EFFECT OF ETHANOLIC EXTRACT OF *Vernoniaamygdalina* ON HAEMATOLOGICAL PARAMETERS IN ALBINO RATS

ABSTRACT

It has been demonstrated that *Vernoniaamygdalina*, often known as bitter leaf, offers a range of medical benefits that can improve human health.This study was designed to the effects of *V. amygdalina*(VA) ethanolic leaf extracts on some haematological parameters in albino rats.A total of thirty six albino rats 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 in addition to receiving growers' mash in all other groups. 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 packed full blood count autoanalyzer. The results showed that although the mean neutrophil count differed significantly ($p<0.05$) 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 *Vernoniaamygdalina* had no negative effects on the hematological indicators studied.

KEY WORDS: *Vernoniaamygdalina*, bitter leaf, haematological parameters, *Rattus albus*.

INTRODUCTION

Comment [CC1]: State of the existing knowledge on the research question has been well described, justifying the need for the study

Comment [CC2]: Add those

Medicinal plants are thoseplants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Abolaji*et al*., 2007; Adebayo *et al*., 2010a).Medicinal plants have been used by man since 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 (Pierangeli*et al*., 2009; Ammara*et al*., 2009).The reasons for this, especially in developing nations, include ease and cost of assessing orthodox medicine as well as cost of procuring prescribed medications (Dyson, 1998;Chan, 2003).

Over 5000 plants are known to be used for medicinal purposes in Africa, but only a few have been described or studied (Taylor *et al*., 2001). Only 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 (Adebayo *et al*., 2010b). This therefore brings about the increasing recognition of herbal medicine as an alternative form of health care. The Herbal prescriptions and natural remedies is therefore 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 (Sofowora, 1993; Zhu *et al*., 2002).

Vernoniaamygdalina is a perennial herb belonging to the family, Asteraceae. *V. amygdalina* is commonly known as bitter leaf because of its bitter taste. It has been shown to possess a number of medicinal values including anti-diabetic effect (Reuben *et al*., 2017; Yazid*et al*., 2020), hypolipidemic effect (Omede*et al*., 2018), and hepatoprotective activity (Tokofai*et al*., 2021).

Also, Ademola and Eloff, (2011), 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 (Alawa *et al.*, 2003; Farombi and Owoeye, 2011).However, other studies have noted inconsistent results regarding the impact of *V. amygdalina* leaf on various hematological indicators (Nubila*et al*., 2013; Kadiri, 2017; Chike*et al*., 2018).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 hematological effects (Amah *et al*., 2018).

The increase demand for herbal products coupled with the erroneous impression by the people that herbal products are natural and thus less harmful to the body (Ripoll *et al.*, 2002) 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 (Ladokun*et al*., 2015). Cellular component of blood is valuable in immunotoxicology to evaluate 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 (Joshi *et al*., 2002). 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 (Sharif *et al*., 2013). The study therefore is aimed at providing information on the effects of *V. amygdalina*ethanolic leaf extracts on some haematological parameters in Albino rats.

Comment [CC3]: Objective

MATERIALS AND METHODS

Study Area

This study was carried out in Orlu, Imo State, South-Eastern Nigeria. Imo State shares boundary 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.

Plant Material

Vernoniaamygdalina was the medicinal plant employed in this investigation.Fresh *Vernoniaamygdalina* 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.

Laboratory Animals:

The laboratory animals used for this study were Wistar strain of *Rattus albus*of 2 to 3 months old and body weights of 120 to 180g. The Albino rats were purchased from 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.

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 (NIH publication, 1985).

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.

Processing of Plant Materials

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

Extraction of Active Principles of the Plant Material

The active principle of the selected plant materials was extracted with ethanol at 78^0C using soxhlet extraction method as in Harborne, (1998);Obiajuru and Ozumba, (2009). The extracts were recovered and stored at $+8^0$C in screw capped MacCarteny bottles until required for use.

Experimental Design

Comment [CC4]: Needs reference

A total of thirty six (36) apparently healthy Laboratory animals were used for the studyto 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:

Comment [CC5]: Ideally there should have been 7 groups, 7th being the group for determination of baseline haematological parameters in the rats before intervention. Why this was not considered?

Group 1: received 10mg of*V. amygdalina*ethanolicleaf extract/Kg body weight

Group 2: received 20mgof *V. amygdalina*ethanolic leaf extract/Kg body weight.

Group 3: received 30mgof*V. amygdalina*ethanolic leaf extract/Kg body weight.

Group 4: received 40mg of *V. amygdalina*ethanolic leaf extract/Kg body weight.

Group 5: received 50mg of *V. amygdalina*ethanolic leaf extract/Kg body weight.

Group 6: received feed and 0.5ml 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 dessicator.
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.

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 packed full blood count autoanalyzer.

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Statistical analysis

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

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 TWBCcount 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 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 comparison 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.

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;	37.00±1.41	12.33±0.47	6.17±0.24	10.30±0.42	277.50±3.54

n=6)					
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
3Vs 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(10mg/Kg bwt; n=6)	35.50±5.26	61.50±6.61	3.00±2.01
Group 2 (20mg/Kg bwt; n=6)	31.00±5.48	64.50±6.03	3.50±1.52
Group 3 (30mg/Kg bwt; n=6)	52.50±2.12	46.50±2.12	1.00±0.57

Group 4 (40mg/Kg bwt; n=6)	32.75±9.07	64.00±8.41	2.45±1.64
Group 5 (50mg/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
3Vs 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 $p<0.05$.

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 (Abdala*et al*., 2012). Due to its acclaimed therapeutic potentials, medicinal plants are gaining recognition on a Global scale (Maduka*et al*., 2021). As a result, several medicinal plants are continuously being researched for this same objective especially in developing countries (Ogbodo*et al*., 2017; Ezeugwunne*et al*., 2018; Analike*et al*., 2018).

There were no statistically significant differences found in the current investigation when the mean packed cell volume, red blood cell countand hemoglobin level werecompared between the groups administered with different doses of *V. amygdalina* and the control laboratory animals.

This result demonstrates that *V. amydalina*, sometimes known as bitter 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 isrequired for red blood cell formation. Since RBC and hemoglobin (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 hemoglobin, an iron-containing oxygen transport metalloprotein.

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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 (Biagioli*et al*., 2009). The current result is in consonance with the reports of Nubila*et al*., (2013), Momoh*et al*., (2011), and Oyedeji*et al*., (2013)which found no significant alterations in red blood cell count, packed cell volume and hemoglobin 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 of the dose is able to improve the hematological parameters (Kadiri, 2017). Additionally, Chike*et al*. (2018) 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 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*Vernoniaamygdalina*on the hematological profile in albino wistar rats (Nubila*et al*., 2013). It is possible that the immune system has not been weakened based on the non-significant change in TWBC count caused by the ethanolicleaf 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*does not have the ability to increase thrombopoietin production, as platelets are involved in the blood clotting process otherwise termed hemostasis. Some other previous studied have also documented similar results to the present study (Oyedeji*et al*., 2013) 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 (Nubila*et al*., 2013) 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 (Nathan, 2006).They play a significant role in tissue injury-induced inflammation as effector cells as well (Weiss, 1989).People with a neutrophil deficiency (such as neutropenia) are more vulnerable to bacterial and fungal infections

Comment [CC8]: Write down the full name before putting acronym

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because neutrophils are highly potent and effective at detecting and eliminating microbial infections (Tak*et al*., 2017). 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 (Leliefeld*et al*., 2015; Rodriguez and Novak, 2017).

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. amgdalina* did not negatively impact the body's ability to perform phagocytic functions.

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.

Comment [CC11]: Lymphocyte count is not a valid indicator of acquired immune system it is the immunoglobulin level which is used as an indicator of immune status

Comment [CC12]: This could have been a species specific finding, other studies have shown increase in neutrophils? Kindly comment on this in the discussion , giving the reference

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