test. Mortality data were used to obtained the LC_{50} using the Probit Software (2000).

3. RESULTS

Results obtained for the physico – chemical parameter indicated that pH, total hardness, dissolved oxygen and temperature were 6.91, 56.48 mg/l, 3.23 mg/l and 26.06℃, respectively. Results also showed that there was no mortality in the control and group of fish exposed for 24 h. However, both the different concentrations of the treatment and durations of exposure had significant (P =.05) effect on the mortality of fish with the lowest percentage mortality (45.83%) observed in group of fish treated with 25 mg/L of the extract for 48 h while the highest percentage mortality of 70.86% was observed in fish treated with 100 mg/L for 96 h. The LC_{50} for 48, 72 and 96 hours are 90.03, 50.00 and 42.38 mg/L, respectively (Table 1).

In addition, some abnormal behaviours were observed in groups of fish exposed to the graded concentrations of the extract such as erratic swimming, respiratory distress, gulping of air and loss of balance. The degree of the various abnormal behaviours was also dose dependent.

4. DISCUSSION

The present study showed that *A. cissampeloides* extract significantly increased the mortality of the exposed fish in a dose – dependent manner which suggests acute toxic effect of the extract on the fish. This agrees with the findings of Ibiam et al. [11] who reported that *A. cissampeloides* has acute toxic effect on another *Clarias* genus species: *Clarias batrachus*.

The fish exhibited a range of abnormal behaviours at higher doses of the extract which is corroborated by the observations of Svecevicius [14] and Absalom et al. [15] who studied the acute toxicity of water soluble fractions (WSF) of kerosene on Nile tilapia, *Oreochromis niloticus* fingerlings. Previous studies [16–18] have shown that fish, when exposed to sub-lethal doses of toxicants, exhibit different behavioral changes to adapt them to the toxins. However, an increase in toxicant concentrations and exposure times usually leads to more severe reactions and ultimately to death, in line with the findings of this study.

Respiratory distress, erratic swimming and loss of balance observed in exposed fish might be due to neurotoxicity and/or mucous coagulation as a result of the treatment which resulted in higher respiratory rate, affected the physiology and caused death of the fish. This assertion is substantiated by Banerjee [19]. Studies have shown that animal behaviour has neurotropic regulation mechanism which is mediated by neurotransmitter substances [20–21]. The difficulty in breathing observed among the fish exposed to the treatment might also be as a result of respiratory impairment due to the effect

Table 1. Effect of *A. cissampeloides* on African catfish at different durations of exposure

Duration of exposure	Concentration of treatment		
	25 mg/L	50 mg/L	100 mg/L
24	0.00	0.00	0.00
48	45.83[a]	54.17[b]	54.17[b]
72	58.33[a]	66.67[b]	66.67[b]
96	66.67[a]	70.83[b]	70.86[b]
LC_{50} (mg/L) at 96 h	90.03 (50.18 – 161.47)	50.00 (31.54 – 83.34)	42.38 (22.20 – 80.88)

Values across the table with similar superscripts are not significantly different at 5% based on ANOVA

of the extract on the gills [18]. It was also observed that the LC_{50} values were within the 95% confidence limit with the longest duration of exposure (96 h) having LC_{50} of 42.38 mg/L which indicates the concentration at which that toxicity was glaring. This implies the lower the LC_{50} value, the higher the concentration and effect of the toxicant and the possibility that such extracts may represent a hazard for the environment and drinking water sources and they may accumulate in the meat of caught fish and represent a food safety hazard for the consumer.

5. CONCLUSION

The findings of the study indicate that *A. cissampeloides* leaf extract has acute toxic effect on farmed African catfish (*C. gariepinus*). Therefore, it is imperative to discourage its use in fishing in different water bodies as this can pose serious threat to the environment and drinking water sources. It can also affect humans when fish exposed to this toxicants are consumed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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