

RESEARCH ARTICLE

Crude methanolic extract of *Moringa oleifera* leaves improves the efficacy of Diminazene Aceturate in the treatment of Trypanosome infected rats

Aremu Abdulfatai^{1,*}, K.I. Eghianruwa², K.T. Biobaku¹, A.O.Ahmed³ and A. Basiru⁴

¹Department of Veterinary Pharmacology and Toxicology, University of Ilorin, Nigeria

²Department of Veterinary Physiology, Biochemistry and Pharmacology University of Ibadan, Nigeria

³Department of Veterinary Microbiology, University of Ilorin, Nigeria

⁴Department of Veterinary Physiology and Biochemistry, University of Ilorin, Nigeria

Received:05/06/2017; Accepted:29/08/2017

Abstract: A trypanocidal efficacy study of *Moringa oleifera* leaf supplement alone and in combination with diminazene aceturate on Wistar rats was conducted using *Trypanosoma brucei*. Thirty five rats were randomly allotted into seven groups (A-G) with include five rats each. Groups B and C were treated with methanolic extract of *M. oleifera* leaves at 200mg/kg for 14 days prior to infection. The E and F were treated with *M. oleifera* for 90 days at 200mg/kg prior to infection. Group A and G are negative and positive controls, respectively. All rats in groups, B - G were individually infected with 3×10^6 of *Trypanosoma brucei* per ml of blood and the prepatent period was monitored from the second day. At day five post infection, infected rats in group C, D and F were treated with diminazene aceturate at 7mg/kg while those in group B and E treated with *M. oleifera* leaf extract. Parasites were cleared within 72 hours post treatment from blood of rats in groups C and F which were treated with both *M. aoleifera* and diminazine aceturate. However, it took 96 hours post treatment for parasites to be cleared from rats in group D which was treated only with diminazene aceturate. There were significant ($P < 0.05$) increase in the erythrocyte count, haematocrit and mean corpuscular volume (MCV) in *M. oleifera* treated groups. Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) decreased significantly ($P < 0.05$) in groups E, F and G. There was neutropenia in groups E, F and G when compared with the negative control, while other leucocytes manifested leukocytosis. Serum chemistry showed that all groups had significantly increased ($P < 0.05$) globulin, blood urea nitrogen (BUN) and liver enzymes when compared with the negative control group "A". Histopathological findings showed that congestion in the spleen and lymph nodes were reduced in the treated groups when compared to the untreated groups. Conclusively, our study gives credence that *M. oleifera* does not possess trypanocidal effect but however could be co-administered as a supportive.

Keywords: *M. oleifera*, Diminazene aceturate, *Trypanosoma brucei*, parasitaemia and wister rats.

INTRODUCTION

African trypanosomiasis a major factor that has devastated the livestock industry in about 10 million km² in the Southern part of the equator of tropical Africa where trypanosomiasis is an endemic, which left the health of most affected societies precarious and economically improvised (Griffin and Allanby, 1999).

Animal African *Trypanosomiasis* is caused by different species of trypanosomes; *Trypanosoma evansi*, *T. brucei*, *T. congolense* and *T. simiae*. The disease hinders livestock production in many parts of Africa (Freiburghaus *et al.*, 1998). Resistance of the disease to the limited available trypanocides in the fields, clinic and other places are on the rise (Shaba *et al.*, 2006). *Trypanosoma congolense*, *T. vivax* and *T. brucei* are among the commonest pathogenic tsetse transmitted trypanosome species which are the causative agents to the animal trypanosomiasis in tropical regions of Africa, where the vector is prevalent (Nantulya, 1990).

The disease is one of the major haemoparasitic diseases in Sub-Saharan Africa in domestic animals which is characterized by severe anaemia, weight loss, reduced productivity, infertility and abortion, even death occurring in some animals during the acute phase of the disease (Adeyemi *et al.*, 2012). Chemotherapy and chemoprophylaxis still remain the main methods of controlling trypanosomiasis (Dolan *et al.*, 1996). These control measures are still the most reliable in spite of drug resistivity and toxicity (Reiter *et al.*,

*Corresponding Author's Email: aremu.a@unilorin.edu.ng

2011) since the development of an effective vaccine against the disease has been unsuccessful due to variant antigenic property of the parasites (Kinabo and Bogan, 1998). Diminazene aceturate however, is one of the few drugs available for both treatment and prophylaxis of African trypanosomosis in livestock especially in cattle infected with *T. congolense*, *T. vivax* and *T. brucei* (Eghianruwa *et al.*, 2004).

Conventional chemotherapeutic treatment of trypanosomosis is no longer befitting as a result of side effects associated with the drugs and the development of many resistance trypanosome in many part of the world. Research now focuses on finding new compounds for treating trypanosome infection (Kibona *et al.*, 2002). Due to the aforementioned reasons and implications this study is aimed at assessing the effect of *M. oleifera* on the efficacy of a conventional trypanocidediminazene aceturate.

MATERIALS AND METHODS

Drug

Diminazeneaceturate (Kepro[®] Veterinary Company, Holland) at 7mg/kg was used, and was administered in the quadriceps using 23 gauge syringe and needle.

Plant Authentication and Preparation

The leaves of *M. oleifera* were procured at Ajibode, Akinyele Local Government area of Oyo State, located in the South Western part of Nigeria, which is about 2 Km from the University of Ibadan, Ibadan, Nigeria. It was taxonomically identified and authenticated in the herbarium of the Department of the Forest Research Institute of Nigeria (FRIN), with voucher number UIH-10847. The leaves of *M.oleifera* were washed thoroughly, dried in shade and pulverized to powder. The methanolic extraction was done at the Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Science, University of Ibadan using the method of Adeyemi (2000). The concentration of the extract in the final preparation was 7.5 % of the stock solution, and it was diluted in soya oil as a vehicle. The percentage yield of the extract was also obtained using a formulae.

M. oleifera Phytochemical Screening

Phytochemical screening of methanol extract of *M. oleifera* leaves was done by qualitative chemical screening for identification of the various classes of the chemical constituents present, as previously described by Sofowora (2006).

Ethics, Experimental Animals and Study Design

Ethical approval was sought from ACUREC, University of Ibadan, Nigeria with approval code number, UI-ACUREC/APP/2016/011. Thirty five adult male and female Wistar rats between the ages of 8-12 weeks, weighing 90-105g were used for this study. The rats were housed in the animal house of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria. The animals were kept in cages under normal environmental temperature (20-22°C) and fed with standard pelleted feed (Livestock[®]Lagos, Nigeria) and water given *ad libitum*. The rats were allowed to acclimatize to the laboratory environment for two weeks before the experiment commenced. Rats were grouped into seven with five rats each and the extracts were administered orally using an oral cannula.

The experimental dosages were as follows: Group A (n = 5 rats): Control and the rats were uninfected and untreated. Group B: Dosed prophylactically with *M. oleifera* at 200 mg/kg daily for 14 days before infection and *M. oleifera* was used for treatment post infection. Group C: Dosed prophylactically with *M. oleifera* orally at 200 mg/kg daily for 14 days before infection and treated with diminazene aceturate at 7mg/kg post infection. Group D: Infected rats and treated with only diminazene aceturate at 7 mg/kg. Group E: Dosed prophylactically with *M.oleifera* by oral route at 200 mg/kg for 90 days and *M.oleifera* was used for treatment post infection. Group F: Dosed prophylactically with *M.oleifera* at 200 mg/kg daily for 90 days and treated with diminazene aceturate at 7 mg/kg post infection. Group G: Infected and untreated rats (positive control).

Trypanosoma Stock and Inoculation

Trypanosoma brucei was used in this study and was procured from Nigeria Institute for Trypanosomiasis and Onchocerciasis Research Institute, Vom, Plateau state, Nigeria. The dose of

inoculum was estimated to be 3×10^6 of *Trypanosoma brucei* per ml of blood using the "Rapid Matching method" described by Herbert and Lumsden (1976). The parasites were maintained by serial passage in rats. To infect the rat, 1 ml of blood was collected from heavily infected rat and then mixed with 2 ml of normal saline. The mixture was viewed under microscope at $\times 40$ magnification. The blood containing the parasites was then injected into rats intraperitoneally.

Parasitaemia Assessment and Prepatent Period

The presence of parasites in the blood of the infected rats was monitored from the second day post inoculation. Estimation of parasitaemia was conducted using the method of Herbert and Lumsden (1976). A number of fields (10-15) of each drop blood or incubated media, were counted for parasites in triplicate, using glass slides under inverted microscope ($\times 400$). An average mean trypanosome count was taken as number of trypanosomes per field.

Blood Collection and Analyses

Blood samples were collected from the median canthus of the eye through the ocular vein by insertion of heparinized capillary tube. 2-3ml of blood was collected into EDTA sample bottles. Blood sample was analyzed using the method of Schalm *et al.*, (1975). Haematological parameters analyzed were total Blood Count (TBC), Red Blood Cell (RBC), White Blood cell (WBC), Packed Cell volume (PCV), Haemoglobin (Hb), Platelet (PLT), and differential count including Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC).

The Serum chemistry analyses were carried out using commercial test kits Biorex[®] (Diagnostic Limited, United Kingdom) total protein, alanine aminotransferase (ALT), aspartate aminotransferase (ASP), alkaline phosphatase (ALP), creatinine, albumin, globulin and total Bilirubin.

Histopathological procedures

After harvesting the brain, spleen and lymph node from the rats, the organs were promptly and adequately treated with 10% formaldehyde (fixation) in order to preserve its structure and molecular composition. After fixation, the piece of organ was dehydrated by bathing it successively in graded mixture of ethanol and water (70 – 100%). The ethanol was then replaced with a solvent miscible with the embedding medium. As the tissues were infiltrated with xylene, it became transparent (clearing). The impregnated tissue by xylene was placed in melted paraffin in an oven, maintained at 58 – 60°C (embedding). The heat caused the solvent to evaporate and the spaces within the tissues became filled with paraffin. The tissue together with its impregnating paraffin hardened after removal from the oven. The sections (5 μ m) were then floated on water and transferred to a glass slide, and stained with haematoxylin and eosin stains. The slides were viewed under light microscope with a magnification of $\times 100$.

Data analysis

All data generated were expressed as mean \pm SEM. The differences between the groups were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc multiple comparison test using Graphpad Prism 5.03 statistical package, San Diego, California, U.S.A (www.Graphpad.Com). P value considered was ($P \leq 0.05$).

RESULTS

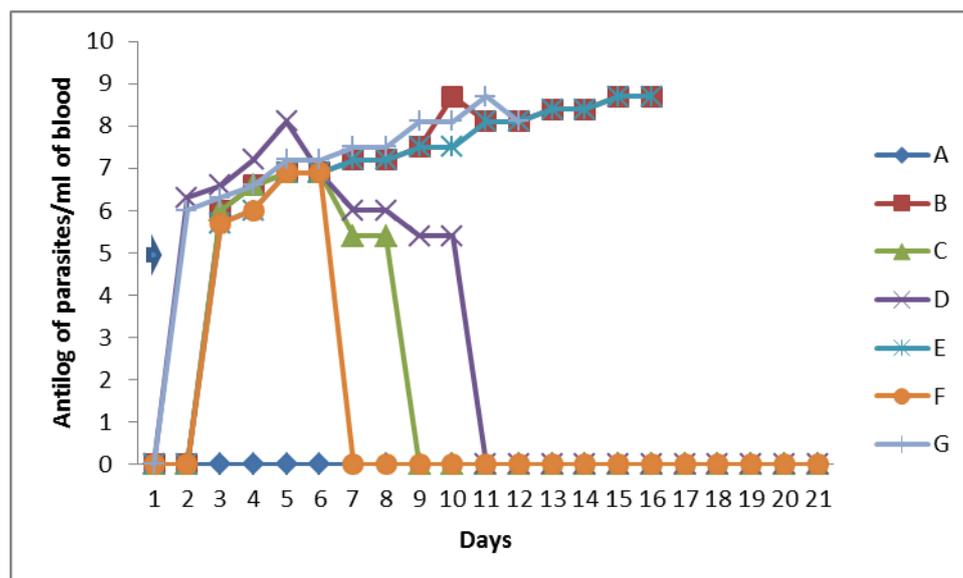
The percentage yield of the extract was 75% W/W, and colour was dark to black. It was soluble in vegetable oil.

Phytochemical

Phytochemical analysis of methanolic extract of *M. oleifera* revealed the presence of flavonoid, tannin, saponin, steroid, phenol carbohydrate and glycoside as shown in Table 1.

Table 1: Phytochemical analysis of methanolic extract of *Moringa oleifera* leaves.

Component	Test	Observation	Scoring
Alkaloid	Dragendorffs	Brownish-red colour	+
Tannins	Ferric chloride	Deep red colour	+
Flavonoid	Pew's	Red colour	+
Saponin	Frothing	Persistence foam	+
Phenol	Buchard	Violet colour	+
Carbohydrate	Molisch's	Red colour	+
Glycosides	Salkowski's	Reddish brown	+



➡ Treatment with diminazene aceturate

Figure 1: The pattern of parasitaemia curve in rats infected with *T. brucei* and treated with diminazene aceturate alone and in combination with the methanolic extract of *Moringa oleifera* leaves. Treatments: A-Uninfected and untreated; B- *M. oleifera* alone for 14 days before infection and after infection; C- *M. oleifera* (14days) + diminazene aceturate; D-diminazene aceturate alone treatment after infection; E - *M. oleifera* alone for 90days before infection and after infection; F-*M. oleifera* (90 days) + diminazene aceturate and G- Infected and untreated.

Parasitaemia

The parasites were detectable in the blood of infected rats in group D and G after 48 hours following inoculation of *T. brucei*. Parasites were detectable by 72 hours post infection (pi) in groups B, C, E and F that received daily doses *M. oleifera*. The level of parasitaemia persistently increased in all groups and reached (25×10^8 trypanosomes/ml of blood) by day 5 before treatment with diminazene aceturate.

Parasites were cleared within 72 hours post treatment (pt) from blood samples of rats in groups C and F those treated with both *M. oleifera* and diminazene aceturate. However, it took 96 hours pt for parasites to be cleared from rats in group D which was treated only with diminazene aceturate. Parasitaemia persistently

increased in group G that was not treated and reached 8.7×10^8 trypanosomes/ml of blood by day 13 and all the rats in the group were dead by day 14 post infection. Similarly, parasitaemia in groups B and E, those were treated with *M. oleifera* extract only, reached antilog of 8.7×10^8 /ml of blood by day 15 and the rats died by day 17 as seen in figure 1.

Effects of Infection and Treatment on the Haematology

The decrease in number of red blood cell induced by *T. brucei* infection responded favourably to treatment with diminazene aceturate alone or in combination with *M. oleifera* leaves. The result presented in table 2 showed that RBC of rats in groups B, C, D, E and F were non-significant ($P > 0.05$) when compared with the control. The

rats in group G (infected and untreated) remain significantly lower ($P<0.05$) when compared with the control as observed in table 1.

The decrease in haemoglobin concentration induced by *T. brucei* infection responded favourably to treatment with diminazine alone and in combination with *M. oleifera* extract. The haemoglobin concentration of rats in groups E and G were significantly lower ($P<0.05$) when compared with the control as stated in table 1.

The differential white blood cell counts; Lymphocytes, Monocytes, Eosinophils and Basophils were non-significant ($P>0.05$) in all groups when compared with the control. The Neutrophils of rats in groups E, F and G were significantly lower ($P<0.05$) when compared with the control as seen in table 1.

Effects of Infection and Treatment on Serum chemistry

Table 3 shows the serum chemistry of rats infected with *T. brucei* and treated with diminazine acetate alone and in combination with *M. oleifera* extract. All rats treated either with diminazine acetate alone or in combination with *M. oleifera* were non-significant to total protein ($P>0.05$). Rats in groups D and G were significant to albumin ($P<0.05$) when compared with the control as stated in table 2. Rats in all the groups showed no significant in globulin, AST, ALT, ALP and BUN ($P>0.05$) in comparison to the control as observed in table 2. Creatinine and total bilirubin were significant ($P<0.05$) in group G which are infected and untreated when compare with the negative control as seen in table 2.

Table 2: Haematology result of rat infected with *T. brucei* treated with *Moringa oleifera* and diminazine acetate.

Parameters	A (Control)	B	C	D	E	F	G
PCV (%)	42.2±0.86	39.0±1.05	40.62±1.29	43.6±0.98	37.7±1.11*	39.5±1.5	36.2±0.49*
RBC (x10 ⁶ /μl)	7.07±0.15	6.42±0.22	6.71±0.20	7.24±0.18	6.29±0.23	6.75±0.29	5.35±0.13*
H.b (g/dl)	14.02±0.16	12.9±0.34	13.5±0.34	14.3±0.38	12.5±0.34*	13.3±0.44	11.9±0.11*
PLT (x10 ⁹ /L)	145.2±8.9	164±15.2	138±6.3	158±6.9	143±4.3	142±5.0	123±6.2
MCV (fl/cell)	53.6±5.6	60.9±0.59	60.4±0.47	60.2±0.89	60.2±0.98	58.5±0.72	62.4±2.48
MCH (pg/cell)	19.8±0.25	20.1±0.24	20.1±0.25	19.7±0.21	19.9±0.25	33.1±.15*	20.6±0.8
MCHC (g/dl)	33.2±0.34	33.1±0.22	33.3±0.27	32.8±0.21	27.7±2.7*	19.8±0.2*	20.6±0.7*
WBC (x10 ³ mcL)	5.66±1.03	4.82±0.62	4.95±0.39	4.14±0.44	4.09±0.33	4.3±0.27	3.76±0.2
L (x10 ³)	3.38±0.57	3.06±0.30	3.35±0.31	2.82±0.29	2.82±0.26	2.29±0.3	2.49±0.10
N (x10 ³)	2.19±0.48	1.59±0.35	1.37±0.07	1.16±0.14*	1.1±0.11*	1.28±0.09	1.13±.09*
M (x10 ³)	0.13±0.04	0.12±0.03	0.06±0.02	0.06±0.02	0.09±0.01	0.07±0.01	0.07±0.01
E (x10 ³)	0.16±0.05	0.07±0.01	0.13±0.02	0.08±0.02	0.06±0.01	0.04±0.01	0.08±0.01

Histopathology

Effects of T. brucei infection on the brain of rats

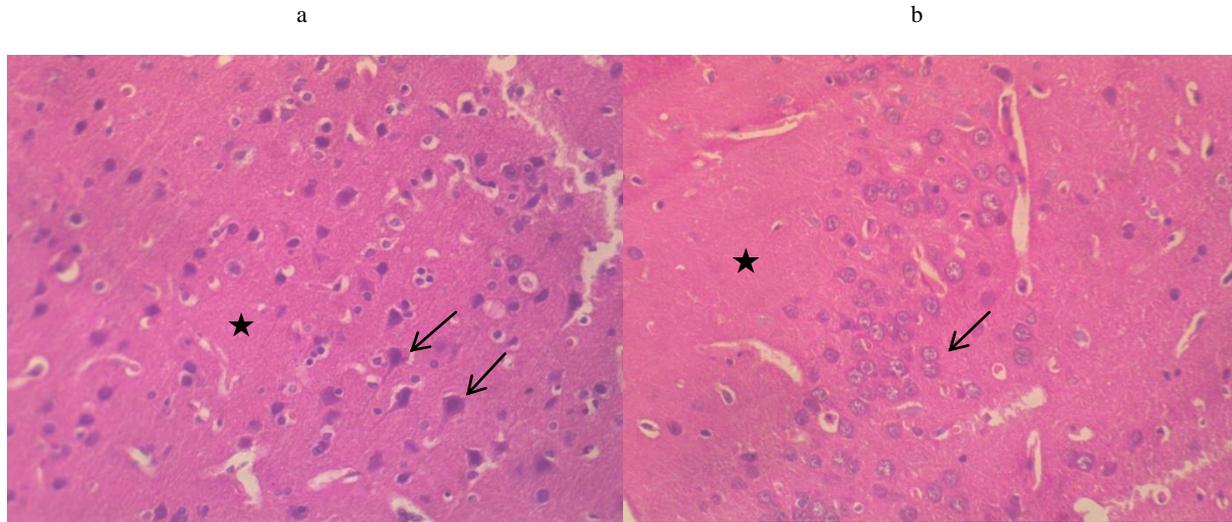


Figure 2a Photomicrograph of brain in uninfected and untreated rats (H&E) the neurons (arrow) and neutrophil (star) appear normal and show no visible lesion (NVL); **2b.** Photomicrograph of brain in rats treated with M.O alone for 14 days (H&E) the neurons (arrow) and neutrophil (star) appear normal and show no visible lesion (NVL).

Effects of T. brucei infection on the spleen of rats

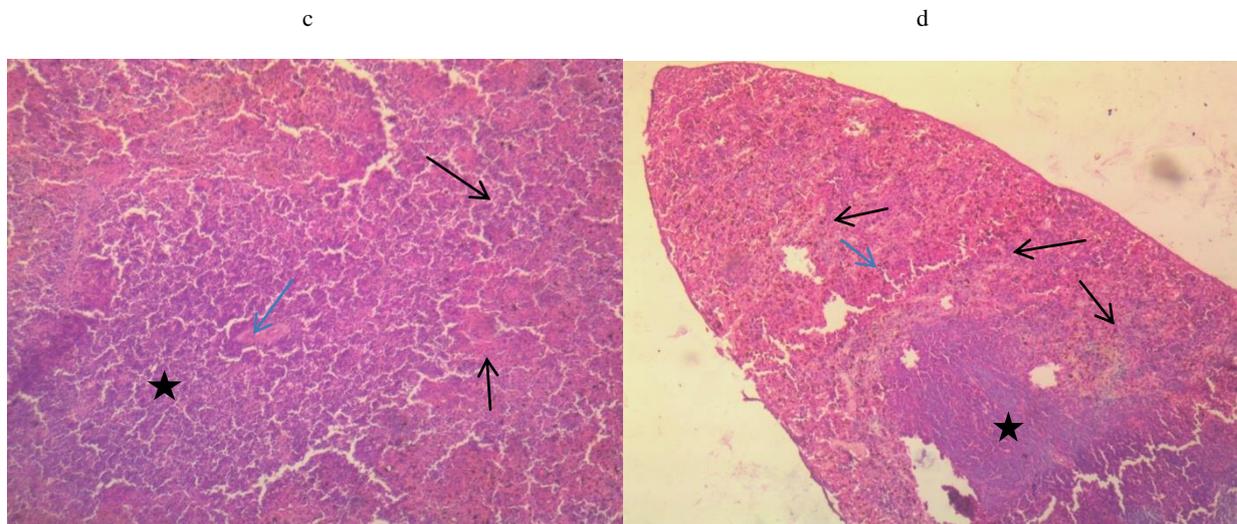


Figure 2c: Photomicrograph of spleen in rats untreated and uninfected (H&E), moderate numbers of fairly-large PALs (star). Congestion of the splenic sinuses and sinusoids; **2d:** Photomicrograph of spleen in rats treated with M.O alone for 14 days (H&E), few large discrete PALs (star). Marked congestion of the splenic sinuses and sinusoids. Multiple foci of macrophages laden with golden brown pigments [haemosiderosis] (thin arrows).

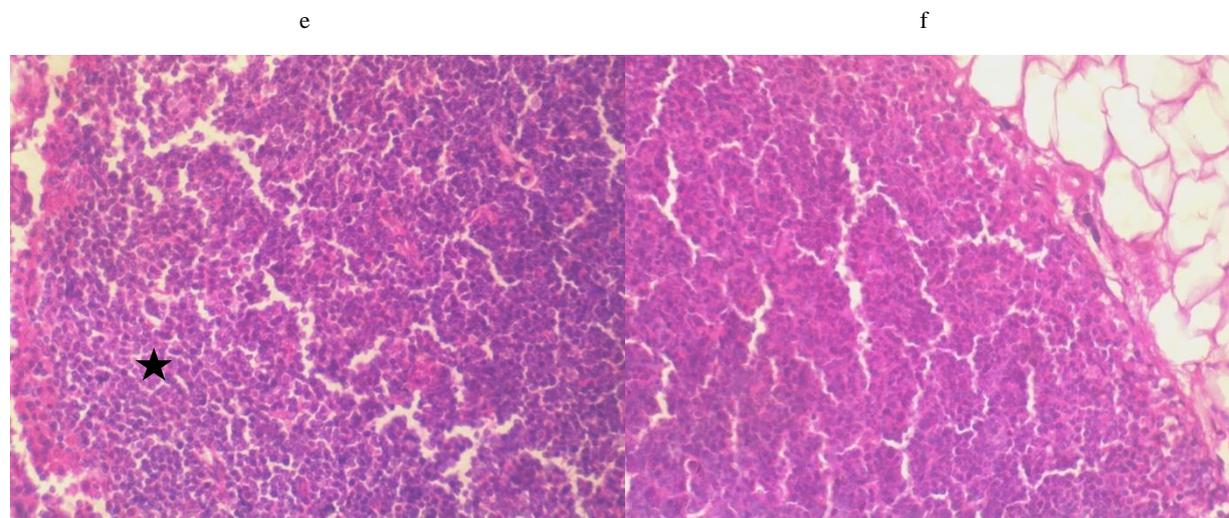
Effects of *T. brucei* infection on the lymph node of rats

Figure 2e: Photomicrograph of lymph node in rats untreated and uninfected (H&E), the cortex, paracortex and medulla consist of densely-packed lymphoid cells. NVL; **2f:** Photomicrograph of lymph node in rats treated with both diminazene and M.O (H & E), the cortex, paracortex and medulla consist of densely-packed lymphoid cells. Discrete follicles are present.

Table 3: Serum chemistry result of rat infected with *T. brucei* treated with *Moringa oleifera* and diminazene aceturate.

Parameters	A(Control)	B	C	D	E	F	G
Total protein	82.4±1.6	84.4±1.3	84.2±1.2	78.2±1.5	82.5±2.8	82.5±1.8	77.8±1.6
Albumin	33±1.8	34.6±1.3	33±0.71	28±1.3*	32±2.7	31±1.9	26.8±1.5*
Globulin	49.4±0.68	49.8±2.06	52.6±0.68	50.2±0.92	52.5±0.87	51.5±0.29	51±0.63
AST	45±0.63	39.8±1.43	42±1.23	42.8±2.39	37.5±1.4	40.8±1.6	42.6±0.93
ALT	32.8±0.37	29.2±0.97	31±1.27	31.8±1.16	29±0.82	29.8±1.65	31±1.09
ALP	106±7.01	111±4.36	99.8±7.07	111±4.01	102±5.56	91.3±10.3	92.6±3.34*
BUN	16.54±0.27	17.12±0.19	16.96±.19	17±0.35	16.28±.24	16.63±.27	16.08±.49
Creatinine	0.9±0.06	0.82±0.09	0.78±0.07	0.82±0.05	0.7±0.0*	0.80±0.12	0.66±0.11*
Total BL	0.42±0.08	0.42±0.08	0.52±0.16	0.38±0.11	0.35±0.05	0.30±0.07	0.26±0.02*

All values are express in mean ± standard error of mean

* Significantly lower (P<0.05)

AST: Aspartate aminotransferase

BUN: Blood urea nitrogen

ALT: Alanine aminotransferase

BL: Bilirubin

DISCUSSION

The qualitative phytochemical screening of the methanolic extract of *M. oleifera* leaves use in this study indicated the presence of tannins, alkaloids, flavonoids, saponins and glycosides. This is in agree with previous work described by other workers (Tijjan *et al.*, 2009, Bannetti *et al.*, (2003).

This study showed that methanolic extract of *M. oleifera* leaves does not possess trypanocidal activities against *T. brucei* infected rats. The result of this study is in contrast to the work of Atawodi *et al.*, (2005) that concluded that different parts of *M. oleifera* possess anti-trypanocidal effect against different species of trypanosome such as *T. brucei* and *T.*

congolensis. Though *M. oleifera* supplement decreased the parasitaemia level and increased the survival period of the rats for extra 2 days when compared with group G that was not supplemented with *M. oleifera*. The ameliorative effects of *M. oleifera* extract was apparently due to early clearance of the parasites in the extract treated groups (C and F) compared to the group D that were only treated with diminazene aceturate. The result of this study also showed that there was no difference in the ameliorative effect between acute and chronic administration of *M. oleifera* seen in group C and F considering the pattern of recovery and clearance of the parasites. The study also observed the mortality in groups B and E, those treated with *M. oleifera* alone, occurred at the same day. This observation further confirmed the fact that *M. oleifera* lacks trypanocidal activities.

The outcome of this study showed that *M. oleifera* supplement improved the haematological parameters of rats infected with *T. brucei*. It has been reported that anaemia in trypanosomiasis is caused by a combination of factors which includes haemolysis of erythrocytes, haemodilution and erythrophagocytosis by the trypanosomes (Mackenzie, 1990). *Trypanosome brucei* cause anaemia through disruption of erythrocytes membrane integrity (Huan *et al.*, 2000). Erythrocytic peroxidation has also been reported to be one of the factors which play an important role in the pathogenesis of anaemia in mice infected with *T. brucei* (Igbokwe *et al.*, 1994).

The Pcv, Rbc, Hb, Mcv, Mch and Mchc were greatly improved in all groups treated with *M. oleifera* extract when compared with the rats in group G (infected and untreated) which were significantly lower ($P < 0.05$) as seen in table 1. This result is similar to the result of Ueno *et al.*, (2000) and Weber (2009). However, the differential WBC showed lymphocytopenia, neutrophilia and monocytopenia which were evident especially in groups that were not supplemented with *M. oleifera*. (Table 1)

The results of this study indicated non-significant ($P > 0.05$) alterations in several biochemical parameters in the *T. brucei*-infected rats as stated in table 2. The results indicated that there was hypoproteinaemia in the groups that were not treated with *M. oleifera* extract (group A and G). This implies that *M. oleifera* extract prevent hypoproteanemia in infected rats as

observed in previous work reported by Atawodi *et al.* (2005).

Hepatocellular damage caused by trypanosome was monitored by serum activities of ALT and AST (Umar *et al.*, 2000) which had leaked from hepatic tissues (Zilva and Pannall, 1990). The result of enzyme assays showed elevation of both ALP and AST in all the groups of infected rats seen in table 2. The elevation of these enzymes is in consonance with earlier reports by Gray (1986) and Adah *et al.*, (1997). This has been reported to be due to inflammation and necrosis in the infected host particularly in organs like liver, kidney muscle and even heart (Losos and Ikede, 1972). This is possible due to the ability of *T. brucei* to invade soft tissue especially the liver, kidney and heart thereby localizing and causing tissue damage which subsequently leads to the release of these enzymes from damaged tissue (Igbokwe *et al.*, 1994).

The renal damage caused by the trypanosome infection was reflected by an increase in serum concentrations of urea and creatinine. This has also been observed by Umar *et al.* (2010). In this study, blood urea nitrogen, creatinine and total bilirubin decrease across all the groups infected with *T. brucei* with marked decrease in group G which were untreated or unsupplemented; significantly lower ($P < 0.05$). This was in line with the work by Adejinmi and Akinbolade (2000) where they observed lower serum biochemical changes in West African dwarf goats infected with *T. brucei*.

Splenomegaly was a prominent feature of infection with both *T. brucei* and *T. congolensis*. This observation has also been reported by others (Kaikabo and Salako, 2006) associated splenomegaly with acute or parasitemic phase of trypanosome infection and attributed its cause to red blood cell sequestration and expanded macrophage population. The hyperplasia of germinal center and proliferation of macrophages and plasma cells were observed in the present study (Figure 2d) in support with the findings of other workers.

Clyton *et al.*, (1992) reported an increase in splenic macrophages and the profound effects of trypanosomes on mature splenic lymphocytes. The presence of trypanosomes has been demonstrated to stimulate and cause proliferation of splenic B-lymphocytes (Malik *et al.*, 2008).

Some of the proliferating B-cells differentiate and secrete large amount of immunoglobulins (ILRAD, 2008) of diverse specificity (Hunter *et al.*, 1991). The proliferation of immune competent splenic cells may be responsible for prominent increase in size following infection with *T. brucei*. Treatment using diminazene aceturate with or without *M. oleifera* gave variable results in related to the responses of the spleen to treatments. Diminazene aceturate in combination with *M. oleifera* were more in reducing spleen weight than diminazene treatment alone in *T. brucei* infection. However, it is difficult to compare this observation with those of other workers as result of the dearth of data on *M. oleifera* supplementation following trypanocide treatment in trypanosomosis. Thus report of Kaikabo and Salako, (2006) showed that the spleen of vitamin E supplemented rats had less lesions. The photomicrograph of spleen in this study showed marked congestion of the splenic sinuses and sinusoids suggesting marked splenic depletion as seen in figure 2d. This is as a result of erythrocytes destruction by the trypanosome organisms which is in line with the report of Hausteen *et al.* (2009) that showed the pathogenesis of anaemia in *T. congolensis* infected mice.

The histopathology of this study revealed that the brain of the rats that were infected and untreated had multiple neuronal degeneration that leads to congestion of the cerebral blood vessels. Trypanosome parasites penetrate the brain leading to vascular degeneration. This is in consistent with the previous work of Diwao and Roy (2000) which confirm the brain invasion of trypanosome parasites in dogs. The brain of the groups dosed with *M. oleifera* extract had lower neuronal degeneration and the neuron appears normal with no visible lesion as observed in rats that are in group B,C, E and F (Figure 2b) when compared with the positive control

The histopathology result of this study showed that methanolic extract of *M. oleifera* leaves is a potent ameliorative agent that reduces neuronal degeneration in the brain of rats infected with *T. brucei*.

The congestion in the spleen and lymph nodes were considerably reduced when compared with the positive control group. This result is in line with the findings of Shetty and Naryana (2007) that compare the histopathology in rats infected with *T. brucei* and *T. congolensis*.

CONCLUSION

The results obtained from this present study showed that *M. oleifera* does not possess anti-trypanosomal activities but can be used as an ameliorative supplement to reduce the level of parasitaemic pattern and prompt clearance of the parasites when combined with potent conventional trypanocides such as diminazene aceturate.

The study also showed that *M. oleifera* supplement reduce tissue and organ damage caused during trypanosomal infection and this probably occurs as result of the potent antioxidant effect of the extract as reported in previous work by Jaquelin *et al.* (2015) when the antioxidant effect of *M. oleifera* was established during oxidative stability of fish subjected to accelerated storage.

ACKNOWLEDGEMENT

We wish to acknowledge the technologists in the Physiology and Pharmacology laboratory for the technical assistance rendered in the course of this work.

REFERENCES

- Adah, M. I., Rohwedder, A., Olaleye, O. D. and Werchau, H. (1997). Nigerian rotavirus serotype G8 could not be typed by PCR due to nucleotide mutation at the 39 end of the primer binding site. *Archives of Virology*, **142**: 1881-1887
- Adejinmi, J. O. and Akinbolade (2000). Serum biochemical changes in West African Dwarf goats with experimental mixed *T. brucei* and *cowdria ruminantum* infection. *Journal of Tropical veterinary science*, **18**(1-2): 111-120.
- Adeyemi, O. S., Akanji, M. A., and Ekanem, J. T. (2000). Ethanolic extract of *Psidium guajava* influences protein and bilirubin level in *Trypanosomabrucei* infected rats,. *Journal of Biological science* **12**:111-116
- Anosa V. O (1998). Haematological and biochemical changes in human and animal trypanosomosis. Part 1 *Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux (Paris)*, **41**: 65-78
- Atawodi, S. E. (2005) Comparative *in vitro* trypanocidal activities of the petroleum ether, chloroform, methanol and aqueous extracts of some Nigerian Savannah Plants. *African Journal of Biotechnology*, **4**: 177-182.
- Awotunde, A. J., Ibrahim, S., and Ameh, D. A., (2002). In vitro trypanocidal effects of methanolic extract of some Nigeria savanna plants. *African journal of biotechnology*, **2**: 317-321.
-

- Bailey, N. M. (1998). Oral Berenil in the treatment and prophylaxis of human trypanosomiasis. *Tropical journal of Medicine*, **62**: 122-130.
- Bannetti, M.P., Burchmore, R.J., and Stich, A. (2003): "The trypanosomiasis" in Africa, human African trypanosomiasis. *Journal of Applied Neurobiology*, **12**:81-94.
- Clyton, E. G., Caceres, A., Saravia, A. and Rizzo, S. (1992): Pharmacologic properties of *Moringaoleifera*. Screening for antispasmodic, anti-inflammatory and diuretic activity. *Journal of ethno pharmacology*, **36**(3): 233-237.
- Diwao, A. R., and Roy, B. D. (2000). Studies on binding of berenil to microsomal protein and its significance with respect to microsomal metabolism of trypanocidal drug. *Indian journal of experimental biology*, **26**: 464-466.
- Dolan A. C., Damper, D. and Patton, C. L. (1996). Pentamidine transport in *Trypanosomabrucei* pharmacokinetics and pharmacodynamic sequence, **8**: 400-420. Blackwell publishers, London.
- Eghianruwa, K.I, Odiaka P.C.G. and Ogunpolu. J. (2004). A preliminary comparative efficacy study of isometamedium following oral and intramuscular administration in mice experimentally infected with *T. congolense*. *Sahel journal of veterinary science*, **3**: 33-37.
- Freiburghaus, F, Steck, A., Ptander, H., Brun R., Cunningham, M.P., Van Hove, K. and Lumsden, W.H. R. (1998). A bio-assay technique for determination of trypanocidal drug levels, and its application in estimating the duration of activity of berenil in treated cattle. *Procure at International Congress of Parasitology*, **1**: 301-303.
- Gray, A. S., Genet, G., Mancebo, O. A. and Monzon, C.M. (1986). Accion deldiminazene en ratones y equinos experimentalmente infectados con *Trypanosomaequinum*. *Veterin. Argentina* **3**, 844-849. maintained by chemoprophylaxis under trypanosomiasis risk. *Journal of agricultural Science*, **105**: 147-166.
- Griffin, A. R. and Allanby, B. J. (1999). Epidemiology of bovine trypanosomiasis in the Ghibe valley, southwest Ethiopia. *Journal of Accra tropical medicine*, **53**: 151-163.
- Hausteen, A, R., Hampal, V., Hug. L., Leigh, J. W., Dacks, J. B., Lang, B. F., Simpson, A. G. and Roger, A. J. (2009). "Phylogenomic analyses support the monophyly of Excavata and resolve relationships among eukaryotic "supergroups"". *Procure: National academic Science*,. U.S.A. **106** (10): 3859-64.
- Herbert W.J. and Lumsden W.H.R. (1976) *Trypanosomabrucei*: A Rapid "Matching" method for Estimating the host parasitemia. *Journal of experimental parasitology*, **40**: 427-431.
- Huan, S., Weisz, K., James, T.L. and Shafer, R. H. (2000): H-NMR studies on the (GCTTAAGC) and its complex with berenil. *European journal of biochemistry* **204**, 31-38.
- Hunter, C.A., Gow, J.W., Kennedy, P.G.E., Jennings, F. W. and Murray, M (1991). Immunopathology of experimental African sleeping sickness: detection of cytokine mRNA in the brains of *Trypanosomabrucei*-infected mice. *Journal of infectious immunology*, **59**, 4636-4640.
- Igbokwe, I. O., Esiebo, K., A., N., Saror, D. I. and Obagaiye, O. K. (1994). Increased susceptibility of erythrocytes to *invitro* peroxidation in acute *Trypanosomabrucei* I infection in mice. *Journal of Veterinary parasitology* **55**, 247-86.
- Igweh, A.C., Aguiyi, J.C. and Okwuaasaba, F.K. (2002). Antitrypanosomal Effect of the Aqueous Extract of *Brassica oleracea*. infections after chemotherapy. *Journal of Parasitology*, **97**: 51-61.
- Jaqueline, A. N., Marciane, M., Janaina, M. B. S., Kassandra, L.G.V., Araujo, A., Poliana, S. E., Alline, S. S., Antonia, L. S., Silva, M. C. D. and Antonia, G. S. (2015): Assessment of the antioxidant effects of *Moringaoleifera* extract in fish oil during storage. *Journal of food processing and preservation*, **40**: 29-36.
- Kaboré, J., Camara, M., Cooper, A., Tait, A., Jamonneau, V., Bucheton, B., Berriman, M and MacLeod, A. (2016). Population genomics reveals the origin and asexual evolution of human infective trypanosomes. *E life journal*, **5**: 14-73. doi: 10.7554/eLife.11473.
- Kagira, J. M., Thuita, J. K., Ngotho, M., Mdachi, R., Mwangangi, D. M. and Ndung'u, J. M. (2006). Haematology of experimental *Trypanosomabrucei rhodesiense* infection in vervet monkeys. *African journal of health science*, **13**: 59-65.
- Kaikabo, A. B. and Salako, E. Y. (2006). Comparative Antitrypanosomal Activity of *Terminalia iachebula* dried fruit. Therapeutic Effects of *Zanthoxy lumalatum* leavea and *Eugenia caryophyllata* Buds (Fruits) against *Trypanosoma evansi*. *Journal of Veterinary parasitology*, **2**: 91-97.
- Kibona, J., Camara, M., Cooper, A., Tait, A., Jamonneau, V., Bucheton, B., Berriman, M and MacLeod, A. (2002). Population genomics reveals the origin and asexual evolution of human infective trypanosomes. *E life journal*, **5**: 14-73. doi: 10.7554/eLife.11473.
- Losos, G.J. and Ikede, B.O. (1972). Review of pathology of diseases in domestic and laboratory animals caused by *T. congolense*, *T. vivax*, *T. brucei*, *T. rhodesiense* and *T. gambiense*. *Veterinary Pathology Supplement*, **9**: 1 - 71.
- Mackenzie, B. C. (1990): Berenil (diminazene aceturate) resistant *Trypanosoma congolense* in cattle under natural tsetse

- challenge at Kibaha, Tanzania. *Actatropical journal*, **45**:239-244.
- Malik, S. B., Pightling, A. W., Stefaniak, L. M., Schurko, A. M. and Logsdon J. M. (2008). "An expanded inventory of conserved meiotic genes provides evidence for sex in *Trichomonas vaginalis*". *PLoS ONE journal*, **3** (8): 110-120.
- Nantulya, V. M. (1990). Trypanosomiasis in domestic animals: problems of diagnosis. *Revue. Scientifique et technique. International office of epizootic*, **9**: 357-367.
- Schalm, O. W, Jain, N. C and Carrol, E. G. (1975). *Veterinary haematology*, 3rd edition, Lea and Fabiger publishers, Philadelphia, 20-280.
- Shaba, P., Pandey, N.N., Sharma, O.P., Rao, J.R. and Singh, R.K. (2006). Antitrypanosomal activity of Methanolic Research. *Journal in Engineering and Applied Sciences*, **3**(1):43-49.
- Shetty, B. C. and Naryana, A. R. (2007). Molecular analysis of archived blood slides reveals an atypical human *Trypanosoma* infection. *Diagnostic Microbiology and Infectious Disease*, **61** (4): 428-433.
- Reiter, I., Biittner, M. and Seitz, A. (2011). *Trypanosoma theileri* Laveran in naturally and Anti-trypanosomal Potential of Methanolic Extract of *Calotropis gigantea* leaves against *Trypanosoma evansi* and its Cytotoxicity. *International journal of Biosource and Stress Management*, **73**: 121-124.
- Sofowora, E. A., (2006). Medicinal plant and traditional use in African. Spectrum books Ltd, Ibadan Nigeria, 134-7, 195-248.
- Taiwo, V.O., Olaniyi, M.O. and Ogunsanmi, A. (2003). comparative plasma biochemical changes and susceptibility of erythrocytes to *in vitro* peroxidation during experimental *trypanosome congolense* and *T. brucei* infection in sheep. *Israel journal of veterinary medicine*, **58**: 4-20.
- Tijjan, A. B., Tahiliani, P. and Kar, A. (2009). Role of *Moringa oleifera* leaf extract in the regulation of thyroid hormone status in adult male and female rats. *Pharmacology Reserves*, **41**: 319-323.
- Ueno A. R., Van Hoeve, K. and Cunningham, M. P. (2000). Prophylactic activity of Berenil against trypanosomes in treated cattle. *Veterinary Records*, **76**:260-280.
- Umar, I.A., Ibrahim, M.A., Fari, N.A., Isah, S, and Balogun, D.A. (2010). *In-vitro* and *in-vivo* anti-trypanosoma *evansi* activities of extracts from different parts of *khaya senegalensis*. *Journal of cell and animal biology*, **4**: 91-95.
- Weber, B. C. (2009). Extra vascular foci of *Trypanosoma vivax* in goats: the central nervous system and aqueous humor of the eye as potential sources of relapse. *Journal of Protozoology*, **13**: 234-239.
- Zilva, A. R. and Pannall, B. C. (1990). *In vivo* and *in vitro* sensitivity of *Trypanosoma evansi* and *T. equiperdum* to diminazene, suramin,; The treatment of *T. simiae* in pigs with diminazene aceturate in combination with a 5-substituted 2-nitroimidazole compound *Journal of Tropical Medical Parasitology*, **38**: 175-176.
-

