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ORIGINAL ARTICLE

Effects of aqueous leaf extracts of *Loranthus micranthus* Linn. on hematological profile of albino rats infected with *Trypanosoma brucei brucei*

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Abstracts
African trypanosomiasis has continued to threaten human health and economical development (Kuzoe Acta Trop 54:153–162, 1993; WHO J Tsetse Tryp Info Quart 3:4–9, 2000). It is caused by parasitic protozoa of the genus *Trypanosoma* (Adeiza et al. J Med Plant Res 4(17):1770–1777, 2010), and the distribution corresponds with that of tsetse flies. Effects of aqueous leaf extracts of *Loranthus micranthus* on hematological parameters of albino rats infected with *Trypanosoma brucei brucei* were investigated for 28 days using standard methods. The HB, PCV, RBC, and its indices (MCH, MCV, and MCHC) of the infected rats significantly decreased ($P < 0.05$) at various dose levels of the extracts when compared with the control groups. The WBC counts of the treatment groups and those of the negative control group showed significant increases ($P < 0.05$) in all the weeks when compared with the normal control. The WBC differentials revealed that neutrophils were significantly higher ($P < 0.05$) in the test group in comparison with the positive control group at week 3; however, lymphocytes, eosinophils, and basophils were not significantly different ($P > 0.05$) from the positive control in week 3. Furthermore, minimal increases in the WBC differentials were observed in the group administered 800 mg/kg of the plant extract. The present study showed that all test rats and the negative control group died from the resultant overwhelming parasitemia at week 4 unlike the case of those administered the standard drug, which is an indication that the extract lacks anti-trypanocidal activity. Thus, the aqueous leaf extract of *Loranthus micranthus* is an inadequate anti-trypanosomal agent.

Keywords Parasitaemia · Trypanosomiasis · Hematological profile · Albino rats · Aqueous leaf extracts · *Loranthus micranthus*

Introduction

African trypanosomiasis has been a threat to human health and economical development (Kuzoe 1993; WHO 2000). Trypanosomiasis is a lethal disease which affects both man and animals, and it is caused by a parasitic protozoa of the genus *Trypanosoma* (Adeiza et al. 2010). The distribution of trypanosomiasis corresponds roughly with that of tsetse flies. Trypanosomiasis, which is also known as sleeping sickness, is caused by *Trypanosoma brucei gambiense* and or *Trypanosoma brucei rhodesiense* following an infective bite from tsetse fly (Welburn et al. 2001; Haydon et al. 2002; WHO 2006). However, up to 80% of the Nigerian land mass is infested by the vector of the parasite, tsetse fly. Presently, the disease in cattle has been on the increase due to the menace of the vector, drug resistance and presence of other hematophagous flies (Holmes 2000). Transmission of this parasite is mostly through the bite of an infected tsetse fly; other ways include mother to child transmission through the placenta, mechanical transmission through other blood sucking insects (though it is difficult to assess the epidemiological impact of this mode of transmission), accidental transmissions/infection due to pricks from contaminated needles in the laboratory (Seed 1998; WHO 2006; Kennedy 2006).

Pathogenic trypanosome infections of domestic animals in sub-Saharan African largely account for the low livestock productivity in the continent thus, making it an important priority for the agricultural and biomedical and public agencies (Aliyu et al. 2010). The parasites infestation constitutes the greatest

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single constraint to livestock and crop production thereby directly contributing to hunger, poverty, protein malnutrition, and suffering of entire communities in Africa. This is as its name suggest, infected people tend to sleep a lot, leading to loss of man hours that could have been applied to productive farm work (Murray 1994; Aroke et al. 1998; Jodi et al. 2011).

Chemotherapy of trypanosomiasis infection is dependent on the stage of infection (that is whether it is chronic and or acute infection). Normally, the drugs used in the initial stage of the disease infection are of lower toxicity and easier to administer (Burri et al. 2000; Chappuis et al. 2005). The earlier the disease is identified, the better the prospect of a cure. Treatment success in the second stage of infection (that is, the advanced stage) depends on a drug that can cross the blood–brain barrier to reach the parasite. Some of the drugs used include pentamidine, suramin, and eflornithine (Burri et al. 2000; Chappuis et al. 2005; WHO 2008). Despite the efficacious nature of these drugs, researchers have directed their energies to screen local medicinal plants as potential trypanocides. Although some herbal formula such as Jubi herbal formula and African herbal formula among others are already in circulation, efforts are made to develop effective drugs from medicinal plants for both the management and treatment of trypanosomiasis (Okochi et al. 2003; Erah et al. 2003).

Plant extract and its parts are good sources of medicine in traditional African societies and beyond (Obasi et al. 2011). The dependence on herbal medicine apparently diminished with the advent of orthodox medication. The benefit from alternative medicine, especially in the developing parts of the world were limited by lack of scientific basis, warranting that the quality and consistency of the alternative medicines be ascertained and maintained for their maximal use and efficacy (Ukoha et al. 2011). The African mistletoe, *Loranthus micranthus* Linn., is a ubiquitous hemi parasitic plant that thrives well in tropical climates (Obatomi et al. 1996). It depends on its host for mineral salts and water but can photosynthesize its own carbohydrates. Mistletoe grows on a wide range of evergreen and deciduous trees. Host plants of mistletoe include *Persea americana*, *Kola accuminata*, *Baphia nitida*, *Treculia africana*, etc. (Nzekwe et al. 2009). Mistletoe has a long history of traditional use for a wide range of diseases such as diabetes, diarrhea, and epilepsy.

Due to its medicinal value/uses and pharmacological activities, mistletoe has been revealed to have a great potential for its use in various systemic and non-systemic infections due to bacteria and fungi (Osadebe and Ukwueze 2004).

Objective of the study Due to the medicinal value/uses and pharmacological activities of mistletoe, this study is therefore designed to investigate the effects of aqueous leaf extracts of African mistletoe, *Loranthus micranthus*, on hematological profile of albino rats infected with *Trypanosoma brucei brucei.*

Materials and methods

The reagents used for this research were all of analytical grades.

Collection and preparation of *L. micranthus* extract

Fresh leaves of *L. micranthus* were procured from the forests around Obukpa, a community in Nsukka Local Government area of Enugu State from the host tree (*Kola accuminata*). The plant was identified by a plant biologist in the Department of Plant Biology and Biotechnology, University of Nigeria, Nsukka. The leaves were collected, weighed, and shade dried for 2 weeks. The weight of the dried leaves was reweighed using an electronic weighing balance (Metller, PC 2000). After shade drying, the dry leaves were pulverized into fine powder using a laboratory milling machine (Honda: model 622, China). About 500 g of the powdered materials was soaked in 1000 ml of distilled water and allowed to stand for 24 h at room temperature, with intermittent shaking to increase the extraction capacity. The decoction was filtered using a muslin cloth (60-mesh sieve), concentrated with rotary evaporator at 60 °C and then oven-dried at the same temperature to completely evaporate the water. Weighed samples of the extract was redissolved in normal saline and used to prepare the stock solution for oral administration to the animals according to their body weights.

Procurement and management of experimental animals

Adult male albino rats were obtained from Genetic and Animal Breeding Laboratory of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were kept in stainless wire-rat cages equipped with drinkers and fecal collecting trays, in a clean experimental animal house. The rats were fed commercial growers chick mash (18% crude protein) made by Vital Feeds Nigeria Limited and clean drinking water, and allowed to get acclimatized for 14 days before the start of the experiment. The animals were allowed free access to food and water ad libitum. The fecal droppings in the tray were removed daily. The experimental rats were handled in strict compliance with recommendation by the Committee and the International Guidelines for Handling of Laboratory Animals (Derrell 1996).

Experimental design

Seventy-two rats were assigned into six groups (A, B, C, D, E, and F) of 12 rats per group with each group comprising 3 replicates of 4 rats per replicate. Groups A, B, and C served as the treatment groups while groups D, E, and F were the negative, positive, and normal control groups, respectively.

Three different concentrations of the aqueous extract were administered to the different treatment groups according to their body weight. Group A was given 400 mg/kg body weight of the leaf extract, while groups B and C were administered 800 mg/kg and 1200 mg/kg, respectively. The negative control (group D) also regarded as infected and untreated group and normal control groups (group F) were given 1 ml/kg body weight of normal saline according to their body weights. The positive control groups (group E) were inoculated and treated with a standard drug (dimenazene aceturate) also known as berenil, according to their body weight. All doses were administered once daily orally for 28 days (4 weeks) for all the groups using gastric gavage.

Determination of hematological profiles

Hemoglobin (Hb), red blood cells (RBCs), and white blood cells (WBCs) were determined using the method of Sood (2006) and packed cell volume (PCV) using the method of Coles (1986). Red cell indices are determined using the method of Bakers et al. (2001), and differential white blood cells were determined using the method of Cheesbrough (2005).

Statistical analysis

Data accumulated was analyzed using the GENSTAT (VSN International, Hemel Hempstead, Herts, UK). One-way ANOVA was used to test the effect of treatment, whereas a two-way ANOVA was used to determine the interactive effects of treatment and duration. Duncan's multiple range test was used in the separation of means of the different treatment groups. All results were expressed as mean ± standard error of mean (SEM), while values were considered significant at $P < 0.05$.

Results

Effects of aqueous leaf extract of *L. micranthus* on PCV, Hb, RBC, and WBC of albino rats on weekly basis

Table 1 shows the weekly effects of aqueous leaf extract of *L. micranthus* on the PCV, Hb, RBC, and WBC levels of albino rats. From the table, there were significant decreases ($P < 0.05$) in PCV levels of all the groups (A–D) when compared with week 0, though they were not dose dependent. However, group E showed a significant increase ($P < 0.05$) at week 1 when compared with the baselines (week 0). Taking into account the effect of duration of treatment on the rats, there was no significant difference ($P > 0.05$) at weeks 1 and 2 when compared with the control except groups E and B which showed a significant decrease ($P < 0.05$) at weeks 1 and 2, respectively. However, at week 3, the PCV levels of all the groups (A–D) significantly decreased ($P < 0.05$) when compared with the control. There was a time-dependent and significant decrease ($P < 0.05$) in Hb levels of rats in groups A, B, and D from weeks 1 to 3 while group C showed a significant decrease ($P < 0.05$) at weeks 1 and 3 when compared with the baseline, though not time dependent. However, there was a minimal increase in group E from weeks 1 to 4 when compared with the baselines (week 0). On the effect of treatments, there was an overall dose-independent and significant decrease ($P > 0.05$) in groups A–D in all the weeks when compared with normal control groups except A, C, and D which showed no level of significance ($P > 0.05$) at week 2. No significant difference was observed in group E when compared with the control. The RBC levels in the rats showed a time dependent and significant decrease ($P < 0.05$) in groups A–D from weeks 1 to 3 when compared with week 0 (baseline). However, group E significantly decreased ($P < 0.05$) at weeks 1 and 2 and increased from weeks 3 to 4 when compared with week 0. On the effect of treatment, there was no dose-dependent significant difference ($P > 0.05$) for all the treatment groups when compared with the normal control except groups A and E which showed a significant decrease ($P < 0.05$) at week 2. However, at week 3, there were significant decreases in the RBC levels of all the groups (A–E) when compared with the control. Also, there was significant increases ($P < 0.05$) in the WBC levels of rats in groups B and D at weeks 1 and 3 and group C at weeks 1 to 3, when compared with the baselines (week 0). The standard control (group E) showed a significant increase ($P < 0.05$) at weeks 1, 2, and 4. In the effect of treatment, there was no significant increase ($P > 0.05$) though dose dependent in groups A–D except group B which showed a significant increase ($P < 0.05$) at weeks 2 and 3 and group C at week 2 only, when compared with the normal control. However group E showed significant decreases ($P < 0.05$) at weeks 1, 2, and 4 when compared with the control.

Effects of aqueous leaf extract of *L. micranthus* on red cell indices of albino rats on weekly basis

Table 2 shows the weekly effects of the aqueous extracts *L. micranthus* on the mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) in the albino rats. However, time-independent significant differences ($P < 0.05$) were observed in MCV levels in all the groups (A, C, and D) at weeks 2, 3, and 2 when compared with week 0. Group E showed a marked significant difference ($P < 0.05$) from weeks 1 to 4 when compared with the control. Similarly, in the effects of treatment, no overall significant difference ($P > 0.05$) was observed in the groups from weeks 1 to 4 when compared with the normal control. Moreover, there were significant increases ($P > 0.05$)

Table 1 Effects of aqueous leaf extract of *L. micranthus* on PCV, Hb, RBC, and WBC of albino rats on weekly basis

Parameters	Treatments	Duration (weeks)				
		0	1	2	3	4
PCV (%)	A (400 mg/kg)	38.17 ± 0.62^{1a}	26.67 ± 1.20^{2b}	27.00 ± 9.00^{2ab}	0.00 ± 0.00^{3c}	*
	B (800 mg/kg)	37.67 ± 0.71^{1a}	30.67 ± 2.18^{2b}	20.00 ± 1.00^{3b}	20.50 ± 0.50^{3b}	*
	C (1200 mg/kg)	38.00 ± 0.57^{1a}	27.00 ± 4.61^{2b}	32.00 ± 1.00^{12ab}	28.00 ± 5.00^{2b}	*
	D (infected and untreated)	38.42 ± 0.55^{1a}	$30.00 + 2.08^{2b}$	26.00 ± 2.00^{2ab}	20.50 ± 0.50^{3b}	*
	E (standard drug)	37.25 ± 0.71^{2a}	46.33 ± 2.02^{1a}	43.67 ± 6.48^{12a}	39.67 ± 2.02^{12a}	43.67 ± 2.186^{12a}
	F (normal control)	38.31 ± 0.64^{1a}	38.69 ± 2.41^{1b}	39.12 ± 2.01^{1a}	39.28 ± 1.00^{1a}	38.33 ± 2.100^{1a}
Hb (g/dl)	A (400 mg/kg)	13.083 ± 0.25^{1a}	9.000 ± 0.36^{2b}	9.05 ± 2.95^{2ab}	*	*
	B (800 mg/kg)	12.967 ± 0.25^{1a}	10.37 ± 0.71^{2b}	7.20 ± 0.50^{3b}	6.475 ± 0.02^{3c}	*
	C (1200 mg/kg)	13.00 ± 0.24^{1a}	9.17 ± 1.58^{2b}	11.00 ± 0.00^{12ab}	10.00 ± 1.00^{2b}	*
	D (infected and untreated)	13.08 ± 0.22^{1a}	9.93 ± 0.63^{2b}	8.75 ± 0.75^{2ab}	6.950 ± 0.05^{3c}	*
	E (standard drug)	12.82 ± 0.27^{1a}	15.33 ± 0.88^{1a}	14.90 ± 2.15^{1a}	13.66 ± 0.88^{1a}	14.67 ± 0.66^{1a}
	F (normal control)	13.76 ± 0.25^{1a}	13.83 ± 0.33^{1a}	13.58 ± 0.64^{1a}	$13.3021 \pm .50^{1a}$	13.51 ± 0.51^{1a}
RBC ($\times 10^6$/mm^3)	A (400 mg/kg)	$7.90 \pm 0.291a$	$5.14 \pm 1.492a$	$4.07 \pm .5003b$	*	*
	B (800 mg/kg)	7.25 ± 0.81^{1a}	6.53 ± 1.13^{2a}	5.70 ± 1.00^{3a}	3.11 ± 0.51^{4c}	*
	C (1200 mg/kg)	7.78 ± 0.13^{1a}	5.11 ± 0.55^{3a}	6.01 ± 1.19^{2a}	4.41 ± 0.24^{4bc}	*
	D (infected and untreated)	6.62 ± 0.38^{1a}	5.78 ± 2.93^{2a}	5.81 ± 1.00^{2a}	3.20 ± 0.23^{3c}	*
	E (standard drug)	8.11 ± 0.01^{1a}	6.83 ± 0.81^{2a}	4.22 ± 0.67^{3b}	5.58 ± 1.23^{3b}	6.70 ± 0.64^{2a}
	F (normal control)	7.39 ± 1.14^{1a}	7.27 ± 0.56^{1a}	7.59 ± 0.72^{1a}	7.30 ± 1.18^{1a}	7.21 ± 2.07^{1a}
WBC ($\times 10^3$/mm^3)	A (400 mg/kg)	$4.96 \pm .27^{1a}$	$5.07 \pm .58^{1b}$	$4.10 \pm .90^{1b}$	*	*
	B (800 mg/kg)	$4.33 \pm .27^{3a}$	7.60 ± 1.92^{2b}	6.80 ± 2.20^{23a}	$24.75 \pm .25^{1a}$	*
	C (1200 mg/kg)	$4.19 \pm .39^{2a}$	$8.93 \pm .52^{2b}$	8.90 ± 5.30^{2a}	10.00 ± 4.00^{1b}	*
	D (infected and untreated)	$4.30 \pm .26^{3a}$	$9.07 \pm .58^{2b}$	4.50 ± 1.50^{3b}	$11.95 \pm .05^{1b}$	*
	E (standard drug)	$4.22 \pm .26^{3a}$	14.40 ± 1.83^{1a}	8.87 ± 1.86^{2a}	6.07 ± 1.29^{23b}	8.33 ± 2.33^{2a}
	F (normal control)	$4.43 \pm .27^{1a}$	$4.40 \pm .12^{1b}$	$4.41 \pm .36^{1b}$	$4.22 \pm .27^{1bc}$	$4.21 \pm .38^{1b}$

Values with different alphabetic (lowercase) superscripts differ significantly ($P < 0.05$) between different concentrations within the same exposure duration. Similarly, values with different numeric superscripts differ significantly ($P < 0.05$) between different exposure periods within the same concentration. Results are expressed as mean ± SEM of triplicate determination

*The animals that have undergone mortality

observed in MCH levels in groups A, C, and D at weeks 2, 1, and 2, respectively, when compared with week 0 (Table 2). However, significant increases ($P < 0.05$) were also observed in group E from weeks 1 to 3 but decreased at week 4 when compared with week 0. On the effect of treatment, there was a dose-independent and significant decrease ($P > 0.05$) in groups B at weeks 1and 2, and significant increase ($P > 0.05$) in group E at week 2 and groups B to E at week 3 when compared with the normal control group. Also, there was a significant decrease ($P < 0.05$) in groups A and D and increase in group B at weeks 1 to 3 when compared with week 0 (Table 2). Also, there was a significant decrease ($P < 0.05$) in the level of MCHC in group E at week 1 when compared with week 0. Similarly, except group A, there was significant decreases ($P < 0.05$) in all the groups at week 1 in the effects of treatment when compared with the control, same occurred in A and D at week 2 and B at week 3 when compared with the normal control.

Effects of aqueous leaf extract of *L. micranthus* on differential white blood cell of albino rats on weekly basis

The results of the weekly effects of the aqueous extracts of *L. micranthus* on the neutrophil, lymphocyte, eosinophil, basophil, and monocyte levels of albino rats are shown in Table 3. There was no overall time-dependent and significant different ($P > 0.05$) in neutrophil levels of the treatment groups (A–C) when compared with week 0. Moreover, there were significant increases ($P < 0.05$) in group D at weeks 1 and 3 and group E at weeks 1, 2, and 4 when compared with week 0. In the effect of treatment, there was no significant increase ($P > 0.05$) in groups A–D except group B which showed a significant increase ($P < 0.05$) at week 3 when compared with the normal control. However, group E showed significant increase ($P < 0.05$) at week 1 when compared with the control. Furthermore, there was no significant difference

Table 2 Effects of aqueous leaf extract of *L. micranthus* on red cell indices of albino rats on weekly basis

Parameters	Groups	Duration (weeks)				
		0	1	2	3	4
MCV (u^3)	A (400 mg/kg)	5.4 ± 0.28^{2a}	5.2 ± 0.00^{2a}	6.7 ± 0.50^{1a}	*	*
	B (800 mg/kg)	5.2 ± 0.85^{12a}	5.0 ± 0.00^{12a}	4.0 ± 1.00^{2a}	6.6 ± 0.66^{1a}	*
	C (1200 mg/kg)	5.4 ± 0.28^{2a}	5.4 ± 0.00^{2a}	5.3 ± 0.33^{2a}	7.0 ± 0.00^{1a}	*
	D (infected and untreated)	6.3 ± 0.33^{1a}	6.0 ± 1.00^{1a}	5.2 ± 0.00^{2a}	6.6 ± 0.66^{1a}	*
	E (standard drug)	4.6 ± 0.25^{2a}	7.6 ± 0.66^{1a}	7.5 ± 1.00^{1a}	7.8 ± 0.00^{1a}	7.1 ± 0.66^{1a}
	F (normal control)	5.4 ± 0.28^{1a}	5.4 ± 0.28^{1a}	5.5 ± 0.71^{1a}	5.5 ± 0.71^{1a}	5.4 ± 0.28^{1a}
MCH (Pg)	A (400 mg/kg)	18 ± 0.57^{2a}	18 ± 0.00^{2a}	22 ± 0.50^{1b}	*	*
	B (800 mg/kg)	17 ± 0.14^{12a}	16 ± 0.66^{12b}	14 ± 1.00^{2c}	20 ± 0.00^{1a}	*
	C (1200 mg/kg)	18 ± 0.57^{2a}	18 ± 0.10^{2a}	18 ± 0.33^{2b}	25 ± 0.10^{1a}	*
	D (infected and untreated)	21 ± 0.66^{1a}	18 ± 0.00^{12a}	16 ± 1.00^{2b}	21 ± 0.72^{1a}	*
	E (standard drug)	15 ± 1.00^{3a}	25 ± 0.00^{12a}	35 ± 1.00^{1a}	26 ± 0.00^{12a}	23 ± 0.33^{2a}
	F (normal control)	18 ± 0.57^{1a}	18 ± 0.57^{1a}	18 ± 0.57^{1b}	18 ± 0.50^{1b}	18 ± 0.57^{1a}
MCHC (g/dl)	A (400 mg/kg)	351 ± 0.35^{1a}	346 ± 0.15^{2a}	333 ± 0.33^{3b}	*	*
	B (800 mg/kg)	308 ± 0.33^{4b}	333 ± 0.33^{2b}	360 ± 0.00^{1a}	320 ± 1.00^{3b}	*
	C (1200 mg/kg)	342 ± 0.10^{2a}	337 ± 0.03^{3b}	343 ± 0.75^{2a}	357 ± 0.14^{1a}	*
	D (infected and untreated)	342 ± 0.10^{1a}	333 ± 0.33^{2b}	334 ± 0.62^{2b}	345 ± 0.00^{1a}	*
	E (standard drug)	345 ± 0.94^{12a}	332 ± 0.60^{3b}	346 ± 0.51^{1a}	348 ± 0.72^{1a}	339 ± 0.53^{2b}
	F (normal control)	360 ± 0.52^{1a}	363 ± 0.15^{1a}	346 ± 0.15^{2a}	341 ± 0.02^{2a}	355 ± 0.26^{2a}

Values with different alphabetic (lowercase) superscripts differ significantly ($P < 0.05$) between different concentrations within the same exposure duration. Similarly, values with different numeric superscripts differ significantly ($P < 0.05$) between different exposure periods within the same concentration. Results are expressed as mean ± SEM of triplicate determination

*The animals that have undergone mortality

($P > 0.05$) in the duration of treatment in lymphocyte level of groups A–C at weeks 1 to 3 except B and C which showed significant increase at weeks 2 and 3, respectively. Groups D and E significantly increased ($P < 0.05$) at weeks 1 and 3, and 1, 2, and 4, respectively, when compared with week 0. On the effect of treatment, there was no overall significant increase ($P > 0.05$) in all the groups when compared with the control except B and E which significantly increased ($P < 0.05$) at weeks 3 and 1, respectively. No overall significant difference ($P > 0.05$) in the duration and effects of treatment in eosinophil and basophil levels of all the groups from weeks 1 to 4 when compared with week 0 and normal control, respectively, except A and B of eosinophils and basophil that significantly decreased ($P < 0.05$) at week 1. More so, there was a significant decrease ($P < 0.05$) in monocyte level of group A at week 2 when compared with week 0. Also, there was a time-dependent and significant increases ($P < 0.05$) in groups B and C at weeks 2 to 3 when compared with week 0, while groups D and E did not exhibit any level of significance ($P > 0.05$) at weeks 1 to 4. On the effect of treatment, there was no dose-dependent significant difference ($P > 0.05$) for all the treatment groups when compared with the normal control except groups A, B, and C which showed significant decrease ($P < 0.05$) at week 3.

Discussion and conclusion

Hematolgical parameters are important physiological indices used to evaluate the extent of deleterious effects of foreign agents on the blood constituents of both animals and humans. They could also be used to explain blood-related functions of chemical compounds of plant extracts (Yakubu et al. 2007; Ashafa et al. 2009). The significant reductions observed in the red blood cells (RBCs), packed cell volume (PCV), and hemoglobin (HB) of the infected animals treated with *L. micranthus* leaf extracts is very instructional. This may be attributed to the enormous number of circulating parasites which could not be cleared by the aqueous leaf extract. This however agreed with a similar works by Sam-Wobo et al. (2010) and Yakubu et al. (2014), who noted that RBC and its associated parameters (PCV and HB) of animals infected with *T. brucei brucei* were lower than that of non-infected animals, and attributed this to the enormous number of circulating trypanosomes causing hemolysis of the red blood cells. This inference further supports the work of Igbokwe and Mohammed (1992), who opined that the decreases observed in the RBC, PCV, and hemoglobin levels causing severe anemia is a cardinal feature associated with *T. brucei brucei* infection in comparison with other species of trypanosomes.

Table 3 Effects of aqueous leaf extract of *L. micranthus* on differential white blood cell of albino rats on weekly basis

Parameters	Groups	Duration (weeks)				
		0	1	2	3	4
Neutrophils (μL)	A (400 mg/kg)	2.600 ± 0.16^{1a}	2.6300 ± 0.34^{1b}	2.250 ± 0.25^{1a}	*	*
	B (800 mg/kg)	2.608 ± 0.16^{2a}	4.267 ± 1.09^{2b}	3.900 ± 0.90^{2a}	13.250 ± 0.25^{1a}	*
	C (1200 mg/kg)	2.330 ± 0.21^{2a}	5.100 ± 0.37^{1b}	5.050 ± 2.95^{1a}	5.100 ± 2.10^{1b}	*
	D (infected and untreated)	2.291 ± 0.16^{3a}	5.167 ± 0.55^{2b}	2.750 ± 0.75^{3a}	6.5950 ± 0.00^{1b}	*
	E (standard drug)	2.291 ± 0.16^{3a}	8.00 ± 1.02^{1a}	5.000 ± 1.00^{2a}	3.333 ± 0.76^{23bc}	4.600 ± 1.53^{2a}
	F (normal control)	2.323 ± 0.24^{1a}	2.668 ± 0.51^{1b}	2.515 ± 0.31^{1a}	2.633 ± 0.30^{1bc}	2.602 ± 0.12^{1a}
Lymphocyte (μL)	A (400 mg/kg)	2.075 ± 0.12^{1a}	2.066 ± 0.26^{1b}	1.750 ± 0.55^{1b}	*	*
	B (800 mg/kg)	1.616 ± 0.12^{3ab}	3.200 ± 0.80^{2b}	2.500 ± 1.30^{2a}	12.250 ± 0.25^{1a}	*
	C (1200 mg/kg)	1.591 ± 0.14^{2b}	3.600 ± 0.40^{12b}	4.000 ± 2.80^{1a}	4.950 ± 0.05^{1b}	*
	D (infected and untreated)	1.783 ± 0.11^{3ab}	3.466 ± 0.29^{2b}	1.500 ± 0.50^{3b}	4.950 ± 0.05^{1b}	*
	E (standard drug)	1.691 ± 0.10^{3ab}	6.033 ± 0.53^{1a}	3.533 ± 1.03^{2a}	2.433 ± 0.61^{23bc}	3.600 ± 0.94^{2a}
	F (normal control)	1.641 ± 0.12^{1ab}	1.881 ± 0.19^{1b}	2.133 ± 0.73^{1a}	1.891 ± 0.50^{1c}	1.811 ± 0.11^{1a}
Eosinophil (μL)	A (400 mg/kg)	0.033 ± 0.02^{1a}	0.067 ± 0.03^{1a}	0.000 ± 0.000^{1a}	*	*
	B (800 mg/kg)	0.000 ± 0.00^{1a}	0.000 ± 0.00^{1b}	0.000 ± 0.000^{1a}	*	*
	C (1200 mg/kg)	0.017 ± 0.01^{1a}	0.000 ± 0.000^{1b}	0.000 ± 0.000^{1a}	*	*
	D (infected and untreated)	0.008 ± 0.01^{1a}	0.000 ± 0.000^{1b}	0.000 ± 0.000^{1a}	*	*
	E (standard drug)	0.033 ± 0.01^{1a}	0.000 ± 0.000^{1b}	0.067 ± 0.03^{1a}	*	*
	F (normal control)	0.030 ± 0.01^{1a}	0.029 ± 0.01^{1b}	0.031 ± 0.01^{1a}	*	*
Basophils (μL)	A (400 mg/kg)	0.075 ± 0.02^{1a}	0.067 ± 0.03^{1a}	0.050 ± 0.05^{1a}	*	*
	B (800 mg/kg)	0.017 ± 0.01^{2a}	0.000 ± 0.00^{2b}	0.100 ± 0.00^{1a}	0.1250 ± 0.02^{1a}	*
	C (1200 mg/kg)	0.067 ± 0.02^{1a}	0.033 ± 0.03^{1a}	0.000 ± 0.00^{1a}	0.1000 ± 0.10^{1a}	*
	D (infected and untreated)	0.033 ± 0.01^{1a}	0.067 ± 0.03^{1a}	0.050 ± 0.05^{1a}	0.000 ± 0.00^{1a}	*
	E (standard drug)	0.067 ± 0.02^{1a}	0.067 ± 0.03^{1a}	0.100 ± 0.05^{1a}	0.0333 ± 0.03^{1a}	0.033 ± 0.03^{1a}
	F (normal control)	0.067 ± 0.02^{1a}	0.067 ± 0.02^{1a}	0.050 ± 0.05^{1a}	0.0333 ± 0.01^{1a}	0.033 ± 0.03^{1a}
Monocyte (μL)	A (400 mg/kg)	0.242 ± 0.02^{1a}	0.267 ± 0.06^{1a}	0.050 ± 0.05^{2a}	*	*
	B (800 mg/kg)	0.100 ± 0.02^{3b}	0.200 ± 0.11^{23a}	0.350 ± 0.05^{2a}	1.375 ± 0.02^{1a}	*
	C (1200 mg/kg)	0.183 ± 0.02^{2ab}	0.200 ± 0.15^{12a}	0.250 ± 0.05^{12a}	0.4500 ± 0.05^{1b}	*
	D (infected and untreated)	0.183 ± 0.03^{1ab}	0.367 ± 0.12^{1a}	0.200 ± 0.20^{1a}	0.3750 ± 0.25^{1bc}	*
	E (standard drug)	0.233 ± 0.03^{1a}	0.367 ± 0.23^{1a}	0.167 ± 0.08^{1a}	0.2667 ± 0.03^{1c}	0.433 ± 0.12^{1a}
	F (normal control)	0.180 ± 0.02^{1ab}	0.183 ± 0.05^{1a}	0.181 ± 0.01^{1a}	0.181 ± 0.68^{1c}	0.181 ± 0.32^{1a}

Values with different alphabetic (lowercase) superscripts differ significantly ($P < 0.05$) between different concentrations within the same exposure duration. Similarly, values with different numeric superscripts differ significantly ($P < 0.05$) between different exposure periods within the same concentration. Results are expressed as mean ± SEM of triplicate determination

*The animals that have undergone mortality

Ekanem and Yusuf (2007) also ascribed the decrease in RBC, PCV, and HB as a measurement of acute anemia in *T. brucei brucei* infection as an indicator of the severity of the proliferating parasites.

Total white blood cells (WBCs) contribute to the host defense mechanism by defending the body against infectious diseases and foreign invaders (Barry and Turner 1992). The host's defense system consists of an intricate network of cytokines and progenitor cells which maintain basal myelopoiesis (formation of WBCs) and allow rapid adjustments in the rates of production of WBCs in response to acute and chronic infections (Stock and Hoffman 2000). The results of this study showed significant elevations in the levels of WBCs of the trypanosome infected rats from the first week to the last week of the leaf extract administration. This observed significant increase in WBC counts of the treated rats is not surprising as such is indicative of a disease condition. Furthermore, observations made in this present study further suggest that the infected rats experienced severe protozoan infection and myeloproliferative disorders, leading to the mass production of WBCs. It is also worthy of note here to suggest that the increased number of the circulating WBCs may also be as a result of the overwhelming number of parasites (trypanonsomes) circulating in the blood. This general

increase in all the infected groups may be due to immune response to the presence of *T. brucei brucei* in rats, thus, an indication that the parasite could not be control by the plant extracts. This result corroborates the views of Yusuf et al. (2013), who reported increase in the WBCs following acute and chronic *T. brucei brucei* infection of bone marrow and peripheral blood cells of Wistar rats. Sulaiman and Adeyemi (2010) and Adeyemi et al. (2009) noted that increased WBC count in animals affected by *T. brucei brucei* infection is attributed to the efforts of the animals' defense system in eliminating the invading parasites.

The calculated blood indices such as mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), and mean cell hemoglobin concentration (MCH) evaluated in this study are of crucial importance in anemia diagnosis in most animals (Coles 1986). The non-significant effects of these indices relating to the RBC suggest that there was no effect on the average size of RBC and also in the hemoglobin. Similarly, the absence of observable significant effect of the extract on MCHC, MCV, and MCH among the treatment groups and all the control groups throughout the period of experiment suggests that neither the incorporation of hemoglobin into the red blood cells nor the morphology and osmotic fragility of the red blood cells were altered. This result therefore suggests that the aqueous leaf extract may not possess any potential for inducing anemia following prolonged period of administration. This result corroborates that of Yakubu et al. (2007) which reported the non-significant effects of these indices as it relates to the average size of RBC microcytes of rats infected with *T. brucei brucei*.

White blood cell differentials determine the number of each type of WBC present in the blood and how each of these WBC provides immunity against infections. This present investigation revealed elevated levels of neutrophils, whereas lymphocytes, basophils, eosinophils, and monocytes were lower in the differential counts. The elevated neutrophil count in the infected and treated rats may be indicative or suggestive of severe viral infections, tissue death, or chronic myeloid leukemia (Sternberg 2004). The lower lymphocyte, monocyte, basophil, and eosinophil counts further suggests that the animals infected with *T. brucei brucei* may have experienced autoimmune disorders, hairy cell leukemia, acute viral infection, and bone marrow damage which could be arrested by the plant extracts. This inference is supported by the observations made by Abubakar et al. (2005) who reported that high neutrophil count in animals infected with trypanosomiasis is as a result of leucocytosis which has been implicated in the wax and wear syndrome on the animals immune system caused by the ever changing variable surface glycoprotein of the infecting trypanosome. His work also corroborate a similar work by Anosa and Kaneko (1983), who reported lower counts of basophils, lymphocytes, and eosinophils which they narrowed down to lymphocytosis. In addition, Sulaiman and Adeyemi (2010) reported higher counts of neutrophils and lower counts of lymphocytes, monocytes, and basophils on *T. brucei brucei*-infected rats and suggested that these lower counts to infection may be as a result of induced immunosuppression.

Conclusion

Data presented in the present study have clearly demonstrated that the aqueous leaf extract of *L. micranthus* appeared to have no therapeutic properties in organisms infected with *T. brucei brucei*. This is more so as findings from this study showed that all infected and treated rats as well as the infected and untreated control group died from the resultant overwhelming parasitemia unlike the case of those administered the standard drug. This is an indication that the extract lacks antitryopanosomal activities.

It is therefore advocated that other methods of utilizing this important medicinal plant should be explored. The available documented evidence of African mistletoe, *L. micranthus*, being a good medicinal plant with good medicine potentials may probably not have been exhausted. Hence, more studies are advocated into the molecular constituents of its bioactive ingredients to ascertain its suitability in the management of trypanosomiasis (Monthana et al. 2000; Monthana et al. 2003).

Compliance with ethical standards

Ethical approval Handling of animals in this research was in accordance with that recommended by the Committee and the International Guidelines for Handling of Laboratory Animals (Derrell 1996).

Conflict of interest The authors declare that they have no conflicts of interest.

Informed consent Informed consent was acquired from each author participant enclosed in this study.

References

Abubakar A, Iliyasu B, Yusuf AB, Igweh AC, Onyekwelu NA, Shamaki BA, Afolayan DO, Ogbadoyi EO (2005) Antitrypanosomal and haematological effects of selected Nigerian medicinal plants in Wistar rats. Biokemistri 17:95–99

Adeiza AA, Mohammed A, Mamman M (2010) Comparative *in vivo* evaluation of the trypanocidal activities of aqueous leaf, stem-bark, and root extracts of *Khaya senegalensis* on *Trypanosoma evansi*. J Med Plant Res 4(17):1770–1777

Adeyemi OS, Akanji MA, Oguntoye SA (2009) Ethanolic leaf extract of *Psidium guajava:* Phyto-chemical and trypanocidal activity in rats infected with *Trypanosome brucei brucei*. J Med Plant Res 3(5): 420–423

Aliyu SM, Shetty SN, Asuzu IU, Chime AB (2010) Effects of some trypanosides and inflammatory agents in experimental *Trypanosoma brucei* in mice. Zariya Vet J 2:9–15

Anosa VO, Kaneko JJ (1983) Pathogenesis of *Trypanosoma brucei* infection in deer mice (Peromyscus maniculatus): hematologic, erythrocyte, biochemical and iron metabolic aspects. Am Vet Res 44(4):639–644

Aroke AH, Asonganyi T, Mbonda E (1998) Influence of a past history of Gambian sleeping sickness on physical growth, sexual maturity and academic performance of children in Fontem, Cameroon. Ann Trop Med Parasitol 92:829–835

Ashafa AO, Yakubu MT, Grierson DS, Afolayan AJ (2009) Effects of aqueous leaf extract from the leaves of *Chrysocoma ciliate* L. on some biochemical parameters of Wistar rats. Afr J Biotechnol 8:1425–1430

Bakers FJ, Silverton RE, Pallister CJ (2001) Introduction to medical laboratory technology. Bounty Press Limited, Nigeria

Barry JD, Turner CM (1992) The dynamic of antigenic variation and growth of African trypanosomes. Parasitol Today 7:207–211

Burri C, Nlimli S, Merolle A, Smith T, Brun R (2000) Efficacy of new concise schedule for melarsoprol in treatment of sleeping sickness caused by *Trypansosoma brucei gambiense:* a randomized trial. Lancet 355(9213):1419–1425

Chappuis F, Udayraj N, Stietentroth K, Meussen A, Bovier PA (2005) Eflornithine is safer than melarsoprol for the treatment of second-stage *Trypanosoma brucei gambiense* human African trypanosomiasis. Clin Infect Dis 41(5):748–751

Cheesbrough M (2005) District laboratory practice in tropical countries (Part 1). Cambridge University Press, Cambridge

Coles EH (1986) Veterinary clinical pathology, Fourth edn. W. B. Saunders Company, Philadelphia

Derrell C (1996) Guide for the care and use of laboratory animals. Institute of Laboratory Animal Resources. National Academy Press, Washington DC

Ekanem JT, Yusuf OK (2007) Some liver function indices and blood parameters in *T. brucei*-infected rats treated with honey. Biochem 19(2):81–86

Erah PO, Asonye CC, Okhamafe AO (2003) Response of *Trypanosoma brucei brucei*-induced anaemia to a commercial herbal preparation. Afr J Biotechnol 2(9):307–311

Haydon DT, Cleaveland S, Taylor LH, Laurenson MK (2002) Identifying reservoirs of infection: a conceptual and practical challenge. Emerg Infect Dis 8:1468–1473

Holmes P (2000) Programme against African trypanosomiasis. J Tsetse Tryp Info Quart 23:1–6

Igbokwe IO, Mohammed A (1992) Some plasma biochemical changes in experimental Trypanosoma brucei infection of Sokoto red goats. Rev Elev Med Vet Pays Trop 45(3–4):287–290

Jodi SM, Adamu T, Abubakar U, Abubakar MG, Chafe UM, Ukatu VE, Sani DM, Adamu S (2011) Effects of treatment with ethanol extract of *Gardenia sokotensis* on haematological and biochemical changes in *Trypanosoma brucei brucei* infected rabbits. J Med Plant Res 5(16):3839–3845

Kennedy PGE (2006) Diagnostic and neuropathogenesis issues in human African trypanosomiasis. Int J Parasitol 36:505–512

Kuzoe FAS (1993) Current situation of African trypanosomiasis. Acta Trop 54:153–162

Monthana RAA, Awadh NAA, Jensen R, Wenger U, Mentel R, Lindequest U (2003) Antiviral lanostanoid triterpenes from the fungus *Ganoderma pfeifferi BRES*. Fitoterapia 74:177–180

Monthana RAA, Jensen R, Julich WD, Lindquest U (2000) Ganomycin A and B, new antimicrobial farnesyl hydroquinones from the basidiomycete *Ganoderma pfeifferi*. BRES J Nat Pro 63:416–418

Murray CJL (1994) Quantifying the burden of disease: the technical basis for disability-adjusted life years (DALYs). Bull World Health Organ 72:429–445

Nzekwe U, Ugwuoke CEC, Uju GC (2009) Anatomical studies and preliminary phytochemical screening of the leaves of mistletoe, *Loranthus micranthus* Linn.(Loranthaceae) parasitic on *Citrus sinensis*. Int J Bot 1(1):43–48

Obasi NL, Ejikeme PM, Egbuonu ACC (2011) Antimicrobial and phytochemical activity of methanlic extracts and its fractions of jatropha curcuas Linn. (Eurphorbiaceae) stem bark. Afr J Pure Appl Chem 5(5):92–96

Obatomi DK, Aina VO, Temple VJ (1996) Effect of African mistletoe on blood pressure in spontaneously hypertensive rats. Int J Pharm 34(2):124–127

Okochi VI, Okpuzor J, Okubena MO, Awoyemi AK (2003) The influence of African herbal formula on the haematological parameters of trypanosome infected rats. Afr J Biotechnol 2(9):312–316

Osadebe PO, Ukwueze SE (2004) A comparative study of the phytochemical and anti-microbial properties of the eastern Nigerian specie of African mistletoe (*Loranthus micranthus*) sourced from different host trees. J Biol Res Biotechnol 2(1):18–23

Sam-Wobo SO, Igenezoa AJ, Idowu OA, Otesile EB, Ekpo UF, Kehinde OO (2010) Bovine trypanosomosis and its impact on cattle in derived savanna areas of Ogun State, Nigeria. J Pub Health Epidemiol 1(3):43–47

Seed JR (1998) African trypanosomiasis. Parasitol 5:267–282

Sood R (2006) Medical laboratory technology. Jaypee brothers medical publishers limited, New Delhi

Sternberg JM (2004) Human African trypanosomiasis: clinical presentation and immune response. Parasite Immunol 26:469–476

Stock W, Hoffman R (2000) White blood cells 1: non-malignant disorders. Lancet 355:1351–1357

Sulaiman FA, Adeyemi OS (2010) Changes in haematological indices and protein concentrations in *trypanosome brucei* infected rats treated with homidium chloride and diminazene aceturate. Exp Clin Sci J 9:39–45

Ukoha PO, Egbuonu ACC, Obasi NL, Ejikeme PM (2011) Tannins and other phytochemicals of the *Samanaea saman* pods and their antimicrobial activities. Afr J Pure Appl Chem 5(8):237–244

Welburn SC, Fevre EM, Coleman PG, Odiit M, Maudlin I (2001) Sleeping sickness: a tale of two diseases. Trends Parasitol 17:19–24

World Health Organization (WHO) (2000) Sleeping seekness treatment and drug resistance. J Tsetse Tryp Info Quart 3:4–9

World Health Organization (2008) Global Burden of Disease (http://www.who.int/healthinfo/globalburdendisease/en/index.html) . Accessed on 20th June 2012

World Health Organization (2006) Human African trypanosomiasis (sleeping sickness): epidemiological update. Weekly Epidemiol Rec 81:71–80

Yakubu DP, Dawet A, Olaleye NA (2014) Effective of vitamin E and selenium of some blood parameters of *Trypanosoma brucei brucei* rats. Br J App Sci Technol 4(7):1100–1108

Yakubu MT, Akanji MA, Oladiji AT (2007) Haematological evaluation in male albino rats following chronic administration of aqueous extract of *Fadogia agrestis* stem. Pharmacogn Mag 3:34–43

Yusuf OS, Oseni BS, Olayanju AO, Hassan MA, Ademosun AA, Akele RY (2013) Acute and chronic effects of *trypanosome brucei brucei* experimental infection on bone marrow and peripheral blood cells in wistar rats. Sch J Appl Med Sci 1(6):1036–1040