

Effect of Aqueous Root-Bark Extract of *Vitex Doniana* Sweet on Haematological Parameters in Rats

F. I. Abdulrahman¹, J.C. Akan, O.A.Sodipo² and P.A. Onyeyili³

1. Department of Chemistry, University of Maiduguri, P.M.B 1069, Maiduguri, Borno State, Nigeria

2. Department of Clinical Pharmacology and Therapeutics, College of Medical Sciences, University of Maiduguri, P.M.B 1069, Maiduguri, Borno State, Nigeria.

3. Faculty of Veterinary Medicine and Pharmacology, University of Maiduguri, P.M.B 1069, Maiduguri, Borno State, Nigeria

E-mail: joechemakan@yahoo.com

ABSTRACT: Acute and sub-acute toxicities of aqueous extract of *Vitex doniana* was carried out in rats. The LD50 following intraperitoneal administration estimated at 95% confidence interval was 980 mg/kg. The oral administration of the extract for 21 days at 50,100 and 200mg/kg had beneficial effects on the haematological parameters. There were significant ($P<0.05$) increases in red blood cell count (RBC) haemoglobine (HB) concentration and packed cell volume (PCV) values in treated rats. The treated animals had leucocytosis, which may be due to increase lymphocyte count observed. The i.p LD50 (980 mg/kg) indicated that the extract is moderately toxic, though the prolong oral administration of the extract under the condition of this study shows that the extract may be toxic at higher doses. Nevertheless, the extract appear to be more beneficial at lower doses and significantly ($p< 0.05$) improves RBC, HB and PVC values and this effect has potential application as anti-anaemic agent. This seems to provide justification for its use as anti-anaemic agent in African traditional medicine. [Journal of American Science. 2010;6(12):8-12]. (ISSN: 1545-1003).

Keywords: *Vitex doniana*, Red blood Cell Count, Haemoglobin Concentration, Packed Cell Volume, Anaemia, Aqueous Extract.

1. INTRODUCTION

There is little doubt the positive roles that herbal medicines have and continue to have on the lives of Nigerians and of all people throughout the world. Herbs are known to have sustained mankind not only as sources of food but also as medicines and poisons utilized in various ways for varied purposes (Abdu-aguye, 1997). The use of medicinal plants in West Africa is probably as old as the duration of human settlement in the region.

Vitex doniana is one of the agents used for folklore medicinal purposes. Although parts of the plant are used by traditional healers for the treatment of various ailments (Akiniyi and Sultanbawa 1987) information on the toxicity of the extract in man animal are lacking. The present study investigated the acute and chronic toxicity of the aqueous extract of *vitex doniana* in rats. This is important since science requires the validation of drugs by medicinal practitioners and drug regulatory authorities demand that all potential drugs should pass through a rigorous series of study and scrutiny (Abdulrahman, 2004).

2. MATERIALS AND METHODS

2.1 Plant collection and preparation

The root-bark of *Vitex doniana* was collected from the outskirts of Maiduguri, Borno State and identified by Prof. S.S. Sanusi a plant

taxonomist in the Biological Sciences Department of University of Maiduguri, Nigeria. The air-dried root-bark was subsequently ground into powder. Five hundred grammes (500g) of the powdered root- bark was exhaustively soxhlet extracted with distilled water (Mittal *et al.*, 1981 and WHO, 1992). The extract was concentrated *in vacuo* and stored at 4°C until required. A fresh solution was prepared from the residue on each day of extract administration.

2.1.1 Animals

Rats of both sexes weighing between 94. 8-1 20 g was used for the experiments. They were obtained from a colony of rats maintained at the animal house of the Institute for Trypanosomiasis Research, Vom, Nigeria. The animals were housed in clean plastic cages and had access to feed (ECWA Feeds Nigeria LTD, Jos, Nigeria) and water ad libitum. They were allowed to acclimatize for two weeks in the Veterinary Physiology and Pharmacology Laboratory before the commencement of the studies. The animals were handled according to the international guiding principles for Biochemical Research (CIOMS, 1985) as certified by the Animal ethics committee of the Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Nigeria.

2.2 EXPERIMENTAL PROCEDURE

2.2.1 Acute Toxicity of Aqueous Extract of *Vitex doniana* in Rats

Six groups of five rats each were used. The animals in groups A to E were dosed intraperitoneally (i.p.) with varying doses (400, 600, 1000, 1200 and 1600 mg/kg) of aqueous extract of *vitex doniana* while the rats in group F (control) received distilled water only by same route. The rats were allowed access to food and water *ad libitum* and observed over a period of 24 hours for clinical signs and death. The LD₅₀ with 95% confidence interval was determined using the arithmetic methods of Aliu, and Nwude (1987).

2.2.2 Effect of Extract on Haematology

The rats (100) were separated into four equal groups. Animals in groups I-III were treated with 50, 100 and 200 mg/kg of the root-bark extract orally respectively for three (3) weeks, while group IV served as control and was given distilled water only by the same route. Clinical signs and haematological parameters were used to assess the effects of the various doses of the extract on the rats

following prolonged administration. The animal were bled weekly and the blood used for the determination of the total red blood cell counts (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), white blood cell counts (WBC) and differential leucocyte counts (DLC) using standard procedure (Kelly, 1977 and Pflanzner, 1990).

3 RESULTS AND DISCUSSIONS

3.1 Acute Toxicity Study

The dose of water root-bark extract of *Vitex doniana* that produced mortality was 3000 mg/kg, while the dose that causes 100% death was 1600 mg/kg (Table 1). The symptoms of toxicity observed with extract administration were dose dependent. Ten to fifteen minutes after administration of extract all the rats in the various groups were very weak. Those that received 600 mg/kg dose and above were deeply sedated and slept. Signs observed before death included loss of appetite, lethargy, paralysis of hind limb, which progressed to fore limbs, difficulties in respiration and coma. Mortality was recorded five hours after 1600 mg/kg extract of *Vitex doniana* treatment. The LD₅₀ with 95% confidence limit of the extract was estimated to be 980 mg/kg.

Table 1. The dose of water root-bark extract of *Vitex doniana*

Group (n=5)	Extract dose (mg/kg)	Number of death	% Mortality
A	400.00	0	0.00
B	600.00	0	0.00
C	1000.00	3	60.00
D	1200.00	4	80.00
E	1600.00	5	100.00
F	Control (distilled water)	0	0.00

3.2 Effect on haematology

The rats treated with 100 and 200mg/kg of the water extract of *Vitex dtmiana* were depressed and less active compared to the rats in the other groups. The percentage weight gain decreased significantly with increase in extract dose (Table 2). Twenty one days following administration of the extract at 50, 100 and 200 mg/kg respectively to rats, there was a percentage weight gain of 56.0, 40.0 and 5.6 percent, Fourteen days following termination of treatment resulted in percentage weight gain of 52.3, 53.4 and 28.9 percent respectively in the group treated with 50, 100 and 200 mg/kg of *Vitex doniana* extract. The control group showed a percentage weight gain of 112.8 percent by the end of the experimentation period. The mean RBC counts of rats treated with the

aqueous extract of the root back of *vitex doniana* orally for 21 days (3 weeks) are shown in Table 3. The RBC counts of rat treated with the extract increased significantly ($p < 0.05$) and at 21 days of treatment the percentage increase were 93.3, 38.1 and 36.7 percent respectively in groups treated with 50, 100 and 200 mg/kg of the extract. RBC counts decreased with the termination of treatment but were still above the pre-treatment values.

The mean Hb concentration (Table 4) increased from the pretreatment values of 1.4 ± 0.11 , 12.2 ± 0.17 , 13.2 ± 0.13 g/dl respectively in groups treated with 50, 100, 200mg/kg of the root bark extract to 14.6 ± 0.13 , 15.0 ± 0.37 and 14.4 ± 0.38 g/dl at 21days of treatment. Following the termination of extract treatment there was gradual decrease in Hb

concentration.

The mean PVC value of rats treated with various doses of the root back extract of vitex doniana showed that at 21 days of treatment the PCV value were significantly ($P < 0.05$) increased (36.4 ± 0.13 ,

37.4 ± 0.27 and 37.4 ± 0.34 , however at 14 days post-treatment the values decreased to 30.0 ± 0.21 , 28.0 ± 0.44 and 29.0 ± 0.55 per cent in the groups treated with 50, 100 and 200 mg/kg of extract doses (Table 5).

Table 2: Effect of aqueous extract of the root-back of *Vitex doniana* on mean body weight of albino rats percent respectively) for the treatments

Extract Dose (mg/kg)	Days of Treatment					
	0	7	14	21	28	35
50.0	68.2±3.20	11.0±10.0	102.5±2.41	106.4±1.54	114.8±2.60	104.0±4.54
100.0	110.0±0.82	134.0±7.33	148.0±4.80	154.0±4.20	164.0±8.00	160.7±1.48
200.0	115.6±2.86	120.0±5.40	120.50±4.60	122.5±2.10	137.5±2.80	134.0±3.70
Control	48.4±2.32	62.2±1.70	57.20±8.70	74.0±2.00	90.0±2.00	103.0±4.70

Table 3: Effect of aqueous extract of the root-back of *Vitex doniana* on red blood cells (RBC) count

Extract Dose (mg/kg)	Days of Administration of extract					
	0	7	14	21	28	35
50.0	3.26±0.34	3.18±0.65	4.11±0.12*	6.30±0.25*	5.72±0.52 *	5.26±0.29*
100.0	5.30±0.28	4.90±0.34	7.04±0.24*	7.32±0.51*	5.60±0.50*	5.40±0.17*
200.0	4.96±0.19	4.94±0.56	5.52±0.28 *	6.78±0.38*	5.82±0.46*	5.84±0.44*
Control	4.96±0.19	4.28±0.60	4.36±0.25	4.24±0.54	4.34±0.59	4.44±0.15

*Significant increased ($p < 0.05$) compared with mean of the control

Day 0= immediate ly before extract administration Day 34 – 14 days post treatment

Control = Given distilled water only

Table 4: Effect of aqueous extract of the root-back of *Vitex doniana* on haemoglobin concentrations in rats

Extract Dose (mg/kg)	Days of Extract Treatment					
	0	7	14	21	28	35
50.0	11.4±0.11	11.6±0.23	14.2±0.09*	14.6±0.13*	13.4±0.30	12.74±0.15
100.0	12.2±0.17	12.4±0.42	14.6±0.35 *	15.0±0.37*	13.2±0.19*	12.6±0.19
200.0	13.2±0.13	13.2±0.35	14.2±0.14*	14.4±0.38 *	13.0±0.07	12.8±0.27
Control	12.6±0.10	13.0±0.15	12.8±0.13	12.7±0.29	12.6±0.37	13.0±0.23

*Significant increased ($p < 0.05$) compared with mean of the control

Day 0= immediate ly before extract administration Day 34 – 14 days post treatment

Control = Given distilled water only

Table 5: Effect of aqueous extract of the root-back of *Vitex doniana* on packed cells volume (PCV)

Extract Dose (mg/kg)	Days of Extract Treatment					
	0	7	14	21	28	35
50.0	29.9±0.21	29.0±0.33	33.6±0.17 *	36.4±0.13*	30.0±0.33	30.0±0.21
100.0	30.7±0.14	31.8±0.40 *	34.0±0.41*	37.4±0.27*	29.6±0.51	28.0±0.44
200.0	30.8±0.29	13.8±0.40	36.2±0.42 *	37.4±0.34*	29.2±0.38	29.0±0.55
Control	29.8±0.30	31.0±0.37	31.0±0.14	31.0±0.02	31.0±0.11	29.8±0.06

*Significant increased ($p < 0.05$) compared with mean of the control

Day 0= immediate ly before extract administration Day 34 – 14 days post treatment

Control = Given distilled water only

Table 6: Effect of aqueous extract of the root-bark of *Vitex doniana* on white blood cells count in rats

Extract Dose (mg/kg)	Days of Extract Treatment					
	0	7	14	21	28	35
50.0	4.34±0.55	4.36±0.67	4.88±0.94	4.85±0.78	6.34±0.56*	4.02±0.99**
100.0	4.28±0.44	4.16±0.49	6.08±0.08*	6.48±0.71*	6.78±0.38*	3.50±0.60**
200.0	4.86±0.29	4.88±0.57	6.12±0.77*	6.34±0.57*	6.53±0.47*	4.36±0.58**
Control	4.74±0.38	4.64±0.37	4.72±0.76	4.69±0.51	4.68±0.51	4.74±0.76

*Significant increased ($p < 0.05$) compared with mean of the control

** Significant decreased ($p < 0.05$) compared with mean of the control

Day 0= immediately before extract administration Day 34 – 14 days post treatment

Control = Given distilled water only

The total leucocyte (WBC) counts (Table 6) in rats treated with 100 and 200mg/kg doses of the water extract of *Vitex doniana* root-bark were significantly increased ($P < 0.05$) from day 14 of treatment. However, 14 days following withdrawal of the extract the high leucocyte values were reduced and were below the values for the control. The mean WBC counts of the control were statistically the same throughout the experimentation period. The differential leucocyte counts (DLC) of rats treated with various doses of the aqueous extract of root-bark of *Vitex doniana* showed some fluctuations. Following treatment with the extract, neutrophil significantly decreased ($P < 0.05$), while the percentage lymphocytes increased significantly ($P < 0.05$) when compared to the control. When treatment was withdrawn the percentage lymphocyte/neutrophil ratio improved. The percentage basophil, monocyte and eosinophil were also decreased following extract treatment but improved with the termination of treatment.

The LD50 of the aqueous extract of *Vitex doniana* following intraperitoneal administration was 980 mg/kg using the arithmetic method of Aliyu and Nwude (1987). The calculated LD50 showed that the extract is not very toxic. Clark and Clark (1977) were of the opinion that any substance whose LD50 in rats that falls between 50-500 mg/kg should be regarded as very toxic, while substances with LD50 above 500 mg/kg but below 1000 mg/kg are classified as being moderately toxic. The signs observed before death following the administration of *Vitex doniana* extract in this study included loss of appetite, paralysis of hind limbs. This progressed to fore limbs, dyspnea and cornea. The toxicity observed may have resulted from the various organic chemicals like saponins, tannins, glycosides and phenolic compounds present in the extract (Abdulrahman, 2004).

The administration of the extract to rats for 21 days at 100 and 200 mg/kg doses produced depression, with the animals being less active. The percentage weight gain also decreased with increasing

extract dose. The overall weight gain in the control rats was much higher than those of the extract treated animal. The decreased weight gain in extract treated rats may be due to decreased feed consumption since the animals were depressed and inactive. It also may be due to the indirect effect of saponin present in the extract. Saponins are known to cause bloating, thereby reducing the appetite of the treated animals (Trease and Evans, 1992).

The aqueous root-bark extract of *Vitex doniana* administered at various doses for 21 days to albino rats appears to have some beneficial effects on haematological parameters. The results of the haematological study revealed significant increase in RBC, Hb and PCV values of the treated rats (Table 3, 4 and 5) when compared with the control. The increase in haematological parameters also appears to decrease with increased concentration of the extract. This may be an indication that the extract could be more beneficial at low dosages than at higher concentration. The improvement of RBC, Hb and PCV values of treated animals is an indication of the anti-anaemic effect of the extract. Substance with anti-anaemic effect is known to stimulate increase production of RBC and improve the values of Hb and PCV (Brown, 1976). The higher percentage increase in RBC of rats treated with 50 mg/kg of the aqueous extract of *Vitex doniana* compared to those of rats treated with 100 and 200 mg/kg of the extract could be an indication of the concentration of saponin absorbed following treatment.

It is suggested that the saponin component of the extract at 50 mg/kg would be very low to exact any appreciable effect after administration on RBC. However, at 100 and 200 mg/kg doses higher saponin contents occur and could be responsible for the lower percentage RBC values when compared to the group with 50 mg/kg dose. Saponins are known to cause lysis of the RBC and/or inhibition of blood cell synthesis (Effraim, et al., 1999 and Irvine, 1961). Furthermore orally administered saponins are known to indirectly affect the haematological parameters by

reducing the appetite of the animals (Trease and Evans, 1989). Interaction of saponins with micronutrients makes the nutrients unavailable and could also affect haemopoiesis (Xing et al., 1995).

The improvement of RBC, Hb and PCV values of treated rats in this study may be an indication that the extract could be useful for treatment of anaemia, hence the justification for its use by natives.

The administration of the extract at various doses stimulated increased production of WBC significant ($P < 0.05$) from day 14 of treatment. This could be a result of possible stimulation of immune system (Kashinath, 1990). Furthermore, reports have shown that persistent antigen load in the body results in lymphocytosis (Schalm, et al., 1975). Lymphocytosis may be primarily responsible for the increases in WBC count in the present study.

4. Conclusion

In conclusion, the aqueous extract although used by traditionalists for treatment of ailments in man was observed in rats under the conditions of this study to be toxic. Therefore, caution must be exercised in its usage especially in high doses. In low dosage the extract improved the haematological parameters and could be used to treat anaemia.

Corresponding Author:

Dr. Joseph Clement Akan
Department of Chemistry, University of Maiduguri,
Maiduguri, P.M.B 1069,
Maiduguri, Borno State, Nigeria.
Tel: +2348036000506

References

1. Abdu-aguye I. Medicinal herbal research in West Africa. Annual Regional Conference of West Africa society of Pharmacognosy, Usmanu Danfodio University, Sokoto, Nigeria. 1997; 22-25.
2. Abdulrahman FI. Studies on the chemical and pharmacological activities of the root bark extract of vitex doniana Guill and pers. (Black Plum). Ph.D Thesis, Department of chemistry, University of Maiduguri, Maiduguri, Nigeria. 2004; pp 1-4.
3. Akiniyi JA, Sultanbawa MUS. A glossary of kanuri names of plants with botanical names, distribution and uses. Annals of Borno. 1987;1:85-98.
4. Aliu YO, Nwude N. Veterinary pharmacology and toxicology experiments. 1st Ed. Baraka press and Publishers, Zaria. 1987; pp 43-45.
5. Brown BA. Haematological, principles and procedures. 2nd ed. Lea and Ferbinger, Philadelphia. 1976; pp 56-81
6. Clark E. GC, Clark ML. Veterinary toxicology. 2nd ed. Nailliere Tindall, New York. 1977; pp10
7. CIOMS: Council for International Organizations of Medicinal Sessions. International Guiding Principles for Biomedical Research Involving Animals C/O WHO 1211, Geneva 27, Switzerland. 1985.
8. Effraim K.D, Salami HA, Nwafor P. The effect aqueous seed of Trigonelli foenum graecom (Fenugreek) on haematological parameter in albino rats. Afri. J. Biomed. Res. 1999; 47-51.
9. Irvine FI. Woody plants of Ghana with special reference to their uses. Oxford University Prsss, London. 1961; pp868
10. Kelly WR. Veterinary clinical diagnosis. Baelliere Tindall, London. 1977; pp 271-282.
11. Kashinath RT. Hypolipimedic effect of disulphide in rate fed with high lipid and/or ethanol. Ph.D Thesis, University of Bangalore. 1990; pp 221-225.
12. Mittal GC, Aguwa CN, Akube AA. Preliminary Pharmacological studies on the anti-venom action of Diochia scandens leaves. Nig. J. Pharm. 1981;12: 432-436.
13. Pflanze RG. Experimental and applied physiology 4th ed. W.M.C Brown publishers, London. 1990; pp 187-218.
14. Schalm OW, Jain NC, Carol, EJ. Veterinary haematology. 3rd ed. Lea and Ferbinger Philadelphia. 1975; pp 200-280.
15. Trease GE, Evans WC. Pharmacognosy. Brailliere Tindal, London. 1989; pp 687-689.
16. Trease GE, Evans WC. Pharmacognosy. 11th ed. Brailliere Tindal. London, 1992; pp 176-180.
17. WHO. Quality control methods for medicinal plants materials. Geneva. 1992; pp 26.
18. Xing D, Du K, Ung L, (1995) Effects of ginseng flower saponin on blood and levels of five metals elements in tissue of dogs with hemorrhagic shock. Zhongyino Bingli Shengli Zazhilas. 1995; 11: 83-85.

15/06/2010