

Acute Toxicity and Effects of Aqueous Pod Extract of *Acacia nilotica* on Some Haematological Parameters and body weight in Rats

Umaru, B.
Saka, S.
Mahre, M. B.
Ojo, N. A.
Dogo, H. M.
Onyeyili, P. A.

ABSTRACT

The aqueous pod extract of *Acacia nilotica* was studied for its acute toxicity and its effects on some haematological parameters due to prolonged oral administration in rats. The pod extract of *A. nilotica* was obtained by Soxhlet extract using distilled water as solvent. The administration of the extract at 3000 mg/kg body weight in the acute toxicity study did not produce any death in the treated rats, indicative of the low toxicity of the extract. Prolonged administration of the extract for 21 days did not significantly ($p > 0.05$) alter the levels of red blood cells (RBC), haemoglobin (Hb) concentration and packed cell volume (PCV) when compared to the control group. The treatment of the rats with the extract at varying doses for 21 days resulted in increase in body weight. The observed increase were significant ($p < 0.05$) in the groups treated with various doses, especially at 14 and 21 days. It is therefore concluded that *A. nilotica* aqueous pod extract was relatively safe and its usage for medicinal purpose is justified.

Keywords: Toxicity, Haematological parameters, *A. nilotica* aqueous Pod extract.

INTRODUCTION

Acacia nilotica: (English Names; thorn tree, wattles, Babul, Black babul, Indian Arabic gum), is a member of the Family; *Fabaceae*. Its tender branches are used as fodder for goats and sheep; stem bark crude extract is used to treat toothache. *Acacia nilotica* inner bark contain tannin (18-23%) used for tanning and dying of leather (National

Umaru B., Mahre M. B., Ojo, N. A. are Lecturers in the Department of Veterinary Physiology, Pharmacology and Biochemistry, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria. *Saka, S.* is a Lecturer in the Department of Veterinary Pharmacology and Toxicology, University of Abuja, P.M.B. 117, Gwagwalada, Abuja, Nigeria. *Dogo H. M.* is a Lecturer in the Department of Surgery, Urology Division. University of Maiduguri Teaching Hospital, P.M.B. 1414, Maiduguri, Borno State, Nigeria. *Onyeyili, P. A.* is a Lecturer in the Department of Veterinary Pharmacology and Toxicology, Federal University of Agriculture, P.M.B. 2373, Makurdi, Benue State. E-mail: bukamar@yahoo.co.uk.

Academy of Science, 1980). Tender pods and shoots are used as vegetables and are fed to camels, sheep and goats especially in Sudan, where it is said to improve milk production from these animals. In South Africa, the Zulus take the stem bark extract for cough, and the Chipi use root bark for treatment of tuberculosis, while the Masai use the stem bark and root decoction, to alleviate mood. In Ayurvedic medicine, the stem bark is considered a remedy for treating premature ejaculation (Pande, Talpada, Pebel and Shukla, 1981). Today herbal medicine has been brought into focus to meet the goal of a wider coverage of primary healthcare not only in Africa but also in other countries of the world (Elujoba, 2005), as a result of high cost of the orthodox drugs and increased toxicities resulting from administration of synthetic drugs. Many of the drugs used today are herb-based. Some like digoxin, reserpine, physostigmine and quinine are obtained from plants, while countless others are manufactured in the laboratory from structural activity-relationship studies of natural molecule isolated from plants, only few are manufactured from raw materials (Sofowora, 1984, Abdu-Aguye, 1997). Thus, medicinal plants play a key role in the development and advancement of modern studies of biological activities of substances. In Northern Nigeria, indigenes use the pod of *A. nilotica* and decoction for treatment of animal and human ailments in folk medicine but no information is available on its toxicity. Therefore this study was design to determine its acute toxicity and effects on some haematological parameters in rats.

MATERIALS AND METHOD

Plant Collection, Identification and Extract Preparation: Fresh pods of *Acacia nilotica* were collected from Lai –Lai grazing reserve, Potiskum Local Government Area of Yobe State and submitted to Professor S. S. Sanusi, Department of Biological Sciences, University of Maiduguri, and a voucher specimen was deposited at the Department of Veterinary Physiology and Pharmacology herbarium, University of Maiduguri, Nigeria. The pods were air dried at room temperature for three weeks. The crushing of the pods was done in the laboratory using pestle and mortar, after which it was ground into powder. Two hundred grammes (200gm) of the powdered pod was weighed and introduced into a conical flask and 1 litre of distilled water was added thereafter. The mixtures was then shaken and allowed to stand for 30 minutes, after which it was boiled for one hour, cooled and shaken vigorously, before filtration using whatman No. 1 filter paper. The filtrate was concentrated in a rotatory evaporator and stored at 4°C until used, and the yield was 6.75% (w/w).

Experimental Animals: Wister albino rats of both sexes were used for the experiments. They were kept in plastic cages and allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the experiments. They were fed with growers mash (Sanders Nig. Ltd, Jos) and water provided *ad libitum*.

Acute Toxicity Studies: The up and down procedure as described by Dixon and Mood (1984) and modified by Dixon (1985 and 1991) was used to evaluate the oral acute toxicity of the aqueous pod extract of *Acacia nilotica*. Five (5) adult rats, weighing between 130-180g were randomly selected for the experiment. They were housed in individual cages for two weeks prior to the treatment to allow them acclimatize to laboratory condition. The rats were fasted overnight, but allow free access to water and were given aqueous pod extract orally at a dose of 3000 mg/kg (limit dose) and were observed for 48 hours after administration of the extract for signs of toxicity or death. The same procedure was adopted until all the five rats were treated at the same dose (Bruce, 1985), and the animals were handled according the international guiding principles for biomedical research involving animals (C.I.O.M.S., 1985)

Effects of Prolonged Oral Administration of Aqueous Pod Extract of *A. nilotica* on some Haematological Parameters: Twenty five (25) Wister albino rats of both sexes (weighing between 140-160g) randomly selected and divided into five groups of five rats each, were used for the studies. Groups II, III, IV and V were treated orally with 50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg of aqueous pod extract of *Acacia nilotica* respectively for a period of 21 days. While Group I (control) received distilled water for the same period. The body weights of the rats were obtained prior to the extract administration and thereafter at weekly interval. Blood samples were collected from the tail vein of the rats prior to extract administration using the method of Coles (1986), and weekly thereafter, the blood samples collected were used for the determination of haematological parameters such as the red blood cells count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV) and weight gain was determined weekly.

Statistical analysis: All values were expressed as Mean \pm Standard Deviation. One way analysis of variance (ANOVA) was used to analyse the extent of variation between groups and p values equal to or less than 0.05 were considered significant (Mead and Curnow, 1982). Graphpad instat 3.0 for windows USA[®] computer software was used to analyse the data.

RESULTS AND DISCUSSION

Acute toxicity of aqueous pod extract of *A. nilotica* at the dose of 3000mg/kg did not produce any mortality in the treated animals. The administration of such high dose to animals without death or serious toxicity may be an indication of relative safety of the extract. According to Clarke and Clarke (1977), substances with the LD₅₀ of 50-500 mg/kg body weight are regarded as being highly toxic, while those above 500 mg/kg body weight but not more than 1000 mg/kg are moderately toxic and those substances whose LD₅₀ are above 1000 mg/kg body weight are regarded as being relatively safe (low toxicity). However, clinical signs of depression, anorexia and dyspnoea were seen in the rats treated with the extract these were indicative of respiratory and nervous

impairments. Plants rich in flavonoid and alkaloids are known to cause the above signs in mammals (Markings, 1988; Umaru *et al*; 2011). Prolonged administration of the extract at various doses (50, 100, 200, and 400 mg/kg) to the rats did not significantly ($p>0.05$) increase the RBC, Hb and PCV values (Table 1, 2 and 3). The slight increase in RBC counts (Table 1) at various doses of the treatment groups implied that the aqueous pod extract of *A. nilotica*, (although not statistically significant) most likely contain some active ingredient that can stimulate the formation of erythropoietin like agents in the stem cells of the rats (Oyedemi *et al*, 2011). Erythropoietin is a glycoprotein hormone that can stimulate the bone marrow to produce more RBC. There was no significant increase in the values of Hb and PCV. These parameters are used mathematically to define concentration of haemoglobin and to suggest restoration of oxygen carrying capacity of the blood, though the mechanism of action of the aqueous pod extract was not investigated in this study.

The result of the effect of *A. nilotica* administration on the mean body weight of treated rats is presented in Table 4. The treatment of the rat with the extract at varying doses for 21 days resulted in significant ($p<0.05$) increases in body weight. The observed increases were significant ($p<0.05$) in the groups treated with various doses, especially at days 14 and 21 following extract treatment when compared with the control. The enhanced body weight gain in the rats could be attributed to increase body metabolic activity and is clear indication that the plant extract of *A. nilotica* could increase glucose metabolism. This observation is corroborated with earlier report by Ravi *et al* (2004) that kernel seed of *Eugenia jambolana* increased body weight in rats.

Table 1: Effect of aqueous pod extract of *Acacia nilotica* on mean* red blood cell ($\times 10^9/L$) count of rats treated with the extract for 21 days respectively

Extract treatment(mg/kg)	Treatment Days			
	0	7	14	21
Control	4.32 ± 0.11	4.30 ± 0.10	4.19 ± 0.10	4.12±0.11
50	4.49 ± 0.39	4.67±0.36	4.30 ± 0.10	4.170.33
100	4.50 ± 0.35	4.73± 0.24	4.60 ± 0.32	4.23±0.24
200	4.49 ± 0.27	4.63±0.32	4.50 ± 0.21	4.75±0.22
400	4.47 ± 0.27	4.62±0.32	4.63 ± 0.25	4.55±0.23

*Mean ± Standard deviation based on five observations

Source: Experimentation, 2014

Table 2: Effect of aqueous pod extract of *Acacia nilotica* on mean* haemoglobin concentration Hb (g/dl) in rats treated for 21 days respectively

Extract treatment(mg/kg)	Treatment Days			
	0	7	14	21
Control	9.40±0.20	9.42±0.21	9.44±0.22	9.44±0.22
50	9.10±0.27	9.24±0.22	9.02±0.33	9.70±0.29
100	9.20±0.98	9.82±0.87	9.60±0.30	9.70±0.35
200	9.30±0.27	8.38±1.08	9.16±0.22	9.20±0.28
400	8.90±0.41	8.68±0.96	8.46±0.40	8.78±0.99

*Mean ± Standard deviation based on five observations

Source: Experimentation, 2014

Table 3: Effect of aqueous pod extract of *Acacia nilotica* on mean* packed cell volume PCV (%) in rats treated for 21 days respectively

Extract treatment(mg/kg)	Treatment Days			
	0	7	14	21
Control	49.2±0.45	48.1±0.44	45.0±1.58	46.2±0.45
50	49.6±1.90	48.0±1.58	45.0±2.51	46.8±1.30
100	47.8±1.64	49.6±1.52	46.4±2.51	48.6±1.72
200	47.2±1.64	48.8±4.38	46.0±2.92	48.6±1.73
400	48.6±1.14	47.4±2.34	46.4±2.40	47.4±2.72

*Mean ± Standard deviation based on five observations

Source: Experimentation, 2014

Table 4: Effect of aqueous pod extract of *A. nilotica* on mean^b body Weight (grams) of Wister albino rats respectively

Extract treatment(mg/kg)	Treatment Days			
	0	7	14	21
Control	148.5±11.2	155.8±6.30	158.3±7.50	160.3±11.50
50	149.8±7.2	170.1±9.40*	187.9±1.7*	193.4±14.80*
100	152.1±10.2	165.2±11.6*	177.1±8.30*	180.9±9.20*
200	146.5±12.3	151.6±6.70	165.7±3.10*	170.0±5.13*
400	149.3±3.90	150.9±9.10	161.1±9.03	

*P<0.05 means significantly different from the control

b = Mean ± Standard deviation based on five observations

Source: Experimentation, 2014

CONCLUSION

The aqueous pod extract of *Acacia nilotica* was studied for its acute toxicity and its effects on some haematological parameters due to prolonged oral administration in rats. The pod extract of *A. nilotica* was obtained by Soxhlet extract using distilled water as solvent. Fresh pods of *Acacia nilotica* were collected from Lai –Lai grazing reserve, Potiskum Local Government Area of Yobe State. Wister albino rats of both sexes were used for the experiments. The up and down procedure as described by Dixon and Mood (1984) and modified by Dixon (1985 and 1991) was used to evaluate the oral acute toxicity of the aqueous pod extract of *Acacia nilotica*. The results showed that *A. nilotica* aqueous pod extract was relatively safe and its usage for medicinal purpose is justified. It has no effect on haematological parameters but was found to significantly increase body weight.

ACKNOWLEDGMENTS

We thank Dr. U. S. Hassan, Department of Veterinary Pathology, Mr. Tanko Usman, Bitrus Wampana, Mal. Isa Gulani and Ibrahim Wiam for their technical assistance.

REFERENCES

- Abdu-Aguye, I.** (1979). Medicinal Herbal Research in West Africa. Paper presented at the 24th Annual Regional Conference of the West African Society for Pharmacology (WASP) at Usmanu Danfodiyo University Sokoto, Nigeria.
- Bruce R. D.** (1985). An up and down procedure for acute toxicity testing. *Fundamental and Applied Toxicology*, 5, 151 – 157.
- Clarke, E. G. C. and Clarke, M. L.** (1977). *Veterinary Toxicology* (1st ed). New York: Baelliere Tindal.
- Coles, E. H.** (1986). *Veterinary clinical pathology* (14th ed). Philadelphia: W.B Sanders Company.
- Council for International Organization of Medical Sciences (CIOMO)** (1985). *International Guiding Principles for Biomedical Research Involving Animals*. Switzerland: WHO 1211, Geneva 27.
- Dixon W. J.** (1985). The Up and Down method for small samples. *Journal of American Statistical Association*, 60, 967 – 978.
- Dixon W. J. and Mood, A. M.** (1984). A method for obtaining and analyzing sensitivity data. *Journal of American Statistical Association*, 43, 109 – 126.
- Dixon W. J.** (1991). *Design and Analysis of Quantal Dose Response Experiments (with Emphasis on stain case Design)*. Los Angeles: Dixon Statistical Association.
- Elujoba, A. A.** (2005). Medicinal properties of plants with Oral Health Implications. Proceedings of the 2nd Dr. David Barmes' Memorial Public Health Symposium, 25th March 2003, organized by the Regional Centre for Oral Health Research & Training for Africa. Jos in collaboration with WHO Regional Office, Brazzaville
- Markings, L.** (1988). *Oral Toxicity of Rotenone to Mammals*. Kansas City: U.S. Fish and Wildlife Services, Missouri.
- Mead, R. and Curnow, R.N.** (1982). *A simple Experiment in Statistical Method in Agriculture Biology*. London: Chapman Hall.
- National Academy of Science (NAS)** (1980). *Firewood Crops, Shrubs and Tree species for Energy Production*. Washington D.C: National Academy of Sciences.
- Oyedemi S.O., Adewusi E. A., Aiyegoro O. A and Akinpelu D. O.** (2011). Antidiabetic and Haematological effects of aqueous stem bark of *Azzeria Africana* (Smith) on Streptozocin-induced diabetic Wister rats. *Asian Pacific Journal of Tropical Biomedicine*, 1(5), 353-358.
- Pande M. B., Talpada P. M., Pebel J. S. and Shukla P. C.** (1981). Note on the nutritive value of babul (*Acacia nilotica* Linn) Seeds (extracted). *Indian Journal of Animal Science*, 51(1), 107-108.
- Ravi K. Rajasekaran S. and Subramanian S.** (2005). Antihyperlipidemic effect of *Eugenia jambolana* seed kernel on streptozotocin-induced diabetes in rats. *Food and Toxicology*, (43), 1433-1439.
- Sofowora, E.A.** (1984). *Medicinal plants and Traditional in Africa* (1st ed). London: John Wiley and Sons Ltd.
- Umaru B., Onyeyili P. A., and Saka, S.** (2011). Anti-diarrhoeic and Antibacterial effects of aqueous pod extract of *A. nilotica* in rats. *Nigerian Veterinary Journal*, 32(1), 30-35.