# Effect of Methanolic Extract of (*Vernonia amygdalina*) on the Haematology and Lipid Profile of Rabbit Does

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***Abstract—*** *This study investigated the effects of methanolic extract of Vernonia amygdalina on the haematological and lipid profiles of rabbit does. A total of 24 rabbit does, aged 8–12 months, were randomly allocated to four treatment groups (T1, T2, T3, and T4) in a Completely Randomized Design (CRD), with six does per group. T1 served as the control and received no extract, while T2, T3, and T4 were administered 50 mg/kg, 100 mg/kg, and 200 mg/kg body weight of the extract, respectively, over a four-week period. Blood samples were collected at three time points: prior to extract administration, midway through the experiment, and at the end of the study. Haematological parameters were evaluated, and sera were analyzed for lipid profile components using standard laboratory procedures. The results revealed that Vernonia amygdalina extract exhibited dose-dependent immunostimulatory effects, maintained red blood cell indices, and significantly improved lipid profile parameters. Specifically, the extract reduced levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and very-low-density lipoprotein cholesterol (VLDL-C) while increasing high-density lipoprotein cholesterol (HDL-C). These changes occurred without adverse physiological effects on the experimental rabbit does. In conclusion, the methanolic extract of Vernonia amygdalina demonstrated potential as a natural immunostimulant and lipid-modulating agent, suggesting its application in enhancing the health and productivity of livestock.*

***Keywords— Haematology, Methanolic extract, Rabbit does, Lipid profile, Vernonia amygdalina.***

## I. INTRODUCTION

Lipid profiling involves a panel of blood tests used to measure lipids, including cholesterol and triglycerides, which are synthesized in the liver and play critical roles in cell membrane integrity, hormone synthesis, immune function, energy storage, vitamin transport, and neural health (Cleveland Clinic, 2024; Eliza, 2023). It is a valuable diagnostic tool for detecting genetic disorders, cardiovascular diseases, pancreatitis, and liver diseases (Nigam, 2011; Cleveland Clinic, 2024).

Haematology, the study of blood's cellular components—erythrocytes, leucocytes, and thrombocytes—assists in diagnosing and managing diseases of the blood, bone marrow, and vascular systems (Merck Manual, 2012; Washington and Van Hoosier, 2012). Changes in haematological parameters are often indicative of environmental, nutritional, or pathological stress, providing insights into an animal's physiological status and responses to administered substances (Togun *et al*., 2007; Khan and Zafar, 2005).

Vernonia amygdalina (bitter leaf) is a widely available perennial shrub in the African tropics, valued for its pharmacological properties, including antimicrobial, antioxidant, antimalarial, and antibacterial effects (Anibijuwon *et al*., 2012; Ayoola *et al*., 2008). These properties are attributed to its bioactive compounds such as flavonoids, saponins, alkaloids, and tannins (Jisaka *et al*., 1993; Kiplimo *et a*l., 2011). Additionally, its leaves are rich in vitamins, minerals, and proteins, making it a potential feed supplement.

Given its phytochemical composition and availability in Nigeria, V. amygdalina holds promise as a functional ingredient in animal feed. This study aims to evaluate the physiological effects of methanolic extract of V. amygdalina on the haematological and lipid profiles of rabbit does, offering insights into its potential application in rabbit nutrition

## II. MATERIALS AND METHODS

### 2.1 Experimental site:

The research was carried out at the Livestock Teaching and Research Farm of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, Abia State. Geographically, Umudike is located in the Rain-forest zone of Southeastern Nigeria and lies on an altitude of 122m above sea level on latitude 5°29' North and longitude 7°21' East. The average highest daily temperature of the region ranges between 27°C and 36°C during the dry, hot season, while the average lowest temperature is from 20 to 26 during the rainy season. Average annual rainfall of the region is about 2100mm with double Maxima pattern (NRCRI, 2021).

### 2.2 Experimental Animals and Management:

A total of twenty-four (24) does aged between 8 months and one (1) year were purchased from a reputable rabbit farm. They were housed in standard cages under normal room temperature (25°C) with cross ventilation. The animals were acclimatized for a period of four (4) weeks before commencement of treatment during which they were dewormed and monitored. They were fed a compounded diet and good quality forage and provided with good quality portable water ad libitum. The extract was administered orally using a gavage, for a period of 4 weeks following the four-week acclimatization period.

**TABLE 1**
**GROSS COMPOSITION AND CALCULATED NUTRIENTS OF EXPERIMENTAL DIETS FOR RABBIT DOES**

| **Ingredients** | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** |
|---|---|---|---|---|
| Maize offal | 15 | 15 | 15 | 15 |
| Wheat offal | 60 | 60 | 60 | 60 |
| Palm kernel meal | 9 | 9 | 9 | 9 |
| Groundnut cake | 12 | 12 | 12 | 12 |
| Bone meal | 3 | 3 | 3 | 3 |
| Common salt | 0.5 | 0.5 | 0.5 | 0.5 |
| Vit./min. Premix* | 0.5 | 0.5 | 0.5 | 0.5 |
| **Total** | **100** | **100** | **100** | **100** |
| **Calculated nutrients** | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** |
| Crude protein (%) | 15.96 | 15.96 | 15.96 | 15.96 |
| Metabolizable Energy (ME) (Kcal/kg diet) | 2009.6 | 2009.6 | 2009.6 | 2009.6 |
| Crude fibre (%) | 8.58 | 8.58 | 8.58 | 8.58 |
| Lysine (%) | 0.50 | 0.50 | 0.50 | 0.50 |
| Methionine (%) | 0.1 | 0.1 | 0.1 | 0.1 |

**$T_1$= 0mg/kg; $T_2$= 50 mg/kg; $T_3$= 100 mg/kg and $T_4$= 200 mg/kg of *Vernonia amygdalina* leaf extract.**
***Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 50.00mg; vitamin K3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6, 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotin, 0.08mg; vitamin B12, 0.25mg; folic acid, 1.00mg; chlorine chloride,300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; iodine, 1.55mg; cobalt, 0.25mg; selenium, 0.10mg; antioxidant, 200mg.***

### 2.3 Collection and Extraction of Plant Materials:

Fresh specimens of *V. amygdalina* were sourced from Umudike, Ikwuano Local Government Area, Abia state. The fresh leaves collected were air dried and then pulverized into a fine powder. The dried leaves were milled and grounded into coarse powder using Wiley machine. The powdered plant sample were soaked in methanol for 24 hours and filtered with Whatmann no. 1 filter paper to obtain the extract. The methanol extract was concentrated using rotary evaporator. The percentage yield (w/v) of the extract was be calculated using the formula:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of material extracted}}{\text{Weight of plant material}} \times 100 \quad (1)$$

(Source: Brian and Turner, 1975)

The extract was reconstituted to form a solution for oral administration by dissolving one gram (1g) of the extract in 100ml of distilled water. The dose (ml) of the liquid suspension of the extract was determined using the formula:

$$\text{Dosage} = \frac{\text{Administration rate} \times \text{concentration}}{\text{Body weight of the animal}} \quad (2)$$

(Source: Ukar *et al*, 2023)

### 2.4 Experimental Design:

The experimental design was a Completely Randomized Design (CRD) having four (4) treatments with three (3) replicates each. Each treatment group had six (6) does per treatment. Animals in treatment groups were fed the test ingredient as follows: treatment one ($T_1$) served as the Control; treatment two ($T_2$) received 50mg of the test ingredient per kilogram of body weight; treatment three ($T_3$) received 100mg of the test ingredient per kilogram body of weight; treatment four ($T_4$) received 200mg of the test ingredient per kilogram of body weight.

The following model was used:

$$Y_{ij} = \mu + T_i + e_{ij} \quad (3)$$

**Where:**

$\mathbf{Y_{ij}}$ = Single observation. = overall mean.

$\mathbf{T_i}$ = treatment effect,

$\mathbf{e_{ij}}$ = experimental error.

### 2.5 Data collection and Analysis:

Three does were randomly selected from each treatment group. Blood samples were collected via venipuncture from the major ear vein at three intervals: a day before extract administration (baseline), on day 12, and on day 26. The samples were analyzed for haematological parameters, including hematocrit, blood cell count, hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and differential leukocyte count. Lipid profile parameters analyzed included total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C).

### 2.6 Statistical Analysis:

The data obtained were analyzed by ANOVA using Statistical Software Package System (SPSS version 20) software. All values were expressed as the mean value ± standard error of the mean and the level of significance $P<0.05$ was considered statistically significant difference between tests and control groups for measured values. All statistical analysis were according to Steel and Torrie, (1980).

## III. RESULTS AND DISCUSSION

**TABLE 2**
**EFFECT OF METHANOLIC EXTRACT OF *VERNONIA AMYGDALINA* ON HAEMATOLOGICAL PROFILE OF RABBIT DOES**

| Treatment group | | | | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **Collection** | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** | **SEM** |
| Haemoglobin (g/dl) | 1st | 11.26$^{ab}$ | 10.93$^{b}$ | 11.80$^{a}$ | 11.40$^{ab}$ | 0.13 |
| | 2nd | 12.06 | 12.66 | 11.8 | 12.06 | 0.14 |
| | 3rd | 11.7 | 12.8 | 13.19 | 12.53 | 0.29 |
| Packed Cell Volume (%) | 1st | 29.66 | 28.33 | 34.33 | 31 | 1.12 |
| | 2nd | 31.33 | 34.33 | 30.33 | 31.66 | 0.67 |
| | 3rd | 30.00$^{b}$ | 33.36$^{ab}$ | 38.96$^{a}$ | 33.33$^{ab}$ | 1.23 |
| Red Blood Cell ($\times 10^6/\mu l^3$) | 1st | 4.82 | 4.57 | 5.56 | 5.02 | 0.18 |
| | 2nd | 5.16 | 5.56 | 4.99 | 5.19 | 0.1 |
| | 3rd | 5.16 | 5.56 | 4.99 | 5.19 | 0.2 |
| TWBC ($\times 10^3$ cells/µl) | 1st | 7.98 | 6.88 | 7.45 | 7.31 | 0.32 |
| | 2nd | 8.11$^{b}$ | 8.80$^{ab}$ | 7.80$^{b}$ | 9.93$^{a}$ | 0.33 |
| | 3rd | 8.21 | 8.25 | 8.65 | 8.65 | 0.4 |
| MCV ($\mu m^3$) | 1st | 62.86 | 63.21 | 62.93 | 62.91 | 0.11 |
| | 2nd | 61.84 | 62.78 | 61.92 | 62.14 | 0.18 |
| | 3rd | 62.36 | 61.4 | 61.75 | 62.01 | 0.16 |
| MCH (pg/cell) | 1st | 23.56 | 23.99 | 21.45 | 22.75 | 0.53 |
| | 2nd | 23.37 | 22.75 | 23.66 | 23.25 | 0.19 |
| | 3rd | 23.53 | 22.64 | 20.48 | 22.65 | 0.41 |
| MCHC (g/dL) | 1st | 38.29 | 36.87 | 34.71 | 36.91 | 0.85 |
| | 2nd | 38.54 | 36.91 | 39 | 38.14 | 0.37 |
| | 3rd | 38.44 | 37.59 | 34.27 | 37.77 | 0.64 |

***$^{a-b}$: means along the same row with different superscript letters across groups indicate significant ($p<0.05$) difference. SEM: Standard Error of Mean. TWBC: Total White Blood Cell; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration. $T_1$= 0mg/kg; $T_2$= 50 mg/kg; $T_3$= 100 mg/kg and $T_4$= 200 mg/kg of Vernonia amygdalina leaf extract.***

The results presented in Table 2 highlight the effects of treatment and duration on the haematological parameters of rabbit does administered graded doses of Vernonia amygdalina leaf extract. Haemoglobin (Hb) values ranged from 10.93 g/dl to 13.19 g/dl, with significant differences observed only in the first collection. Packed cell volume (PCV) values showed no significant differences in the first two collections but were significantly higher in the third collection, with T3 (100 mg/kg) recording the highest value (38.96%) and the control (T1) the lowest (30.00%).

Red blood cell (RBC) counts ranged from 4.57 to 5.56 × $10^6$/µl, showing no significant differences across treatments. Total white blood cell (TWBC) counts ranged from 6.88 to 9.93 × $10^3$, with significant increases observed in T4 (200 mg/kg) during the second collection. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) values remained consistent across treatments, showing no significant alterations.

In the first collection, PCV values for T1 (29.66%) and T2 (28.33%) were below the normal range (31–50%) (Washington and Van Hosier, 2012), indicating mild anemia. However, PCV values improved significantly in subsequent collections, demonstrating the anti-anaemic activity of V. amygdalina, consistent with findings by Tom-Otu *et al*. (2023). Similarly, RBC counts initially showed signs of hemolysis (Melillo, 2007) but normalized over time.

Overall, V. amygdalina did not cause significant alterations in Hb or RBC counts, while PCV and TWBC values improved significantly in T2 and T4 during the second and third collections, suggesting a dose-dependent immune-stimulatory effect.

These findings align with Pascal *et al*. (2021) and Johnson *et al*. (2014). The extract maintained erythropoiesis, exhibited anti-anaemic and immune-stimulatory properties, and showed no deleterious effects on haematological parameters, thus confirming its safety.

**TABLE 3**
**EFFECT OF *VERNONIA AMYGDALINA* ON DIFFERENTIAL WHITE BLOOD CELL COUNT OF EXPERIMENTAL RABBITS DOES**

| Parameters | | Treatment group | | | | SEM |
|---|---|---|---|---|---|---|
| | Collection | $T_1$ | $T_2$ | $T_3$ | $T_4$ | |
| Lymphocyte (%) | 1st | 60 | 59 | 59.33 | 59.66 | 0.35 |
| | 2nd | 60 | 61.33 | 57.66 | 58.33 | 0.71 |
| | 3rd | $59.00^{b}$ | $63.38^{a}$ | $61.01^{ab}$ | $60.00^{b}$ | 0.6 |
| Neutrophils (%) | 1st | 35.33 | 35.33 | 33.66 | 35.33 | 0.55 |
| | 2nd | 33.66 | 32.66 | 35 | 34.33 | 0.63 |
| | 3rd | $35.03^{a}$ | $29.00^{b}$ | $32.07^{ab}$ | $34.03^{a}$ | 0.82 |
| Monocyte (%) | 1st | 3 | 3.66 | 4.66 | 3.66 | 0.27 |
| | 2nd | 4.33 | 4.66 | 4.33 | 4.66 | 0.33 |
| | 3rd | $3.90^{b}$ | $5.34^{a}$ | $4.63^{ab}$ | $4.53^{ab}$ | 0.19 |
| Eosinophils (%) | 1st | 1.66 | 2 | 2.33 | 1.33 | 0.2 |
| | 2nd | $2.00^{ab}$ | $1.33^{b}$ | $3.00^{a}$ | $2.66^{a}$ | 0.26 |
| | 3rd | 1.99 | 1.33 | 2.03 | 1.63 | 0.21 |
| Basophil (%) | 1st | 0 | 0 | 0 | 0 | 0 |
| | 2nd | 0 | 0 | 0 | 0 | 0 |
| | 3rd | 0 | 0 | 0 | 0 | 0 |

***[a-b]: means along the same row with different superscript letters across groups indicate significant (p<0.05) difference. SEM: Standard Error of Mean.***

The results presented in Table 3 reveal the effects of methanolic Vernonia amygdalina leaf extract on the differential leukocyte counts of rabbit does. Lymphocyte percentages ranged from 57.66% to 63.38%, with significant differences observed in the final collection. T2 (50 mg/kg) recorded the highest lymphocyte value (63.38%), while the control group (T1, 0 mg/kg) had the lowest (59.00%). Neutrophil percentages followed a similar trend but exhibited an inverse relationship, with T2 significantly ($p < 0.05$) lower compared to T1. Monocyte percentages aligned with the trend seen in lymphocytes, while basophilic cells were not detected in any samples. Eosinophil counts fluctuated across treatment groups but remained within normal values ($p > 0.05$) in the first and third collections.

Lymphocyte counts are known to increase in Vitamin B-12/folic acid anemia and decrease in iron/B-6 deficiency anemia or internal bleeding (Krishna Kumari et al., 2007). Lymphocytes are the most abundant leukocytes in healthy rabbits, with fluctuations influenced by stress, disease, and time of day (Washington and Van Hoosier, 2012). Typically, lymphocyte values peak in the early morning and dip in the late afternoon and evening. Acute stress can cause lymphocytosis, while chronic stress may lead to lymphopenia (Melillo, 2007). The dose-dependent increase in lymphocyte counts observed in this study suggests that the methanolic extract of Vernonia amygdalina exhibits immune-stimulatory effects.

Neutrophil counts, which are lowest in the morning and peak in the afternoon (Melillo, 2007), were lower in this study due to the morning timing of blood sample collection rather than the extract's effect. Similarly, monocyte counts, the largest white blood cells in rabbits, also follow a diurnal rhythm, being lowest in the morning and highest in the afternoon (Washington and Van Hoosier, 2012). Elevated monocyte levels are linked to chronic inflammation and conditions like mastitis or empyema, though such increases were not observed in this study (Melillo, 2007).

Eosinophil counts, influenced by daily rhythm, stress, and disease, can indicate abscess presence or wound healing (Melillo, 2007). In this study, eosinophil counts remained within normal ranges, aligning with observations in healthy rabbits where low or absent eosinophil counts are typical (Washington and Van Hoosier, 2012).

Overall, the methanolic extract of Vernonia amygdalina demonstrated immune-stimulatory properties, as evidenced by the increased lymphocyte counts, while other leukocyte parameters remained within normal physiological ranges. These findings highlight the extract's potential as a safe immunomodulatory agent in rabbit does

**TABLE 4**
**EFFECT OF METHANOLIC EXTRACT ON LIPID PROFILE OF RABBIT DOES**

| Parameters | | Treatment group | | | | SEM |
|---|---|---|---|---|---|---|
| | Collection | $T_1$ | $T_2$ | $T_3$ | $T_4$ | |
| Cholesterol (mg/dl) | 1st | 122.69$^{a}$ | 100.67$^{b}$ | 94.96$^{c}$ | 90.31$^{d}$ | 3.74 |
| | 2nd | 121.92$^{a}$ | 99.90$^{b}$ | 94.25$^{c}$ | 89.94$^{d}$ | 3.71 |
| | 3rd | 122.18$^{a}$ | 100.16$^{b}$ | 94.49$^{c}$ | 90.06$^{d}$ | 3.72 |
| Triglycerol (mg/dl) | 1st | 78.00$^{a}$ | 64.48$^{b}$ | 62.23$^{c}$ | 61.29$^{c}$ | 2.04 |
| | 2nd | 78.53$^{a}$ | 64.20$^{b}$ | 62.26$^{c}$ | 60.87$^{c}$ | 2.13 |
| | 3rd | 78.35$^{a}$ | 64.29$^{b}$ | 62.25$^{c}$ | 61.01$^{c}$ | 2.09 |
| HDL (mg/dl) | 1st | 41.93$^{b}$ | 42.88$^{b}$ | 43.67$^{b}$ | 46.37$^{a}$ | 0.56 |
| | 2nd | 40.92$^{c}$ | 43.15$^{b}$ | 43.28$^{b}$ | 46.04$^{a}$ | 0.55 |
| | 3rd | 41.25$^{c}$ | 43.06$^{b}$ | 43.41$^{b}$ | 46.15$^{a}$ | 0.53 |
| LDLC (mg/dl) | 1st | 64.70$^{a}$ | 44.05$^{b}$ | 38.44$^{c}$ | 31.04$^{d}$ | 3.78 |
| | 2nd | 65.25$^{a}$ | 44.46$^{b}$ | 38.00$^{c}$ | 31.51$^{d}$ | 3.81 |
| | 3rd | 65.06$^{a}$ | 44.32$^{b}$ | 38.15$^{c}$ | 31.35$^{d}$ | 3.8 |
| VLDL (mg/dl) | 1st | 13.13$^{a}$ | 13.13$^{b}$ | 12.36$^{c}$ | 12.19$^{c}$ | 0.31 |
| | 2nd | 15.77$^{a}$ | 13.09$^{b}$ | 12.33$^{bc}$ | 12.04$^{c}$ | 0.45 |
| | 3rd | 15.44$^{a}$ | 13.11$^{b}$ | 12.40$^{c}$ | 12.09$^{c}$ | 0.39 |

***$^{a\text{-}b\text{-}c}$: means along the same row with different superscript letters across groups indicate significant ($p<0.05$) difference. SEM: Standard Error of Mean. HDL: High-density lipoprotein; VLDL: Very-low-density lipoprotein; LDLC: Low-density lipoprotein cholesterol.***

The result presented in table 4 showed the effect of the methanoloc extract of *Vernonia amygdalina* on the lipid profile of rabbit does. The sera levels of Cholesterol, Triglyceride, LDLC and VLDL ranged from 89.94 to 122.69 mg/dl, 60.87 to 78.53 mg/dl, 31.04 to 65.25mg/dl and 12.04 to 15.77mg/dl respectively and they followed the same trend, with significant ($p<0.05$) reduction (below the allowable amount in normal rabbit doe) across the extract administered groups in a dose dependent manner. However, serum HDL-cholesterol level, had values ranging from 40.94 to 46.37 mg/dl, was noticeably ($p<0.05$) increased, above the normal serum level, as the extract doses were increased, suggesting that the extract exhibited better protection, with no compromise to the membrane integrity (its stability and fluidity) and their functions.

According to Basavanthappa *et al*, (2021), when serum lipids (cholesterol and triglyceride) values are elevated above reference range with low HDL value, it may suggest a possible risk of heart disease, circulatory problem as well as hazards on liver, kidney functions. From the lipid parameters profiled, *Vernonia amygdalina* extract improved the sera lipids, evidently possessed significant hypolipidemic effect, hepato- and cardio-protection by possibly preventing isoproterenol-induced myocardial damage in rabbits, as well as possess no hazards on liver functions. The lowering of cholesterol mean level in the sera of the experimental rabbit does in groups $T_2$, $T_3$ and $T_4$ compared with group $T_1$ might suggest that the ingredients (bioactive phyto-constituents) contained in the leaf extract at higher doses (dose dependently) was capable of inhibiting the activities of hepatic lipogenic and cholesterogenic enzymes, such as malic enzyme, fatty acid synthase, glucose 6-phosphate dehydrogenase and HMG-CoA reductase (Yeh and Yeh, 2003) which are all required for cholesterol synthesis. The low levels of serum triglyceride, LDLc and VLDLc in the extract treated groups may be as a result of some activity of the *Vernonia amygdalina* extract such as its ability to decrease the availability of fatty acids for esterification and encourage tissues lipases, activation of acetyl-CoA carboxylase (McCarty, 2021), or the production of triglycerides precursors such acetyl-CoA and glycerol phosphate (Campillo *et al.*, 2014). The result also demonstrated hypolipidemic potential, by influencing the liver metabolism towards decreasing the synthesis of lipids as the graded doses of extract were increased.

## IV. CONCLUSION

The methanolic extract of *Vernonia amygdalina*, from this study showed immunostimulatory activity in a dose-dependent manner; had no significant effect on red blood cells and showed significant hypolipidemic activity by reducing the values of Cholesterol, Triglyceride, LDLC and VLDL while improving HDL values.

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