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Toxicological Evaluation of the Median Lethal Concentration (LC 50) of Aqueous Extract of *Adenium obesum* Stem Bark in African Catfish, *Clarias gariepinus* (Burchell 1822) Juveniles

Muyiwa BO[1*], Sambo JS[1] and Oniye SO[2]

[1] *Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

[2] *Department of Biological Sciences, Faculty of Natural Sciences, Ahmadu Bello University, Zaria, Nigeria*

[*]**Corresponding author:** Muyiwa BO, Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, Tel: +234-8050296826; E-mail: bmuyiwa2571@gmail.com

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Abstract

The study evaluate the acute toxicity of aqueous extract of *Adenium obesum* stem bark to determine the median lethal concentration (LC50) in exposed *Clarias gariepinus* under laboratory conditions using static non-renewal bioassays over a 96-h exposure with continuous aeration. The fish (N=180, mean weight and length 21.48 ± 3.32 g and 11.37 ± 1.23 cm), were randomly distributed 10 (ten) fish per group in triplicates. There were six (6) experimental groups G1 (Control), G2 (6.5 mg/l), G3 (7.8 mg/l), G4 (8.5 mg/l), G5 (9.5 mg/l) and G6 (11.5 mg/l). Exposed fish showed clinical signs of changed behaviors with adaptive responses, respiratory distress and nervous compromise, including mortality in some of the exposed fish. The clinical signs observed and their severity was concentration and exposure period-dependent. The LC 50 value of 8.38 mg/l was established for the extract in the exposed fish where mean mortality was significantly ($p<0.05$) concentration and exposure period-dependent. The phytochemical constituents and LC 50 of aqueous extract *Adenium obesum* stem bark evaluated will assist toxicologists and aquatic researchers in determining the safety concentrations of *Adenium obesum* in exposed *Clarias gariepinus* juveniles and aquatic studies.

Keywords: *Adenium obesum*; *Clarias gariepinus*; Toxicity; Median Lethal Concentration; Toxicology

Introduction

Adenium obesum (Forssk) Roem and Schult with synonyms: *Adenium somalense* Balf. f (1888); *Adenium socotranum* Vierh, (1904); *Adenium arabicum* Balf. f; *Adenium coetaneum* Stapf; *Adenium honghel* A. DC, *Nerium obesum Forssk*, belongs to the Family *Apocynaceae* [1-3]. The plant was discovered and described in Kenya in 1752 by a German Scientist, P. Forsskal. *Adenium* is an Arabic name of the plant, *Oddaejn,* which means Aden, which is the former name of Yemen [1] while obesum was derived from the swelling of the basal part of the plant stem [4] displayed in Figure 1. However, *A. obesum* is known locally in Nigeria as "Kariya" amongst the Hausa ethnic group [5,6] just as it is also called "Akpalataa" amongst the Igbo ethnic group of south-eastern zone of the country. Aqueous extract of *Adenium obesum* stem bark have been reported to have effect against some bacteria such as *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus* [6].

Figure 1: The plant *Adenium obesum.*

Phytochemical constituents of *Adenium obesum*

Several cardiac glycosides have been reported in *A. obesum* [7]. The main cardiac glycoside in the plant is Oleandrigenin β-gentiobiosyl (1 →4) β-D- thevetoside [8,9]. In addition, Oleandrigenin-β-D-glucosyl (1 →4)-β-D- digitalose was isolated from the chloroform fraction of the plant [10]. Hoffman and Cole reported the presence of other active cardenolides (Somalin, honghelosides A, 16-acetylstrospeside and

honghelin) and an active flavonol (3, 3-bis [o-methyl] quercetin) from the ethanol extract of *A. obesum.* Ethanol extract of *A. obesum* have been reported to contain an inactive triterpene (dihydroifflaionic acid) and an inactive flavonol 38 (3-0-methylkaempferol). The methanol extract of *A. obesum* stem bark has been reported to contain some alkaloids, flavonoids, saponins, tanins, glycosides, anthroquinones and steroids [11]. However, only saponins, tannins, steroids and glycosides were reported from the petroleum spirit extract of *Adenium obesum* stem bark [12]. Similarly, a triterpenoid named botulin (Lup-20 (29)-ene-3, 28-diol) was reportedly isolated from the stem bark of the plant [12]. Studies has shown the potential of *Adenium obesum* as a biological reducing agent and capping agent for the synthesis of Silver Nano particles

Adult *Clarias gariepinus* showed various signs of toxicity ranging from uncoordinated movements, repeated attempts to jump out of reconstituted extracts and excessive mucous secretions to increased opercula movements, exposed snouts, adoption of different postures and sudden darts when exposed to the ethanolic extract of *Adenium obesum* stem bark [13].

This study investigates the toxic effect of aqueous extract of *Adenium obesum* stem bark on *Clarias gariepinus* juveniles by determination of 96-hour LC 50 value using probit analysis in SPSS version 20.

Materials and Methods

Plant collection

The *Adenium obesum* stem bark was collected from Bassawa area within Zaria, Kaduna State Nigeria around November-December, 2016, and authenticated at the Herbarium section of the Department of Biological Sciences, A.B.U, Zaria, where a specimen was deposited and a voucher number 01386 was assigned. The leaves was picked and dried under shade until constant weight was obtained. The dried leaves were crushed into coarse powder using a pestle and mortar and stored for the extraction process.

Plant extraction

The stem bark of *Adenium obesum* was dried under shade until constant weight is obtained, stem bark were crushed into coarse powder using a pestle and mortar and stored for the extraction process. The fine powder was added into distilled water and shaken gently for ten minutes using a shaker to make a homogenous mixture. The mixture was left for 24 hours and then filtered. The filtrate was used for the study; the extraction process was carried out as described by Saravanan [14].

Preliminary phytochemical screenings was conducted on the aqueous extract of *Adenium obesum* stem bark in order to confirm the presence of phytochemical constituents following the methods described by [15,16].

Experimental animals

An ethical clearance approval was given by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with approval number ABUCAUC/2017/014 for this study.

The live juvenile of the African catfish, *C. gariepinus* (N=180, average weight and weight of (21.48 ± 3.32) g and length of (11.37 ± 1.23) cm respectively were purchased from a commercial catfish farm of reputable standing 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. The fish were fed to their satisfaction (ad libitum) twice daily with 2 mm Coppens® fish feed for aquaculture (Coppens® International by Helmond, Holland). A range finding test to determine the five extract concentrations as described by Fafioye et al. [17] was performed. Mortality was used as an end point of toxicity and this was determined as described in the OECD guideline No. 423.

Toxicity bio-assay

After acclimatization experimental fishes were selected at random and were kept in a static system of water. The feeding was stopped one day prior to exposure to aqueous extract *Adenium obesum* stem bark and fishes were not fed throughout the test. The acute toxicity tests were performed according to the static nonrenewable bioassay procedure [18]. The experimental design consisted of a control and five concentrations (6.5, 7.8, 8.5, 9.5, and 11.5 mg/l) with 10 (ten) fish per group in triplicate. A glass aquarium of 40 litres capacity with aeration was used per each group in replicate. Each glass aquarium was covered with nylon mesh tied firmly with rubber strap to prevent the fish from jumping out. Fishes showing no respiratory movement and response to tactile stimuli were considered as dead and removed immediately. During the exposure in different concentrations of aqueous extract *Adenium obesum* stem bark, the behavioral changes of the fishes were also recorded. Daily physicochemical characteristics, temperature and pH of fish culture water were ascertained using a Hanna ''Combo portable hand instrument (Hi 98129, Hanna Instrument, Mauritius) while their dissolved oxygen contents were similarly established using the modified Winkler-Azide method [19,20].

Statistical analyses

Data was expressed as mean ± SEM and then subjected to Two-way Analysis of Variance (ANOVA) for statistical significance at $p<0.05$. Tukey's multiple comparison tests for means was used to compare differences between the various means using SPSS version 20.

Results

The extraction process of 3 kg *Adenium obesum* stem bark yielded a total of 207.53 g, *Adenium obesum* stem bark aqueous extract and an extractive yield of 6.91% w/w was obtained. The qualitative constituents of aqueous extract of *Adenium obesum* stem Bark is presented in Table 1.

Constituents	Test	Qualitative analysis
Carbohydrates	Molisch test	+
Anthraquinones	Borntrager test	-
Glycosides	Fehling test	+
Cardiac glycosides	Kellar-Killant test	+
Saponins	Frothing test	+
Steroids and Triterpenes	Lieberman-Burchard	+

Tanins	Ferric chloride test	+
Flavonoids	Shinoda test	+
Alkaloids	Dragendorff test	+
Keys: +=present, -=absent		

Table 1: Phytochemical constituents of aqueous extract of *Adenium obesum* stem bark.

The physico-chemical parameters across the groups was nonsignificant ($p>0.05$) for oxygen and temperature, while there was a nonsignificant ($p>0.05$) increase between the control and the other groups for pH, there was a significant ($p<0.05$) increase in the total dissolved solids (TDS) and electric conductivity (µs/cm) is presented in Table 2. The behavioral display of the exposed fish is presented in Table 3.

Group	PH	TEMP (°C)	DO (mg/l)	TDS (mg/l)	Electric Conductivity (µs/cm)
1	7.01 ± 0.01a	27.01 ± 0.01a	4.87 ± 0.01a	3.37 ± 0.01a	35.02 ± 0.12a
2	8.00 ± 1.01b	27.02 ± 0.01a	4.88 ± 0.03a	46.01 ± 3.01b	124.22 ± 1.42b
3	8.01 ± 0.11b	27.00 ± 0.00a	4.79 ± 0.12a	53.02 ± 3.22c	231.10 ± 2.67c
4	8.02 ± 0.01b	27.00 ± 0.00a	4.89 ± 0.11a	77.13 ± 6.13d	354.22 ± 4.11d
5	8.07 ± 0.02b	27.00 ± 0.00a	4.91 ± 0.20a	86.05 ± 7.55e	397.11 ± 4.87e
6	8.13 ± 0.01b	27.01 ± 0.02a	4.88 ± 0.03a	107.02 ± 9.65f	499.81 ± 6.31f

Table 2: Physicochemical parameters of the different concentrations for acute toxicity test (Data are presented as means ± SEM. KEYS: G1 (Control), G2 (6.5 mgl), G3 (7.8 mg/l), G4 (8.5 mg/l), G5 (9.5 mg/l), G6 (11.5 mg/l); Values in each column with different superscripts are significantly different at $p<0.05$).

Variables	Extract	Exposure period			
Clinical signs	(mg/l)	24-h	48-h	72-h	96-h
Exposed snout	G1	-	-	-	-
	G2	-	+	±	+
	G3	-	+	±	+
	G4	-	+	±	+
	G5	-	+	-	+
	G6	-	+	-	+
Motionless	G1	-	-	-	-
	G2	-	+	+	+
	G3	-	+	+	+
	G4	-	+	+	+++
	G5	-	+	+	+++
	G6	-	+	++	+++
Different postures (Vertical, Angular, Flat)	G1	-	-	-	-
	G2	-	+	+	+
	G3	-	+	+	++
	G4	-	+	++	+++
	G5	-	+	++	+++
	G6	-	++	++	+++

Sudden dart	G1	-	-	-	-
	G2	-	+	+	+
	G3	-	+	+	++
	G4	-	+	++	+++
	G5	-	+	++	+++
	G6	-	++	++	+++
Swirling and/or sluggish movements	G1	-	-	-	-
	G2	-	+	+	+
	G3	-	+	+	++
	G4	-	+	++	++
	G5	-	+	++	+++
	G6	-	++	++	+++
Loss of balance	G1	-	-	-	-
	G2	+	+	+	+
	G3	+	+	+	++
	G4	+	+	++	+++
	G5	+	+	++	+++
	G6	++	++	++	+++
G1, Control (No Extract), G2 (6.5 mgl), G3 (7.8 mg/l), G4 (8.5 mg/l), G5 (9.5 mg/l), G6 (11.5 mg/l)					

The analysis showing probit mortality responses and concentration is presented in Table 4.

Group	Extract Conc (mg/l)	Log Conc	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
1	0	0	30	0	0	0	0
2	6.5	.813	30	10	9.813	.187	.327
3	7.8	.892	30	14	15.601	-1.601	.520
4	8.5	.929	30	22	19.478	2.522	.649
5	9.5	.978	30	23	24.225	-1.225	.808
6	11.5	1.061	30	29	28.865	.135	.962

Table 4: The mortality of *Clarias gariepinus* at 96 h after exposure to different concentrations of aqueous extract *Adenium obesum* stems bark.

The LC 50 value of 8.38 mg/l with a lower confidence limited (LCL) of 5.218 mg/l and an upper confidence limit (UCL) of 9.374 mg/l with 95% confidence limited determined by SPSS version 20 is presented in Table 5. The relationship between the probit mortality to logarithm of concentration is presented in Figure 2.

Probability	95% Confidence limits for concentration			95% Confidence Limits for log (Concentration)[a]		
	Estimate	Decrease Bound	Upper Bound	Estimate	Decrease Bound	Upper Bound

LC1	5.355	1.183	7.035	0.729	0.073	0.847
LC2	5.644	1.411	7.262	0.752	0.149	0.861
LC3	5.836	1.577	7.411	0.766	0.198	0.870
LC4	5.984	1.714	7.526	0.777	0.234	0.877
LC5	6.108	1.835	7.620	0.786	0.264	0.882
LC6	6.215	1.945	7.702	0.793	0.289	0.887
LC7	6.310	2.046	7.775	0.800	0.311	0.891
LC8	6.397	2.141	7.841	0.806	0.331	0.894
LC9	6.477	2.231	7.901	0.811	0.349	0.898
LC10	6.551	2.317	7.957	0.816	0.365	0.901
LC15	6.868	2.711	8.196	0.837	0.433	0.914
LC20	7.131	3.071	8.394	0.853	0.487	0.924
LC25	7.365	3.415	8.371	0.867	0.533	0.933
LC30	7.581	3.757	8.735	0.880	0.575	0.941
LC35	7.788	4.102	8.893	0.891	0.613	0.949
LC40	7.988	4.457	9.050	0.902	0.649	0.957
LC45	8.188	4.827	9.209	0.913	0.684	0.964
LC50	8.388	5.218	9.374	0.924	0.718	0.972
LC55	8.394	5.636	9.50	0.934	0.751	0.980
LC60	8.809	6.088	9.743	0.945	0.784	0.989
LC65	9.036	6.581	9.966	0.956	0.818	0.999

Table 5: Probit analysis aqueous extract of *Adenium obesum* stem bark.

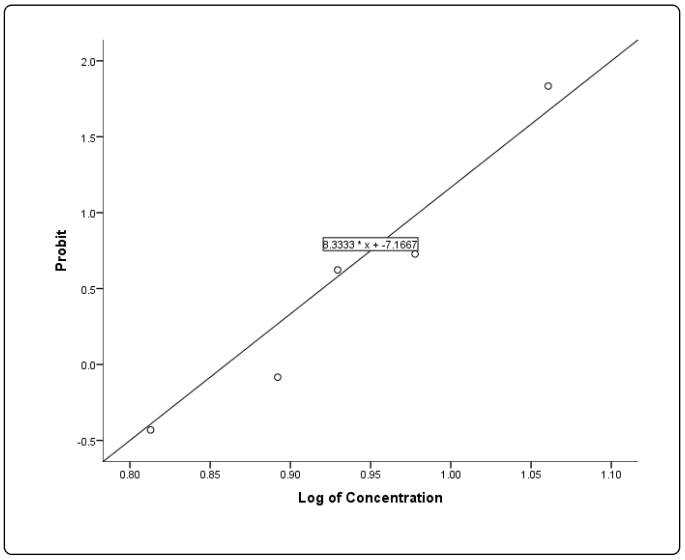

Figure 2: Linear relationship between mean probit mortality and log concentration of *C. gariepinus* juveniles exposed to aqueous extract *Adenium obesum* for 96 hours.

Discussion

Acute bioassay of toxicants/botanicals is an important procedure in aquatic ecotoxicology and toxicopathological fields. The aim of such study is to determine and establish the lethal concentration of toxicants/botanicals or their mixtures that can be tolerated by aquatic organisms in an acute exposure and also assist in establishing/setting the sub lethal concentrations of such toxicants/botanicals in research studies. The phytochemical findings in this study revealed the presence of alkaloids, saponins, tannins, cardiac glycosides, glycosides and flavonoid similar finding was reported by Abalaka et al. [13] who reported that the ethanol extract and aqueous extract of *Adenium obesum* stem bark, respectively contained carbohydrates, glycosides, tannins, cardiac glycosides, saponins and flavonoids, steroids and triterpenes in addition to resin. Authors of Tijjani et al. [11,12,21] reported the presence of alkaloids, saponins, tannins, glycosides, anthraquinones and steroids in addition to triterpenoid, betulin from

methanol, petroleum spirit and the petroleum ether extracts of *A. obesum* stem bark. The differences noticed in the phytochemical constituents of *Adenium obesum* extract extract among these authors, especially as it relates to the presence/absence of resins, botulin and anthraquinones may be due to the extraction methods used, the age and parts of the plants used, genetic variability between species, climatic conditions and the nature of the soil profile upon which the plant was cultivated [22].

Physico-chemical parameters such as temperature, pH, dissolved oxygen, pH, electric conductivity and total dissolved solids are important aquatic parameters that determine fish health, growth and reproduction. In this study, the TDS and the electric conductivity are different from the control except for temperature, pH and the DO. The pH of the water samples varied from concentration to concentration and the values obtained for the different treatments where lower than the standard of 6.5 to 8.5 [23], the increase in pH with time may be due to the production of basic products of metabolism. In the acute bioassay of *Clarias gariepinus* exposed to sponge plant fruit extract recorded a low pH. TDS and Electrical conductivity also increased across the different treatments, this may be due to the chemical composition of *Adenium obesum.* DO is one of the most important factor for all living organisms especially fish survive. The DO in this study did not decreased as was recorded but rather was steadily maintained throughout the study, this may be due to the continuous aeration provided by aerators within the system. The reduction in the DO content in a bioassay media has been reported to reduce as toxicant concentration increases which may be due to antioxidant property of the toxicant [24]. The physico-chemical parameters monitored in this study tend to have contributed little or none to the toxicity of *Adenium obesum* stem bark extract.

The behavioral responses noticed due to the toxicity of the aqueous extract of *Adenium obesum* in this study is similar to the findings of previous investigators on *Clarias gariepinus* juveniles exposed to different plant extracts [13,25-27]. Similarly, Ubahe et al. [28] observed irrational behavior in *Clarias gariepinus* exposed to *Hypoestes forskalei* extract, and these include vigorous movement, fast back stroke movement, restlessness, increased opercular movement and jumping. The erratic behaviour prior to death in the present and past studies may be associated with the impact of toxicants on fish. The excessive mucus secretions observed in the exposed fish in this study has also been reported [29,30]. Excessive mucus secretions are part of natural defense mechanisms by exposed fish to coat their body surfaces in order to prevent and/or reduce the absorption of the offending toxicant. However, such excessive mucus secretions are reported to reduce respiratory activity in fishes. This present observation might be due to bio-transformation of the active toxic constituents of the plant's extract over time, especially as the extract concentrations were not maintained daily throughout the exposure period [31,32]. The appearance and intensity of clinical signs in the contact phase of the fish toxicity study of juvenile *Clarias gariepinus* exposed to aqueous *Adenium obesum* extract was concentration-dependent as these increased with increasing extract concentrations. The initial agitations and restlessness characterized by erratic movements and repeated attempts of the fish to jump out of the culture water in the contact phase of the fish toxicity study were natural avoidance response to escape from the toxic aquatic environment. This was possibly to prevent the continuous absorption of the toxicant in the water environment. This is a normal adaptation response, which allows fish to survive in altered environment [33]. There was progressive respiratory distress, characterized by increased opercula movements, air gulping and exposed snouts above water surfaces. Respiratory impairment may arise from increasing gill cellular damage and/or increasing accumulation of elaborated mucous on gill surfaces [17,30,34]. *Oreochromis niloticus* juveniles treated with fresh root bark extract of *Moringa oleifera* also displayed these behavioral and clinical signs.

The established LC 50 value of 8.38 mg/l with a lower confidence limited (LCL) of 5.218 mg/l and an upper confidence limit (UCL) of 9.374 mg/l showed that the extract was very toxic to the exposed fish. There was strong correlation (0.949) between increase in concentrations of aqueous extract *Adenium obesum* stem bark and mortality of the exposed fish [13], reported LC 50 value of 7.15 mg/l in Adult *Clarias gariepinus* exposed to ethanoic extract of *Adenium obesum* stem bark, the difference in the LC 50 values of these studies may be due to extraction methods employed and range finding concentration values of the extract used to determine the concentrations for the definitive test. Studies have reported different LC 50 for various plant extracts. However, the toxicities of other plants' extracts on fish having similar results have been reported such as *Blighia sapida* and *Kigelia africana* on *C. gariepinus, Parkia biglobosa* and *Raphia vinifera* on *C. gariepinus* and Tilapia [17], Tobacco on *O. niloticus* and *C. gariepinus* [35-47], *Raphia hookeri* on *C. gariepinus.*

Conclusion

The cause of mortality in this study was anoxia, as a result of the excessive mucus coating of the secondary lamellae which eventually leads to the suffocation of the fish due to the continuous assault of the toxicant on the exposed fish, respiration is compromised as shown by fish exposed snout with gradual degeneration to other clinical signs. From the results obtained, it can be concluded that the aqueous extract of *Adenium obesum* stem bark is toxic to exposed *Clarias gariepinus* juveniles.

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