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Effect of Ethanolic Leaf Extract of *Vernonia amygdalina* on Haematological Parameters in Albino Rats

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Authors' contributions

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

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Original Research Article

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ABSTRACT

It has been demonstrated that *Vernonia amygdalina*, often known as the bitter leaf, offers a range of medical benefits that can improve human health. This study was designed to investigatethe effects of *V. amygdalina* (VA) ethanolic leaf extracts on some haematological parameters in albino rats. A total of thirty - six albino rats fed with a commercial pelleted poultry grower's mash- diet were used for this study and they were divided into six groups, each containing six rats. Groups 1, 2, 3, 4 and 5 received 10, 20, 30, 40 and 50 mg/kg body weight (bwt) of VA leaf extract three times a week at two-day intervals over a period of three weeks. Group 6 (control) received water only. The haematological parameters (red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), total white blood cell count (TWBC), platelet (PLT) count, and WBC differentials (neutrophils, lymphocytes, and mixed cells) were analyzed using three part full blood count autoanalyzer. The results showed that although the mean neutrophil count differed significantly

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($p<0.05$) when compared between the groups studied, there was no statistically significant ($p>0.05$) difference in the mean levels of PCV, Hb, RBC, TWBC, platelet count, lymphocytes and mixed cell count when compared across and between the groups. This study revealed that *Vernonia amygdalina* had no negative effects on the haematological indicators studied.

Keywords: Vernonia amygdalina; bitter leaf; haematological parameters; Rattus albus.

1. INTRODUCTION

"Medicinal plants are thoseplants that contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs" [1,2]. **"**Medicinal plants have been used by man for ages in traditional medicine as a result of their therapeutic potential. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relied on traditional medicine for their primary healthcare needs" [3,4]."The reasons for this, especially in developing nations, include ease and cost of assessing orthodox medicine as well as thecost of procuring prescribed medications" [5,6].

"Over 5000 plants are known to be used for medicinal purposes in Africa, but only a few have been described or studied" [7]. "Only a few of the plants/herbs used in herbal medicine herbs have been scientifically validated for the claimed medicinal effects, hence slowing down the pace of drug discovery from such plants. Natural products from plants can be another potent source for the discovery of excellent biological activities, that is: anticancer and antioxidant activities" [8]. "This therefore, brings about the increasing recognition of herbal medicine as an alternative form of health care. Herbal prescriptions and natural remedies are consequently a common practice in developing countries for the treatment of various diseases and this practice is an alternative way to compensate for some perceived deficiencies in orthodox Pharmacotherapy" [9,10].

"Vernonia amygdalina is a perennial herb belonging to the family, Asteraceae. *V. amygdalina* is commonly known as a bitter leaf because of its bitter taste. It has been shown to possess a number of medicinal values including theanti-diabetic effect" [11,12], hypolipidemic effect [13] and hepatoprotective activity [14]. Also, Ademola and Eloff, [15], reported that extracts of *V. amygdalina* possess *In vitro* anti-parasitic (anti-helminthic) properties. "Thus, it is effective against amoebic dysentery, gastro-intestinal disorders and has anti-microbial and anti-parasitic activities" [16,17]. However, other studies have noted inconsistent results regarding the impact of *V. amygdalina* leaf on various haematological indicators [18,19,20]. On the other hand, several additional research discovered that *V. amygdalina*leaves are susceptible to contamination by heavy metals and environmental pollutants, which may have a negative effect on its haematological effects [21].

"The increasing demand for herbal products coupled with the erroneous impression by the people that herbal products are natural and thus less harmful to the body" [22] and "has brought concern and fear over the quality, efficiency and safety of some of the available natural heals. Blood is a good indicator to determine the health of an organism. It is also a good pathological mirror of the entire body" [23]. "The Cellular component of blood is valuable in immunotoxicology to evaluate the immunotoxic potential of a compound. To this end, haematological parameters are important in establishing the body's functional status as a result of exposure to toxicants" [24]. "Due to the limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these remedies, there is the need to design works that look into the safety of the commonly used plant in our immediate society to further expose the possible associated effects of the continuous use of the herbs" [25]. The study therefore is aimed at providing information on the effects of *V. amygdalina* ethanolic leaf extracts on some haematological parameters in Albino rats.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Orlu, Imo State, South-Eastern Nigeria. Imo State shares boundaries with Anambra State in the North, Rivers State in the South and West and Abia State in the East. The standard of living is average and most of the populace depends on locally prepared herbs as an alternative medicine for their ailments since they are readily available and affordable.

2.2 Plant Material

Vernonia amygdalina was the medicinal plant employed in this investigation. Fresh *Vernonia amygdalina* leaves were gathered from unused farmland at Amaifeke, Orlu. The plant was identified in the herbarium of Imo State University in Owerri, Nigeria, which houses the Department of Plant Science and Biotechnology. There were also deposited voucher specimens of the plant.

2.3 Laboratory Animals

The laboratory animals used for this study were the Wistar strain of *Rattus albus* of 2 to 3 months old and body weights of 120 to 180 g. The Albino rats were purchased from an accredited animal house. The animals were quarantined and allowed to acclimatize to the laboratory conditions for a period of two weeks. They were fed with a commercial pelleted poultry grower's mash- diet. Potable water was also given at intervals.

2.4 Laboratory Animal Handling

All animals were treated in a manner that complied with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals [26].

Inclusion criteria: Healthy *Rattus albus* without any sign or symptom of cardiac or renal diseases were selected for the study.

Exclusion criteria: *Rattus albus* with cardiac and renal disease markers when tested were excluded from the research.

2.5 Processing of Plant Materials

The leaves of *Vernonia amygdalina* were dried under the shade and finally in a thermostatically controlled hot air oven at 40ºC until each maintained constant weight. Each was ground into fine powder using a warren blender machine and sieved using a 1 mm mesh sieve. The powdered plant materials were stored in labeled screw capped bottles and stored in the fume cupboard until required for extraction.

2.6 Extraction of Active Principles of the Plant Material

The active principle of the selected plant materials was extracted with ethanol at 78ºC using the soxhlet extraction method as in Harborne, [27]; Obiajuru and Ozumba, [28]. The extracts were recovered and stored at +8ºC in screw - capped MacCartney bottles until required for use.

2.7 Experimental Design

A total of thirty-six (36) apparently healthy albino rats were used for the study to determine the effects of the selected plant extract on Haematological parameters. Each albino rat received its normal daily feed and water while experimental groups in addition to their normal feed and water were treated with different doses of the plant extracts three times a week at two days interval for a period of 3 weeks.

The Laboratory animals were divided into six groups each made up of six albino rats according to:

Group 1: Received 10 mg of *V. amygdalina* ethanolic leaf extract/Kg body weight.
Group 2: Received 20 mg of *V. amygdalina* ethanolic leaf extract/Kg body weight.
Group 3: Received 30 mg of *V. amygdalina* ethanolic leaf extract/Kg body weight.
Group 4: Received 40 mg of *V. amygdalina* ethanolic leaf extract/Kg body weight.
Group 5: Received 50 mg of *V. amygdalina* ethanolic leaf extract/Kg body weight.
Group 6: Received feed and 0.5 ml of water only.

The albino rats were anesthetized at the end of the third week by placing them on wire gauze and placing cotton wool soaked in diethyl ether beneath the gauze in a clear glass desiccator.

Each of the albino rats was put to sleep within 34 to 57 seconds (on average 48.7 seconds).

To avoid adhesion proteins (coagulation factors) in cell-cell-matrix interactions for haematological analyses, 2 ml of blood from each animal was drawn through heart puncture and placed into Ethylene-diamine-tetra-acetic acid (EDTA) bottles after sedation.

2.8 Laboratory Estimation of Haematological Parameters

The haematological parameters (red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), total white blood cell count (TWBC), platelet (PLT) count, and WBC differentials (neutrophils, lymphocytes, and mixed cells) were analyzed using three part full blood count autoanalyzer (Biobase hematology analyzer-BK-6190).

2.9 Statistical Analysis

The data obtained from the study were analyzed using the analysis of variance (ANOVA) and posthoc test using IBM SPSS Statistics version 23.0. Results were expressed as mean ± SD and a p-value < 0.05 was assumed significant.

3. RESULTS

When the mean packed cell volume (PCV) was compared within and between the groups, there were no statistically significant differences (F= 1.509; P>0.05) respectively.

The results of the analysis of variance revealed that there was no statistically significant difference in the group's mean hemoglobin (Hb) levels (F=1.509; P=0.216). Additionally, a paired-wise analysis of the mean Hb values revealed no statistically significant differences.

Furthermore, the results of the analysis of variance revealed that there was no statistically significant difference in the group's mean red blood cell count (RBC) levels (F=1.509; P=0.216). Also, a paired-wise analysis of the mean RBC count revealed no statistically significant differences (p>0.05).

There was no statistically significant difference in the group's mean total white blood cell count (TWBC) levels, according to the analysis of variance results (F=1.509; P=0.216). Additionally, there were no statistically significant differences in the mean TWBC count after a paired-wise analysis (p>0.05).

The results of the analysis of variance (F=1.090; P=0.403) showed no statistically significant difference in the mean platelet count compared across the groups. After a paired-wise analysis, the mean platelet count showed no statistically significant differences (p>0.05).

However, there was a statistically significant mean difference in neutrophil count compared using the analysis of variance (F=2.783; p=0.031). The mean neutrophil count was statistically significantly decreased in group 2 albino rats than in those in group 3 (31.00±5.48 Vs 53.50 ±2.12; P=0.034). Also, the mean neutrophil count was statistically significantly increased in group 3 albino rats than in those in group 5 (53.50 ±2.12 Vs 31.25±9.07; P=0.038), although all other paired-wise comparisons did not differ statistically significantly (p>0.05).

However, when comparing the mean lymphocyte count as well as the mixed cell count between and among the study groups, there was no statistically significant difference (F=2.470, 2.678;p>0.05) respectively.

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Fig. 1. Diagram of *Vernonia amygdalina* leaf (bitter leaf)

Table 1. Levels of PCV, Hb, RBC, TWBC and platelet count in the albino rats administered with different doses of *V. amygdalina* (bitter leaf) leaf extracts (Mean±SD, n=36)

Groups	PCV (%)	Hb (g/dl)	RBC (pg/L)	TWBC (cells/µL)	Platelet (cells/µL)
Group 1(10mg/Kg bwt; n=6)	37.75±2.75	12.58±0.92	6.29±0.46	6.45±3.04	197.25±7.56
Group 2 (20mg/Kg bwt; n=6)	34.50±4.20	11.50±1.40	5.75±0.70	5.85±1.85	236.25±4.50
Group 3 (30mg/Kg bwt; n=6)	37.00±1.41	12.33±0.47	6.17±0.24	10.30±0.42	277.50±3.54
Group 4 (40mg/Kg bwt; n=6)	37.00±1.83	12.33±0.61	6.17±0.30	7.50±0.36	305.25±5.50
Group 5 (50mg/Kg bwt; n=6)	34.75±4.43	11.58±1.48	5.79±0.74	6.48±1.78	280.50±14.99
Group 6 (control; n=6)	35.75±0.96	11.92±0.32	5.96±0.16	8.46±1.86	274.00±6.83
f-value	1.509	1.509	1.509	1.499	1.090
p-value	0.216	0.216	0.216	0.219	0.403
1 Vs 2	1.000	1.000	1.000	1.000	1.000
1 Vs 3	1.000	1.000	1.000	0.819	1.000
1 Vs 4	1.000	1.000	1.000	1.000	0.803
1 Vs 5	1.000	1.000	1.000	1.000	1.000
1 Vs 6	1.000	1.000	1.000	1.000	1.000
2 Vs 3	1.000	1.000	1.000	0.370	1.000
2 Vs 4	1.000	1.000	1.000	1.000	1.000
2 Vs 5	1.000	1.000	1.000	1.000	1.000
2 Vs 6	1.000	1.000	1.000	1.000	1.000
3 Vs 4	1.000	1.000	1.000	1.000	1.000
3 Vs 5	1.000	1.000	1.000	0.846	1.000
3 Vs 6	1.000	1.000	1.000	1.000	1.000
4 Vs 5	1.000	1.000	1.000	1.000	1.000
4 Vs 6	1.000	1.000	1.000	1.000	1.000
5 Vs 6	1.000	1.000	1.000	1.000	1.000

**Statistically significant at p<0.05*

Table 2. Levels of neutrophil, lymphocyte and mixed cell count in the albino rats administered with different doses of *V. amygdalina* (bitter leaf) leaf extracts (Mean±SD, n=36)

Groups	Neutrophil (%)	Lymphocyte (%)	Mixed cells (%)
Group 1(10 mg/Kg bwt; n=6)	35.50±5.26	61.50±6.61	3.00±2.01
Group 2 (20 mg/Kg bwt; n=6)	31.00±5.48	64.50±6.03	3.50±1.52
Group 3 (30 mg/Kg bwt; n=6)	52.50±2.12	46.50±2.12	1.00±0.57
Group 4 (40 mg/Kg bwt; n=6)	32.75±9.07	64.00±8.41	2.45±1.64
Group 5 (50 mg/Kg bwt; n=6)	31.25±9.07	67.50±7.59	2.50±0.71
Group 6 (control; n=6)	37.00±9.97	60.50±10.47	1.75±0.96
f-value	2.783	2.470	2.678
p-value	0.031*	0.050	0.116
1 Vs 2	1.000	1.000	1.000
1 Vs 3	0.200	0.406	1.000
1 Vs 4	1.000	1.000	1.000
1 Vs 5	1.000	1.000	1.000
1 Vs 6	1.000	1.000	1.000
2 Vs 3	0.034*	0.066	1.000
2 Vs 4	1.000	1.000	1.000
2 Vs 5	1.000	1.000	1.000
2 Vs 6	1.000	1.000	1.000
3 Vs 4	0.069	0.166	1.000
3 Vs 5	0.038*	0.096	1.000
3 Vs 6	0.352	1.000	1.000
4 Vs 5	1.000	1.000	1.000
4 Vs 6	1.000	1.000	1.000
5 Vs 6	1.000	1.000	1.000

**Statistically significant at p<0.05*

4. DISCUSSION

Medicinal plants have long been utilized as medicine throughout human history to cure a variety of illnesses. According to estimates, traditional medicine is relied upon by around 80% of people who reside in developed nations [29]. Due to their acclaimed therapeutic potentials, medicinal plants are gaining recognition on a Global scale [30]. As a result, several medicinal plants are continuously being researched for this same objective especially in developing countries [31,32,33].

There were no statistically significant differences found in the current investigation when the mean packed cell volume, red blood cell count and hemoglobin level were compared between the groups administered with different doses of *V. amygdalina* and the control laboratory animals. This result demonstrates that *V. amygdalina*, sometimes known as thebitter leaf, is not hematotoxic and may not have hematinic value when consumed on a short-term basis. Furthermore, it shows that there was no loss of red blood cells and no alteration in the rate of red blood cell formation (erythropoiesis) as a result of its inability to stimulate the production and release of erythropoietin which is required for red blood cell formation. Since RBC and haemoglobin (Hb) are crucial for transporting respiratory gases, the non-significant effects of the *V. amygdalina* ethanol extract suggest that there were no changes in the blood's ability to carry oxygen and the amount of oxygen delivered to tissues. All vertebrate red blood cells and some invertebrate tissues contain haemoglobin, an iron-containing oxygen transport metalloprotein.

It transports oxygen from the lungs to the rest of the body, where it is released to oxidize nutrients and supply energy to regulate the organism's functions [34]. The current result is in consonance with the reports of Nubila et al. [18], Momoh et al. [35] and Oyedeji et al. [36] whofound no significant alterations in red blood cell count, packed cell volume and haemoglobin level following the administration of *V. amygdalina* in experimental animals compared to the control groups. However, this result is in contrast with the results of some other previous studies which observed that bitter leaf in respective the dose is able to improve the haematological parameters [19]. Additionally, Chike et al. [20] found that following 28 days of treatment of *V. amygdalina*, there was a dose-dependent significant decrease in the blood levels of erythrocyte parameters, particularly for RBC, Hb, and PCV counts, which is inconsistent with the current finding.

The present study found no statistically significant mean difference in the total white blood cell count when compared between the experimental groups and the control. This is in agreement with the finding of Nubila et al. that recorded “no significant effect of *V. amygdalina* on white blood cell count following their study which evaluated the sub-acute effects of the methanolic crude leaf extract of *Vernonia amygdalina* on the haematological profile in albino wistar rats” [18]. It is possible that the immune system has not been weakened based on the non-significant change in total white blood cell count (TWBC) caused by the ethanolic leaf extract of *V. amygdalina*.

Furthermore, this study recorded no significant difference in mean platelet count when compared between the experimental groups and the control respectively. This might be a sign that ethanolic leaf extract of *V. amygdalina* did not have the ability to increase thrombopoietin production, as platelets are involved in the blood clotting process otherwise termed hemostasis. Some other previous studies have also documented similar results to the present study [36] although some other studies found that the mean value of the platelet count was statistically significantly decreased following 25 mg/kg body weight administration of *V. amygdalina* for six days when compared with the control [18] which does not agree with our current finding.

Additionally, the mean neutrophil count in group 2 (20 mg /Kg body weight) albino rats was statistically significantly lower than that in group 3 (30 mg /Kg body weight). Also, the mean neutrophil count in group 3 (30 mg/Kg body weight) albino rats was statistically significantly higher than in group 5 (50 mg /Kg body weight) albino rats, despite the fact that all other paired-wise comparisons did not show statistically significant differences. This suggests that *V. amygdalina* may have the capacity to significantly modify neutrophil count. The first line of defense used by the host immune system against invading pathogens is made up of neutrophils, which are polymorphonuclear and phagocytic leukocytes [37]. They play a significant role in tissue injury-induced inflammation as effector cells as well [38]. “People with a neutrophil deficiency (such as

neutropenia) are more vulnerable to bacterial and fungal infections because neutrophils are highly potent and effective at detecting and eliminating microbial infections" [39]. Neutrophils interact with other immune cells, such as lymphocytes and antigen-presenting cells (APC), to influence the immune response in addition to killing pathogens by phagocytosis, degranulation, and the production of NETs [40,41].

Additionally, the non-significant change in lymphocyte count seen in this study implies that *V. amygdalina* did not impair the body's acquired immune response. Similarly, there was no statistically significant difference in the mixed cell count; this suggests that the albino rats' treatment with the ethanolic leaf extract of *V. amygdalina* did not negatively impact the body's ability to perform phagocytic functions.

5. CONCLUSION

This study found no significant changes in packed cell volume, hemoglobin, platelet count, white blood cell count, red blood cell count, lymphocyte count, or mixed cell count after albino rats were given an ethanolic leaf extract of *V. amygdalina* for three weeks. However, neutrophil counts showed significant alterations.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no completing interests exist.

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