

Haematological parameters of nematode infected goat treated three medicinal plants

Bada, A. A.^{1*}, Adewole, O. S.² and Olofintoye, L. K.²

¹Department of Biological Sciences, Elizade University, Ilara- mokin, Nigeria.

²Department of Zoology, Ekiti State University, Ado-Ekiti, Ekiti-State, Nigeria.

*Corresponding author. Email: glorynew.20@gmail.com

Copyright © 2023 Bada et al. This article remains permanently open access under the terms of the [Creative Commons Attribution License 4.0](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received 23rd March 2023; Accepted 10th May 2023

ABSTRACT: This study investigates haematological parameters in goat treated with three medicinal plants (*Vernonia amygdalina*, *Ocimum grattissimum* and *Talinum triangulare*). Twenty goats were infected with L3 larval stage of nematode parasite and the experiment lasted for 22 days. Standard procedures were employed to investigate the extraction of medicinal plants, animal infection and treatment and blood parameters of the treated goat with medicinal plants. The goat treated with *Vernonia amygdalina* and *Ocimum grattissimum* showed high reduction in Packed Cell Volume (PCV), Red Blood Cell (RBC) and Haemoglobin (HB). This showed that the goat treated with these medicinal plants were anaemic. The goat treated with *Talinum triangulare* showed little reduction in PCV, RBC and HB. This signified that *Talinum triangulare* can be used to improve vitality of goat. Other parameters like White Blood Cell (WBC), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) had no significant difference from control. Erythrocyte Sedimentation Rate (ESR) had significant difference from control. Hence the research can be used for increasing the economy status of the goat.

Keywords: Blood, economy, goat, medicinal plants.

INTRODUCTION

The medicinal plants to treat infected goat with nematode *in vivo* and *in vitro* were *Vernonia amygdalina*, *Ocimum grattissimum* and *Talinum triangulare* (Adeola *et al.*, 2014). These botanicals showed anthelmintic activity towards infected goat with nematode. The phytochemicals inside these botanicals include alkaloid, flavonoid, saponin and tannin (Adeola *et al.*, 2015a).

The blood parameters of goat include packed cell volume (PCV), white blood cell (WBC), red blood cell (RBC), haemoglobin (Hb), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) (Carlson, 1996). They improve the physiological status of the goat (Madan *et al.*, 2016), and alter the body temperature (Mazzuca Pizetti *et al.*, 2021). White cell blood types include: granulocytes, neutrophils, eosinophils, basophils, monocytes and lymphocytes. Red blood cell indices include MCV, MCH and MCHC.

Red blood cell count (RBC) indicates the number of red

blood cells in an animal has (Diog,2017). White blood cells or leucocytes are cells of the immune system involved in defending the body against disease and foreign materials (Janeway, 2001). Haemoglobin is the iron containing oxygen-transport metalloprotein in all red blood cells of vertebrates (Maton *et al.*, 1993). Packed cell volume is the volume percentage of red blood cells in the blood (Purves *et al.*, 2004). Low PCV is associated with high strongyle EPG (Egg Per gram) count in cosmopolitan goat breed (Zanzani *et al.*, 2020). Low PCV signifies low number of red blood cell and the goat can suffer from anaemia. PCV is normally the percent of blood that is in red blood cell. Ahmad (2022) showed no significant difference between PCV and haemoglobin. Haemoglobin and PCV were blood parameters that can be used to signify anaemia in goat. Mean cell volume expresses the amount of haemoglobin in one red cell expressed as pictogram (pg). Mean cell haemoglobin concentration expresses the concentration of

haemoglobin in the red cells, as compared to the concentration haemoglobin in 100 ml of whole blood.

Olosunde *et al.* (2020) showed that West Africa Dwarf goat fed *Vernonia amygdalina* for 20 weeks had no effect on haematological and biochemical parameters of the goat. RBC, PCV and HB were higher than control in goat fed *Ocimum grattisimum* (Adebayo *et al.*, 2019). Haematological parameters were deranged in rat fed *Talinum triangulare* (Olorunnisola *et al.*, 2016)

Few studies have investigated the effect of hematological parameters of infected goat treated with *Vernonia amygdalina* and *Ocimum triangulare*, with no study that had investigated the effect of *Talinum triangulare* treated goat on haematological parameters. In other to know the effect of medicinal plants on hematological parameters of the goat.

MATERIALS AND METHODS

Extraction of phytochemicals from medicinal plant

The leaves of the medicinal plants were air dried for 2 months. After which they were grinded into powder using a blender. 74.8 g of *Vernonia amygdalina Del* was soaked in 262 ml ethanol, 74.64 g of *Ocimum grattisimum L* was soaked in 262 ml ethanol while 21.57 g of *Talinum triangulare Wild* was soaked in 75 ml of ethanol. They were soaked for 72 h after which it was filtered using Whatman 75 mmm filter paper. The filtrate was left to stand for 4 days for the ethanol to evaporate leaving the extract of the plants.

The method of extraction described in Edeoga *et al.*, (2005) was used in this study. The leaves of the medicinal plants (*Vernonia amygdalina*, *Ocimum grattisimum* and *Talinum triangulare*) were air dried for 2 months after which they were grinded into powder using a blender. 500g, 400g and 250g of *V. amygdalina*, *O. grattisimum* and *T. triangulare* were soaked in 2.50, 2.00, and 1.25 L of ethanol respectively. The ratio of weight of the plants to ethanol was 1:5 i.e 100g of plant material: 500ml of ethanol. They were soaked for 72 h, after which they were filtered using muscline cloth. The filtrate was left to stand for 11 days for the ethanol to evaporate leaving a concentrate of the extract of the plants.

Culturing and harvesting of L3 larval stage parasite were done according to the study of Adeola *et al.* (2016).

Animal infection

Twenty small African goats (3-4 months old) weighing 3-6.4 kg of both sexes (16 males and 4 females) were purchased from local market (Mimiko Market) in Akure. They were infected on the 10th day of purchase with 10 ml of L3 larval of nematode parasite solution containing approximately about 4000 larvae and left for 7 days before the faecal sample was collected from the floored pen. Each

collection was put in container labelled with the number of the animal. The qualitative analysis was done through floatation method to determine the type of eggs and quantitative analysis was done to determine the quantity of eggs using McMaster counting technique (Adeola *et al.*, 2015 a or b?).

In vivo assay

In vivo assay used was as described by Bachaya *et al.* (2009) and Adeola *et al.* (2015b).

Haematological parameters

About 1 0ml of blood was collected from jugular veins of the necks of the treated goats with medicinal plants on day 22 of the experiment. The blood was stored in EDTA vials and later taken to the laboratory for analysis.

Red cell count

The blood was drawn up to 0.5 mark in a pipette and it was mixed well and the outside of the pipette was wiped with the tissue paper. The tip of the pipette was immersed into the diluting fluid containing 3 g of sodium citrate, 1 ml formaldehyde and dissolved in 100 ml of distilled water. And carefully drawn up exactly to the 101 mark. The blood dilution was mixed well by shaking for 30 seconds and ¼ of the contents was expelled before filling the counting chamber.

An improved Neubauer counting chamber was cleaned including the cover slip. The blood dilution was filled in to the chamber and allowed to stand for one minute for the red cells to settle. All the red contained in 80 of the 400 smallest squares were counted with x 40 objective and x8 eye pieces. Cell touching the centre line bordering the top and right side of each group of small squares were omitted. Those touching the centre at the bottom and left of the ruled area were included.

Red cell count (cells/cubic millimeter) = number of cells counted x correction factor as only 0.2 sq.cm counted x correction factor as depth of dilution (0.1mm) x correction factor as blood dilution (1:200)

White blood count

The blood was diluted in the same way as was done for red cells but using a white cell pipette to give a 1:20 dilution. White cell diluting fluid contained acetic acid 3 ml, distilled water 97 ml and 0.5% crystal violet. The Neubauer chamber was filled with blood dilution. X 10 objective lens and x8 eyepiece count all the white cells contained in the 4 outer large squares. At each corner of square has an

area of 1 sq.mm and is divided into 16 smaller squares.

White cell count (cells/cub.mm) = number of cells counted x correction factor as 4 sq.mm area was counted x correction factor as depth of dilution (0.1mm) x correction factor as blood dilution (1:20)

Packed cell volume

The blood samples collected from the treated goat were mixed gently for 2 minutes. The well mixed blood was then drawn up a 75 x 1.5mm capillary tube through $\frac{3}{4}$ of its length. One end was sealed with sealant and placed in the micro haemocrit centrifuge and it was ensured that the sealant was at the outer end. The centrifuge lid was closed. The blood was centrifuge at 12,000rpm for 4 minutes. The tubes were placed in the reader and readings taken. The reading were expressed as a percentage of packed red cells to total volume of the whole blood.

Haemoglobin

The collected blood was mixed well for one minute. The mouthpiece, sucker and 0.02 ml (20 micro litre) pipette were used to draw blood past the mark. Excess blood was withdrawn by touching the pipette tip on the palm of the hand until the blood level is exactly opposite the pipette graduation. The outside of the pipette was wiped with tissue paper. The blood was expelled into Drabkin's solution containing potassium cyanide 0.2 gm, potassium ferricyanide 0.2 gm, sodium bicarbonate 1 g dissolved in distilled water 1000 ml. The pipette was flushed completely by repeated filling and ejection of the Drabkin's solution. Stopper was used for the tube and the blood was mixed and kept to stand for 5 minutes to allow for full colour development. A standard was prepared as above using a blood sample of known haemoglobin concentration. A green (624) filter was used to set the colorimeter to zero using Drabkin's solution as a blank. The samples and standard blood dilutions was read on the colorimeter.

Calculation

$$\text{Hb} = \frac{\text{Reading of test} \times \text{standard haemoglobin}}{\text{Reading of standard}}$$

$$\text{MCH} = \frac{\text{Rb (g/dl)} \times 10}{\text{No red blood cells (million}/\mu\text{L)}}$$

$$\text{MCHC} = \frac{\text{RHb (g/dl)}}{\text{HTO}} \times 100$$

Where: Hb = Sample Haemoglobin concentration. MCH = Mean Cell Haemoglobin, MCHC = Mean cell Haemoglobin concentration.

MCV (Mean Cell Volume) = is the average size of each Red Blood Cell (Mazzuca Pizetti *et al.*, 2021).

Statistical method

Hematological parameters were assessed using T-test at $p < 0.05$ significant level.

RESULTS

The PCV result for *Vernonia amygdalina* and *Ocimum grattissimum* medicinal plants treated goat were presented in Table 1. The PCV was low but not significant difference from positive control. ESR results for the medicinal plants treated goats were presented in Table 1 as well. ESR for *Vernonia amygdalina* and *Ocimum grattissimum* were significantly different from positive control. The ESR for *Talinum triangulare* was the same as positive control. RBC results for the medicinal plants were presented in Table 1. RBC *Vernonia amygdalina* was low but not significant difference from positive control. RBC for other medicinal plants were not significant difference from positive control. WBC and HB for the medicinal plants treated goat were presented in Table 1. For WBC and HB, no statistically significant difference between the medicinal plants and positive control. The comparison of PCV, ESR, RBC, WBC, HB for the medicinal plants treated goat with standard control were almost the same as the positive control as shown in Table 1.

The result for MCHC, MCH and MCV for the medicinal treated goat were presented in Table 2. MCHC for medicinal treated goat were not statistically significant difference from positive control. MCH and MCV for *Vernonia amygdalina* treated goat were high but not significantly different from positive control. While for the other medicinal plants treated goat, they were not significantly difference from positive control.

DISCUSSION

PCV for *Vernonia amygdalina* treated goat was low, ESR high, RBC low, but not statistically significant difference in comparison with positive and standard control. The MCH and MCV are high and MCHC normal in goat treated with *Vernonia amygdalina* in comparison to *Ocimum grattissimum* and *Talinum triangulare* goat treated with these medicinal plants. It can signify anaemia (loss of blood) in this animal. This could be due to active ingredient present in this medicinal plant that can cause this anaemia as discussed by Ahmad *et al.* (2022). The PCV result in this study for *Vernonia amygdalina* treated goat correlate with PCV value from Ajayi *et al.* (2017), but RBC and WBC values were higher than the value in this study and HB value was lower than this study value. This could be due to the type of concentrates (*Vernonia amygdalina* and

Table 1. Haematological parameters of control and experimental goat.

Parameters	PCV (%)	ESR (mm)	RBC (cubic /mm)	WBC (cubic/mm)	Hb g/100ml
<i>Vernonia amygdalina</i>	24	3.0	8240000	17800	8.0
<i>Ocimum grattissimum</i>	27	1.0*	11,080000	17100	9.0
<i>Talinum triangulare 1</i>	33	0.5*	12,810000	16300	11.0
<i>Talinum triangulare 2</i>	32	0.5*	11,960000	14900	10.7
Positive control	36	0.5*	14,930000	19250	12.0
Standard control	30.2		11900000	14300	10.1

Table 2. Blood parameters of control and experimental goat.

Goats treated with the different plant extracts	MCHC (%)	MCH (pg of haemoglobin)	MCV (Cubic microns)
<i>Vernonia amygdalina</i>	33.3	9.7	29.1
<i>Ocimum grattissimum</i>	33.3	8.1	24.4
<i>Talinum triangulare 1</i>	33.3	8.6	25.8
<i>Talinum triangulare 2</i>	33.4	8.9	26.8
Positive control	33.3	8.0	24.1

Tithonia diversifolia) that they fed goat with in Ajayi *et al.* (2017) when compared to this study.

A bit high in white blood cells number in treated goat with *Vernonia amygdalina* in comparison to standard control but low to positive control signifies that the goat might have low immunity to fight infection depending on environmental conditions surrounding the goat (Janeway, 2001).

Low PCV, RBC, HB and high ESR in goat treated with *Ocimum grattissimum* in comparison with positive control and standard control might signifies that the goat is anaemic in nature. This contradict the result of Olorunnisola *et al.* (2016) that showed high values of RBC, PCV and HB for goat fed *Ocimum grattissimum* probably because the medicinal plant was added as additive in their diet compared to as raw diet in this study. No effect of MCHC and MCH values on control for *Ocimum grattissimum* treated goat in this study correlate with no effect on rat from Ofem *et al.* (2012).

For blood parameters, PCV was a bit low for positive control but high for standard control in *Talinum triangulare* treated goat. RBC in *Talinum triangulare* were a bit lower than the positive control but almost the same as standard control. This signifies that the goat might not be anaemic as compared to *Vernonia amygdalina* and *Ocimum grattissimum*. For *Talinum triangulare*, the blood parameter (HB) was not significant difference from positive and standard control, which can be due to the fact that the medicinal plant does not have effect on haemoglobin part of the blood of the goat. This is true for *Talinum triangulare* that is used for feeding the goat to improve the vitality of the goat as mentioned by Adeola *et al.* (2014). This result showed that the medicinal plant can be used to improve the physiological status of the goat. ESR of goat treated with *Talinum triangulare* was the same as positive control.

There was no difference in comparison of result with both positive and standard control. This signifies that were no difference between them.

WBC was low for positive control but a bit higher than standard control in goat treated with *Talinum triangulare* and *Ocimum grattissimum*. This signifies that the goats might be prone to infection or not (Janeway, 2001) depending on the environmental conditions surrounding the treated goat.

Erythrocyte values (MCV, MCH, MCHC) did not show any significant difference to the positive control in *Ocimum grattissimum* and *Talinum triangulare* treated goat. This signifies that no effect on oxygen transport capacity on blood as discussed by Tsai *et al.* (2010).

Conclusion

Talinum triangulare treated goat did not had much altered haematological parameters. This showed that it can be used to improve vitality and physiological status of the goat and hence help to improve the economy status of the infected goat with nematode.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

- Adebayo, K. O., Aderinboye, R. Y., K. A., Oyewusi, I. K., & Isah, O. A. (2019). Microbial population and blood parameters of West African dwarf goats fed scent leaf (*Ocimum grattissimum*)

- as additive. *Nigerian Journal of Animal Production*, 46(1), 225-235.
- Adeola, A. O., Adewole, S. O., & Olofintoye, L. K. (2014). Studies on ethnoveterinary practice of ruminants in Ekiti State Nigeria. *Research Journal of Agriculture and Environmental Management*, 3(12), 632-645.
- Adeola, A. O., Adewole, S. O., & Olofintoye, L. K., (2015a). Phytochemical screening of four different medicinal plants. *Unique Research Journal of Agricultural Sciences*, 3(1), 1-6.
- Adeola, A. O., Osho, I. B., Adewole, S. O., & Olofintoye, L.K (2016). *In vitro* anthelmintic effects of three medicinal plants. *Journal of Health Sciences and Nursing*, 2(7), 55-63.
- Adeola, A. O., Osho, I. B., Adewole, S. O., & Olofintoye, L. K., (2015b). *In vivo* effects of four medicinal plants on nematodes of goat. *Journal of Biology and Nature*, 4(2), 122-126.
- Ahmad, S., Lashari, M. H., & Farooq, U. (2022). A preliminary study on devising a hematological formula for estimation of hemoglobin from packed cell volume in beetal goats. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 74(1), 77-82.
- Ajayi, F. T., Abegunde, T. D., Olona, J. F., Balogun, F. A. (2017). Haematological and Serum indices of West African Dwarf Goats fed *Panicum maximum* hay and leaf meal. *Journal of American Science*, 13(9), 74-78.
- Carlson, G. P. (1996). Clinical chemistry tests. In: Smith, B. P. (ed.). *Internet Animal Medicine*, 2nd Edition. Mosby Publisher, USA, p. 183.
- Janeway, C. A. (2001). Immunobiology: The immune system in Health and Disease. 5th edition. Retrieved from <http://ncbi.nlm.nih.gov/books/NBK27092/>
- Madan, J., Sindhu, S., Gupta, M., & Kumar, S. (2016). Hematobiochemical profile and mineral status in growing beetal goat kids. *Journal of Cell & Tissue Research*, 16(1), 5517-5522.
- Maton, A., Jean, H., Charles, W., Mclaughlin, S. J., Maryanna, Q. W., David, L., Wright, J. D., (1993). *Human biology and health*. Engewood Cliffs, New Jersey, USA: Prentice Hall. p. 256.
- Mazzuca Pizetti, A. J., Sarmiento, R. O., Pintos, L. A., Trova, G. B., Binda, J. A., & Sánchez Negrette, O. (2021). Haematological and protein profile of goat rodeo in extensive productions of different regions in the province of S alta, Argentina. *Journal of Applied Animal Research*, 49(1), 239-246.
- Ofem, O. E., Ani, E. J., & Eno, A. E. (2012). Effect of aqueous leaves extract of *Ocimum gratissimum* on hematological parameters in rats. *International Journal of Applied and Basic Medical Research*, 2(1), 38-42.
- Olorunnisola, O. S., Adetutu, A., Afolayan, A. J., & Owoade, A. O. (2016). Effect of methanolic leaf extract of *Talinum triangulare* (Jacq). willd. on biochemical parameters in diet induced dyslipidemia wistar rats. *Pharmacognosy Magazine*, 12(48), 333-339.
- Tsai, A. G., Hofmann, A., Cabrales, P., & Intaglietta, M. (2010). Perfusion vs. oxygen delivery in transfusion with “fresh” and “old” red blood cells: the experimental evidence. *Transfusion and Apheresis Science*, 43(1), 69-78.
- Zanzani, S. A., Gazzonis, A. L., Alberti, E., Neilly, T. M., Villa, L., & Manfredi, M. T. (2020). Gastrointestinal nematode infections in goats: Differences between strongyle faecal egg counts and specific antibody responses to *Teladorsagia circumcincta* in Nera di Verzasca and Alpine goats. *Parasitology Research*, 119, 2539-2548.