Effects of Aqueous Extract of Sleepy Morning Leaves (*Waltheria indica*) on Hematological Profile of Rabbit Buck.

Uruku N.N.[1*]

1. *Department of Animal Production and Health, Federal University Wukari P.M.B 1020, Taraba. Nigeria.*

Abstract

This experiment was carried out to determine the effect of aqueous extract of Waltheria indica leaves on haematology profile of rabbit buck. The study lasted a period of 8 weeks. A total of 36 mixed breed grower male rabbits with an initial body weight of 1.17±0.07 kg and were fed with commercial feed (Top feed), the rabbit was assigned to 6 treatments at random in a 2 x 3 factorial arrangement in a Completely Randomized Design. Each treatment was replicated thrice (two bucks per replicate). The factors were; the extract processing method (cold and hot extraction) and the levels of inclusion of the extract (T1 [0 ml], T2 [0.5 ml] and T3 [1.0 ml]). The treatment combinations used were C x T1, C x T2, C x T3, H x T1, H x T2 and H x T3. Data collected were subjected to analysis of variance using R statistical packages and significant different means were separated using Duncan's new multiple range test. Results of this experiment showed a higher and significant (p < 0.05) effect of the extract amount was observed in the WBC, HB, MCV and PCV concentration for rabbits fed 0.5 ml and 1.0 ml extract compared to the control (0 ml extract). The interaction gave rise to effect on the mean **corpuscular volume (H x T2 was lowest compared to others) and packed cell volume (C x T3) is** higher. Therefore, this study concluded that it will be ideal to recommend hot extract, 0.5

Email Address: nnuruku@gmail.com (**Uruku N.N.[1*]**)

ml or Hot x 1.0 ml aqueous leaves extract of Waltheria indica for the proper functioning of the blood haematology of rabbit buck.

INTRODUCTION

Waltheria indica, commonly referred to as sleepy morning, is a shrub recognized for its wide range of traditional medicinal uses across tropical and subtropical regions. This plant is traditionally employed to treat various ailments, including respiratory infections, wounds, fever, and anemia [9]. The pharmacological effects of *Waltheria indica* are largely credited to its diverse phytochemical constituents, such as flavonoids, alkaloids, tannins, saponins, and sterols, which have demonstrated antioxidant, anti-inflammatory, and hematopoietic properties [7]. Several studies have investigated the hematological effects of *Waltheria indica* extracts in experimental animal models. For instance, hydro-ethanolic extracts of the plant have shown a significant anti-anemic effect in albino rats infected with Trypanosoma brucei brucei, resulting in a marked increase in packed cell volume (PCV), hemoglobin concentration, and red blood cell (RBC) counts compared to untreated controls . These findings suggest that *Waltheria indica* may improve blood parameters and support erythropoiesis, potentially through its antioxidant and bioactive components. Despite the known medicinal benefits of *Waltheria indica* and its influence on blood profile parameters in rodents, there remains limited research assessing its impact specifically on the hematological profile of male rabbits (bucks). Rabbits are important animal models and valuable livestock, Rabbits are efficient producers of meat, fur, and sometimes pets due to their high feed-to-protein conversion, rapid reproduction, and small space requirements. Rabbit farming offers significant income and livelihood opportunities, particularly for smallholder or resource-limited farmers, by providing affordable, sustainable protein and multiple revenue streams with low startup costs [13].

Understanding the effects of medicinal plants on their hematology is essential for animal health and productivity. This study aims to explore the effects of aqueous extracts of *Waltheria indica* leaves on the hematology of rabbit bucks to provide further evidence on its safety and potential therapeutic benefits in animal physiology.

MATERIALS AND METHODS

Experimental Site and Duration

The study was conducted at the rabbitry section of the Livestock and Research Teaching Farm of Federal University Wukari, Taraba State. It is located on latitude 7°89N and 9°78E of the equator, with topography of 189m above the sea level (Sources: Taraba State 2025 Diary). The study also lasted for a period of 2 months (8 weeks).

Sample Collection and Identification

The leave of sleepy morning (Waltheria indica) was collected from Takum Local Government Area of Taraba State. The plant was identified and authenticated by a taxonomist at the Department of Forestry and Wild Life Management of Federal University Wukari, Nigeria.

Experimental Animals and Management

Thirty-six (36) mixed breed of grower male rabbits were obtained from Wukari local Government after which they were transported to the rabbitry section of the livestock and research teaching farm of Federal University Wukari, Taraba State. The rabbits were fed with commercial grower ration (Top Feed) and water.

Experimental Treatment and Preparation of Aqueous Extracts of Sleepy Morning

Six (6) treatments replicated 3 times were sampled in this study and two buck each per replicate, with a total of 36 rabbits of 8 − 10 weeks, were used. Table 4 showed the treatments and different combinations.

Table 4: Treatment combinations in this study

Extraction methods	T_1 (0ml)	T_2 (0.5ml)	T_3 (1ml)
Cold (C)	C x T_1	C x T_2	C x T_3
Hot (H)	H x T_1	H x T_2	H x T_3

Preparation of the Cold Aqueous Extracts of Sleepy Morning

The leaves were washed and dried under room temperature (25ºC) for two weeks, after which it was ground into powder. One hundred grams (100/g) of the powder was soaked in 150 ml of distilled water. The extracts were obtained by maceration method with distilled water for 24

hours to obtain a cold final aqueous concentration. The extract was kept in an air tight container throughout the experiment inside the refrigerator as described by [12].

Preparation of the Hot Aqueous Extracts of Sleepy Morning

The leaves were washed and dried under room temperature (25ºC) for two weeks, after which it was ground into powdered form. One hundred grams (100/g) of the powder was soaked in 150 ml of hot distilled water. The extracts were obtained by the maceration method with distilled water for 24 hours to obtain a hot final aqueous concentration. The extract was kept in an air tight container throughout the experiment inside the refrigerator as describe by [12]

Blood Collection and Haematological Analysis

Blood collection was done at the end of the experiment (at the $8^{th\ week}$). Blood samples were collected from the prominent ear vein with the aid of a hypodermic needle and syringe. Blood sample was collected into plain sterile bottles labeled with Ethylene-diamine-tetra-acetic (EDTA) treated with tubes for haematological analysis as described by Ahemen *et al*. (2013).

Statistical Analysis

Data were analyzed using R packages version 0.1.0 for analysis of design of experiments for biological research (DoEBioResearch) (Popat and Banakara, 2022) in a 2 x 3 factorial arrangement of treatment in a complete randomized design (CRD). Significant means among the treatments were separated by Duncan new multiple range test as found in the statistical package at $P < 0.05$. The model is as shown below:

$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$; Where,

Y_{ijk}	=	Response variable
μ	=	Overall mean
α_i	=	Effect of the ith level of the first factor (level of inclusion of extract)
β_j	=	Effect of the jth level of the second factor (extraction method)
$(\alpha\beta)_{ij}$	=	Interaction effect between factors
ε_{ijk}	=	Random error associated with k^{th} replicate of the ij^{th} treatment.

RESULTS AND DISCUSSION

Effect of Aqueous Extract of Sleepy Morning Leaves on Haematology of Rabbit Bucks

From the findings on the effect of *Waltheria indica* leaves extract on haematological indices, it was observed that cold or hot extract as a factor did not have significant ($p > 0.05$) effect on the male rabbit's haematology. The treatment effects showed the existence of significant ($p < 0.05$) differences on the mean red blood cells (RBC), haemoglobin concentration (HB), mean corpuscular volume (MCV) and packed cell volume (PCV), with no significant ($p > 0.05$) effect on the white blood cells. The highest concentration of RBC was shown on the rabbit bucks fed 0.5 ml extract (T2) and 1.0 ml (T3). The RBC concentration for both groups significantly differed from the control (T1) with the lowest level of RBC. PCV followed the same trend as in RBC concentration of rabbit bucks. With respect to haemoglobin concentration, highest concentration was recorded in buck fed 1.0 ml extract (T3). Rabbit bucks on T1 and T2 with the lowest concentration were similar ($p > 0.05$). The MCV showed highest mean on the T3 fed rabbit bucks, and followed by those rabbits on T1 ($p > 0.05$). The MCV of rabbits on T2 was lowest and different ($p < 0.05$). The interaction between the extraction methods and levels of inclusion showed effects ($p < 0.05$) on the HB, MCV and PCV. Highest HB concentration was recorded for rabbit bucks fed C x T3 compared to other interaction combinations ($p < 0.05$). Rabbit bucks fed H x T2 had lower MCV compared to other combinations with the higher volumes. PCV of the rabbit bucks showed highest percentage for those on C x T3 compared ($p < 0.05$) to the lowest percentages recorded for rabbit bucks fed C x T1, H x T1 and H x T3 combinations.

In tandem with the significant difference reported in this study [4] also reported similar outcome in haematology when weaner rabbits were fed *Ficus sycomorus* and *Parkia biglobosa* leaf meals. In another study involving phytogenic leaf extract [11] reported significant changes in packed cell volume, haemoglobin, mean cell volume and white blood cell when rabbits were fed *Carica papaya* leaf meal. The reported range of haematological values in this study are in agreement with the normal ranges reported by [8] Based on this, it can be inferred that *Waltheria indica* leaf extract had no detrimental effect on the blood haematology of rabbit bucks rather, there was an improvement up to 1.0 ml of the extract. For instance, the improved PCV recorded for the treatment groups may be attributed to the biologically active compounds such as phenols, alkaloids or tannins which may have enhanced nutrient utilization or oxygen carrying capacity of the blood [11]. This further indicated that the diets or the leaf extract in water were nourishing, non-toxic and influenced adequate blood supply at 1.0 ml *Waltheria indica* extract. The medicinal properties of the *Waltheria indica* leaf extract maybe due to the presence of phytogenic compounds like alkaloids, saponins, tannins and flavonoids [6]. These compounds have been reported [6] to have antibacterial, anticoagulant, antiparasitic, antiviral, antifungal, anti-inflammatory effects and promote digestive and lung health. These together with the

antioxidant properties which remove free radicals from the body may have resulted to the significant improvement on the parameters reported in this study.

In other study [15] the PCV, Hb and RBC were improved following treatment with *W. indica* ethanol fraction in albino rats. The authors further noted that restoration was higher in animals treated with 300mg/kg of *W. indica*. According to [15], this positive improvement was perhaps an indication that the plant extract helped the animals to improve their depressed state of trypanosome induced anaemia. Phytogenic antioxidants improved RBC count and haemoglobin concentration by enhancing erythropoiesis (red blood cell production) and protecting the RBCs from oxidative damage [1]. For example, *Curcuma longa* (turmeric) supplementation has been shown to improve haemoglobin levels and haematocrit in broiler chickens due to its high curcumin content, which has potent antioxidant effects just like *Waltheria indica* [3]. The flavonoids and phenolic compounds in *Waltheria indica* act as natural antioxidants. By scavenging free radicals and reducing oxidative stress, these antioxidants protect red blood cells from oxidative damage. This action could lead to improved erythropoiesis (red blood cell production), resulting in a higher RBC count and better maintenance of haemoglobin levels. Alkaloids present in *Waltheria indica* may promote erythropoiesis as well. In animal studies, some alkaloid-containing plants have been linked to increased RBC count and haemoglobin concentration, which are essential for efficient oxygen transport in the body [14]. The presence of these compounds makes *Waltheria indica* a potential candidate for improving blood parameters, especially in conditions like anaemia or recovery from disease. The presence of iron and other minerals in *Waltheria indica* could contribute to improved haemoglobin synthesis. Haemoglobin is vital for oxygen transport in the blood, and its deficiency can lead to anaemia and poor animal performance. By enhancing haemoglobin production, *Waltheria indica* can improve the oxygen-carrying capacity of the blood, leading to better growth and overall performance in animals [10]. The results of the effect of aqueous sleepy morning leaves extract on haematological indices of rabbit bucks is presented in Table 8.

Table 8: Effect of Aqueous Extract of Sleepy Morning Leaves on Haematology of Rabbit Bucks

Parameters	WBC ($\times 10^6$/mm^3)	RBC ($\times 10^6$/mm^3)	HB (g/dl)	MCV (fl)	PCV (%)
Cold extract	3.86±0.52	3.95±0.50	81.32±13.59	76.08±1.44	1.22±0.47
Hot extract	4.83±0.82	3.75±0.38	86.11±8.37	69.98±7.14	0.88±0.33
P. value	0.13^{NS}	0.57^{NS}	0.57^{NS}	0.16^{NS}	0.25^{NS}
T1	3.48±1.01	2.75±0.39^b	67.33±9.33^b	77.88±1.93^a	0.33±0.18^b
T2	4.53±1.09	4.24±0.92^a	73.32±25.32^b	64.67±10.89^b	1.44±0.65^a
T3	5.02±1.1	4.56±0.36^a	110.50±8.61^a	77.88±3.58^a	1.38±0.64^a
P. value	0.15^{NS}	0.38***	0.00***	0.03*	0.01**
C x T1	2.27±0.07	2.33±0.59	61.67±15.45^c	77.13±1.65^a	0.27±0.33^c
C x T2	4.87±0.97	4.60±0.67	63.63±25.99^c	77.43±3.28^a	1.10±0.48^{bc}
C x T3	4.43±0.67	4.91±0.47	118.67±16.02^a	73.67±2.68^a	2.31±1.16^a
H x T1	4.70±1.95	3.17±0.18	73.0±3.21^c	78.63±2.20^a	0.39±0.03^c
H x T2	4.20±1.2	3.87±1.17	83.00±24.64^{bc}	51.90±18.50^b	1.78±0.82^{ab}
H x T3	5.60±1.53	4.21±0.24	102.33±1.20^b	79.40±4.48^a	0.45±0.11^c
P. value	0.15^{NS}	0.12^{NS}	0.02**	0.01**	0.00**

^{a,b,c}: Means on the same column with different superscripts are significantly different (p < 0.05), WBC: white blood cell, RBC: Red blood cell, HB: Hemoglobin, MCV: Mean corpuscular volume, PCV: Pack cell volume C: Cold extract, H: Hot extract and T: Treatment.

Conclusion

This study attempted to determine the effect of aqueous leaf extract of *Waltheria indica* on the haemalogical profile of rabbit buck. However, the result showed Significant effect of treatments on the haematology of rabbit bucks was reported and this evident was in the improved red blood cells, haemoglobin concentration, mean corpuscular volume and packed cell volume for rabbit bucks on 0.5- and 1.0-ml extracts. The interaction gave rise to significant effect on the mean corpuscular volume (H x T2 was lowest compared to others) and packed cell volume (C x T3).

REFERENCES

[1] Adewale, A. O., Alagbe, J. O. and Adeoye, A. O. (2021). Dietary supplementation of *Rauvolfia vomitoria* root extract as a phytogenic feed additive in growing rabbit diets: Haematology and serum biochemical indices. *International Journal of Orange Technologies*, 3(3), 1-12.

[2] Ahemed, T., A.H., Abu, V.Gbor (2013). Haemotological and serum biochemical parameters of rabbits fed varying dietary level of water spinach (Ipomeo aquatic) leaf meal. Advances in applied science rsesearch 4(2):370-373

[3] Alagawany, M., Abd El-Hack, M. E., Farag, M. R., Tiwari, R., Dhama, K. (2019). Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health – A comprehensive review. *Veterinary Quarterly*, 39(1): 1-29

[4] Audu, R., Tijjani, A., Ibrahim, A. A., Amin, A. B., Gumel, I. A., and Suleiman, A. T. (2018). Evaluation of haematology and serum biochemistry of weaner rabbits fed diets containing *Ficus sycomorus* and *Parkia biglobosa* leaf meals. *Nigerian Journal of Animal Production*, 45(5), 30-38.

[5] Bala Aminu Yabo, Adamu Tukur and Muhammad Imrana Arzika (2021). Anti-anaemic potential of the hydro-ethanolic extract of Waltheria indica (L) in albino rats with parasitaemia – induced anaemia.World Journal of Advanced Research and Reviews, 2021, 11(03), 291–299https://doi.org/10.30574/wjarr.2021.11.3.0454

[6] Boshra, V. and Tajul, A.Y. (2013). Papaya – An Innovative Raw Material for Food and Pharmaceutical Processing Industry. *Health and the Environment Journal*, vol. 4, no. 1, pp. 68–75.

[7] Chitra M,Venkatalakshmi K,Sivakumar P,Arulselvi S,shwariya. S and Velayutha A(2022).Nutritional   and therapeutic potential of Waltheria indica linn: A review. The Pharma   Innovation Journal 2022; 11(4): 977-983

[8] Etim, N. N., Enyenihi, G. E., Akpabio, U., & Offiong, E. E. (2014). Effects of nutrition on haematology of rabbits: a review. *European Scientific Journal*, 10(3): 413-424.

[9] F. Zongo, C. Ribuot , A. Boumendjel , I. Guissou (2013).Botany, traditional uses, phytochemistry and pharmacology of Waltheria indica L. (syn. Waltheria americana): A review.https://doi.org/10.1016/j.jep.2013.03.080

[10] John AO. (2024). Novel phytogenics' impact on weaned pigs' growth performance, haematology and serum biochemical indicators. *BSJ Agri,* 7(2): 82-89.

[11] Jiwuba, P. D. C., & Kadurumba, O. E. (2019). Nutritional and phytogenic properties of pawpaw (Carica papaya) leaf meal on blood characteristics of growing rabbits. *Acta Fytotechnica et Zootechnica*, 22(2), 46-51.

[12] Marikinyo, A.O., Ahmed O., Yinusa R., & Adegoke A.O. (2009). Effect of aqueous extract of garlic (Allium Sativa) on testicular functions in the rat. *Nigerian journal of health and biochemical science.* 10(3): 42-49.

[13] Mutai, P.N, Ondiek, J.O.(2025). Assessing the Role of Rabbits in Circular Food Systems: A Review. ISRG Journal of Agriculture and veterinary Science. Key Title: ISRG. J. Agri.Vet.Sci.   ISSN: 3048-8869   (Online)   Journal   homepage: https://isrgpublishers.com/gjavs/

[14] Pasri, P., Rakngam, S., Gérard, N., Mermillod, P. and Khempaka, S. (2024). Synthetic and phytogenic antioxidants improve productive performance, antioxidant activity, gene expression, and offspring quality in breeder hens subjected to heat stress. *Poultry Science*, 103(3), 103390.

[15] Yabo, B. A., Tukur, A. and Arzika, M. I. (2021). Anti-anaemic potential of the hydro-ethanolic extract of *Waltheria indica* (L) in albino rats with parasitaemia–induced anaemia. *World Journal of Advanced Research and Reviews*, 11(3), 291-299.