

## Original Research Article

# Haematotoxicity of Ethanol Extract of *Adenium obesum* (Forssk) Roem & Schult Stem Bark in Wistar Rats

Samson Eneajo Abalaka<sup>1\*</sup>, Muhammad Yakasai Fatihu<sup>1</sup>, Najume Doguwar Giginya Ibrahim<sup>1</sup> and Suleiman Folorunsho Ambali<sup>2</sup>

<sup>1</sup>Department of Veterinary Pathology, <sup>2</sup>Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria

\*For correspondence: **Email:** [seabalaka@yahoo.co.uk](mailto:seabalaka@yahoo.co.uk); **Tel:** +234-8037863462

Received: 11 January 2014

Revised accepted: 12 October 2014

### Abstract

**Purpose:** To investigate the acute toxicity of ethanol extract of *Adenium obesum* (Forssk.) Roem. & Schult stem bark in Wistar rats in relation to haematological parameters.

**Methods:** This involved the administration of single dose of 300 mgkg<sup>-1</sup>, 2000 mgkg<sup>-1</sup> and 5000 mgkg<sup>-1</sup> of the extract by oral gavage separately to three different groups of female rats (n = 3) one after another based on the absence of mortality and/or morbidity during a 14-day observation period. The control group was administered distilled water as placebo (1 mL per 100 g body weight).

**Results:** The exposed rats did not show any obvious signs of toxicity, morbidity or mortality. Median lethal dose (LD<sub>50</sub>) of the extract was ≥ 5000 mgkg<sup>-1</sup> or ∞ (unclassified) based on the fixed LD<sub>50</sub> cut-off values. Final body weight of control rats (196.00 ± 3.06 g) was significantly (p < 0.05) higher than the initial body weight (184.30 ± 1.45 g) and weight gain in extract treated groups was not significant (p > 0.05). Packed cell volume, red blood cell count and haemoglobin concentrations in the rats (42.67 ± 1.33 %, 5.10 ± 0.20 × 10<sup>12</sup> L<sup>-1</sup> and 130.70 ± 2.96 gL<sup>-1</sup>, respectively) did not change significantly (p > 0.05). However, the white blood cell count significantly increased from 7.50 ± 0.63 × 10<sup>9</sup> to 11.63 ± 0.50 × 10<sup>9</sup> L<sup>-1</sup> while the lymphocyte count significantly increased from 5.81 ± 0.43 × 10<sup>9</sup> to 9.99 ± 0.42 × 10<sup>9</sup> L<sup>-1</sup> (p < 0.05) at the highest extract dose (5000 mgkg<sup>-1</sup>) compared to their respective controls.

**Conclusion:** *Adenium obesum* might not be haematotoxic and is considered a safe medicinal plant administered orally.

**Keywords:** *Adenium obesum*, Haemoglobin, Blood count, Mortality, Morbidity, Haematotoxicity

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

## INTRODUCTION

*Adenium obesum* (Forssk) Roem & Schult is an ornamental plant that is cultivated worldwide because of its characteristically pink “showy” flowers that confers on it the name “Desert rose” [1]. It is a deciduous pachycaul with half buried swollen base and twisted branches [1], which may grow up to six meters high [2]. Despite the fact the plant can be found worldwide, it is indigenous to the Sahel region of Africa and

Central Africa, and the Arabia [3]. The plant is locally known as “Kariya” and “Akpalataa” amongst the Hausa and Igbo ethnic groups of northern and south-eastern Nigeria, respectively. The plant is used to kill fish in addition to being a potent arrow poison where targeted large animals normally die within two kilometers from the place of first contact [2]. This notwithstanding, *Adenium obesum* is also a reputable medicinal plant that is chewed as an abortifacient to produce miscarriages or induce

abortions [3]. Its latex is used to treat decaying teeth, boils and septic wounds while a decoction of its roots is used as nose drops for rhinitis in Somalia [2]. However, there is dearth of information on structured investigations into acute toxicity of the plant in exposed animals, especially as it relates to its haematotoxicity in exposed rats thereby necessitating the present study. This is because the haematopoietic system is one of the most sensitive targets of toxic compounds and also an important index of physiological and pathological status in man and animals [4]. Therefore, the study aimed to evaluate haematotoxicity of ethanol extract of *Adenium obesum* stem bark in Wistar rats.

## EXPERIMENTAL

### Plant collection and extraction

Following approval by Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) (reference number ABUCAUC/2014/006) in compliance with the Guide for the Care and Use of Laboratory Animals [5], *A. obesum* was gathered from the open fields of Rurum town in Rano Local Government Area of Kano State, Nigeria between January – April, 2011. Plant authentication was carried out by Mallam Musa Mohammed of the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria where a voucher specimen no. 1386 was deposited. The barks were sun-dried and pounded into powder for use after they were removed from the stems.

Ethanol extraction was performed by maceration of 3.95 kg of the powdered stem bark in 21 litres of ethanol (96.0 % vol. Sigma-Aldrich® Inc., St. Louis, MO 63178, USA) over a 72-h period to obtain a filtrate that was concentrated to dryness in an evaporation dish at room temperature until a constant weight was obtained [6].

### Rat toxicity bioassay

Twelve female rats of  $180.80 \pm 4.55$  g mean weight were sourced from the Animal Unit of National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. These were acclimatized for seven days in clean metal cages in a well ventilated room under natural photoperiod (12/12-h). Experimental rats were provided with fresh drinking water and fresh NVRI locally formulated pelleted rat feed (crude protein - 19.97 %, crude fibre - 6.89 %, Ash - 7.60 %, nitrogen free extract - 59.21 % and moisture - 12.98 %) *ad libitum* during the period.

OECD guideline no. 423 [6] was used to perform the rat acute toxicity bioassay. The extract was administered by oral gavage to the recommended three female rats per group in a step-wise procedure (one group after another) depending upon the morbidity and/or mortality status of the preceding treated rats as follows:

**Table 1:** Oral administrations of ethanol extract of *Adenium obesum* stem bark to Wistar rats by gavage

Experimental group	Number of female rats	Extract dose
I	3	Control
II	3	300 mgkg <sup>-1</sup>
III	3	2000 mgkg <sup>-1</sup>
IV	3	5000 mgkg <sup>-1</sup>

Control rats were administered distilled water placebo (1 mL per 100 g body weight). The protocol provided for the use of 5 mgkg<sup>-1</sup>, 50 mgkg<sup>-1</sup>, 300 mgkg<sup>-1</sup> and 2000 mgkg<sup>-1</sup> fixed doses. However, a starting dose of 300 mgkg<sup>-1</sup> was recommended where there is no scientifically proven information on the substance to be tested for animal welfare reasons as was the case in this study. This is in addition to allowing the use of 5000 mgkg<sup>-1</sup> dose only if a strong likelihood exist that the result have a direct relevance for human, animal and environmental protection due to same animal welfare reasons, especially as *A. obesum* is used to kill fish for human consumption.

Experimental rats were observed within the first 30 minutes of administration and daily thereafter throughout the 14-day observation period for signs of toxicity. Adverse changes in body weights were also used as a measure of toxicity [8] while mortality was used as an end point of toxicity. Determination of the median lethal dose (LD<sub>50</sub>) of the extract was based on OECD guideline no. 423 [7].

### Haematological evaluation

Blood (10 ml) were collected into sample bottles containing EDTA anticoagulant from all the experimental rats at the end of the 14-day observation period via vene-section after light chloroform anaesthesia. The packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) count, white blood cell (WBC) count, WBC differential count, including RBC indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were analyzed based on standard procedures [9] using an auto-analyzer (Sysmex XT 2000i. S/No. 12386. Sysmex Corporation, Kobe 650=8691, Japan).

## Data analysis

Data were analyzed (mean  $\pm$  SEM) and subjected to a one-way analysis of variance (ANOVA) for statistical significance at  $p < 0.05$  as well as Tukey's multiple comparison test for differences between the various means using GraphPad Prism 4 (GraphPad Prism, version 4.0, San Diego, California, USA).

## RESULTS

### Toxicity bioassay

The extract treated rats did not show obvious signs of toxicity nor was death recorded in any of the groups treated with the extract. Thus there were no changes in skin, fur, eyes and mucous membranes. Tremors, salivation, diarrhea, sleep, convulsions or coma did not also occur. Therefore, an LD<sub>50</sub> value  $\geq 5000$  mgkg<sup>-1</sup> or  $\infty$  (unclassified) based on the fixed LD<sub>50</sub> cut-off was established for ethanol extract of *A. obesum* stem bark in the treated rats. Changes in body weights of experimental rats are as shown in Table 2. Although weight gains were recorded in

experimental rats, this was significant ( $p < 0.05$ ) only in control rats.

### Haematological profile

Haematological changes in rats exposed to the extract are as shown in Table 3. Significant changes ( $p < 0.05$ ) were recorded in the leukocytosis and lymphocytosis only at the highest extract dose (5000 mgkg<sup>-1</sup>) compared to the controls.

## DISCUSSION

The toxicity of ethanol extract of *A. obesum* stem bark was very low in the exposed rats from the observed absence of obvious signs of toxicity as affirmed by the very high LD<sub>50</sub> of  $\geq 5000$  mgkg<sup>-1</sup>. The very low toxicity of the extract was further reinforced by the respective weight gains in the exposed rats instead of adverse effects on their body weights, which is indicative of toxicity [7]. This is in spite of the fact that *A. obesum* is a globally recognized arrow poison [1] where the toxic phytoconstituents of the plant are delivered parenterally. Therefore, the oral delivery of the toxic phytochemicals, as in the present study, might have greatly compromised their bioavaila-

**Table 2:** Changes in body weights (mean  $\pm$  SEM) of Wistar rats orally administered ethanol extract of *Adenium obesum* stem bark by gavage

Extract dose	Body weight (g)		
	0-day	7-day	14-day
Control	184.30 $\pm$ 1.45	192.30 $\pm$ 2.73	196.00 $\pm$ 3.06
300 mgkg <sup>-1</sup>	163.30 $\pm$ 9.39	169.00 $\pm$ 12.06	169.0 $\pm$ 13.05
2000 mgkg <sup>-1</sup>	182.30 $\pm$ 4.06	190.00 $\pm$ 4.36	192.00 $\pm$ 2.00
5000 mgkg <sup>-1</sup>	193.30 $\pm$ 10.48	197.70 $\pm$ 7.17	208.00 $\pm$ 4.16

Values with asterisks are significantly ( $p < 0.05$ ) different from the controls

**Table 3:** Haematological profile (mean  $\pm$  SEM) of Wistar rats orally administered ethanol extract of *Adenium obesum* stem bark by gavage

Haematological parameter	Extract dose			
	Control	300 mgkg <sup>-1</sup>	2000 mgkg <sup>-1</sup>	5000 mgkg <sup>-1</sup>
PCV (%)	42.67 $\pm$ 1.33	39.00 $\pm$ 1.00	41.00 $\pm$ 1.00	42.00 $\pm$ 1.16
Hb (gL <sup>-1</sup> )	130.70 $\pm$ 2.96	133.00 $\pm$ 4.36	132.00 $\pm$ 1.00	138.30 $\pm$ 2.03
RBC (x 10 <sup>12</sup> L <sup>-1</sup> )	5.10 $\pm$ 0.20	4.60 $\pm$ 0.10	4.73 $\pm$ 0.13	4.97 $\pm$ 0.23
MCV (fL)	85.00 $\pm$ 1.16	85.00 $\pm$ 1.00	86.50 $\pm$ 0.50	86.00 $\pm$ 1.00
MCH (pg)	26.00 $\pm$ 1.16	27.50 $\pm$ 0.50	28.00 $\pm$ 0.57	28.00 $\pm$ 0.57
MCHC (gdL <sup>-1</sup> )	33.00 $\pm$ 0.57	33.00 $\pm$ 0.57	33.30 $\pm$ 1.00	33.50 $\pm$ 1.50
WBC (x 10 <sup>9</sup> L <sup>-1</sup> )	7.50 $\pm$ 0.63	7.90 $\pm$ 0.30	8.40 $\pm$ 0.70	11.63 $\pm$ 0.50*
Neut. (x 10 <sup>9</sup> L <sup>-1</sup> )	1.68 $\pm$ 0.21	1.33 $\pm$ 0.20	1.32 $\pm$ 0.09	1.51 $\pm$ 0.31
Lymph. (x 10 <sup>9</sup> L <sup>-1</sup> )	5.81 $\pm$ 0.43	6.07 $\pm$ 1.37	5.95 $\pm$ 0.83	9.99 $\pm$ 0.42*

Values with asterisks are significant ( $p < 0.05$ ) different from the controls; PCV – packed cell volume; Hb – haemoglobin concentration; RBC – red blood cell; MCV – mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; WBC – white blood cell; Neut – neutrophil; Lymph. – lymphocyte

bility resulting in the recorded very low toxicity of the extract in the exposed rats. This might be due to the biotransformation of the extract within the gastrointestinal tract or metabolism within the liver. Builders *et al* [10] reported similar LD<sub>50</sub> of above 5000 mgkg<sup>-1</sup> as well as no adverse effect on body weights of rats exposed to extracts of *Parkia biglobosa* stem bark.

The decreased PCV, RBC, MCV, MCH and MCHC as well as the increased Hb concentrations notwithstanding, the fact these changes were non-significant ( $p > 0.05$ ) showed that the extract did not cause anaemia or has considerable effects on the oxygen carrying capacity of the exposed rats. The significant ( $p < 0.05$ ) increase in WBC and lymphocyte counts at the highest extract dose (5000 mgkg<sup>-1</sup>) in the exposed rats was indicative of immunogenic response. Similar response was reported in rabbits exposed to aqueous extract of *Moringa oleifera* and *Caulis bambusae* leaves [11]. Although the non-concentration dependent nature of these haematological changes was not well understood, similar variable patterns of responses were reported in exposed rats exposed to ethanolic extract of *Xylopi aethiopica* [12].

The obtained results showed the non-toxic nature of the extract in treated rats suggestive of the inability of the extract to cause oxidative damage, which might be due to its antioxidant phyto-contents. This is because the extract is reported to contain some alkaloids, saponins, tannins and flavonoids [13], which are all rich in antioxidant activities [14]. The non-toxic nature of the extract affirms the traditional ethnomedicinal use of the plant where its immunogenic property can be further exploited to enhance body defence. However, repeated use of high dose of extracts of the plant over a long time period, as it is always with our traditional healers, should be discouraged until a therapeutic dose is effectively determined for a broader and safer clinical ethnomedicinal use of the plant.

## CONCLUSION

The ethanol extract of *A. obesum* stem bark was not haematotoxic to orally exposed rats in spite of the fact that the plant is a known potent arrow poison when administered parenterally. Therefore, the plant might be a safe oral medicinal plant.

## ACKNOWLEDGEMENT

We appreciate the contributions of Mr Abdulsalam Umar and Dr Chidiebere Uchendu to the work.

## REFERENCES

1. McLaughlin J, Garofalo J. Desert Rose (*Adenium obesum*). Miami-Dade County/University of Florida Cooperation Extension Services Fact-Sheet No. 17, 2002 [cited 2010 Dec 21]. Available from: [http://www.miami-dade.ifas.ufl.edu/pdfs/ornamental/ornamental\\_publications/desert-rose.PDF](http://www.miami-dade.ifas.ufl.edu/pdfs/ornamental/ornamental_publications/desert-rose.PDF).
2. Oyen, Leo PA. *Adenium obesum* (Forssk.) Roem. & Schult. In: Schmelzer, Gaby H.; Gurib-Fakim, Ameenah, Eds. *Plant Resources of Tropical Africa 11 (1): Medicinal Plant 1*. Wageningen: PROTA Foundation/Backhuys Publishers/CTA; 2008; pp 46-49.
3. Arbonnier, Michel. *Trees, Shrubs and Lianas of West African Dry Zones*. GMBH, MNHN: CIRAD, MARGRAF Publishers; 2004; p 161.
4. Adeneye AA, Ajagbonna OP, Adeleke, TI, Bello, SO. Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats. *J Ethnopharmacol* 2006; 105: 374-379.
5. National Research Council (NRC). *Guide for the care and use of laboratory animals*. Washington DC: The National Academic Press; 1996.
6. Abu-Dahab R, Afifi F. Anti-proliferative activity of selected medical plants of Jordan against a breast adenocarcinoma cell line (MCF7). *Sci Pharm* 2007; 75: 121-136.
7. Organisation for Economic Co-operation and Development (OECD). *Guidelines for the Testing of Chemicals No. 423: Acute Oral Toxicity – Acute Toxic Class Method (adopted: 17th December, 2001)*. Paris: Organisation for Economic Co-operation and Development; 2001; pp 1-14.
8. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90-day oral gavage toxicity study of D-methylphenidate and D, L-methylphenidate in Sprague-Dawley rats. *Toxicol.* 2002; 179: 183-196.
9. Dacie JV, Lewis SM. (1975) *Practical haematology*. 5th edition. London, Churchill Livingstone; 1975.
10. Builders MI, Isichie CO, Aguiyi JC. Toxicity studies of the extracts of *Parkia biglobosa* stem bark in rats. *British J Pharmaceut Res* 2012; 2 (1): 1-16.
11. Isitua CC, Ibeh IN. Toxicological assessment of aqueous extract of *Moringa oleifera* and *Caulis bambusae* leaves in rabbits. *J Clin Toxicol* 2013; S12: 003. doi:10.4172/2161-0495.S12-003.
12. Woode E, Abaidoo CS, Alhassan A. An evaluation of the effect of ethanolic extract of *Xylopi aethiopica* on

- haematological and biochemical parameters in male rats. Der Pharmacia Sinica* 2011; 2 (2): 39-45.
13. Abalaka SE, Fatihu MY, Ibrahim NDG, Ambali SF. Exploitation of ethanol extract of *Adenium obesum* stem bark as a potent organic piscicide. *Res J Biol Sci* 2013; 8 (5): 143-149.
14. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *Int J Biomed Sci* 2008; 4: 89-96.