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Effect of Ethanolic Leaf Extract of *Vernonia amygdalina* (Bitter Leaf) Extract on some of the Haematological Parameters in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated some haematological parameters of Wistar rats using *Vernonia amygdalina* extract. The dried leaves of the plant species were extracted and put in 80% Volume/volume butanol dehydrogenase (V/V; BDH) ethanol. Thirty (30) Wistar rats were divided into 3 groups of 10 rats each. Group I served as control and feed with normal food and water. Groups II & III were served as test groups and were administered with VA100 mg/kg and VA300 mg/kg, orally once daily for 28 days. Five (5) animals were sacrificed from each group on the 14th and 28th day. Blood samples were collected through cardiac puncture for analyzing hematological parameters. It was

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observed that (RBC, Hb, PCV, MCV, MCH and MCHC) showed non-significant ($p>0.05$) changes on the 14th day. However, these showed a dose – dependent significant ($p<0.05$) decrease on day 28. There was a dose – dependent significant increase ($p<0.05$) in WBC and Lymphocyte; while neutrophils count decreased significantly ($p<0.05$) with dose dependent manner. Serum platelet level decreased significantly ($p<0.05$) if the level of dose increases. These findings revealed that *V. amygdalina* may have a possible potential to inhibit erythropoietin release or action and may strengthen the immunity. The observed reduced level of neutrophil count may be one of the reasons for the benign ethnic neutropenia which is seen in Africans. However, the study helps to conclude the anti inflammatory and potential effects of bitter leaf. This particular study also depicts that, ethanolic leaf extract of *V. amygdalina*may cause anaemia especially at prolonged exposure.

Keywords: Haematological parameters; Vernonia amygdalina; anaemia; wistar rats.

1. INTRODUCTION

The homeostatic role of body fluids is such that it is very vital in physiological processes of the human body. Blood is a bodily fluid in animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells. Blood accounts for 7% of the human body weight [1], with an average density of approximately 1060 kg/m^3, very close to pure water's density of 1000 kg/m^3. The average adult has a blood volume of roughly 5 liters (1.3 gal) [2], which is composed of plasma and several kinds of cells: these formed elements of the blood are erythrocytes, leucocytes and thrombocytes [3]. Plants are sources of potential therapeutic agents against various diseases due to their biodiversity and presence of a wide array of bioactive phytochemicals and secondary metabolites [4]. *V. amygdalina* is a shrub or small tree of 2-5 m tall with petiolate green leaf of about 6mm in diameter and elliptic shape [5]. *Vernonia amygdalina*, is a member of the Asteraceae family. The leaves are green with a characteristic odour and bitter taste. No seeds are produced and therefore to be distributed through cutting. It grows under a range of ecological zones in Africa and produce large mass of foliage and drought tolerant [6]. *Vernonia amygdalina* has a variety of names in various languages. *Vernonia amygdalina*is popularly known as bitter leaf because of its characteristic bitter taste. In English, it is referred to as bitter leaf [7]. “Ewuro” in Yoruba, “Etidot”in Efik, Ijaw and Ibibio. The Igbos and the Etche people of Rivers State call it “Onugbo” or “Olubu”. The bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides [8]. Pharmacological studies have also shown that the leaf extract of *V. amygdalina*has both hypoglycaemic and hypolipidaemic properties in experimental animals and so could be used in managing diabetes mellitus [9]. Its nutritional, medicinal uses and scientific studies have respectively been articulated in two extensive reviews [4]. However, the present study was designed to evaluate the effect of *Vernonia amygdalina* extract on some haematological parameters in Wistar rats. The results of this study will create public health awareness on the benefits and toxicities of leaf extract of *Vernonia amygdalina* and scientifically direct its use for better therapeutic potential.

2. MATERIALS AND METHODS

2.1 Study Centre and Period

This study was conducted at the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba, Nigeria; between June and August, 2014.

2.2 Collection and Identification of Plant Materials

Fresh leaf samples of *Vernoniaamygdalina* obtained from a private farm in Odufor Community in Etche Local Government Area of Rivers State, Nigeriawere identified and authenticated by Dr. N. L. Edwin–Wosu, of the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria.

2.3 Preparation of Ethanolic Leaf Extracts of *Vernonia amygdalina*

Fresh leaves of *V. amygdalina* were rinsed in clean water to remove dirt, and dried at room temperature (26ºC) for a period of 3 weeks. The dried leaves were milled to fine powder using manual engine grinder (Modelcorene, A.5

lander YCIA S.A) and 500 g of the plant was obtained.
The weighed quantity was soaked in 400 ml of 80% Volume/volume butanol dehydrogenase (V/V; BDH) ethanol. (v/v, BDH) ethanol for 48 hours.) It was then filtered with Whatman No. 1 filter paper to separate the filtrate from the residue. The extract was concentrated under reduced pressure in a vacuum at 45ºC using a rotary evaporator (Searl Instruments Ltd. England). The yield of the crude ethanolic extract of *V. amygdalina* leaves obtained weighed 63.1g. The extract was stored in a refrigerator at 4ºC before use for the study.

2.4 Experimental Animals

Thirty (30) male Wistar albino rats weighing (160 – 270 g) used for this study were purchased from animal farm of Department of Human Physiology and kept at the animal house, Department of Human Physiology, University of Port Harcourt, Nigeria, in spacious and well ventilated cagesat room temperature (28 ± 1ºC) and under natural day/light cycles. They were allowed to acclimatize for 14 days and had free access to feed and water *ad libitum*. "Principles of laboratory animals care" (NIH publication No. 85, revised 119 (1985), were followed as well as specific national laws where applicable [10].

2.5 Experimental Design

Thirty (30) experimental rats were randomly grouped into three (3) groups (I - III) of 10 animals (n=10) each after the period of acclimatization. Group I: Served as the Control, received water and normal feed. Group II: Received 100mg/kg body weight of ethanolic leaf extract of *V. amygdalina*. Group III: Received 300 mg/kg body weight of ethanolic leaf extract of *V. amygdalina.* The different animal groups received 1 ml of their respective extract doses using an oral gavage, once daily (9 am – 10 am), for a period of 28 days.

The doses of 100 mg/kg and 300 mg/kg for *Vernonia amygdalina* was chosen based on a previous study that extracts from the *Vernonia amygdalina* leaves have LD_{50} of 500 mg/kg [7].

Five (5) Animals from each group were sacrificed on the 15^{th} and 29^{th} day respectively, after 14^{th} and 28^{th} day of extract administration and blood was collected by cardiac puncture into EDTA tubes and estimated for haematological parameters viz, packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell counts (WBC), lymphocytes, neutrophil and platelet using an automated analyzer machine (Beacon by Mindray Haematological Analyzer, Bc 2300, US) available in the Haematology unit, University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Nigeria. Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated from the values of RBC, PCV and Hb obtained as follows:

MCV (fL) = PCV (%) X 10/ RBC Count
MCH (pg) = Hb (g/dl) X 10/ RBC Count
MCHC (g/dl) = Hb (g/dl) X 100/ PCV (%)

2.6 Statistical Analysis

Data were expressed as Mean ± SEM and data were analyzed using one way analysis of variance (ANOVA). Differences were considered significant at $p<0.05$.

3. RESULTS

The mean body weight changes of Wistar rats before and after administration of ethanolic leaf extracts of *V. amygdalina* are shown in Fig. 1.

From the result, the mean body weight of animals' administered with VA 300 mg/kg (184.00 ± 5.21) for day 14 recorded a non-significant increase when compared to day 0. There was a significant ($p<0.05$) decrease in mean body weight of rats on day 28 for VA 300 mg/kgwhen compared to day 0. However, the mean values for control (210.00 ± 5.48) and VA 100 mg/kg (175.00 ± 2.24) increased significantly ($p<0.05$) when compared to the day 0.

As summarized in Table 1, the mean values of erythrocyte parameters (Red blood cell, Haemoglobin, Packed cell volume, Mean corpuscular volume, Mean corpuscular haemoglobin and Mean corpuscular haemoglobin concentration) showed non-significant ($p>0.05$) changes on the 14^{th} day of extract administration. However, this was different on the 28^{th} day (Table 2); mean values of RBC, PCV and Hb counts at the tested doses of 100 and 300 mg/kg b w showed a dose – dependent significant ($p<0.05$) decrease when compared to the control.

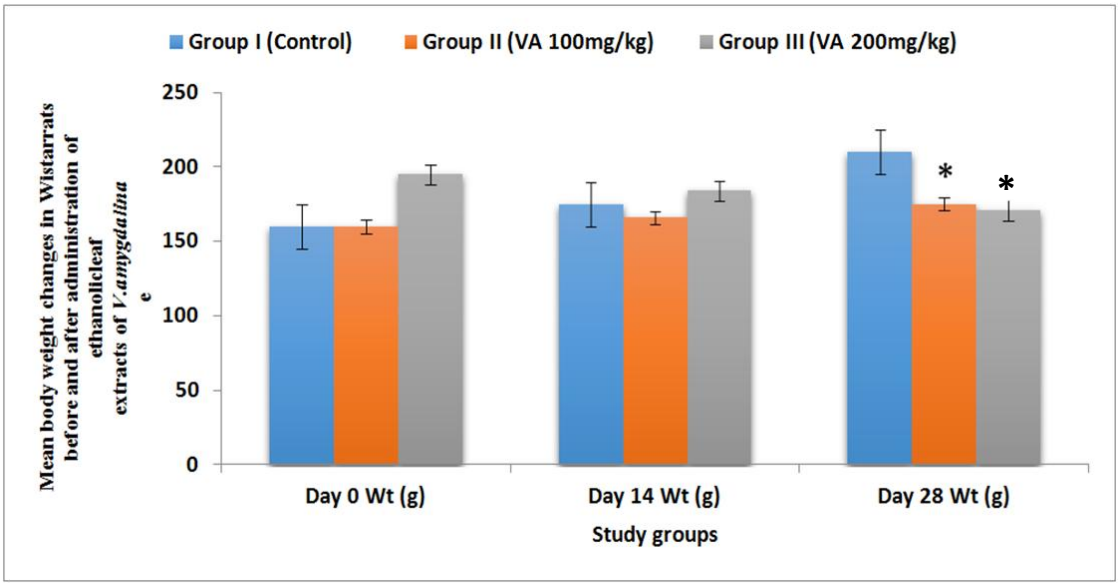

Fig. 1. Mean body weight changes in Wistarrats before and after administration of ethanolic leaf extracts of *V. amygdalina*

** Significant at P<0.05; VA = Vernonia amygdalina*

Table 1. Effects of ethanolic leaf extract of *V. amygdalina* on erythrocyte parameters after 14 days of administration in wistar rats

Parameters	Group I (Control)	Group II (VA 100 mg/kg)	Group III (VA 300 mg/kg)
RBC ($X10^{12}$/L)	7.31 ± 0.23	7.42 ± 0.41	6.52 ± 0.26
Hb(g/dl)	12.48 ± 0.32	11.52 ± 0.31	13.00 ± 0.77
PCV (%)	45.00 ± 1.05	44.40 ± 0.98	48.00 ± 1.87
MCV (FL)	61.94 ±3.14	60.69 ± 4.13	74.03 ± 4.04
MCH (pg)	17.12 ± 0.67	15.65 ± 0.62	20.07 ± 1.46
MCHC (g/dl)	27.79 ± 0.99	25.98 ± 0.76	27.29 ± 2.01

Values are expressed as Mean ± SEM; n=5; VA = Vernoniaamygdalina

Table 2. Effects of ethanolic leaf extract of *V. amygdalina* on erythrocyte parameters after 28 days of administration in wistar rats

Parameters	Group I (Control)	Group II (VA 100 mg/kg)	Group III (VA 300 mg/kg)
RBC ($X10^{12}$/L)	7.24 ± 0.16	6.33 ± 0.04*	5.30 ± 0.08*
Hb(g/dl)	11.20 ± 0.29	9.52 ± 0.11*	9.22 ± 0.06*
PCV (%)	43.80 ± 1.07	40.40 ± 1.03*	36.40 ± 0.81*
MCV (FL)	60.65 ± 2.29	63.81 ± 1.85	68.67 ± 1.64*
MCH (pg)	15.51 ± 0.62	15.03 ± 0.20	17.40 ± 0.26*
MCHC (g/dl)	25.59 ± 0.20	23.63 ±0.66*	25.39 ± 0.65

*Values are expressed as Mean ± SEM; n=5; * Significant at P<0.05; VA = Vernoniaamygdalina*

On the other hand, treatment of the animals with *V. amygdalina*at tested dose of 300 mg/kg b w produced a significant increase ($p<0.05$) in the mean values of MCV and MCH count with a non-significant increase ($p>0.05$) observed for MCHC value when compared to the control. The mean values of MCV and MCH for animals administered with *V. amygdalina* at tested dose of 100 mg/kg b w produced a non-significant change ($p>0.05$); with a significant increase (<0.05) observed for MCHC value when compared to the control.

Table 3. Effects of ethanolic leaf extract of *V. amygdalina* on white cell parameters and platelets after 14 days of administration in wistar rats

Parameters	Group I (Control)	Group II (VA 100mg/kg)	Group III (VA 300mg/kg)
WBC ($X10^9$/L)	12.10 ± 0.17	29.92 ± 0.57*	17.88 ± 0.67*
LYMPHOCYTE (%)	34.20 ± 1.99	73.80 ± 2.46*	76.60 ± 0.93*
NEUTROPHIL (%)	65.80 ± 1.99	24.20 ± 1.11*	23.40 ± 0.93*
PLATELET ($X10^9$/L)	406.40 ± 4.31	466.00 ± 2.72	417.40 ± 5.52

*Values are expressed as Mean ± SEM; n=5; * Significant at P<0.05; VA = Vernonia amygdalina*

Table 4. Effects of ethanolic leaf extract of *V. amygdalina* (VA) on white cell parameters and platelets after 28 days of administration in wistar rats

Parameters	Group I (Control)	Group II (VA 100 mg/kg)	Group III (VA 300 mg/kg)
WBC ($X10^9$/L)	11.92 ± 0.20	24.52 ± 2.39*	21.74 ± 0.82*
LYMPHOCYTE (%)	31.20 ± 1.83	89.00 ± 0.71*	88.40 ± 0.51*
NEUTROPHIL (%)	66.80 ± 2.04	11.00 ± 0.71*	11.60 ± 0.51*
PLATELET ($X10^9$/L)	402.20 ± 3.98	526.20 ± 50.42	157.00 ± 5.24*

*Values are expressed as Mean ± SEM; n=5; * Significant at P<0.05; VA = Vernonia amygdalina*

4. DISCUSSION AND CONCLUSION

The study investigated the effect of ethanolic leaf extract of *Vernonia amygdalina* on some of the haematological parameters using normal Wistar rats as models.

Hematological parameters such as erythrocyte markers (Red blood cell, Packed cell volume, Haemoglobin, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration) and leukocyte markers.

*V. amygdalina*at tested doses of 100 and 300mg/kg b w produced a dose – dependent significant increase ($p<0.05$) in blood level of lymphocyte count; anda dose – dependent significant decrease ($p<0.05$) in blood level of neutrophil count when compared to the control. There was significant ($p<0.05$) decrease in the level of platelets for the high dose.

White blood cells, platelet, neutrophil, and lymphocytes are used to provide useful information for diagnosis in routine clinical evaluation of the state of health of a patient.

Changes in the haematological system have a higher predicative value for human toxicity.

The results obtained on the day 14, showed a non-significant change ($p>0.05$) in the blood levels of erythrocyte parameters (red cell count, haemoglobin concentration, packed cell volume, MCV, MCH and MCHC).

This is an indication that there may be no destruction of red blood cells and no pronounced change in the rate of production of RBC (erythropoiesis) therefore, suggesting the non - toxic nature of the plant extracts to red blood cells at this period.

Blood level of Platelet count for animals treated with *V. amygdalina* at the dose of 100 mg/kg b w increased non-significant ($p>0.05$), but as the concentration increases to 300mg/kg b w, the blood level of Platelet count decreases; however, this was also non-significant ($p>0.05$) when compared to the control (Table 3). However, a significant reduction ($p<0.05$) in the blood level of Platelet count was observed for treated dose of 300 mg/kg b w when compared to the control (Table 4).

However, on the 28^{th} day of administration, a dose – dependent significant reduction ($p<0.05$) in the blood levels of erythrocyte parameter was observed, especially for RBC, Hb and PCV counts, this finding revealed that *V. amygdalina* may have a possible potential to inhibit erythropoietin release from the kidneys, which is the humoral regulator of RBC production and also affect the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since red blood cells and haemoglobin (Hb) are very important in transferring respiratory gases [11,12]. It is therefore possible the excessive consumption of *Vernonia amygdalina* by humans can lead to anaemic state especially in menstruating and pregnant women. Further

collaborative work is ongoing with the Department of Obstetrics and Gynecology, University of Port Harcourt Teaching Hospital, Nigeria.

The reduction might be due to the presence of saponin, which has been reported to reduce haematological parameters probably due to lysis of blood cells or suppression of blood synthesis [13].

The high significant increase ($p<0.05$) in blood level of WBC count observed at the dose of 100mg/kg b w, as compared to the significant decrease ($p<0.05$) in WBC count for 300mg/kg b w. The increase in WBC and lymphocyte counts may be due to the presence of anti-nutritional compounds such as saponins, sesquiterpenes, flavonoids and steroid glucosides such as vernoniosides in *V. amygdalina* [5] It has been stressed by [14], that the high percentage of WBC especially lymphocytes are associated with the ability of the animals to perform well under very stressful condition. These increases in the WBC and percentage lymphocyte counts suggest that the phytochemical compounds present in the extracts elicited stress responses. The effect of the extracts on the total WBC count could be due to the presence of glycosides. This compound has an anti-inflammatory property and so has vital effect on inflammatory processes of some pathological states such as bacterial infection, malaria and liver diseases [15].

This finding is in agreement with the work of [16] which suggested that *V. amygdalina* extract may strengthen the immune system through many cytokines regulation. The result on reduced level of neutrophil count caused by the plant extracts may be one of the reasons for the benign ethnic neutropenia experienced by Africans. This probably indicates that the body's ability to attack and destroy invading bacteria, viruses and other injurious agents (Phagocytosis) was compromised [16]. The non-significant increases ($p>0.05$) in platelet count observed for treated dose of 100 mg/kg bw on the 14 day, and the subsequent significant decrease ($p<0.05$) observed for tested dose of 300 mg/kg bw on the 28 day, is an indication of the probable ability of the extract to inhibit the actions of platelet activating factor (PAF) especially when administered at a longer duration, hence reducing the blood clotting potentials.

In conclusion, the present study which investigated the effect of ethanolic leaf extract of *Vernonia amygdalina* on some of the haematological parameters have showed that ethanolic leaf extract of *V. amygdalina* may cause anaemia especially at prolonged exposure, this is depicted by the observed effect on the body weights of the animals. However, the anti-inflammatory and potentials of the plant extract was shown.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, ethical rules and regulations for animals have been followed by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Alberts, Bruce: Table 22-1 blood cells. Molecular Biology of the Cell. NCBI Bookshelf; 2012.
2. Elert, Glenn and his students': Volume of Blood in a Human. The Physics Fact book. Archived from the original on 2012-11-01.
3. Austin CC, Perkins, SL. Parasites in a biodiversity hotspot: A survey of hematozoa and a molecular phylogenetic analysis of plasmodium in new guinea skinks. J. of Parasitol. 2006;92(4):770-7.
4. Farombi EO. African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents Afr. J. Biotechnol. 2003;2(12):662-671.
5. Argheore EM, Makkar HPS, Becker K, Feed value of some plants; 1998. Available:http://www.nih.gov/news/health/may2011/nhlbi-26.htm
6. Bonsi GO, Lessen WA, Burda S, Jurszsta M. Nutritional assessment of *Vernonia amygdalina* leave in growing mice. J. of Agric. food chem. 1995;(43):2162-2166.
7. Ojiako OA, Nwanjo HU. Is *Vernonia amygdalina* hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats. Afri. J. of Biotech. 2006;5 (18):1648-1651.
8. Ologunde BO, Akinpelu D, Aregheoro EM. Antimicrobial activity of *Vernonia*

amygdalina leaves. Fitoterapia J. Stud. Med. Plant. 1999;(70):432.

9. Izevbigie EB, Bryant JL, Walker A. Narural inhibitor of extracellular signal regulated kinases and human breast cancer cells. Exp. Bio. Med. 2004;(229):163-169.
10. National Institute of Health (N.I.H). Guide for the care and use of laboratory 343 animals. DHEW Publication. Office of Science and Health Reports. 1985; Bethesda, 344 U.S.A.
11. Polenakovic M, Sikole A. Is erythropoietin a survival factor for red blood cells? J. Am. Soc. Nephrol. 1996;7(8):1178-1182.
12. Oyedeji KO. Bolarinwa AF, Akintola AM. Effect of methanolic extract of *Vernonia amygdalina* on haematological and plasma biochemical parameters in male albino rats. J. Dental Med. Sci. 2013;3(5):64-67.
13. Schneider CR, Sheidt K, Brietmaier E. Four new pregnant glycosides from *Gongrenema latifolium* (Asclepidaceous). J. Parkische Chem Chenisker – Zutung. 2003;(353):532–536.
14. Mitruka HM, Rawnsley SK. Chemical, biochemical and haematological reference in moral experimental animal. Mason, N.Y. 1997;287-380.
15. Ugochukwu NH, Babady NE. Antihyperglycemic effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. Life Sci. 2002;73(15):1925-1938.
16. Sweeney CJ, Mehrotra S, Sadaria MR, Kumar S, Shortle NH, Roman Y, Sheridan C, Campbell RA, Murray DJ, Badve S, Nakshatri H. The sesquiterpene lactone parthenolide in combination with docetaxel reduces metastasis and improves survival in a xenograph model of breast cancer. Molecul. Can. Therap. 2005;4(6):1004.

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