



ISSN 2278 – 0211 (Online)

Determination of Haematological Effects of Aqueous Leaf Extract of *Cadaba Farinosa* in Adult Wistar Rats

Abdulrahman A. Musa

Associate Professor, Department of Pharmacognosy,
Usman Danfodiyo University Sokoto, Nigeria

Abdulaziz Aisha

Laboratory Scientist, Department of Histopathology,
Usman Danfodiyo University, Nigeria

Kabir Hauwa

Head, Medical Laboratory Services, General Hospital
Yabo, Sokoto State, Nigeria

Abubakar Aliyu Khadijah

Head, Medical Laboratory Services,
General Hospital Bodinga, Sokoto State, Nigeria

Solomon Matthias Gamde

Head, Medical Laboratory Services, General Hospital
Silame, Sokoto State, Nigeria

Anthony Perede

Senior Scientist, Department of Haematology,
Specialist Hospital, Sokoto, Nigeria

Halilu E. Mshelia

Associate Professor, Department of Pharmacognosy
Usman Danfodiyo University, Nigeria

Abstract:

Haematological parameters are frequently used to evaluate functional characteristics of blood and blood forming organs. Assessments of these parameters are not limited to established diagnosis of deleterious diseases nor prognosis, but also relating the blood functions of plant extracts. Plant extracts are alternative remedies in the managements of blood related disorders in developing countries including anaemia, neutropenia and thrombocytopenia. Anti-sickling property modelling entities that inhibit and reverse sequence of pathological processes leading to sickling was reported in *Fagara zanthoxyloides*, *Khaya senegalensis* and *Garlic*, possessing similar phytochemicals as *Cadaba farinosa* forsk is a rich natural source of alkaloids, flavonoids and terpenoids with antioxidant properties against oxidative damage. It's possess antithrombotic and vasoprotective properties. Bioflavonoids of *Cadaba farinosa* forsk demonstrated significant cytotoxicity against panel of cancerous cells. However, a possible haematological effect of *Cadaba farinosa* forsk is unevaluated. This study investigated effects of oral administration of aqueous leaf extracts of the plant. Twelve adult Wistar rats were divided into four groups of three rats ($n=30$). Group 1 served as negative control. Extract administered to groups (2, 3 and 4) following 28 days consecutively at dose levels 100, 200 and 300 mg/kg, showed no significant change in RBC count compared with control group ($p>0.05$). PCV, PLT and HGB were not significant ($P>0.05$). However, there was significant elevation of WBC at 300mg/kg ($p=0.02$, 4.28 ± 2.38) ($p<0.05$). Our finding also revealed statistically significant difference at 200 and 300mg/kg of MCHC (304.50 ± 3.54 , 303.00 ± 5.66) compared to control ($P<0.05$). Hence, chronic toxicity studies are needed to further support the use of this plant for treatments.

Keywords: Haematological parameters, *cadaba farinosa*, leaf, aqueous extract, Wistar rats

1. Introduction

Haematological parameters (such as packed cell volume, platelet, neutrophils, lymphocytes, haemoglobin, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume, red blood cell count and white blood cell count) are frequently used to evaluate functional characteristics of blood and blood forming organs [1,2]. Assessments of these parameters are not limited to established diagnosis of deleterious diseases nor prognosis, but also relating the blood functions of plant extracts [3,4]. Extracts of plant are alternative remedies in the managements of blood

related disorders including anaemia, neutropenia and thrombocytopenia than most conventional medicines in poor developing countries [5,6]. The biochemical basis of herbal management of sickle has been reported [7]. Isomeric divanilloylquinic acids of *Fagara zanthoxyloides* root extract possess anti-sickling property modelling entities that inhibit or reverse the sequence of pathological processes leading to sickling was reported [8]. The anti-sickling effects of Garlic were also documented [9] and *Khaya senegalensis* [10].

Cadabafarinosa Forsk is a rich natural source of alkaloids, flavonoids and terpenoids [11]. Its antioxidant properties against oxidative damage was scientifically investigated [12,13]. And its antithrombotic and vasoprotective properties were also reported [13]. The bioflavonoids of *Cadabafarinosa* demonstrated significant cytotoxicity against panel of cancerous cells [14]. In Ayurvedic medicine, leaves of *Cadabafarinosa* is used to detoxify snake bite as well for fevers; a laxative to rid intestinal parasites and to treat diabetes [15]. Our previous studies has shown that *Cadabafarinosa* induces the synthesis of prostaglandins which possess wide range of body functions including contraction and relaxation of smooth muscle and control of blood pressure [16]. This study was aimed at evaluating the possible haematological effects of aqueous leaf extract of *Cadabafarinosa forsk* on normal adult Wistar rats as a preliminary step towards development of more efficacious plant-derived agent to manage haematological disorders and boost health.

2. Materials and Method

2.1. Study Site

This experiment was conducted at the Haematology Unit of Specialist Hospital Sokoto in conjunction with the Department of Medical Laboratory Science, Usman Danfodiyo University Sokoto, Sokoto State, Nigeria.

2.2. Ethical Consideration

The ethical approval for this research was obtained from Ethics Committee on Research and Experiment, Faculty of Pharmaceutical Sciences, Usman Danfodiyo University Sokoto, Nigeria.

2.3. Experimental Animals

Twelve (12) adult Wistar rats of both sexes, weight 180-200g were used for this study. The rats were housed in metal cages and maintained in 12 hour light and 12 hour dark regimen in an environment of normal ambient temperature. The rats were fed *ad libitum* with standard commercial feed (Vital feed, Grand Cereals Nigeria PLC, Jos) and supplied with clean drinking water.

2.4. Source of Plant Material

The inner stem bark of *Cadaba farinosa Forsk* were harvested fresh at the Faculty of Pharmaceutical Science, Usman Danfodiyo University Sokoto, Sokoto state, Nigeria with a voucher number PCG/UDUS/CAPP/0002. Phytochemical extraction was done at the Department of Pharmacognosy, Usman Danfodiyo University Sokoto, Sokoto State, Nigeria.

2.5. Plant Extraction

Fresh leaves of plant was harvested and shade-dried after which they were reduced into fine powder by grinding and soaked in distilled water for 24 hours at room temperature. The mixture was filtered with Whatman No. 1 filter paper to obtain particle free solution that was evaporated to dryness at 45°C in water bath [17]. The solid extract was then re-dissolved in distilled water and stored in capped bottles in a refrigerator at 4°C until required.

2.6. Experimental Design

Groups of Animals	Daily Dose of Extract. (mg/kg body weight)	Duration of Administration (days)
I	Distil water	28
II	100	28
III	200	28
IV	300	28

. Table 1: Shows Experimental Animals and the Daily Doses of Extract Administered Consecutively for 28 Days (n=12)

2.7. Blood Collection for Analysis

At the end of intervention, animals were sacrificed using chloroform. Blood was culled by cardiac puncture using 5ml sterile plastic syringes and needles from each animal aseptically; about 2.5ml of blood was poured into Ethylene Diamine Tetracetic Acid (EDTA) bottle that was mixed and kept at 4°C before haematological analysis on same day of collection.

2.8. Haematological Investigations

The blood collected into EDTA anticoagulant bottles were subjected to complete blood cell count, platelets count and the red cell indices using the standard methods [18].

2.9. Haemoglobin Estimation

Whole blood was diluted 1 in 201 modify Drabkin's solution containing Potassium ferricyanide and Potassium cyanide. The red cells were haemolysed while haemoglobin was oxidized by ferricyanide to methaemoglobin which was converted to stable haemoglobinocyanide (HiCN) by the cyanide. Absorbance of the HiCN solution was read with a spectrophotometer at wavelength 540nm.

2.9.1. Total Leukocyte Count

Whole blood was diluted 1 in 20 in an acid reagent which haemolyses the red cells (not the nucleus of nucleated red cells) leaving the white cells to be counted.

2.10. Erythrocyte Sedimentation Rate (ESR)

Citrated blood in Westergreen's pipette was vertically positioned and left undisturbed. The red cells aggregates and stuck together (Rouleaux formation) and sediment through the plasma. ESR is the rate at which red cells sedimentation occurs in an hour, indicated by the length of the column of clear plasma above the red cell measured in mm. High temperature (over 25°C) increases red cells sedimentation.

2.11. Platelet Count

Blood was diluted 1:20 into filtered solution of Ammonium oxalate which lyses the red cells. Platelets are counted microscopically using Improved Neubauer Ruled Counting Chamber expressed as number of platelets per litre of blood.

2.12. Differential White Cell Count (DWCC)

DWCC provide vital information on different White Cells present in circulating blood (Neutrophils, Lymphocytes, Monocytes, Eosinophil and the rarely seen Basophils). Provided total WBC count is known, the absolute number of each White Cell type, the number of each cell per litre of blood is calculated to determine whether particular cell type is increased or decreased. Thin blood film was made on clean grease free glass slide and smears were stained using Leishman stain and viewed by oil immersion for different cell types.

2.13. Statistical Analysis

Data entry and validation was done using Microsoft excel version 13. SPSS 23.0 software was employed for analysis. Statistical analysis was carried out between the treatment group and control using multivariate analysis of variance (MANOVA). A multiple comparison test, least significant difference (LSD) was used to test for significant differences between control and the treatment means $p \leq 0.05$ was considered statistically significant.

3. Result

The results showed that there was no significant change in RBC count of animals administered with extract compared to control group respectively ($p > 0.05$, Table 2). The PCV PLT and HGB was not significantly higher ($P > 0.05$) than that of the control unlike WBC at 300mg/kg ($p = 0.02$, CI 1.350-10.500).

There was significant elevation of WBC at 300mg/kg (4.28 ± 2.38) ($p < 0.05$). However, there was no significant difference at 100 and 200mg/kg compared to control. Furthermore, our finding also revealed statistical significant difference at 200 and 300mg/kg of MCHC (304.50 ± 3.54 , 303.00 ± 5.66) compared to control ($P < 0.05$, Table 4).

Doses	RBC ($\times 10^{12}/L$)	WBC $\times 10^9/L$)	PLT ($\times 10^9/L$)	HGB (g/dL)	HCT (%)
100mg/kg	8.19 \pm 0.08	12.98 \pm 0.75	316.00 \pm 312.54	12.60 \pm 0.00	42.20 \pm 0.71
200mg/kg	9.48 \pm 2.60	11.26 \pm 2.15	655.00 \pm 22.63	14.90 \pm 4.10	48.90 \pm 13.01
300mg/kg	7.35 \pm 3.99	4.28 \pm 2.38*	460.50 \pm 625.79	11.00 \pm 5.37	36.45 \pm 18.46
Control	9.39 \pm 0.84	10.20 \pm 0.00	571.00 \pm 96.17	66.80 \pm 75.24	39.10 \pm 11.60
Total	8.60 \pm 2.06	9.68 \pm 3.71	500.63 \pm 299.53	26.33 \pm 37.97	41.66 \pm 10.81

Table 2: Effects of Aqueous Leaf Extract of *Cadaba farinosa* following Oral Administration on Haematological Parameters of Wistar Rats and Their Normal Profile

All values are expressed as mean \pm SEM for 3 animals per group. Asterisk indicates significant difference between treated and control groups ($p < 0.05$).

WBC ×10 ⁹ /L)	(J) Doses	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100mg/kg	200mg/kg	1.720	1.648	0.356	-2.855	6.295
	300mg/kg	8.7050*	1.648	0.006	4.130	13.280
	Control	2.780	1.648	0.167	-1.795	7.355
200mg/kg	100mg/kg	-1.720	1.648	0.356	-6.295	2.855
	300mg/kg	6.9850*	1.648	0.013	2.410	11.560
	Control	1.060	1.648	0.555	-3.515	5.635
300mg/kg	100mg/kg	-8.7050*	1.648	0.006	-13.280	-4.130
	200mg/kg	-6.9850*	1.648	0.013	-11.560	-2.410
	Control	-5.9250*	1.648	0.023	-10.500	-1.350
Control	100mg/kg	-2.780	1.648	0.167	-7.355	1.795
	200mg/kg	-1.060	1.648	0.555	-5.635	3.515
	300mg/kg	5.9250*	1.648	0.023	1.350	10.500

Table 3: A Multiple Comparison Test, Least Significant Difference (LSD) Within WBC Following Oral Administration of *Cadaba Farinosa* on Haematological Parameters of Wistar Rats and Their Normal Profile

All values are expressed as mean ± SEM for 3 animals per group. Values for each parameter are compared among animals administered with extract and negative control group for each dose by MANOVA and Least Significant Difference (LSD). *statistically significantly compared to control, p<0.05.

Group	WBC ×10 ⁹ /L)	PLT (×10 ⁹ /L)	MCV (fL)	MCH (pg)	MCHC (g/L)
Control	10.61±0.58	669.50±43.13	53.00±1.13	15.45±0.07	289.00±1.41
100mg/kg	12.98±0.75	316.00±312.54	51.50±1.41	15.40±0.14	298.50±4.95
200mg/kg	11.26±2.15	655.00±22.63	51.65±0.35	15.75±0.07	304.50±3.54*
300mg/kg	4.28±2.38*	460.50±625.79	50.20±2.26	15.20±0.99	303.00±5.66*
Total	9.78±3.74	525.25±307.74	51.59±1.53	15.45±0.43	298.75±7.21

Table 4: Effects of Aqueous Extraction of *Cadaba Farinosa* on Haematological Parameters of Adult Albino Rats (N=12)

All values are expressed as mean ± SEM for 3 animals per group. Asterisk indicates significant difference between treated and control groups (p < 0.05).

MCHC (g/L)	(J) Doses	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100mg/kg	200mg/kg	-6.000	4.213	0.228	-17.700	5.700
	300mg/kg	-4.500	4.213	0.346	-16.200	7.200
	Control	9.500	4.213	0.087	-2.200	21.200
200mg/kg	100mg/kg	6.000	4.213	0.228	-5.700	17.700
	300mg/kg	1.500	4.213	0.740	-10.200	13.200
	Control	15.50*	4.213	0.021	3.800	27.200
300mg/kg	100mg/kg	4.500	4.213	0.346	-7.200	16.200
	200mg/kg	-1.500	4.213	0.740	-13.200	10.200
	Control	14.00*	4.213	0.029	2.300	25.700
Control	100mg/kg	-9.500	4.213	0.087	-21.200	2.200
	200mg/kg	-15.50*	4.213	0.021	-27.200	-3.800
	300mg/kg	-14.00*	4.213	0.029	-25.700	-2.300

Table 5: A Multiple Comparison Test, Least Significant Difference (LSD) Within MCHC

All values are expressed as mean ± SEM for 3 animals per group. Values for each parameter are compared among animals administered with extract and negative control group for each dose by MANOVA and Least Significant Difference (LSD). *statistically significantly compared to control, p<0.05.

4. Discussion

Normal functioning of the body of an organism requires that the blood composition and constituents are maintained under natural conditions [19]. A severe disorder has direct impact on blood parameters, so it is necessary to study the possible changes in haematological parameters [4]. Haematological evaluation in this study showed a significant increase in the level of white blood cell count (p < 0.05) in rats administered with 300mg/kg compared to normal control rats considering the p-values (F-tests 30.347, p = 0.01) Roy's largest Root (Tables 1 and 2). However, this change does not occur in all hematological parameters. The significant increase in white blood cell and the differential leukocytes counts in the test animal shows that aqueous extracts of *Cadaba farinosa* Forsk may have immune boosting properties from the excitation of defence mechanism similar to those reported for *Allium sativum* [5], *Channa punctatus* [20] and *Solanum*

incanum [19]. Since the extracts of *Cadaba farinosa forsk* caused increases in white blood cell counts, it is possible that the phytochemicals in the extract stimulated the production of these regulatory factors at the highest dose (300mg/kg) by engaging the committed stem cells responsible for the production of white blood cells. The proliferations, differentiation and maturation of committed stem cells responsible for the production of white blood cells is regulated by granulocyte-macrophage colony stimulating factor, macrophage colony stimulating factor, interleukins IL-2 IL-4 and IL-5 [19].

Further finding also revealed that there was statistical significant increase of MCHC at 200 and 300mg/kg compared to the control group ($P < 0.05$, Table 4). Decreased in MCHC (although significantly increased in this study), have been attributed to impairment of the biosynthesis of heme in bone marrow [21]. However erythrocytes, Platelet, Hemoglobin and HCT indices do not show statistical significant variation among the test group and control groups. The finding demonstrated that *Cadabafarinosa* does not cause any significant undesirable alteration on hematological factors of adult Wister rats.

5. Conclusion

Based on the aforementioned results, we concluded that oral administration of aqueous leaf extract of *Cadabafarinosa* significantly increases the white blood cell counts (WBC) and MCHC.

The erythrocytes, haematocrit, haemoglobin and mean corpuscular volume were statistically insignificant.

Hence, this underscores that *Cadaba farinosa forsk* probably stimulate immune defense mechanism.

6. References

- i. Dorcas Bolanle James, Ene Blessing Adejor, Danladi Amodu Ameh, Adetoro Olubukola Kadejo, Taiye Olaba. (2014). Preventive and Therapeutic Effects of Garcinia kola Biflavonoid Fractions on some Haematological Parameters of P407 Induced Hyperlipidemic Albino Rats. *Journal of Natural Remedies*. 14(1):42-47.
- ii. Firas Rashad Al-Samarai and Wathiq Ali Hasson Al-Jbory. (2017). Effect of some environmental factors on haematological parameters in apparently healthy Iraqi Awassi sheep. *Journal of entomology and zoology Studies* 2017; 5(3): 1668-1671.
- iii. Yakubu, M., Akanji, A., Oladiji, A. (2007). Haematological Evaluation in Male Albino Rats Following Chronic Administration of Aqueous Extract of *Fadogia agrestis* Stem. *Pharmacognosy Magazine*, 3:34-38.
- iv. Swati Shrivastava, Neelima Singh, Akshay Kumar Nigam, Sanjay Singh Chandel, Reetika Shrivastava¹, Sarvesh Kumar. (2017). Comparative study of haematological parameters along with effect of chemotherapy and radiotherapy in different stages of breast cancer. *International Journal of Research in Medical Sciences*. 5(1):311-315.
- v. Iranloye, B.O. (2002). Effect of Chronic Garlic Feeding on some Haematological Parameters. *African Journal of biomedical Research*. 5:81-82.
- vi. Njagi J Muriithi*, Gitahi S Maina, Njagi M Mugendi, Mwangi B Maina, Mworio J Kiambi, Juma K Kelvin, Aliyu Umar, Mwonjoria K John, Njoroge W Ann, Abdirahman YA, Ngugi M Piero and Njagi NM Eliud. (2015). Determination of Haematological Effects of Methanolic Leaf Extract of *S. incanum* in Normal Mice. *Pharmaceutica Analytica Acta*. 6(10):2-6.
- vii. Sofowora, A. (1993). Medicinal and Traditional medicine in Africa. Second edition, Spectrum Books Limited, Ibadan, Nigeria. Pp. 130.
- viii. Sofowora A. Medicinal plants and medicine in Africa. 3 rd edition. John Wiley Spectrum Books, Ibadan Nigeria. 2008; p 200-202
- ix. Ohlsson A, Aher SM (2009) Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. *Cochrane Database Syst Rev* 6: 227-251.
- x. A. B. Fall, R. Vanhaelen-Fastre, M. Vanhaelen et al., "In vitro antisickling activity of a rearranged limonoid isolated from *Khaya senegalensis*," *Planta Medica*, vol. 65, no. 3, pp. 209– 212, 1999.
- xi. Siva N, Ganesan, Banumathy N, Muthuchelian. Antifungal effect of leaf extract of some Medicinal Plants against *Fusarium oxysporum* causing wilt disease of *Solanum melongena* L. (Tamil Nadu, India) 2008: 1-8.
- xii. Evans, C. Williams (2009). *Trease and Evans Pharmacognosy*, 6th Edition. China: Saunders Elsevier.
- xiii. Haraguchi, H. (2001). *Antioxidative Plant Constituents: Bioactive Compounds from Natural Sources*. London: Taylor and Francis. Pp 337- 379.
- xiv. Silva, L. Gloria; I. K- Soo Lee and A. Douglas, Kinghorn. (1998). Special Problems with the Extraction of Plants, In: Cannell Corrado, (Ed.), *Methods in Biotechnology, Natural Product Isolation*. Totowa, New Jersey: Humana Press Inc.
- xv. Umesh Telrandhe, Vaibhav Uplanchiwar (2013). Phyto-pharmacological Perspective of *Cadaba farinosa forsk*. *American Journal of Phytomedicine and Clinical Therapeutics*. 1:011-022.
- xvi. [16] Gamde S.M, Umar M, Avwioro O.G, Muhammad A.A. (2019). Effects of Aqueous Stem Bark Extract of *Cadabafarinosa* on the Gastrointestinal Tract of Wistar Rats. Annual General Meeting and Scientific Conference. Association of Specialist Doctors in Academic, Pp.18.
- xvii. Majekodunmi O., Zany K., Olanyaga, I, Shi L and Melanhin J., (1996). Selective Toxicity Diterpene from *Euphorbia Pisonis*. *Journal of Medicinal Chemistry*. 39:1005-1008.
- xviii. Monica Cheesbrough (2006). *District Laboratory Practice in Tropical Countries: Erythrocyte Sedimentation Rate*. New York. Cambridge University press. 2nd Edition. Pp.329-331.
- xix. Njagi J Muriithi*, Gitahi S Maina, Njagi M Mugendi, Mwangi B Maina, Mworio J Kiambi, Juma K Kelvin, Aliyu Umar, Mwonjoria K John, Njoroge W Ann, Abdirahman YA, Ngugi M Piero and Njagi NM Eliud. Determination of Haematological Effects of Methanolic Leaf Extract of *S. incanum* in Normal Mice. *Pharmaceutica Analytica Acta*. 2015, 6:10:2-6.
- xx. Jaya Shahi and Ajay Singh. Effect of Bioactive Compounds Extracted from Euphorbious Plants on Haematological and Biochemical Parameters of *Channa punctatus*. *Rev. Inst. Med. Trop. Sao Paulo*. 53(5):259-263.
- xxi. Isselbacher, K. I., Braunwald, E., Wilson, J. D. (1992). *Harrison's principle of internal medicine*. New York McGraw-Hill.