

Anti-anaemic potential of the hydro-ethanolic extract of *Waltheria indica* (L) in albino rats with parasitaemia – induced anaemia

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Abstract

Anaemia has been reported to be the key feature of trypanosomiasis and is used as the primary indicator of when to treat the infection. Therefore, this study was designed to evaluate the anti-anaemic potential of the hydro-ethanolic extract of *Waltheria indica* in albino rats with parasitaemia – induced anaemia. Three groups of 6 rats each were challenged with *Trypanosoma brucei brucei* to induce anaemia and treated with 300mg/kg bw orally for 5 days, 3.5mg/kg Berenil® once intraperitoneally and distilled water. All the rats developed anaemia, manifested by significantly reduced values of Packed Cell Volume (PCV), Haemoglobin (Hb) concentration and Red Blood Cells (RBC) count within 5 days of infection compared with the pre-infection values. Treatment with Berenil and *Waltheria indica* hydro-ethanolic extract at 300mg/kg bw led to remarkable improvement in the values of the PCV, Hb and RBC, however, the restoration was significantly ($P < 0.05$) higher in the animals treated with the ethanol extract of the plant. Results of the study also indicated the absence of the effect of both parasitaemia and treatment on the levels of erythrocyte indices (Mean Cell Volume, Mean Cell Haemoglobin and Mean Cell Haemoglobin Concentration) of the infected rats. Therefore, the plant could be of use in the management of African sleeping sickness and other ailments related to anaemia.

Keywords: Anti-Anaemia; Hydro-Ethanolic; *Waltheria indica*; Erythrocyte Indices; Parasitaemia

1. Introduction

Anaemia refers to a condition in which the haemoglobin content of the blood is lower than normal as a result of a deficiency of one or more essential nutrients [1], heavy blood loss, parasitic infections, acute and chronic infections [2], and congenital haemolytic diseases [3].

The incidence of anaemia is higher in the third world than in developed countries due to the presence of many aggravating factors such as nutrition, low socio-economic status, and high prevalence of blood parasites such as *Plasmodium* and *Trypanosoma* and helminthic infections. Women are known to be susceptible to anaemia during pregnancy as a result of high demand from the developing foetus [4]. Anaemia is among the top ten causes of death in childhood. The World Health Organization (WHO) estimates that the highest proportions of individuals affected by anaemia are in Africa [5].

Over the years, medicinal plants have been recognised to be of great importance to the health of individuals and communities. In many developing countries, herbal medicines are assuming greater importance in primary health care and their international trade has increased. However, the markets in these countries are not adequately regulated and many herbal products in circulation are unregistered by national regulatory bodies [6].

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African trypanosomes are extracellular parasites that survive in the blood stream of the host. Anaemia has been reported to be the key feature of the disease and persists after the first wave of parasitaemia when parasite numbers have declined to low or undetectable levels. Anaemia rather than parasitaemia is best correlated with productivity and is used as the primary indicator of when to treat the infection [7].

Anaemia is one of the numerous ailments claimed to have been successfully treated with plant materials by traditional medical practitioners [8].

Waltheria indica (Sleepy morning) is short-lived shrub popular among traditional healers in the treatment of skin diseases, malaria and typhoid fever, sickle cell anaemia, and epilepsy [9]. However, no scientific data has been published to show the anti-anaemic activity of the plant in *Trypanosoma brucei brucei* infected animals.

Since resistance to trypanosomiasis has been shown to be determined by at least three main characteristics, namely, the ability to control parasitaemia, the ability to develop an effective immune response and the ability to resist the development of anaemia [10], the main objective of this study was to investigate the anti-anaemic potential of the hydro-ethanolic extract of *Waltheria indica* in rats with parasitaemia-induced anaemia, with the hope of producing an effectively potent plant that is alternative to available synthetic haematinics.

2. Material and methods

2.1. Plant Collection and Identification

This research was conducted between July 2015 and April, 2019 in the Parasitology and Biochemistry Laboratories of the Usmanu Danfodiyo University Sokoto, Nigeria. The plant used in this study was collected from Dabagi Farm of the Usmanu Danfodiyo University, Sokoto and transported to the Botany Unit of the Usmanu Danfodiyo University, Sokoto, for identification. A voucher specimen of the plant was deposited in the herbarium of the Department where it was identified.

2.2. Plant Extraction

Whole plant of *Waltheria indica* was cut into pieces, air-dried (under shade to avoid destruction of the active components) at room temperature and pulverized using mortar and pestle. Forty grams (40g) of the powdered form of the air-dried sample was exhaustively extracted with 1000ml of water and ethanol (50% v/v). It was then left to stand for 24 hours after which it was sieved first with cotton wool and then with Whatmann size 15cm filter paper. The filtrate was dried, weighed and stored until required, a 4.8% yield w/w [11].

2.3. Rat Inoculation and Induction of Anaemia

Trypanosoma brucei brucei were obtained from stabilates maintained at the Nigerian Institute of Trypanosomosis and Onchocerciasis Research (NITOR) Vom Jos, Plateau State.

Twenty-four (24) healthy albino rats, purchased from the animal house of the Usmanu Danfodiyo University, Sokoto, were allowed to acclimatize and subjected to the same physical conditions. At the end of the acclimatization period, parasite infected blood obtained from the tail of an infected rat with high parasitaemia, and appropriately diluted with phosphate buffered saline, was inoculated intraperitoneally into 18 uninfected rats. The mixture was found to contain 3 to 4 parasites per view of the microscope (x 100 magnification) and approximately 1×10^6 trypanosomes per ml of blood.

2.4. Administration of Treatment

Six (6) infected rats received 300mg/kg body weight at the peak of parasitaemia. Administration was done orally and continued for five (5) days consecutively. Another group of 6 rats were treated with full dosage of Berenil® (3.5mg/kg) once intraperitoneally, to serve as positive control. The last group of six rats was left untreated to serve as negative control.

2.5. Haematological analyses

Assessment of haematological parameters started before challenge with the parasites, at the peak of parasitaemia (five days post inoculation), immediately after death (for the untreated rats) and then 24 hours and 7 days after the termination of treatment [12]. Blood was collected from the tail veins of each rat in all groups (before and after infection)

and used for the determination of Packed Cell Volume (%), Haemoglobin concentration (g/dl), Red Blood Cell counts ($10^6/\text{mm}$), Mean Cell Volume (fl), Mean Cell Haemoglobin (pg) and Mean Cell Haemoglobin Concentration (g/dl)¹². Determination of haematological parameters and erythrocyte indices was done using the automated haematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan as described by [13].

Percentage drop in haematological parameters was calculated, for each parameter as follows:

$$\% = 100 \times (X_2 - X_1) / X_1$$

where:

- X_2 : value after the infection
- X_1 : value before infection

Percentage Restoration was calculated, for each parameter as follows:

$$\% = 100 \times (X_2 - X_1) / X_1$$

where:

- X_2 : value after treatment
- X_1 : value before treatment (at the peak of parasitaemia)

2.6. Statistical Analysis

Mean haematological parameters (PCV, Hb, RBC) and erythrocyte indices (MCV, MCH and MCHC) were statistically analyzed using one way ANOVA and Correlation Analysis. Post-test analysis was done using the Duncan's Multiple Range Test. Values of $p < 0.05$ were considered as statistically significant. In all cases data was entered into computer and analyzed using SPSS (Version 20.0) statistical package.

3. Results

3.1. Determination of Anaemia

Results of the study showed that all the infected rats had significant ($p < 0.05$) drop in the values of PCV (%), Hb (g/dl) and RBC ($10^6/\text{mm}$) within five days of infection when compared with the normal uninfected rats and pre-infection levels (Fig. 1). The significant decrease in the values of these parameters is an indication of anaemia in all infected animals. Percentage drops were recorded and shown in Table 1.

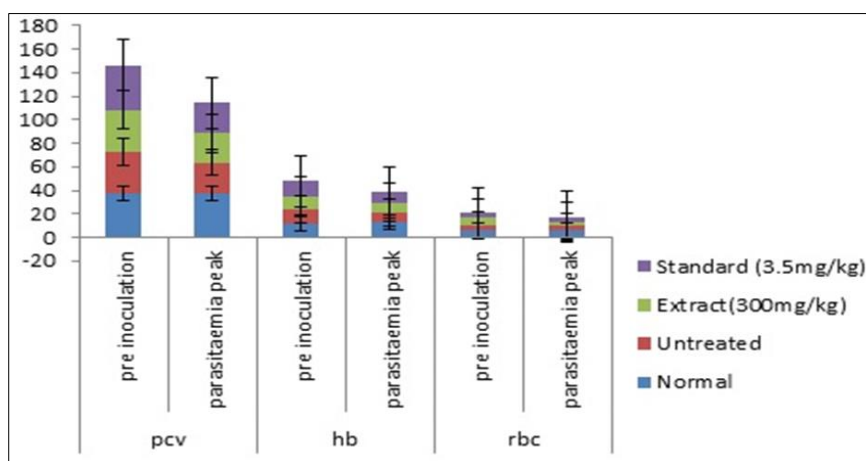


Figure 1 Mean Values of PCV (+), Hb (g/dl) and RBC ($\times 10^6/\text{mm}$) of Rats pre and post infection Infection with *T. brucei brucei* parasites

Table 1 Percentage Decline in PCV (%), Hb (g/dl) and RBC ($\times 10^6/\text{mm}$) of Infected Rats following Infection with *T. brucei brucei*

Rat Grouping	% Decrease		
	PCV (%)	Hb (g/dl)	RBC ($10^6/\text{mm}$)
Untreated	28.52	25.22	27.13
Extract(300mg/kg)	30.82	26.83	33.33
Standard (3.5mg/kg)	31.56	28.77	14.79

3.2. Relationship between Haematological Parameters and Parasitaemia Level

Drops in haematological parameters were recorded with increase in parasite density in all infected experimental animals. The drop in haematological parameters significantly ($P < 0.01$), and inversely correlated with the parasitaemic condition of the infected rats (Table 2).

Table 2 Correlation of Levels of Parasitaemia and Haematological Parameters of *T. brucei brucei* Infected Animals at the Peak of Parasitemia

Parameter		Parasitaemia Level
PCV (%) Level peak of Parasitaemia	Pearson Correlation	-0.759**
	Sig. (2-tailed)	0.000
Hb (g/dl) Level peak of Parasitaemia	Pearson Correlation	-0.753**
	Sig. (2-tailed)	0.000
RBC ($10^6/\text{mm}$) Level peak of Parasitaemia	Pearson Correlation	-0.756**
	Sig. (2-tailed)	0.000

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

3.3. Relationship between Parasitaemia Level and Erythrocyte Indices

Mean post infection levels of erythrocyte indices at the peak of parasitaemia (MCV, MCH and MCHC) did not significantly ($P > 0.05$) differ from those of the normal uninfected rats and with the pre-infection values in all the infected experimental animals (Fig. 2). Similarly, the results of statistical analysis using Pearson Correlation indicated a weak inverse and non-significant ($P > 0.05$) relationship between the level of parasitaemia and the erythrocyte indices of the infected rats (Table 3).

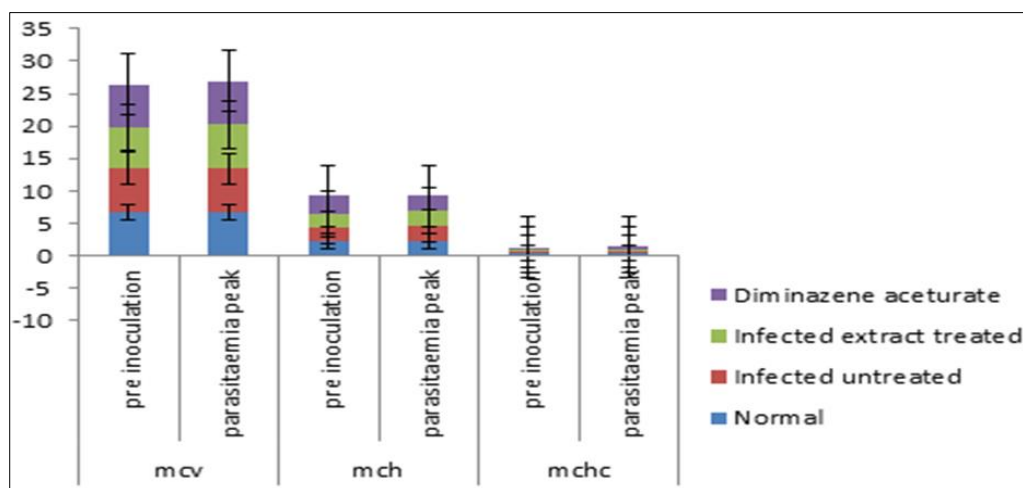


Figure 2 Mean Erythrocyte Indices of Rats Pre and Post Infection with *T. brucei brucei* parasites

Table 3 Erythrocyte Indices of *T. brucei brucei* Infected Rats and their Correlation with Parasitaemia Five Days Post Inoculation

Erythrocyte Indices		Parasitaemia
MCV (fl)	Pearson Correlation	0.014
	Sig. (2-tailed)	0.921
MCH (pg)	Pearson Correlation	-0.067
	Sig. (2-tailed)	0.628
MCHC (g/dl)	Pearson Correlation	-0.225
	Sig. (2-tailed)	0.102

3.4. Anti-Anaemic Effect of the Ethanol Extract of *Waltheria indica* following Treatment of Rats on *T. brucei brucei* Induced Anaemia

Following treatment, there were increases in PCV (%), Hb (g/dl) and RBC ($10^6/mm$) levels of the treated groups of rats (Table 4). However, the PCV (%), Hb and RBC counts in the untreated Group continued to decline to more than 66.67% of their pre-infection values culminating in the death of the rats in this group, suggesting that the death of the untreated animals is correlated with severe anaemic condition of the rats. Although treatment with both Berenil and *Waltheria indica* led to remarkable improvement in the values of the PCV, Hb and RBC, the restoration was significantly ($P < 0.05$) higher in the plant extract treated group. Analysis using Pearson Correlation of the parasitaemia level and the PCV, Hb and RBC counts of the infected and treated animals 24 hours and 7 days post treatment indicated significant ($P < 0.05$) indirect correlation of these parameters with the level of parasitaemia (Table 5).

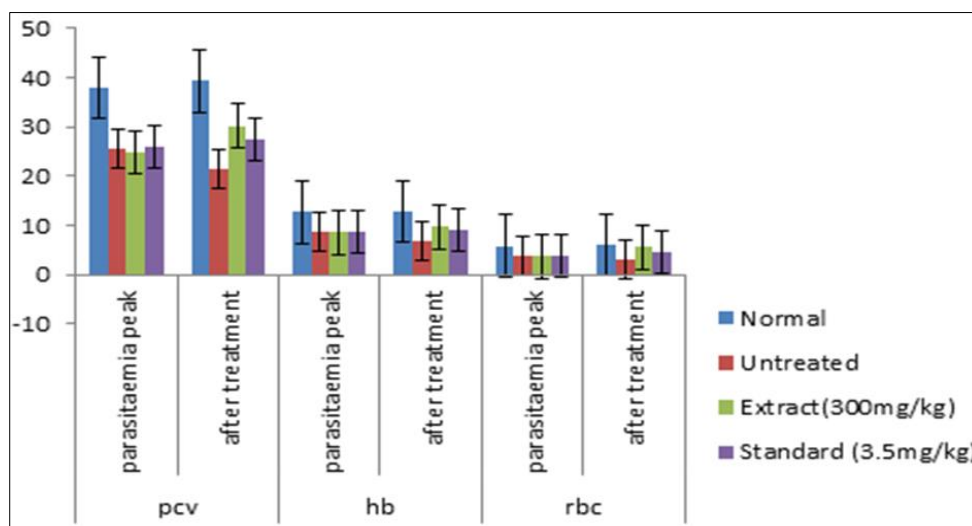


Figure 3 Haematological Parameters of *T. brucei brucei* infected Rats at the Peak of Parasitaemia and after Treatment

Table 4 Percentage Restoration of Haematological indices of *T. brucei brucei* infected rats after Treatment

Animal Group	% RESTORATION		
	PCV (%)	Hb (g/dl)	RBC ($10^6/mm$)
Untreated	-66.0657	-67.7658	-66.6563
Extract(300mg/kg)	21.77419	13.34107	46.54255
Standard (3.5mg/kg)	6.332556	1.696833	13.98964

Table 5 Results of Pearson Correlation of Parasitaemia Level and the Haematological Parameters of the Infected and Treated Animals

Parameter		Parasitaemia Post Treatment	
		24 Hours	7 Days
PCV (%) Post Treatment	Pearson Correlation	-0.446**	-0.436**
	Sig. (2-tailed)	0.006	0.008
Hb (g/dl) Post Treatment	Pearson Correlation	-0.423*	-0.531**
	Sig. (2-tailed)	0.01	0.001
RBC (10 ⁶ /mm) Post Treatment	Pearson Correlation	-0.519**	-0.639**
	Sig. (2-tailed)	0.001	0.001

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

3.5. Effect of the Administration of the ethanol Extract of *W. indica* on Erythrocyte Indices

Significant differences ($p > 0.05$) between the infected and control animals for MCV, MCH and MCHC values were not found following parasite inoculation (Fig 4).

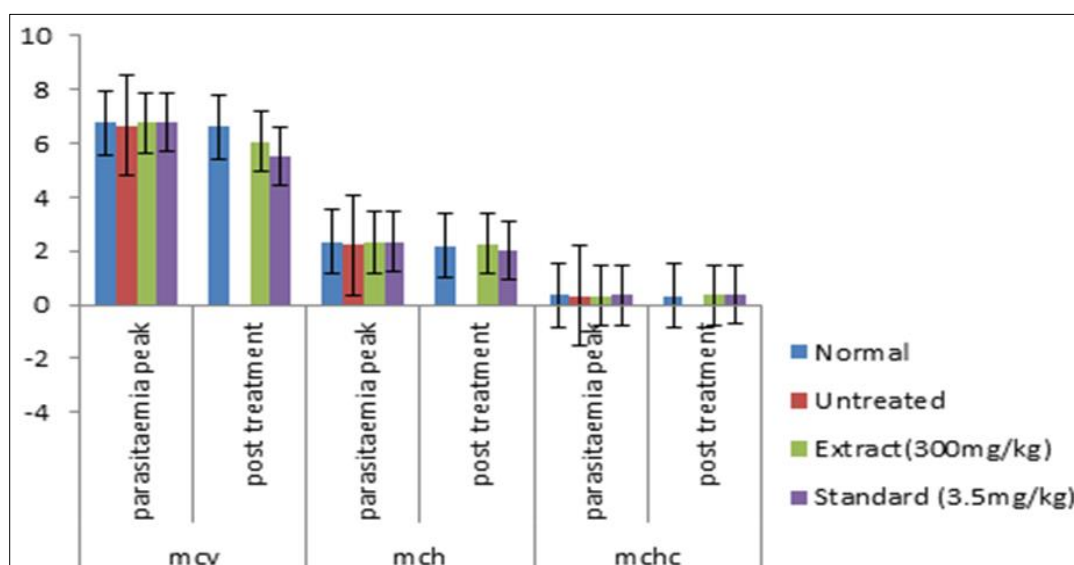


Figure 4 Mean Values of Erythrocyte Indices of *T. brucei* Infected Rats Pre- and Post-Treatment

Table 6 Pearson Correlation analysis of Erythrocyte Indices of *T. brucei* Infected animals with Parasitaemia 24 hours and 7 days post Treatment

Erythrocyte Indices		24 hours PT	7 Days PT
MCHC (g/dl)	Pearson Correlation	0.288	-0.170
	Sig. (2-tailed)	0.088	0.321
MCH (fl)	Pearson Correlation	-0.131	0.540**
	Sig. (2-tailed)	0.445	0.001
MCV (pg)	Pearson Correlation	-0.195	0.438**
	Sig. (2-tailed)	0.255	0.007

**Correlation is significant at the 0.01 level (2-tailed)

Also, no statistical significance ($P > 0.05$) was observed, between treated and control animals in the values of these parameters, after treatment. Thus, indicating the absence of the effect of both parasitaemia and treatment on such parameters. However, statistical analysis indicated the values of MCV and MCH of the treated animals to significantly correlate ($P < 0.05$) with the level of parasitaemia 7 days post treatment (Table 6).

4. Discussion

Haematological parameters and erythrocyte indices remain the tools used by researchers to assess the development of anaemia in parasite-infected animals [14; 15;16;17;18].

The reduced values of PCV (%), Haemoglobin concentration and RBC counts observed in this study following infection have been reported by other workers [19;20;21;22;23]. However, the findings disagreed with those of [24] who recorded no perceptible changes in any of the three blood parameters. The reduction could be attributed to the fact that anaemia is a significant feature of trypanosomiasis and its aetiology according to Yusuf *et al.*, [22] may be haemodilution, osmotic and mechanical fragility of RBCs, extravascular haemolysis, decrease lifespan of RBCs and dyshaemopoiesis.

In the current study, the significant correlation of haematological parameters tested and parasitaemia contradicted the findings of Noyes *et al.*, [25] who found no significant interaction between parasitaemia and trypanosome induced-anaemia from natural tsetse challenge depressed PCV in cattle and in sheep in field studies. However, similar correlations were reported in mice [26], dogs [27] and cattle [28;29;30;31] following infection with trypanosomes.

In the untreated rats, there seem to be a strong correlation between the severity of anaemia and death. Death started when the levels of haematological parameters dropped beyond 67% of their pre-infection values, suggesting the influence of severe anaemia as a possible contributory factor of the death of the animals in this group. A significant linkage between parasitaemia and PCV in cattle, sheep and goat populations has also been reported [32].

In the present study, the PCV, Hb and RBC were observed to improve following treatment with both *W. indica* ethanol fraction and Berenil®. However restoration was higher in animals treated with 300mg/kg of *W. indica*. This positive improvement is perhaps an indication that the plant extract is helping the animals to improve their depressed state of trypanosome induced anaemia. Similar improvements in these indices following treatment have been reported by [33] after treating albino rats with Black seed (*Nigella sativa* seeds). The positive anti-anaemia effect of the plant fraction was further confirmed by the inverse correlation of the level of parasitaemia and haematological indices. This shows that significant improvement in haematological parameters is associated with decline in available parasites in the peripheral blood of the treated animals. Earlier, resistance to trypanosomiasis has been shown to be determined by at least three main characteristics, namely, the ability to control parasitaemia, the ability to develop an effective immune response and the ability to resist the development of anaemia [10]. It may be correct to postulate that the anti-anaemic activity of the plant extract is tied to its trypanocidal activity. This is not surprising because Murray [9] has previously demonstrated the extract of *W. indica* to possess anti-parasitic activity against *T. brucei brucei* in albino rats.

Red cell indexes, MCV, MCH and MCHC, are useful blood tests that provide haematological information about haemoglobin and the size of red blood cells and hence useful in the classification of anaemia. The absence of any change in MCV value observed in the present study, following parasite inoculation, is in contrast to the low and high MCV values reported by [34] and [20] respectively. However, it agrees with observations of eClinPath.com [35] who found unchanged MCV in *Trypanosoma evansi* infection in East African goats. The non – significant alterations in MCHC observed in this study was in contrast to the significant increases observed by Ngeranwa *et al.*,[34]. However, it confirms the statement at [36] that significantly raised MCHC values are not physiologically possible due to limitations on the solubility of the haemoglobin.

5. Conclusion

The study has highlighted the immediate and rapid decline of haematological parameters following trypanosome infection leading to anaemia, which significantly and inversely correlated with parasitaemia level of the infected rats. However, treatment with the ethanol fraction of *W. indica* significantly improves the values of haematological parameters to almost their baseline levels, even better than the standard reference drug Berenil.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they do not have competing interest.

Statement of ethical approval

The ethical approval of this study was obtained from the Ethical Committee of the Usmanu Danfodiyo University Sokoto, Nigeria {UDUS/R/ACA/01/.25}.

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