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ORIGINAL ARTICLE

The Effect of Crude Ethanolic Leaf Extract of *Guiera senegalensis* J. F. gmel (combretaceae) on the Haematological Parameters of Albino Rats Experimentally infected with *Trypanasoma brucei brucei*

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SUMMARY

The effect of *Guiera senegalensis* leaf extract was investigated on the blood parameters of albino rats infected with *Trypanasoma brucei brucei*. Thirty (30) albino rats were randomly divided into six groups (A-F), of five rats each. Rats in groups (A-E) were individually infected with 4×10^6 of *Trypanasoma brucei brucei*, while those in group F remained uninfected control. The infected rats were later treated for four consecutive days starting on day 4 post infection. Groups A, B and C were treated orally with 100, 300 and 600 mg/kg of the extract of *Guiera senegalensis* respectively, while group D was treated with *Diminazine aceturate* at 3.5 mg/kg once. Rats in groups E and F were not treated during the experiment. Rats treated with the extract at 600 mg/kg survived up to day 12 post infection. Haematological parameters (Packed cell volume (PCV), Haemoglobin concentration (Hb), Red blood cell count (RBC) and White blood cell count (WBC) decreased significantly ($p<0.05$) across the days in the extract treated groups compared with the *Diminazine aceturate* treated (group D) and the uninfected control (group F). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) varied significantly ($p<0.05$) in an irregular pattern in the treated groups. Neutrophil, lymphocyte and monocyte decreased significantly ($p<0.05$) in the extract treated groups. However, eosinophil count increased significantly ($p<0.05$) in the *Diminazine aceturate* treated group and the uninfected groups. The extract at the highest dosage tested (600 mg/kg) prolonged the survival of the rats, but did not significantly ($p<0.05$) improve the haematological parameters investigated. Higher dosages are recommended for further studies.

Key words: Haematology parameters, *Guiera senegalensis*, Extract, *Trypanosoma brucei brucei*.

INTRODUCTION

Trypanosoma congolense, T. vivax and *T. brucei* are the major pathogenic tsetse transmitted trypanosome species responsible for the disease trypanosomosis in tropical regions of Africa, where the vector is prevalent (Nantulya, 1990). The disease is one of the major haemoparasitic diseases of domestic animals characterised by severe anaemia, weight loss, reduced productivity, infertility and abortion, with death occurring in some animals during the acute phase of the disease. The trypanosomosis caused by *Trypanosoma brucei* species is fatal in both humans and animals and cannot be combated by vaccination because of extensive parasite antigenic variation (Taylor, 2006), thus resulting in over reliance on other control measures such as chemotherapy, with little or no attention to methods like vector control especially in developing countries including Nigeria. A major setback to the use of chemotherapy for control is the associated drug-induced toxicity and the potential for development of resistance (Adeyemi *et al.,* 2012; Abimbola *et al.,* 2013), hence the need to explore alternative sources including plant-derived compounds.

The haematological changes observed in trypanosomosis drastically influence the pathogenesis of the disease (Kagira *et al.,* 2006), and is associated with a rapid decline in red blood cell (RBC) counts, haemoglobin (Hb) concentration and packed cell volume (PCV) and pallor of mucous membranes in the infected hosts, confirming that anaemia is a critical feature in the pathogenesis of African trypanosomosis. Haematological parameters provide an excellent basis for judgment with respect to the nature of disease, extent of tissue and organ damage, response of defence mechanism of the patient, diagnosis of possible type of anaemia and as an index to characterize health status of animals (Albers *et al.,* 1990).

Among plants commonly used traditionally for the treatment of a variety of ailments particularly among the Fulani pastoralists of northern Nigeria, is *Guiera senegalensis* J. F. Gmel. It is a shrub that grows to about 3 m high and is commonly called 'Sabara' by the Hausas and 'geloki' by the Fulanis. The leaf has a bitter taste and is widely acknowledged as a 'cure-all' medicine (Akuodor *et al.,* 2013). Extracts of the leaves have been reputed to possess anthelmintic effect on *Haemonchus contortus* (Traore *et al.,* 2014) and was previously investigated *in vitro* against *Trypanasoma congolense* (Atawodi *et al.,* 2003), and *in-vivo* against *Trypanosoma brucei brucei*- induced parasitaemia in mice (Aderbauer *et al.,* 2008). These findings coupled with, the recent report by Maikai *et al.* (2010), which showed that the plant; *Guiera senegalensis* is traditionally been used for the treatment of trypanosomosis by livestock owners and herdsmen in some local government areas of Kaduna State, necessitated our quest to investigate this claim in a laboratory model (Wistar rat). The study therefore aimed to evaluate the effect of treatment with graded doses of ethanolic extract of the leaves of *Guiera senegalensis* on *Trypanosoma brucei brucei* induced haematological changes in albino rats.

MATERIALS AND METHODS

Authentication and Preparation of Plant Material

The leaves and leaf powder of *Guiera senegalensis* were obtained in April 2014 from traditional medicine market, opposite NEPA house Maiduguri, Borno state, Nigeria. A botanist at the Department of Biological Sciences, University of Maiduguri, authenticated the plant and voucher specimen (Vet 220 A4) was deposited at the herbarium of the Department of Veterinary Physiology, Pharmacology and Biochemistry of the

Faculty of Veterinary Medicine, University of Maiduguri. Five hundred (500) grams of the powder was exhaustively extracted using soxhlet extractor and condenser utilizing absolute (l00%) ethanol as a solvent at the Department of Chemistry, University of Maiduguri, according to standard method (WHO, 1992). The extract was concentrated *in-vacuo* to remove traces of ethanol. The concentration of the extract was determined and the stock solution was stored in a refrigerator until used.

Determination of phytoconstituents

The ethanolic extract of the leaf of *Guiera senegalensis* was subjected to qualitative chemical screening for the identification of the various classes of the chemical constituents. These analyses were carried out according to the method described by Sofowora (2006).

Acute toxicity testing and safe dose determination

Forty adult Wistar rats of both sexes were randomly separated into eight groups (1-8) of five each. Groups 1-7 were respectively treated intraperitoneally with graded doses (100, 200, 400, 800, 1600, 3200 and 6400 mg/kg) of the ethanolic leaf extract of *Guiera senegalensis,* reconstituted in normal saline, while group 8 (control group) was given only normal saline equivalent to the highest volume of the extract administered. The rats were observed over a period of 24 hours for the development of symptoms of toxicity. The arithmetic method of Karber as modified by Aliu and Nwude (1982) was used to calculate the LD_{50} of the extract.

Experimental Animals

A total of thirty (30) adult Wistar albino rats of both sexes and weighing between 90.4 g and 260.6 g were used for the study. They were obtained from the Animal Breeding Centre, Department of Biochemistry University of Maiduguri, Nigeria and maintained in clean plastic rat cages at the Postgraduate Laboratory, Department of Veterinary Microbiology and Parasitology, University of Maiduguri, Nigeria. Pelleted feed (Vital feeds Nigeria Plc., Jos, Nigeria) and water were provided *ad-libitum*. A fourteen days acclimatization period was observed before the commencement of the experiment. The experiment was approved by Animal Welfare Committee of the Faculty of Veterinary medicine, University of Maiduguri, Nigeria, and carried out according to International guiding principles for biochemical research involving animals (C. I .O. M .S .1985).

Source of Trypanosomes

Trypanosoma brucei brucei (Federe strain) used for the study was obtained from donor rats maintained at the postgraduate laboratory of the Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary medicine, University of Maiduguri. The parasite was earlier sourced from the stabilate maintained at the Nigerian Institute of Trypanosomosis and Onchocerciasis Research, (NITOR), Vom, Nigeria from a previous experiment. It was maintained by serial passages in rats, which produced acute and sub-acute infections with a pre-patent period of 2-3 days.

Experimental Design

After period of acclimatization, the thirty adult Wistar albino rats were randomly divided into six groups (A-F) of five rats each. Rats in groups (A-E) were individually inoculated, intraperitoneally with 0.5 ml of glucose saline diluted with blood containing approximately $4x10^6$ *Trypanosoma brucei brucei* from donor rats, while those in group F remained uninfected control. The number of parasites in the diluted blood was estimated as previously described (Herbert and Lumsden, 1976). Infected rats were

monitored for parasitaemia two days post-inoculation and later on day 3 post-infection. Following establishment of parasitaemia, groups (A-C), were treated with graded doses of the extract of 100 mg/kg, 300 mg/kg, and 600 mg/kg orally, respectively for 4 days, while group D, was treated with *Diminazine aceturate* at a single dose of 3.5 mg/kg intramuscularly. Group E remains infected untreated, while group F is the uninfected untreated. All treatments commenced on day 3 post-infection.

Collection of Blood Sample and Determination of Haematological Parameters

Blood for the determination of haematological parameters was obtained from the tail veins of experimental animals on day 0 and every 4 days until day 20 post-infection when the experiment was terminated. Haematological parameters (Packed cell volume (PCV), Red blood cell count (RBC), Haemoglobin concentration (Hb), White blood cell count (WBC), Differential leucocyte count (DLC)) were determined as described by Schalm *et al.* (1995).

Statistical Analysis

Data generated were expressed as mean ± standard deviation (S.D.) using one-way analysis of variance (ANOVA). Duncan post-hoc test was used to compare within and between groups and $p<0.05$ was considered significant throughout the study using SPSS version 16, (2011).

RESULTS

The yield of the extract was 95.05% w/w, dark green in colour and soluble in water.

Table I: Phytochemical screening of the ethanolic extract of the leaf of *Guiera senegalensis J. F. Gmel (Combretaceae)*

Phytochemical constituents	Tests	Inferences
Carbohydrate	Molisch's	+
	Ketoses	-
	Pentoses	-
	Fehling's	+
	Combined reducing sugars	+
	Soluble starch	-
Tannins	Ferric chloride	+
	Lead acetate test	+
Phlobannins	Hydrochloride acid test	-
Anthraquinones	Free anthraquinones	-
	Combined anthraquinones	-
Cardiac glycosides	Salkowski	+
	Liebermann-Buchard test	+
Terpenoids	Terpenoids	+
Saponin glycosides	Frothing	+
Flavonoids	Shinoda's test	+
	Ferric chloride	+
	Lead aceatete	+
	Sodium hydroxide	-
Alkaloids	Dragendorffs reagent	-
	Mayers test	-

Keys**:** (+) = Present in low concentration, (-) = Absent

Table II: Intraperitoneal LD_{50} of the ethanolic leaf extract of *Guiera senegalensis* in albino rats

Groups (n=5)	Dose	Dose difference(DD)	Dead(D)	Mean Dead (MD)	Mean Dead × Dose difference
1	100	-	0	-	-
2	200	100	1	0.5	50
3	400	200	0	0.5	100
4	800	400	2	1.0	400
5	1600	800	2	2.0	1600
6	3200	1600	4	3.0	4800
7	6400	3200	5	4.5	14400
8	D/H_{20}	-	0	-	-

LD_{50}= Least dose that killed all animals in a group – $\frac{(DD \times MD)}{\text{No. animals/group}}$

$LD_{50} = 6400 - \frac{21350}{5}$

$LD_{50} = 6400 - 4270$

$LD_{50} = 2130$ mg/kg

Phytoconstituents found include; reducing sugars, tannins, cardiac glycosides, terpenoids, flavonoids, saponin glycosides and carbohydrates (Table I).

Mortality pattern of experimental rats

The pattern of mortality of experimental rats during the course of the experiment is presented in Figure I .Two (2) rats died each in groups A, B and E on day 4 post infection, while only one death occurred in group C on the same day. Mortality increased to three (3) on day 5 post infection in group B, while one (1) rat each died in the remaining groups except D and F, where no mortality occurred throughout the experiment. The remaining rats in the extract treated groups (A, B and C) and the infected untreated (E) group died by day 13 post infection (Figure 1).

Effect on haematological parameters (PCV, Hb, RBC and WBC)

The results of the effect of administration of graded concentration of the ethanolic leaf extract of *Guiera senegalensis* on haematological parameters (PCV, Hb and RBC) are presented in Table III. There was a transient decrease in the mean PCV, Hb and RBC on day 4 post infection in all the infected groups. The trend continued, resulting in a significant decrease ($p=0.03$) on days 8 and 12 post infection in all the extract treated groups (A, B and C). However, values for groups D and F did not show any variation throughout the experiment. The pattern of change for WBC is similar to other parameters, where a transient decrease was observed in all the infected groups on day 4 post infection (Table IV).

Neutrophil, Lymphocyte, Monocyte and Basophil

A significant ($p<0.05$) decline was observed in neutrophil count on day 4 post infection in all the groups except A and E (Table V). The decline continued up to day 12 for the extracted treated, while group D remained stable up to day 20 post infection. A significant ($p<0.045$), but irregular pattern of change was observed in group F. Similar trend was observed in lymphocyte count except that there was no statistical significant variation between values of day 0 and 4 (Table V).

Monocyte count showed an irregular pattern that was statistically significant ($p<0.05$) in

Table III: Effect of ethanolic leaf extract of *Guiera senegalensis* on mean (±SD) packed cell volume (L/L), haemoglobin concentration (g/L) and red blood cell count ($\times 10^{12}$/L) of treated and untreated albino rats infected with *Trypanosoma brucei brucei* and their control

Groups/Treatments	Days Post-infection					
	0	4	8	12	16	20
PCV						
Group A	0.50± 0.36^{a}	0.47±0.41^{a}	0.35±0.01^{b}	-	-	-
Group B	0.52± 0.03^{a}	0.50± 0.38^{a}	-	-	-	-
Group C	0.44±0.06^{a}	0.32±0.02^{b}	0.22±0.00^{c}			
Group D	0.46±0.51^{a}	0.43±0.02^{a}	0.45±0.02^{a}	0.45±0.00^{a}	0.47±0.01^{a}	0.46±0.02^{a}
Group E	0.46±0.48^{a}	0.44±0.32^{a}	-	-	-	-
Group F	0.47±0.01^{a}	0.48±0.01^{a}	0.48±0.01^{a}	0.47±0.02^{a}	0.49±0.01^{a}	0.47±0.01^{a}
Hb						
Group A	148.00± 7.64^{a}	142.40±9.52^{a}	88.50±6.36^{b}	-	-	-
Group B	145.40± 5.89^{a}	143.40± 5.27^{a}	-	-	-	-
Group C	139.20±11.21^{a}	134.80±11.21^{b}	82.00±2.82^{c}	61.00±1.41^{d}		
Group D	139.80±11.45^{a}	135.00±9.69^{a}	136.80±9.31^{a}	142.00±3.53^{a}	141.60±4.72^{a}	136.00±6.96^{a}
Group E	129.20±16.36^{a}	123.40±14.58^{b}	-	-	-	-
Group F	139.20±10.35^{a}	139.20±9.03^{a}	146.20±1.30^{b}	141.20±5.16^{a}	145.40±1.51^{ab}	140.40±5.12^{a}
RBC						
Group A	8.82± 0.48^{a}	8.64±0.50^{a}	5.10±0.64^{b}	-	-	-
Group B	8.80± 0.72^{a}	8.75± 0.72^{a}	-	-	-	-
Group C	8.31±0.93^{a}	8.21±1.03^{a}	5.49±0.80^{b}	3.65±0.21^{c}		
Group D	8.36±0.34^{a}	8.31±0.16^{a}	8.52±0.40^{a}	7.89±0.61^{b}	8.50±0.80^{a}	8.17±0.34^{a}
Group E	8.16±0.83^{a}	7.91±0.86^{a}				
Group F	8.06±0.90^{a}	8.08±0.83^{a}	8.42±0.83^{a}	8.31±0.75^{a}	8.64±0.30^{a}	7.98±0.56^{a}

Keys: Group A: extract 100 mg, Group B: extract 300 mg, Group C: extract 600 mg, Group D: Berenil® 3.5 mg, Group E: infected/untreated and Group F: uninfected control. Different superscripts in rows differ significantly ($P<0.05$)

Table IV: Effect of ethanolic leaf extract of *Guiera senegalensis* on mean (±SD) white blood cell (WBC) count ($\times 10^9$/L) of treated and untreated albino rats infected with *Trypanosoma brucei brucei* and their control

Groups/Treatments	Days Post-infection					
	0	4	8	12	16	20
WBC						
Group A	11.14± 1.46^a	11.04±1.26^a	6.75±1.06^b	-	-	-
Group B	11.26± 1.07^a	10.96± 1.16^a	-	-	-	-
Group C	12.04±0.36^a	11.72±0.35^a	6.50±0.28^b	5.10±0.421^c		
Group D	9.28±1.34^a	9.58±0.81^b	9.98±0.75^b	9.38±0.23^a	9.66±0.59^b	10.16±1.31^b
Group E	9.62±1.27^a	9.28±1.11^a	-	-	-	-
Group F	11.38±1.02^a	11.78±0.60^b	12.26±0.26^b	12.12±0.13^b	12.12±0.13^b	11.04±1.21^a

Keys: Group A: extract 100 mg, Group B: extract 300 mg, Group C: extract 600 mg, Group D: Berenil® 3.5 mg, Group E: infected/untreated and Group F: uninfected control. Different superscripts in rows differ significantly (P<0.05)

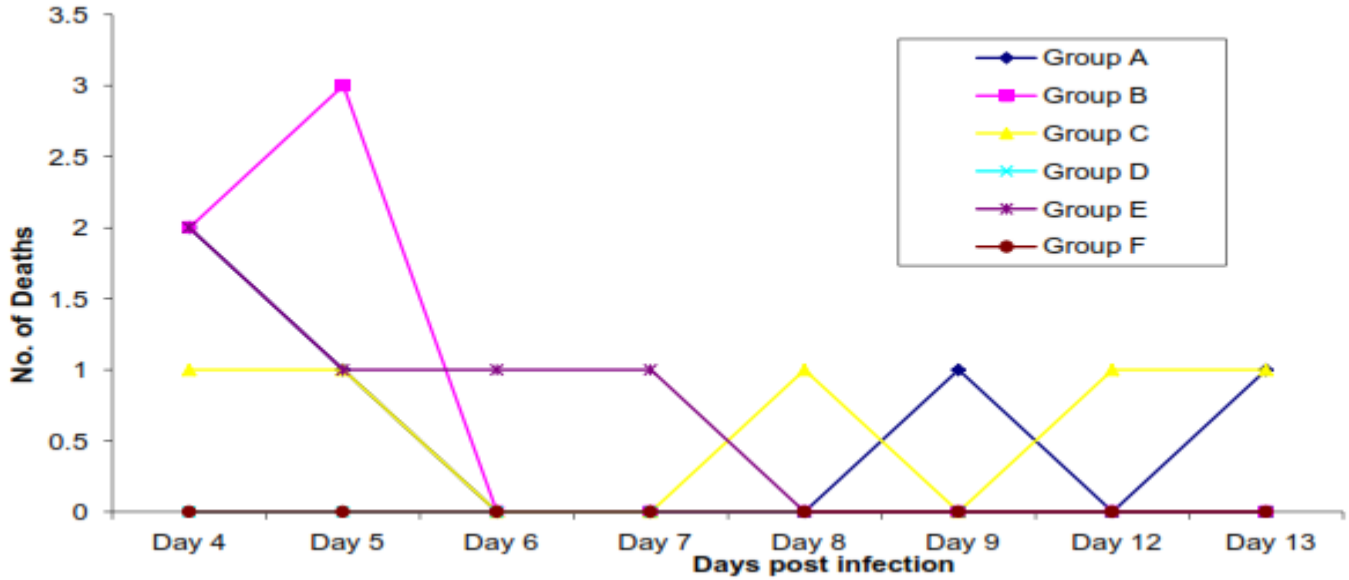

Figure 1: Mortality pattern of rats infected with *Trypanosoma brucei brucei* and treated with extract, diminazine aceturate and their control

Table V: Effect of ethanolic leaf extract of *Guiera senegalensis* on mean (±SD) neutrophil count ($\times 10^9$/L), lymphocyte count ($\times 10^9$/L), monocyte count ($\times 10^9$/L) and eosinophil count ($\times 10^9$/L) of treated and untreated albino rats infected with *Trypanosoma brucei brucei* and their control

Groups/Treatments	Days Post-infection					
	0	4	8	12	16	20
Neutrophil						
Group A	3.40± 0.95^a	2.98±0.96^a	2.55±0.91^b	-	-	-
Group B	3.64± 0.77^a	3.04± 0.63^b	-	-	-	-
Group C	2.68±0.80^a	2.74±0.46^b	1.80±0.42^c	1.65±0.21^c	-	-
Group D	2.08±0.39^a	1.76±0.30^b	2.20±0.33^a	2.10±0.21^a	2.16±0.23^a	2.12±0.32^a
Group E	1.90±0.47^a	1.54±0.20^a	-	-	-	-
Group F	2.20±0.91^a	1.86±0.52^b	2.62±0.34^c	2.98±0.24^d	2.58±0.30^c	2.30±0.49^a
Lymphocyte						
Group A	6.68± 0.82^a	6.80±0.96^a	3.10±0.00^b			
Group B	6.66± 0.85^a	6.66±1.03^a				
Group C	7.86±0.77^a	7.40±0.64^b	3.80±0.42^c	2.50±0.56^d		
Group D	6.02±1.18^a	6.46±0.64^b	6.54±0.46^b	6.26±0.40^a	6.50±0.53^a	7.14±1.05^b
Group E	6.84±1.33^a	6.60±0.94^a				
Group F	7.86±0.87^a	8.06±0.48^b	8.44±0.33^c	7.84±0.37^a	8.44±0.38^b	7.64±1.32^c
Monocyte						
Group A	0.46± 0.11^a	0.45±0.11^a	0.30±0.14^b			
Group B	0.38± 0.13^a	0.42±0.08^a				
Group C	0.80±0.15^a	0.68±0.21^b	0.25±0.07^c	0.25±0.07^c		
Group D	0.50±0.10^a	0.48±0.13^a	0.56±0.24^b	0.46±0.19^a	0.46±0.11^a	0.40±0.01^c
Group E	0.40±0.17^a	0.54±0.11^b	-	-	-	-
Group F	0.60±0.70^a	0.77±0.11^b	0.42±0.8^c	0.60±0.21^a	0.50±0.07^d	0.44±0.19^c
Eosinophil						
Group A	0.60± 0.22^a	0.83±0.20^b	0.80±0.00^b	-	-	-
Group B	0.58± 0.19^a	0.78±0.14^b	-	-	-	-
Group C	0.70±0.10^a	0.88±0.13^b	0.65±0.35^a	0.70±0.14^a	-	-
Group D	0.66±0.15^a	0.88±0.25^b	0.68±0.17^a	0.56±0.11^c	0.54±0.081^d	0.50±0.12^d
Group E	0.48±0.17^a	0.64±0.20^b				
Group F	0.82±0.22^a	0.88±0.17^a	0.78±0.13^b	0.70±0.21^c	0.60±0.07^d	0.66±0.01^d

Keys: Group A: extract 100 mg, Group B: extract 300 mg, Group C: extract 600 mg, Group D: Berenil® 3.5 mg, Group E: infected/untreated and Group F: uninfected control. Different superscripts in rows differ significantly ($P<0.05$)

Table VI: Effect of ethanolic leaf extract of *Guiera senegalensis* on mean (±SD) mean corpuscular volume (MCV) (fL), mean corpuscular haemoglobin (MCH) (pg) and mean corpuscular haemoglobin concentration (MCHC) (g/L) of treated and untreated albino rats infected with *Trypanosoma brucei brucei* and their control

Groups/Treatments	Days Post-infection					
	0	4	8	12	16	20
MCV						
Group A	57.09± 2.73^{a}	54.99±2.69^{a}	69.28±11.49^{b}	-	-	-
Group B	59.68± 4.54^{a}	58.19± 5.09^{a}	-	-	-	-
Group C	58.11±5.77^{a}	54.56±4.37^{a}	59.55±4.87^{b}	61.69±1.651^{b}	-	-
Group D	54.73±1.92^{a}	53.39±2.60^{b}	53.67±1.96^{b}	54.85±0.78^{a}	55.18±1.43^{a}	59.22±3.72^{c}
Group E	57.45±4.19^{a}	56.19±5.27^{a}	-	-	-	-
Group F	59.12±4.01^{a}	59.74±4.10^{a}	57.98±4.16^{b}	57.52±3.91^{b}	56.89±1.02^{b}	59.48±2.57^{a}
MCH						
Group A	16.78± 0.25^{a}	16.44±0.22^{a}	17.39±0.94^{b}	-	-	-
Group B	16.60± 1.62^{a}	16.45± 1.48^{a}	-	-	-	-
Group C	16.82±1.50^{a}	16.52±1.57^{a}	15.06±1.69^{b}	16.72±0.58^{a}	-	-
Group D	16.47±1.22^{a}	16.53±1.23^{a}	16.32±1.10^{a}	17.08±0.24^{b}	16.63±0.72^{a}	17.28±1.09^{b}
Group E	15.81±0.97^{a}	15.61±1.06^{a}	-	-	-	-
Group F	17.32±1.05^{a}	17.28±0.79^{a}	17.47±1.61^{a}	17.06±0.99^{a}	16.82±0.57^{b}	17.62±0.84^{a}
MCHC						
Group A	292.80± 20.16^{a}	299.80±16.69^{a}	253.50±28.99^{b}	-	-	-
Group B	278.20± 17.29^{a}	283.00± 20.39^{b}	-	-	-	-
Group C	290.00±12.78^{a}	303.40±24.04^{b}	252.50±7.77^{c}	271.00±2.82^{c}	-	-
Group D	300.00±16.37^{a}	309.20±9.09^{b}	302.20±8.49^{a}	311.20±7.56^{b}	301.20±10.70^{a}	291.60±4.39^{c}
Group E	275.60±15.27^{a}	278.60±16.74^{a}	-	-	-	-
Group F	293.40±12.40^{a}	289.80±10.73^{a}	300.80±6.72^{b}	296.60±12.70^{a}	295.12±12.52^{a}	295.80±12.08^{a}

Keys: Group A: extract 100 mg, Group B: extract 300 mg, Group C: extract 600 mg, Group D: Berenil® 3.5 mg, Group E: infected/untreated and Group F: uninfected control. Different superscripts in rows differ significantly ($P<0.05$)

groups C, E and F on day 4, when compared to day 0. However, changes in other groups for the same period were not significant. Furthermore, in group C, the decline continued up to day 8 and remained stable to day 12 post infection. Group D declined throughout the experimental period, while group F showed an irregular pattern of change ($p<0.05$).

A significant increase ($p=0.05$) was observed in eosinophil count of all infected groups on day 4 post infection when compared to their respective day 0 (Table V). This was followed by a significant ($p<0.05$) decline in all the extract treated groups (A, B and C) on day 8 and relative stability in group (C) on day 12 post infection. Group D varied significantly ($p<0.05$) across all days, while group F showed irregular, but significant changes ($p<0.05$) throughout the experimental period.

Effect on haematological indices

The effect of the ethanolic extract of *Guiera senegalensis* on haematological indices (MCV, MCH and MCHC) of infected and uninfected rats is presented in Table VI. There was no statistical significant difference in MCV values of days 0 and 4 in all the groups. However, all the extract treated groups (A, B and C) increased significantly ($p<0.05$) on days 8 and 12, when compared to groups D and F. Similar trend was observed in MCH values of groups A and B. The value of MCH in group C decreased significantly ($p<0.05$) by day 8 post infection and later increased by day 12 ($p<0.05$). Group D showed an irregular pattern throughout the experiment.

The MCHC count increased transiently by day 4 in all the infected groups and was followed by a significant ($p<0.05$) decline in groups A and C by day 8 and a further rise ($p<0.05$) by day 12 in group C. Groups D and F showed significant ($p<0.05$), but irregular pattern throughout the experimental period.

DISCUSSION

Recently, plant based studies have received increased attention as evidenced by the volume of information in literatures. This may not be unconnected with the quest to determine toxicity and establish scientific basis for the widespread use of such plants and plants products for both human and animal medicare, especially in developing nations like Nigeria. Screening of the ethanolic extract of the plant, *Guiera senegalensis* revealed the presence of carbohydrate, tannins, cardiac glycosides, terpenoids, saponin glycosides and flavonoids in low concentration. Important secondary metabolites such as alkaloids earlier reported in the root (Shettima *et al.,* 2012) and leaf (Jigam *et al.,* 2011) were not detected in this study. The absence of important metabolites or presence in low concentration may be responsible for the inability of the rats in the extract treated groups to survive beyond day 12 post infection, compared to the *Diminazine aceturate* treated and uninfected groups. Previously, Aderbauer *et al*. (2008) reported 42% reduction in parasitaemia, when *Trypanasoma brucei* infected rats were treated with lipophilic extract of *Guiera senegalensis*, intraperitoneally for three days. This variation observed may be due to geographical site of collection as well as the route of administration of the extract. Orally administered drugs and compounds do undergo some events that potentially decrease the amount reaching systemic circulation for pharmacological effects (Brander *et al.,* 1991), thus producing limited effect, than when administered by intraperitoneal route.

The administration of the ethanolic extract of this plant did not improve the haematological parameters (PCV, Hb, RBC and WBC) of the infected rats post

administration. These parameters decreased significantly ($p<0.05$) across the days in the extract treated and the infected untreated groups. Alam *et al*. (2011) and Kobo *et al*. (2014) reported similar findings in *Trypanasoma brucei brucei* infected rats and gilts respectively. This could be due to either transformation of the active compounds in the extract while passing through the oral route (Brander *et al.*, 1991), thus limiting the amount available for therapeutic action on reaching target sites or due to low quantity of important metabolites in the extract. The mechanism of anaemia in trypanosomosis has been greatly associated with the generation of free radicals and super oxides following lipid peroxidation, thereby resulting in attack on cellular integrity of erythrocytes during trypanosomosis (Ogunsami and Taiwo, 2001; Umar *et al.,* 2007). Furthermore, metabolites such as flavonoids in addition to free radical scavenging effects, may regenerate other antioxidants with known immune-enhancing activity such as vitamin E (Tkayama *et al.*, 1984; Zhu *et al.*, 2000), while saponins and alkaloids have been known to possess potent immune-stimulating and anti-inflammatory effects respectively (Igile, 1995).The absence or presence in low concentrations of these metabolites, in addition to administration of the extract through the oral route may be responsible for the inability of the extract to significantly improve the haematological parameters at the investigated dosages.

From our study, leucocytes counts (neutrophil, lymphocyte and monocyte) decreased significantly ($p<0.05$) across the days, which is consistent with previous reports (Anosa, 1988; Igbokwe *et al.*, 1994).This may be explained by the acute nature of trypanosomosis and the stimulation of the immune system. In addition, sequestration of these cells as seen in acute infection may be a possible explanation. Contrarily, eosinophil count increased significantly ($p<0.05$) in all the extract treated and the infected untreated groups. This may be due to inflammatory response elicited by the presence of the parasite, as eosinophil has been known to be a potent inflammatory cell (Sanderson, 1992). The inability of parameters in the extract treated groups to revert to preinfection value, or at least improve significantly during the course of the experiment, is a further justification for absence or low presence of potent metabolites in the extract used.

The earlier transient fall in MCV and MCH by day 4 post infection, which was followed by a significant increase across the days, in the extract treated and infected untreated groups, agrees with the earlier result of Osuagwuh and Okore (2014), in goats and Kagira *et al.* (2006), in vervet monkeys, both experimentally infected with *Trypanasoma spp*. This however, contrasts the report of Kobo *et al.* (2014), where these parameters were observed to increase. These indices are used to classify type of anaemia (Neiger *et al.*, 2002). The macrocytic anaemia observed may be due to haemolysis of red blood cells, thereby resulting in high reticulocyte count.

Conclusively, the extract at the highest dosage tested (600 mg/kg) prolonged the survival of the rats, but did not improve the haematological parameters investigated. We recommend further studies using other routes of administration and higher dosage of the extract, since the LD_{50} was found to be 2130 mg/kg .

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