African Journal of Biotechnology Vol. 8 (19), pp. 5071-5075, 5 October, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Effects of the aqueous extract of fresh leaves of *Calotropis procera* on haematological and biochemical parameters in female rabbits

Jato Denis Mbako[1], Zainab Adamu[2], Jacob Kotei Afutu[3], Atiku Aliyu[4], Shamaki David[4], M. B. Umar[5] and Chudy Nduaka[6]

[1]Department of Biochemistry, University of Yaoundé I, Yaounde, Cameroon.
[2]Department of Pharmacy, Ahmadu Bello University, Zaria, Nigeria.
[3]Department of Animal Science, University of Ghana, Legon, Ghana.
[4]Biochemistry Department, National Veterinary Research Institute – NVRI Vom, Nigeria.
[5]Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.
[6]Pfizer Inc, Groton, Mystic CT., U.S.A.

Accepted 1 June, 2009

Calotropis procera **has been reported to be medicinal and toxic in animals. A toxicological evaluation of the aqueous extract of its fresh leaves was conducted in female rabbits (*Oryctolagus cuniculus*). Low levels of phytochemicals (alkaloids, saponins, tannins, cardiac glycosides, and flavonoids) were found while 0.23, 0.03, 0.82 and 9.5 mg/g of iron, lead, sodium and potassium, respectively, were detected. Acute toxicity study was conducted with oral administration of 200, 400, 800 and 1600 mg/kg of the extract once to groups I, II, III and IV, respectively. Four rabbits died within 24 h and LD_{50} was estimated (940 mg/kg). 80, 40 and 20 mg/kg of the extract were administered daily to groups I, II, and III, respectively, during sub-acute toxicity study for 14 days. All controls were given water. Statistical analysis of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), albumin and protein showed no significant changes at $P<0.05$. Changes in packed cell volume, white blood cells, haemoglobin, platelets, and differential leucocyte count (lymphocytes, monocytes, eosinophils, heterophils/neutrophils and basophils) were equally statistically not significant at $P<0.05$. It is concluded that the extract had no statistical significance on blood parameters when administered orally at tolerable doses since the values were in the range of control values.**

Key words: *Calotropis procera*, phytochemical, toxicity, haematology, protein and serum enzymes.

INTRODUCTION

Calotropis procera belongs to the family Asclepiadaceae and is a soft wooded, evergreen perennial shrub having few stems, few branches and relatively few leaves concentrated near the growing tip. A copious white sap referred to as the latex flows whenever the stems or leaves are cut. The plant is commonly found in Asian temperate region (Arabian Peninsula), Asia-tropical (Indian subcontinent and Indo-China) and Africa (North, Northeast, East tropical, West Central and West tropical), particularly the semi-arid regions of Bauchi, Borno, Kano, Kaduna and most parts of Northern Nigeria (Adams, 1995; Ahmed et al., 2005; Liogier, 1995; Sharma et al., 1997; Howard, 1989).

C. procera is often found growing in open habitat with little competition and it also grows favorably in dry habitat (Parrotta, 2001). The giant milkweed has been found to be effective in the treatment of leprosy, fever, menorrhagia, malaria and snake bites (Parrotta, 2001).

Traditionally, *C. procera* is used to treat common house hold diseases like fever and diarrhea (Sofowora, 1984). Parrotta (2001) reports that the root bark treats leprosy,

*Corresponding author. E-mail: denison767@yahoo.co.uk. Tel.: +237 77769371.

menorrhagia and snake bite. Mahmoud et al. (1979) also reports larvicidal activity of the latex. The leaves have also been boiled together with fresh milk to obtain cheese.

Despite these uses, *C. procera* poses varying toxic effects in animals through air borne allergies, touch and consumption in livestock. The widespread loss of livestock and low animal production are attributed to the existence of *C. procera* in the arid Northern regions of Nigeria (Burkill, 1985).

Toxicity of *C. procera* is reported in sheep in the form of anorexia and diarrhea, Mahmoud et al. (1979). Consumption of this plant leads to severe poisoning to livestock as well as man (Lewis and Elvin-Lewis, 1977). To better elucidate the toxic effects of *C. procera*, a toxicological evaluation of the aqueous extract of fresh leaves of the plant was conducted on biochemical and haematological parameters.

MATERIALS AND METHODS

Animals

Twelve (24) freshly weaned female New Zealand rabbits, 8 - 10 week old, same weight range were obtained from the Small Animal Unit of the Diagnostic Department, National Veterinary Research Institute (NVRI), Vom and used for this study. Faecal samples were collected and examined for *Eimeria elongata* and *Trichostrongylus retortaeformis* to ensure that they were in good health pending commencement of the experiment. For acute toxicity studies, the animals were housed in four cages in groups of three (Groups I, II, III, IV) with group IV serving as the control. For sub-acute, they were placed in four groups of three in each cage. They were fed daily with pelleted feed obtained from Dagwom Farms, NVRI, Vom. Water was given ad libitum.

Collection and preparation of extract

3.5 kg of the fresh leaves of *C. procera* was obtained from Fadan Karshe, Kaduna, Nigeria and identified at the National College of Forestry, Jos, Nigeria. It was crushed to a pulp using a blender, squeezed and filtered. The fresh extract was then oven-dried at 50°C in a Gallenkamp 300 to obtain a powdered form. 1 g of the dried powdered extract was dissolved in a 100 ml of distilled water to obtain a stock solution.

Elemental analysis

0.2 g of the powdered extract was weighed into a clean Kjerdhal flask. 5 ml of conc. HNO_3, 1ml of conc. H_2S0_4 and 1 ml conc. perchloric acid were added. The mixture was digested on a heater until it turned colourless. After digestion, the clear extract was filtered and the filtrate made up to 100 ml in a volumetric flask with de-ionized water. It was then used to analyze for iron, lead, copper, chromium, cobalt, sodium, potassium and arsenic using the Atomic Absorption Spectrophotometer.

Administration of *C. procera*

200, 400, 800 and 1600 mg/kg of the extract were given to Groups I, II, III and IV respectively within 24 h in acute toxicity study. In sub acute toxicity, test rabbits in groups I, II and III were administered 20, 40 and 80 mg/kg of the extract respectively for 14 days by oral gavage. The control group (Group IV) was given distilled water for the same study period.

Collection of blood sample

7 ml of blood was collected by cardiac puncture and venipuncture on day 0 and day 14. For haematology, 2 ml of the sample was collected directly into a bottle containing ethylene diamine tetra-acetate (EDTA) anticoagulant and centrifuged at 3000 rpm for 5 min using a table centrifuge. The sera obtained were stored at -4°C prior to analysis. For biochemical analysis the sample was collected in universal bottle and then slanted for serum to separate before storage at -4°C.

Haematological analysis

The sample was analysed for packed cell volume (PCV), haemoglobin (Hb) concentration, white blood cell (WBC) count, platelet count and differential leucocyte count as described by Benjamin (1964) and Baker et al. (1998).

Biochemical analyses

For enzymes assay; aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), the methods described by Reithman and Frenkel (1957) were employed. Serum protein and albumin were analyzed as described by Gornall et al. (1949) and Bartholomew and Delaney (1966), respectively.

Statistical analysis

Stat Graphics Plus software was used and data analysis was done using Students t-test. Values obtained were expressed as Mean (± SEM) for all groups, with $P<0.05$ considered statistically significant.

RESULTS

Percentage yield of extract

0.0858 kg of dry powdered extract was obtained from the 3.5 kg fresh leaves, giving a percentage yield of 2.45%.

Clinical signs

The animals were found to show some clinical signs like gasping for breath, convulsion, bradycardia, frothy vomiting and photophobia.

Phytochemical and elemental analyses

Phytochemical analysis results showed presence of alkaloids, saponins, tannins, cardiac glycosides and flavornoids, while elemental analysis showed 0.23, 0.03, 0.82

Table 1. Effect of orally administered aqueous extract of *C. procera* on serum enzymes in rabbits.

Group	Dose administered (mg/kg)	Days of sampling	ALP (IU/l)	AST (IU/l)	ALT (IU/l)
I	80	0	22.72±0.00	27.00±0.00	8.00±0.00
		14	6.01±0.30	56.50±21.67	43.83±14.78
II	40	0	18.22±11.96	18.67±13.56	30.00±18.67
		14	9.63±2.57	168.83±53.44	55.67±21.11
III	20	0	13.88±8.00	23.00±1.00	24.00±9.00
		14	11.95±9.92	232.50±14.33	34.67±11.56
IV	Control	0	143.12±160.00	26.00±22.67	13.33±1.78
		14	5.75±4.02	59.00±25.33	53.50±27.67

Values expressed as mean ± SEM.
ALP, Alkaline phosphatase; AST, aspartate amino transferase; ALT, alanine amino transferase.

Table 2. Effect of orally administered aqueous extract of *C. procera* on serum protein and albumin in rabbits.

Group	Dose administered (mg/kg)	Days of sampling	Protein (g/l)	Albumin (g/l)
I	80	0	52.82±0.00	27.07±0.00
		14	101.00±4.33	18.87±15.69
II	40	0	57.72±3.20	34.83±4.04
		14	96.67±35.00	8.87±5.76
III	20	0	-	-
		14	73.67±8.56	14.50±9.27
IV	Control	0	53.88±2.92	33.24±17.36
		14	82.83±4.22	11.77±4.04

Values expressed as mean ± SEM.
- = Missed values.

and 9.5 mg/g of iron, lead, sodium and potassium, respectively.

Haematological and biochemical analyses

The results for serum enzyme changes are as shown in Table 1. There were no significant changes ($P<0.05$) in values obtained for ALT, AST and ALP. The results for protein and albumin levels are shown in Table 2. There was increase in protein and decrease in albumen levels generally including the control group. Haematological analysis results showed increase in values for eosinophil, basophil, haemoglobin, PCV and platelet which spanned through all test groups and the control group (Tables 3 and 4).

DISCUSSION

The chewing movements of the mouth observed upon administration of the extract may be attributed to palatability; likely due to the bitter and disagreeable taste of phytochemnicals such as alkaloids, cardiac glycosides and tannins (Dada et al., 2002). Lead toxicity could be due to interference with mitochondrial oxidative phosphorylation as well as sodium, potassium and calcium AT-Pases. Iron toxicity causes myocardial disease, commonly causing death (Chapman and Hall, 1995). The levels of these trace elements in the extract were too low to cause any such damage. The presence of cardiac glycosides, alkaloids, saponins, tannins and flavonoids was also reported by Mossa et al. (1991). Sodium exists in blood plasma of rabbits (125-148 mM) (Harkness and Wagner 1989; Ivor Harris, 1994), while levels over 152 nM (hypernaetramia) can result in shrinking of the brain, seizures and eventually death.

Biochemical parameters such as serum enzymes, albumin and total proteins showed slight variations also reported in rats (Dada et al., 2002). These variations were not dose-related and all fell within reference ranges

Table 3. Effect of orally administered aqueous extract of *C. procera* on haematological parameters in Rabbits.

Group	Dose administered (mg/kg)	Days of sampling	PCV (%)	WBC ($X10^9$/l)	PLTS ($X10^9$/l)	HB (g/dl)
I	80	0	42.33±0.44	7.73±1.38	241.33±33.56	12.43±1.82
		14	35.33±1.78	5.37±0.58	245.00±25.33	35.33±1.78
II	40	0	35.67±3.11	6.27±0.44	248.33±22.22	10.80±0.80
		14	32.00±0.67	5.30±1.20	208.67±14.89	32.00±14.89
III	20	0	34.00±2.00	6.07±0.76	241.33±26.89	11.83±0.22
		14	31.33±1.11	4.30±0.33	224.33±19.78	31.33±1.11
IV	Control	0	35.33±2.22	9.40±1.40	254.00±20.67	15.57±4.89
		14	36.33±1.78	4.23±0.16	236.33±32.44	36.33±1.78

Values expressed as mean ± SEM.
PCV = Packed cell volume, WBC = White blood cell, PLTS = platelets, HB = haemoglobin.

Table 4. Effect of orally administered aqueous extract of *C. procera* on differential leucocytes count in Rabbits.

Group	Dose administered (mg/kg)	Days of Sampling	Heterophil/ neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
I	80	0	43.67±9.78	54.33±9.11	1.67±1.11	0.33±0.44	0.00±0.00
		14	51.33±1.11	47.33±0.44	0.00±0.00	0.33±0.44	1.00±0.67
II	40	0	43.33±5.78	54.00±7.33	1.33±1.11	1.33±0.89	0.00±0.00
		14	34.00±6.00	62.67±5.11	1.67±1.11	1.00±0.67	0.33±0.44
III	20	0	50.00±12.67	49.33±13.11	0.33±0.44	0.33±0.44	0.00±0.00
		14	46.67±10.44	52.33±9.11	1.00±1.33	0.00±0.00	0.00±0.00
IV	Control	0	52.33±4.44	46.33±3.56	0.67±0.44	0.67±0.89	0.00±0.00
		14	43.67±3.11	53.00±5.33	2.67±1.78	0.00±0.00	0.67±0.44

Values expressed as mean ± SEM.

in rabbits (Harkness and Wagner 1989) and when analyzed, were found to have no statistical significance at $P<0.05$. This may be because the liver was not greatly damaged to release significant quantities of the enzyme into the blood (Odutola, 2000) due to the quantity of extract administered. The amount of enzyme released into blood is directly proportional to the number of damaged cells and the interval of time between injury and the test (Adedeji et al., 2002). Hence, 14 days may not have been enough for any noticeable increase in serum enzymes to occur.

The fluctuations in values of haematological parameters (PCV, WBC and platelets) as well as differential leucocyte counts were analyzed to have no statistical significance at $P<0.05$. Also, the controls were affected and values still remained within normal reference ranges. All these changes may only be attributed to normal physiological changes associated with growth.

Anaemia reported by Mahmoud et al. (1979) and weight loss by Dada et al. (2002) were not observed. Mild diarrhoea and loss of fluid or serous discharge could be attributed to erosion of mucous lining of the gastro-intestinal tract by tannins, similarly reported by Mahmoud et al. (1979).

In conclusion, the results apparently show that toxicity of *C. procera* is unrelated to changes in blood parameters. However, diarrhoea observed suggests that *C. procera* is not reliable to sustain livestock during the long dry season in semi-arid regions. It is recommended that a chronic study be conducted since changes in these parameters and clinical signs observed were time dependent and dose-related. Also, histopathological studies may better ascertain any organ toxicity and the extent of damage.

ACKNOWLEDGEMENTS

The research work was conducted during the Internship programme in Toxicology, at the National Veterinary Research Institute (NVRI), Vom-Nigeria and sponsored by the Africa Education Initiative (NEF), Mystic Connecticut USA. The authors are also grateful to NVRI's research and laboratory staff for their support and assistance.

REFERENCES

Adams HR (1995). Digitalis and Vasodilator Drugs in Veterinary Pharmacology and Therapeutics. (7th Edition) Adams HR (Editor). Am. Iowa State University Press. pp. 1138-1147.

Adedeji OS, Abubakar MB, Ozegbe PC (2002). Growth-Suppressing Effect of Calotropis procera *(Giant Milkweed*)-Chronic Cynanide toxicity and urinary enzyme excretion in rabbits, Trop. Vet. pp. 1357.

Ahmed KK, Rana AC, Dixit VK (2005). Calotropis Species (*Asclepidaceae*)-A Comprehensive Review. Pharmacognosy Mag. 1(2): 48-51.

Baker FJ, Silverton RE, Pallister CJ (1998). Introduction to Medical Laboratory Technology, 7th Edition.

Bartholomew RJ, Delaney AM (1966). Proceedings Australian Association of Clinical Biochemistry 1: 214.

Benjamin M (1964). Outline of Veterinary Clinical Pathology. The Iowa State University Press Ames, Iowa.

Burkill HM (1985). The Useful Plants of West Tropical Africa 2nd Ed. Royal Botanical Garden, Kew.

Chapman S Hall E (1995). Iron Nutritional and Physiological Significance. The British Nutrition Foundation.

Dada YO, Lamidi MT, Eghianruwa KI, Adepoju F (2002). Effects of Oral Administration of the Latex of Calotropis procera on weights, Hematology and Plasma Biochemistry in Rats. Trop. Vet. 20: 4.

Gornall AG, Bardawill CS, David MM (1949). Determination of Protein and Albumin Using Biuret Reagent. J. Biol. Chem. 177: 751.

Harkness JE, Wagner JE (1989). Rabbit behaviour as related to environmental stress. J. Appl. Rabbit Res. 11(3): 227-236. NAL call number: QL737 L32J6

Howard RA (1989). Flora of the Lesser Antilles, Leeward and Windward Islands -Dicotyledoneae. Arnold Arboretum, Harvard University, Jamaica Plain, MA. 3-2: 658.

Ivor Harris (2004). The Laboratory Rabbit. The University of Queensland, Central Animal Breeding House, Veterinary Science Farm, ANZCCART Facts Sheet (ANZCCART News), 7: 4.

Lewis WH, Elvin-Lewis DMF (1977). Medical Botany: Plants that Affect Man's Health. John Wiley, London.

Liogier HA (1995). Descriptive flora of Puerto Rico and Adjacent Islands. Editorial de la Universidad de Puerto Rico, San Juan, PR. 4 : 617.

Mahmoud OM, Adam SEI, Tartour G (1979). The Effect of Calotropis procera on Small Ruminants. J. Comp. Path. 89: 251-263.

Mossa JS, Tariq M, Moshin A, Ageel AM, Al-Yahya MA, Al-Said MS, Rafatullah S (1991). Pharmacological Studies on the Aerial Parts of Calotropis procera. Am. J. Chin. Med. (An International Journal of Comparative Medicine East and West). 19(3-4): 223-231.

Odutola A (2000). Rapid Interpretation of Routine Clinical Laboratory Tests S. Asekome and Company, Zaria. pp. 24-28.

Parrotta JA (2001). Healing Plants of Peninsular India. CAB International, Wallingford, UK and New York. p. 944.

Reithman S, Frenkel S (1957). Determination of serum transaminase. Am. J. Clin. Pathol. 28: 56-59.

Sharma DK, Tiwari M, Arora M, Behera BK (1997). Microbial Transformation and Biodegradation of Calotropis procera Latex towards Obtaining Value Added Chemicals, Pharmaceuticals and Fuels. Petroleum Science and Technology, Marcel Dekker Inc, 15(2): 137-169.

Sofowora A (1984). Medicinal Plants and Traditional Medicine in Africa. Second Edition, Spectrum Books Ltd, Ibadan, Owerri, Kaduna, Lagos.