Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60071-7

Effect of diminazene aceturate, levamisole and vitamin C combination therapy in rats experimentally infected with *Trypanosoma brucei brucei*

Adieme Ijeoma Chekwube[1], Ezeh Ikenna Onyema[2*], Ugochukwu Emmanuel Ikenna[1], Romanus Chukwuduruo Ezeokonkwo[2]

[1]*Department of Veterinary Medicine, University of Nigeria, Nsukka*
[2]*Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka*

ARTICLE INFO

Article history:
Received 6 November 2013
Received in revised form 18 November 2013
Accepted 15 March 2014
Available online 20 June 2014

Keywords:
Diminazene aceturate
Levamisole
Vitamin C
Therapy
Trypanosoma brucei brucei
Rats

ABSTRACT

Objective: To investigate the effect of diminazene aceturate (DA) alone or in combination with either levamisole and/or Vitamin C in albino rats experimentally infected with *Trypanosoma brucei brucei*. **Methods:** Thirty adult male albino rats, randomly assigned into 6 groups (A–F) of 5 rats each were used. They were either infected with 1×10^6 trypanosomes intraperitoneally (groups A–E) or uninfected (group F). The different groups were treated respectively as follows: group A–with 3.5 mg/kg DA; group B–3.5 mg/kg DA and 7.5 mg/kg levamisole; group C–3.5 mg/kg DA and 100 mg/kg vitamin C; and group D–3.5 mg/kg DA and 7.5 mg/kg levamisole and 100 mg/kg vitamin C. Group E was left untreated. Parameters assessed include: rectal temperature, body weight changes, packed cell volume (PCV), Haemoglobin concentration (Hb), total leucocyte count (TLC) differential leucocyte count (DLC), parasitaemia, clinical signs and survivability. **Results:** Average pre-patent period of 5 days was recorded. Parasites in the blood were cleared in all treated groups (A–D) within 48 hours post treatment (PT). Untreated rats in group E died between 25 and 32 days post infection (PI). Relapse was not recorded in all the treated groups (A–D). The initial reduction in PCV, Hb, TLC and increases in rectal temperature following infection were reversed by the treatments. The rats that received drug combinations (groups B, C and D) showed faster and higher recovery rates than the uninfected control and group A. **Conclusions:** Levamisole and/or Vitamin C combination with DA were more effective in the treatment of rats infected with *Trypanosoma brucei brucei*.

1. Introduction

African animal trypanosomosis is a disease caused by a group of protozoan haemoflagellate parasites of the genus *Trypanosoma*. They produce persistent infection in the blood and induce profound immunosuppression[1]. The disease continues to be a major setback of livestock production in Nigeria despite attempts to control the disease[2]. It has also been known to affect humans causing a condition called sleeping sickness[3]. Currently there is no effective vaccine against the disease. However, the available means to protect and maintain livestock despite the short comings of these measures include tsetse control strategies, chemotherapy and chemoprophylaxis and use of trypanotolerant livestock[4]. In Africa, control relies mainly on chemotherapy and chemoprophylaxis using salts of three compounds – diminazene, homidium and isometbamidium[5]. In Nigeria diminazene aceturate is the most commonly used therapeutic agent while Isometamidium chloride is the most commonly used prophylactic agent[6,7]. Despite this, treatment of trypanosomosis is faced with challenges of drug resistance due to wrong use of drugs, and presence of few trypanocides[8,9]. The inability of readily available drugs to cross the blood–brain barrier and toxicity are also major concerns in chemotherapy of trypanosomosis[10,11]. The challenge therefore lies in the careful management of the few trypanocides in order to minimise resistance and

*Corresponding author: Ezeh Ikenna Onyema, Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka.
Tel: +234 (0) 8034314158
E-mail: ikenna.ezeh@unn.edu.ng ikennaezeh@yahoo.com

prolong the usefulness of these drugs[12,13]. Some of these strategies include sanative pairs[14], combination therapy[15] and high dose and repeat treatment regimen[16].

Trypanosomosis is associated with generalized immunosuppression in infected hosts and the efficiency of the host's immune system is vital in the eventual destruction of the parasite. It is therefore conceivable that co-administration of trypanocides with agents known to have immunostimulatory and antioxidant properties may enhance their therapeutic activity and efficacy, hence the study.

2. Materials and methods

2.1. Experimental animals

Thirty adult male albino rats used for this study were obtained from the breeding colony of the Department of Zoology, University of Nigeria, Nsukka. They were assigned into 6 groups of 5 rats each and kept in metal cages in the laboratory animal unit of the Department of Veterinary Parasitology and Entomology, University of Nigeria Nsukka. The rats were fed standard rat feed (Vital feeds®). The rats were acclimatized for two weeks prior to commencement of the experiment.

2.2. Trypanosomes

The *Trypanosoma brucei (T. brucei) brucei* used in this study was originally isolated from a hunting dog presented at the Veterinary Teaching Hospital, University of Nigeria Nsukka. The parasites were identified morphologically[17] and by the blood incubation infectivity test (BIIT)[18]. They were maintained in mice from which donor rats were first infected.

2.3. Experimental drugs

Three drugs used for this experiment include diminazene aceturate (Trypazen® Pantex Holland), Levamisole hydrochloride (Eagle Company Korea), and Vitamin C (Jinling Pharm China).

2.4. Infection of experimental rats

Infected blood from the donor rats were obtained from the retrobulbar plexus via the median canthus of the eye into sample bottles containing ethylene diamine tetra acetate (EDTA). Infected blood was then diluted in phosphate buffered saline (PBS). Each rat was then infected with 1×10^6 trypanosomes suspended in 0.2 mL of PBS intraperitoneally.

2.5. Experimental design

The thirty Sprague Dawley albino rats used were randomly assigned to six groups (A–F) of five rats each. All rats in groups A–E were infected with 1×10^6 trypanosomes intraperitoneally while group F were left as uninfected control. Rats in group A–D were treated with 3.5 mg/kg diminazene aceturate intramuscularly 7 d post infection (PI). However, rats in groups B, C and D received further treatments with levamisole at 7.5 mg/kg body weight subcutaneously, vitamin C at 100 mg/kg body weight intraperitoneally and a combination of levamisole and vitamin C at same doses respectively. Rats in group E were left untreated as infected control.

2.6. Parameters for assessing efficacy of treatments

The efficacy of treatments was assessed weekly using the following parameters: weight changes, rectal temperature, packed cell volume[19]; haemoglobin concentration, total and differential white blood cell counts[20]. Also clinical signs were observed throughout the period of infection and treatments. Survivability of the rats were also monitored and recorded.

2.7. Statistical analysis

Data obtained were computed into means and analysed using analysis of variance (ANOVA). The means were separated at *post hoc* using Duncan's Multiple Range test[21] at 95% confidence interval.

3. Results

3.1. Parasitaemia

An average pre patent period of 5 days was recorded in all infected rats. Parasitaemia increased until day 7 PI when the rats in groups A–D were treated. All rats in group E died between day 25 and 32 PI (Table 1). The clinical signs observed were anorexia, starry hair coat, cuddling, weakness and depression. These signs disappeared gradually following treatment in groups A–D while the signs remained in the untreated group E in addition to the reluctance to move, facial oedema, enlarged abdomen and death. The treated rats remained aparasitaemic throughout the experiment. No relapse of infection was recorded in any of the treatment combinations.

3.2. Rectal temperature

The mean rectal temperature of infected rats was significantly higher ($P<0.05$) in group E (infected untreated) at days 14 and 21 PI than in the infected treated and uninfected untreated groups (Table 2). However, there was a significantly higher mean rectal temperature ($P<0.05$) of rats in group B (day 28 PI) and group C (days 21, 42, 56 and 70 PI) than in the uninfected control and other treated groups.

3.3. Proportional weight changes

There was a significant decrease ($P<0.05$) in the mean proportional weight change in rats of group B (Table 3) when compared with other infected groups and the uninfected control at day 14 (7 days PI). However, there was a significantly lower ($P<0.05$) weight change in the infected untreated group E, at day 28 PI, with subsequent death of all rats in this group. Similarly, the proportional weight change of rats in group B was significantly lower ($P<0.05$) than the uninfected control at days 14, 21, 28, 35, 42 and 49 PI.

3.4. Packed cell Volume (PCV)

The PCV of rats in the infected groups was significantly lower ($P<0.05$) than those of the uninfected untreated group F at day 7 PI (Table 4). However, 14 days after treatment (day 21 PI) the mean PCV values of rats in group E was significantly lower ($P<0.05$) than those of the uninfected control and other treated groups. This continued till the death of all rats in group E. There was a significantly lower ($P<0.05$) PCV of rats in group A (DA only) than the other treated groups as well as the uninfected control group at days 14, 21, 42, 56 and 70 PT whereas other treated groups (C and D) were not significantly different ($P>0.05$) with the uninfected control group F.

3.5. Haemoglobin concentration (Hb)

The Mean Hb of all infected groups and the control were not significant ($P>0.05$) at 7 days PI (Table 5). However, the infected untreated control had significantly lower Haemoglobin concentration ($P<0.05$) than the uninfected untreated and other treated groups at day 14 PI (7 days PT). This decrease continued for all rats in this group (group E) until death of all rats in the group. However, the mean Hb of rats in all treated groups continued to appreciate, although they were significantly lower ($P<0.05$) than the uninfected control.

Table 1

Parasitaemia of *T. brucei brucei* infected rats treated with diminazene aceturate, and either levamisole or vitamin C combinations.

Days	Group A	Group B	Group C	Group D	Group E	Group F
0	0/5	0/5	0/5	0/5	0/5	0/5
7	5/5	5/5	5/5	5/5	5/5	0/5
14	0/5	0/5	0/5	0/5	5/5	0/5
21	0/5	0/5	0/5	0/5	5/5	0/5
28	0/5	0/5	0/5	0/5	2/2	0/5
35	0/5	0/5	0/5	0/5	0/0	0/5
42	0/5	0/5	0/5	0/5	0/0	0/5
49	0/5	0/5	0/5	0/5	0/0	0/5
56	0/5	0/5	0/5	0/5	0/0	0/5
63	0/5	0/5	0/5	0/5	0/0	0/5
70	0/5	0/5	0/5	0/5	0/0	0/5
77	0/5	0/5	0/5	0/5	0/0	0/5

Numerator: Number of rats positive; Denominator: Number infected and surviving.

Group A: Infected and treated with 3.5 mg/kg DA; Group B: Infected and treated with 3.5 mg/kg DA + 7.5 mg/kg levamisole; Group C: Infected and treated with 3.5 mg/kg DA + 100 mg/kg Vitamin C; Group D: Infected and treated with 3.5 mg/kg DA + 7.5 mg/kg levamisole + 100 mg/kg Vitamin C; Group E: Infected and untreated; Group F: Uninfected and untreated.

Table 2

Mean temperature ± SEM (℃) of rats infected with *T. brucei brucei* and treated with diminazene aceturate alone, or in combination with either levamisole or vitamin C or both.

Days	Group A	Group B	Group C	Group D	Group E	Group F
0	36.36 ± 0.33^{a}	36.84 ± 0.22^{a}	36.76 ± 0.36^{a}	36.44 ± 0.16^{a}	36.7 ± 0.14^{a}	36.60 ± 0.18^{a}
7*	36.56 ± 0.26^{a}	36.52 ± 0.51^{a}	37.08 ± 0.27^{a}	37.08 ± 0.39^{a}	36.66 ± 0.11^{a}	36.33 ± 0.26^{a}
14	37.14 ± 0.15^{ab}	36.44 ± 0.10^{c}	37.62 ± 0.22^{cd}	37.26 ± 0.08^{bc}	37.74 ± 0.07^{d}	36.83 ± 0.06^{a}
21	37.02 ± 0.32^{ab}	36.92 ± 0.12^{ab}	37.06 ± 0.17^{ab}	37.00 ± 0.15^{ab}	37.26 ± 0.22^{b}	36.60 ± 0.12^{a}
28	36.82 ± 0.07^{a}	36.98 ± 0.22^{ab}	37.40 ± 0.28^{b}	37.12 ± 0.09^{ab}	36.98 ± 0.17^{ab}	36.70 ± 0.14^{a}
35	36.12 ± 0.18^{a}	36.88 ± 0.24^{b}	36.58 ± 0.36^{ab}	36.52 ± 0.31^{ab}	0.00 ± 0.00^{b}	35.90 ± 0.13^{a}
42	36.64 ± 0.27^{a}	37.06 ± 0.16^{a}	36.90 ± 0.38^{a}	36.80 ± 0.27^{a}	0.00 ± 0.00^{a}	36.63 ± 0.10^{a}
49	36.76 ± 0.20^{ab}	36.90 ± 0.19^{ab}	37.42 ± 0.36^{bc}	36.52 ± 0.25^{a}	0.00 ± 0.00	36.45 ± 0.21^{a}
56	36.50 ± 0.06^{a}	36.80 ± 0.25^{a}	36.72 ± 0.13^{a}	36.68 ± 0.12^{a}	0.00 ± 0.00	36.53 ± 0.16^{a}
63	36.68 ± 0.20^{bc}	36.80 ± 0.11^{bc}	37.24 ± 0.31^{c}	36.56 ± 0.13^{ab}	0.00 ± 0.00	36.08 ± 0.21^{a}
70	36.64 ± 0.12^{a}	36.32 ± 0.13^{a}	36.62 ± 0.10^{a}	36.62 ± 0.12^{a}	0.00 ± 0.00	36.53 ± 0.15^{a}
77	36.84 ± 0.04^{c}	36.54 ± 0.08^{ab}	37.20 ± 0.18^{d}	36.76 ± 0.12^{c}	0.00 ± 0.00	36.40 ± 0.10^{a}

Different superscript in a row indicates significant difference between the group mean at ($P<0.05$). *Treatment day.

Table 3

Mean proportional body weight changes (g) of rats infected with *T. brucei brucei* and treated with diminazene aceturate alone, or in combination with either levamisole or vitamin C or both.

Days	Group A	Group B	Group C	Group D	Group E	Group F
0	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}
7*	1.02±0.03^{a}	1.01±0.01^{a}	1.02±0.01^{a}	1.01±0.01^{a}	1.05±0.02^{a}	1.05±0.03^{a}
14	1.02±0.02^{b}	0.96±0.01^{c}	1.04±0.02^{ab}	1.03±0.01^{b}	1.09±0.02^{a}	1.08±0.02^{a}
21	1.03±0.02^{ab}	0.97±0.02^{b}	1.11±0.06^{a}	1.05±0.03^{ab}	1.08±0.03^{a}	1.09±0.03^{a}
28	1.04±0.02^{a}	0.98±0.02^{b}	1.08±0.02^{a}	1.06±0.02^{a}	0.86±0.01^{c}	1.10±0.03^{a}
35	1.05±0.02^{a}	0.98±0.03^{b}	1.08±0.02^{a}	1.10±0.01^{a}	0.00±0.00	1.11±0.02^{a}
42	1.03±0.02^{bc}	1.02±0.03^{c}	1.06±0.03^{abc}	1.10±0.02^{ab}	0.00±0.00	1.11±0.02^{a}
49	1.02±0.03^{bc}	0.98±0.04^{c}	1.05±0.03^{abc}	1.08±0.02^{ab}	0.00±0.00	1.11±0.02^{a}
56	0.99±0.02^{b}	1.05±0.03^{ab}	1.04±0.03^{ab}	1.10±0.02^{a}	0.00±0.00	1.09±0.04^{a}
63	1.00±0.02^{c}	1.04±0.03^{bc}	1.05±0.03^{abc}	1.11±0.02^{ab}	0.00±0.00	1.12±0.02^{a}
70	1.04±0.02^{bc}	0.99±0.03^{c}	1.08±0.03^{ab}	1.11±0.02^{ab}	0.00±0.00	1.15±0.03^{a}
77	1.02±0.02^{bc}	0.96±0.03^{c}	1.04±0.04^{bc}	1.07±0.03^{ab}	0.00±0.00	1.14±0.02^{a}

Different superscript in a row indicates significant difference between the group mean at (P<0.05). *Treatment day.

Table 4

Mean packed cell volume (%) of rats infected with *T. brucei brucei* and treated with diminazene aceturate alone, or in combination with either levamisole or vitamin C or both.

DAY	Group A	Group B	Group C	Group D	Group E	Group F
0	44.60±1.69^{b}	44.20±3.09^{b}	44.40±1.94^{b}	43.2±2.08^{b}	53.80±1.46^{b}	43.75±2.06^{a}
7*	37.60±1.96^{b}	40.40±1.86^{ab}	40.40±2.20^{ab}	38.2±1.11^{b}	37.80±1.39^{b}	44.25±1.93^{a}
14	34.40±1.60^{b}	38.20±1.16^{b}	35.80±2.27^{b}	37.8±3.51^{b}	37.80±2.94^{b}	46.00±2.16^{a}
21	40.60±1.21^{b}	43.40±1.25^{ab}	44.00±1.14^{ab}	42.4±1.60^{ab}	32.60±0.24^{c}	45.75±2.17^{a}
28	41.00±2.02^{b}	45.60±0.93^{ab}	44.60±1.63^{ab}	41.6±2.73^{b}	30.40±0.24^{c}	47.75±0.63^{a}
35	41.00±1.26^{a}	43.60±2.54^{a}	42.40±1.54^{a}	39.8±1.43^{a}	0.00±0.00	42.25±1.03^{a}
42	40.60±0.93^{a}	40.40±1.57^{a}	44.60±0.98^{a}	41.4±2.38^{a}	0.00±0.00	43.00±0.91^{a}
49	40.20±1.56^{b}	41.00±1.52^{ab}	40.40±0.68^{b}	41.2±1.93^{ab}	0.00±0.00	44.75±1.03^{a}
56	41.00±1.30^{a}	43.40±0.93^{a}	40.60±0.93^{a}	40.2±1.43^{a}	0.00±0.00	42.75±2.46^{a}
63	34.80±1.11^{b}	37.80±1.50^{ab}	36.20±1.77^{ab}	36.4±1.81^{ab}	0.00±0.00	40.00±2.04^{a}
70	39.60±0.68^{a}	42.20±1.32^{a}	42.00±0.55^{a}	42.4±1.69^{a}	0.00±0.00	42.00±1.08^{a}
77	41.20±0.49^{c}	42.80±2.06^{bc}	45.00±0.45^{ab}	42.4±1.33^{bc}	0.00±0.00	46.50±0.65^{a}

Different superscript in a row indicates significant difference between the group mean at (P<0.05). *Treatment day.

Table 5

Mean haemoglobin concentration (g/dL) of rats infected with *T. brucei brucei* and treated with diminazene aceturate alone, or in combination with either levamisole or vitamin C or both.

Days	Group A	Group B	Group C	Group D	Group E	Group F
0	13.52±0.32^{bc}	13.36±0.52^{c}	14.30±0.28^{abc}	15.26±0.38^{a}	15.10±0.80^{a}	14.90±0.26^{ab}
7*	15.66±0.91^{a}	16.44±0.36^{a}	13.88±0.88^{a}	13.84±1.01^{a}	14.16±0.89^{a}	14.83±0.32^{a}
14	11.60±0.58^{ab}	14.14±0.96^{a}	12.68±1.35^{a}	13.02±0.91^{a}	9.64±0.79^{b}	13.98±0.39^{a}
21	12.76±0.83^{b}	13.18±0.49^{b}	12.28±0.67^{b}	11.46±0.39^{b}	9.48±0.68^{c}	15.65±0.93^{a}
28	11.62±0.71^{a}	13.52±0.32^{a}	13.52±0.51^{a}	13.46±1.24^{a}	9.26±0.64^{b}	13.43±0.83^{a}
35	15.46±1.51^{b}	13.64±0.85^{ab}	13.70±0.96^{ab}	11.62±1.07^{a}	0.00±0.00	11.95±0.23^{a}
42	12.94±0.41^{a}	12.14±0.16^{a}	13.14±0.63^{a}	12.44±0.46^{a}	0.00±0.00	13.33±0.32^{a}
49	11.52±0.25^{a}	11.62±0.77^{a}	12.06±0.54^{ab}	13.60±0.78^{b}	0.00±0.00	11.78±0.14^{a}
56	14.04±0.56^{a}	12.86±0.50^{a}	12.18±0.54^{a}	13.60±0.85^{a}	0.00±0.00	13.60±0.78^{a}
63	12.36±0.51^{ab}	13.76±1.14^{a}	11.84±0.30^{b}	11.18±0.58^{b}	0.00±0.00	13.70±0.10^{a}
70	12.12±0.78^{a}	12.50±0.64^{a}	13.30±0.54^{a}	12.28±0.79^{a}	0.00±0.00	13.78±0.24^{a}
77	13.40±0.41^{a}	12.18±0.55^{a}	13.72±0.37^{a}	12.78±0.76^{a}	0.00±0.00	13.15±0.45^{a}

Different superscript in a row indicates significant difference between the group mean at (P<0.05). *Treatment day.

3.6. Total leucocyte count (TLC)

There was a significantly lower TLC ($P<0.05$) in groups B and D at day 7 PT when compared with the uninfected untreated and other infected groups (Table 6). The significantly lower TLC ($P<0.05$) seen in the infected untreated group at day 14 PT when compared to other groups continued till the death of all rats in that group. Apart from the significantly higher ($P<0.05$) TLC in group B at day 21 PT when compared to other groups and the uninfected control, the TLC of all the treated groups were not significantly different ($P>0.05$) with the control from day 28 PT till the end of the experiment.

3.6.1. Absolute lymphocyte count (ALC)

There was a significantly higher ALC ($P<0.05$) in group B than in other infected groups and the uninfected untreated group at day 7 PI (Table 7). By day 14 PI there was a significantly lower ALC ($P<0.05$) in group B than other infected groups and the uninfected untreated group. By 21 days PI, there was a significantly lower ($P<0.05$) ALC in group E than other treated groups and the uninfected control. This decrease was observed till all the rats in group E died. There was also a significantly lower ALC ($P<0.05$) in group D than the uninfected untreated group. By day 56 post treatment all treated groups were not significantly different ($P>0.05$) with the uninfected untreated group.

3.6.2. Absolute neutrophil count (ANC)

There was a significantly lower ANC ($P<0.05$) in group C at day 7 when compared to group B and the uninfected and untreated group F (Table 8). At day 14 (7 days PT), there was a significantly lower ($P<0.05$) ANC in all the infected groups than the uninfected control group F. By day 21, the ANC of the untreated control (group E) was significantly lower ($P<0.05$) than all the untreated groups (A–D) and the uninfected control. At day 35, there was a significantly lower ANC ($P<0.05$) in groups A and C than other treated groups and the uninfected control. By day 42, the ANC of group B was significantly higher ($P<0.05$) than other treated groups and the control. It was however observed that rats in group A had a significantly lower ANC ($P<0.05$) than the other treated

Table 6

Mean total leukocyte count ($\times10^3$/mm^3) of rats infected with *T. brucei brucei* and treated with diminazene aceturate alone, or in combination with either levamisole or vitamin C or both.

Days	Group A	Group B	Group C	Group D	Group E	Group F
0	15.96±1.95^a	15.34±1.85^a	10.61±0.57^a	13.13±1.96^a	11.70±1.72^a	11.03±1.02^a
7*	13.30±1.44^b	17.38±1.13^c	9.04±0.76^a	10.09±1.57^{ab}	10.12±1.36^{ab}	12.73±1.17^{ab}
14	10.08±0.44^{ab}	7.04±0.57^c	8.10±0.88^{bc}	7.15±1.05^c	8.04±1.12^{bc}	12.38±0.94^a
21	11.70±2.11^a	15.21±2.26^a	12.97±1.12^a	13.93±1.68^a	4.79±0.49^b	13.59±1.56^a
28	10.79±1.34^a	17.86±1.67^b	11.76±1.19^a	11.37±1.60^a	3.67±0.11^c	10.68±2.66^a
35	10.43±0.52^b	12.25±0.69^{ab}	10.85±0.10^b	10.37±0.86^b	0.00±0.00	13.81±0.96^a
42	9.30±0.98^b	12.52±1.09^{ab}	14.67±2.68^a	9.38±1.66^b	0.00±0.00	12.30±1.15^{ab}
49	13.15±2.28^a	12.23±2.12^a	12.52±2.27^a	8.99±0.70^a	0.00±0.00	13.03±1.55^a
56	12.25±1.63^a	11.91±1.62^a	11.51±1.46^a	11.89±1.26^a	0.00±0.00	10.63±2.53^a
63	13.25±0.78^b	12.23±1.31^{ab}	10.54±0.24^b	11.38±0.30^{ab}	0.00±0.00	12.00±0.99^a
70	10.60±1.24^a	12.30±1.41^a	11.84±1.38^a	10.66±0.96^a	0.00±0.00	13.16±0.33^a
77	8.40±0.67^b	11.82±0.73^a	10.84±1.27^a	11.11±0.59^a	0.00±0.00	12.50±0.66^a

Different superscript in a row indicates significant difference between the group mean at ($P<0.05$). *Treatment day.

Table 7

Mean absolute lymphocyte count ($\times10^3$/mm^3) of rats infected with *T. brucei brucei* and treated with diminazene aceturate alone, or in combination with either levamisole or vitamin C or both.

Days	Group A	Group B	Group C	Group D	Group E	Group F
0	8.29±2.33^a	7.00±0.84^a	6.17±0.97^a	5.43±1.93^a	5.24±0.57^a	4.67±1.25^a
7*	7.46±1.53^{ab}	9.31±0.83^b	4.73±0.81^a	4.94±1.38^a	5.19±0.83^a	5.63±0.44^a
14	6.56±0.62^a	2.95±0.26^c	4.87±0.61^{ab}	3.93±0.66^{bc}	3.95±0.52^{bc}	5.30±0.73^{ab}
21	6.57±2.30^{ab}	7.23±2.16^{ab}	7.69±0.97^a	7.92±1.19^a	2.74±0.48^b	5.75±0.55^{ab}
28	6.85±1.08^a	8.16±1.51^a	8.16±1.08^a	7.31±1.07^a	1.48±0.08^b	6.07±1.81^a
35	7.45±0.44^{ab}	6.49±0.26^{ab}	7.85±0.40^{ab}	6.17±0.86^b	0.00±0.00	8.10±1.11^a
42	6.15±1.12^{ab}	5.19±0.66^b	9.07±1.62^a	4.65±1.28^b	0.00±0.00	6.77±1.09^{ab}
49	9.72±1.88^a	8.62±2.32^{ab}	7.07±1.89^{ab}	4.67±0.47^{bc}	0.00±0.00	6.88±0.84^{ab}
56	9.05±0.96^a	7.81±1.44^a	7.72±1.40^a	7.63±0.97^a	0.00±0.00	6.05±1.44^a
63	8.92±1.41^a	6.82±0.74^a	6.66±0.44^a	6.74±0.51^a	0.00±0.00	6.56±1.24^a
70	6.98±1.05^a	6.71±0.61^a	7.49±1.30^a	6.29±0.90^a	0.00±0.00	6.70±0.79^a
77	5.94±0.90^a	7.63±0.53^a	7.96±1.21^a	7.16±0.41^a	0.00±0.00	6.73±1.07^a

Different superscript in a row indicates significant difference between the group mean at ($P<0.05$). *Treatment day.

Table 8

Mean absolute neutrophil count ($\times 10^3/mm^3$) of rats infected with *T. brucei brucei* and treated with diminazene aceturate alone, or in combination with either levamisole or vitamin C or both.

Days	Group A	Group B	Group C	Group D	Group E	Group F
0	6.55±1.45	6.90±1.38	3.25±0.62	6.19±1.65	5.27±1.50	4.92±0.68
7*	4.75±0.67^{bc}	6.94±0.81^{a}	3.91±0.42^{c}	4.67±0.66^{bc}	4.63±0.72^{bc}	6.63±0.70^{ab}
14	3.24±0.40^{b}	3.89±0.39^{b}	2.84±0.51^{b}	3.10±0.41^{b}	3.87±0.73^{b}	6.60±0.44^{a}
21	4.99±1.72^{ab}	7.72±0.85^{a}	4.83±0.23^{ab}	5.92±0.94^{a}	1.97±0.12^{b}	7.56±1.73^{a}
28	3.75±0.50^{a}	9.28±1.32^{b}	3.46±0.65^{a}	4.33±0.63^{a}	2.14±0.03^{a}	4.51±0.93^{a}
35	2.71±0.42^{c}	5.26±0.43^{a}	2.88±0.42^{bc}	4.11±0.70^{ab}	0.00±0.00	5.02±0.32^{a}
42	2.93±0.36^{bc}	7.33±0.70^{d}	5.30±1.17^{a}	4.54±0.70^{ab}	0.00±0.00	5.05±0.24^{a}
49	3.22±0.83^{ab}	3.43±0.37^{ab}	5.20±1.44^{a}	4.17±0.39^{ab}	0.00±0.00	5.64±1.06^{a}
56	2.99±0.78^{a}	3.84±0.60^{a}	3.47±0.55^{a}	4.05±0.41^{a}	0.00±0.00	4.45±1.17^{a}
63	3.98±0.94^{a}	5.14±0.65^{a}	3.57±0.27^{a}	4.27±0.35^{a}	0.00±0.00	4.66±0.63^{a}
70	3.09±0.26^{c}	5.19±0.80^{ab}	4.10±0.69^{bc}	3.98±0.49^{bc}	0.00±0.00	5.91±0.79^{a}
77	2.19±0.40^{c}	3.91±0.61^{ab}	2.73±0.53^{bc}	3.73±0.59^{bc}	0.00±0.00	5.30±0.68^{a}

Different superscript in a row indicates significant difference between the group mean at ($P<0.05$). *Treatment day.

groups and uninfected control group at days 70 to 77 of the experiment.

4. Discussion

Experimental infection of albino rats with *T. brucei brucei* was successful. The parasites were seen in the blood of infected rats from the 4th day PI with an average prepatent period of 5 days. The parasitaemia, which increased progressively, lead to death of all rats in the infected untreated group between 25 and 32 days PI. Parasitaemia in susceptible animals may be influenced by the number of parasite inoculated, stressors such as nutrition/starvation, intercurrent infections, host immune competence and the pathogenicity of the strain of *T. brucei brucei*[22]. The prepatent period of 5 days contrasts with the findings of Sewell and Brocklesby[23] and Anene *et al*[24] where a prepatent period of 3–4 d was reported. However, the findings agree with reports of Ezeokonkwo and Agu[25] in rabbits, Seifert[26] and Anene *et al*[27] in dogs and Anene *et al*[24] in rats. Following treatment, all the treated groups (A, B, C and D) became aparastaemic 48 h post treatment and remained so throughout the end of the experiment. However, in group D parasites cleared in 40% of the rats 24 h PT and by 48 hours PT all the parasites cleared. In other treated groups, parasites cleared 48 hours PT. No relapse infection was recorded in all the treatment groups. This contrasts with the finding of Anene *et al*[24] who recorded relapse infection by day 42 PI in rats treated with DA; but agrees with the findings of Rani and Suresh[28] who treated trypanosomosis in Pomeranian dog with a single dose of DA. It is likely that early treatment (between 3 and 7 days) after infection usually lead to permanent cure whereas late treatment (day 14 or later) PI lead to relapse. Thus, relapse infection observed with early treatment may therefore be due to drug resistance while relapse in late treatment could be due to reappearance of parasites, which may have been sequestered in the brain and therefore were inaccessible to the drugs[29]. Treatment in this work was done on day 7 post infection (early treatment) and thus could be the reason why no relapse infection was recorded all through the observation period.

The clinical signs of anorexia, pale mucous membrane, pyrexia, rough hair coat, dullness, depression, emaciation, swollen face and abdomen observed in the infected rats, were similar to those in mice, dogs and rabbits infected with *T. brucei brucei*[27,30] and in cattle infected with *Trypanosoma congolense*[31]. It is also consistent with the reports of Onyeyili and Anika[32]; Biryomumaisho *et al*[32]; Obidike *et al*[34] and Eze *et al*[35]. Following treatment, the clinical signs gradually disappeared showing that DA as well as the combination treatments was effective in reversing these signs.

Pyrexia, observed between day 7 and 14 of the experiment, is a recognized clinical symptom of trypanosomosis in animals[27,35,36]. This is due to stimulation of the thermoregulatory centre of the hypothalamus by pyrogens released during infection[37]. The treatment given reversed the pyrexia in the treated rats, hence, the return of normal temperature values from day 21 PT as seen in this experiment.

The significant decrease ($P<0.05$) in proportional weight changes of rats observed in the infected untreated group (Group E) is in accordance with other reports that trypanosomosis cause loss of weight[36]. Reduction in weight gain had been reported by Holmes *et al*[38] and is thought to be associated with anorexia and dullness seen with the infection. However, following treatment, there was a gradual increase in the mean proportional weight changes in the treated groups when compared with the uninfected and infected controls. Interestingly, rats in group C and D (treated with DA and vitamin C; and DA, levamisole and vitamin C respectively) showed an overall weight gain which was marked in group D, while Group B (treated with DA and levamisole) showed a significant decrease ($P<0.05$) which did not last long in the course of the experiment. Evidently, combining DA with levamisole and vitamin C gave the best results in terms of weight gain. This may be useful during

convalescence.

Anaemia, reportedly the most prominent feature of animal trypanosomosis[22,39] was seen as a significant reduction ($P<0.05$) in the mean PCV and Hb values of all infected groups by days 7 and 14 PI respectively. The fall in PCV as observed in this study is consistent with the findings of Ezeokonkwo[25], Obidike *et al*[33] and Anene *et al*[24]. Many factors have been reported in the literature to be responsible for these reductions in the packed cell volume, Haemoglobin concentration and total red blood cell count in trypanosomosis of livestock. The factors include a depression of erythropoiesis[38], immunological mechanisms and erythrophagocytosis, increased plasma volume and haemodilution[22] as well as disorders of coagulation[41]. The severity of anaemia has been shown to depend on the level and duration of parasitaemia[42]. By 14th day PT, all the treated groups recovered from the anaemia that resulted from the infection and their PCV and Hb concentration values compared favourably with that of the uninfected control except for Group A which still showed a significantly lower PCV ($P<0.05$) values than other treated groups and the infected untreated group. It could be seen therefore, that the PCV of groups that received drug combinations were not significantly different with the uninfected control group than Group A (DA alone), with Group B showing the best return to normal values.

Reduction in the TLC (leucopenia) in the infected untreated implies that *T. brucei brucei* had an immunosuppressive effect on the infected untreated rats leading to an impaired immune defence mechanism and death of all the rats in that group within 32 days of infection. Leucopenia as observed in this work agrees with the finding of Ezeh *et al*[36] and contrasted with Akpa *et al*[29] who observed leucocytosis in dogs infected with *T. brucei brucei*. The observed leucocytosis in group B by 7 days PI could not be explained. However, since this trend did not continue subsequently, it may be due to individual difference. The increase in TLC was very marked in Groups B and D by day 14 PT when compared with the other treated groups. This could be as a result of the effect of levamisole which was given to these two groups. Kumar *et al*[43] reported that the immunostimulatory activity of levamisole is due to its ability to effect the maturation of leukocyte and stimulation of T-cell differentiation. Singla *et al*[44] also reported that an individual animal's response to levamisole is dependent on the dose, timing and duration of administration as well as the animal's immune status. The early leucopenia observed in this study may be attributed to lymphopenia and neutropenia in the infected groups (A, C, D and E) with exception of Group B where lymphocytosis was recorded. Abenga *et al*[45] observed early leucopenia due to concomitant lymphopenia and neutropenia in Zebu cattle infected with *Trypanosoma vivax*. The lymphopenia and neutropenia noticed in the infected groups (A, C, D and E) returned to normal 14 days post treatment when they showed significant increases ($P<0.05$) than the uninfected untreated group. Though the mean lymphocyte counts of groups C and D significantly increased ($P<0.05$), the mean neutrophil counts of groups B and D were not significantly different ($P>0.05$) with the uninfected control.

In conclusion, early treatment of trypanosomosis using DA alone, and the combinations of either levamisole and or vitamin C was able to effectively clear the parasites from the blood of infected rats and also prevent relapse. Furthermore, the indices of anaemia and cellular response were better in rats treated with the drug combinations. Even so, the cost benefit of such combination therapy should be weighed against the value placed on such animals to be recommended for use, given the rising incidence of repeat treatments due to relapse infection.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Tabel H, Wei G, Shi M. T cells and immunopathogenesis of experimental African trypanosomiasis. *Immunol Rev* 2008; **225**: 128–139.

[2] Omotainse SO, Edeghere H, Omoogun GA, Elhassan EO, Thompson G, Igwe CA, et al. The prevalence of animal trypanosomosis in Konshisha local government area of Benue State, Nigeria. *Is J Vet Med* 2000; **55**(4): 142–144.

[3] Mhalanga JD. Sleeping sickness: Perspectives in African trypanosomosis. *Sci Prog* 1996; **79**(3): 83–214.

[4] Askoy S, Maudlin I, Dale C, Robinson AS, O'Neill SL. Prospects for the control of African ttrypanosomosis by tsetse vector manipulation. *Trends Parasitol* 2001; **17**(1): 29–35.

[5] Uilenberg G. *A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis*. Rome: Food and Agriculture Organisation of the United Nations; 1998.

[6] Anene BM, Onah DN, Nawa Y. Drug resistance in pathogenic African Trypanosomes: What hopes for the future? *Vet Parasitol* 2001; **96**: 83–100.

[7] Ezeokonkwo RC, Kpogban EA, Ogbodo OM, Iheagwam CN, Eseh IO. Efficacy of Samorecide-plus in the treatment of experimental *Trypanosoma brucei brucei* infection of albino rats. *Nig J Exp Appl Biol* 2010; **10**(2): 157–163.

[8] Ezeokonkwo RC, Okoro FC, Eseh IO. The efficacy of increasing doses of Samorenil in the treatment of *Trypanosoma brucei* infected albino rats. *Nig Vet J* 2007; **28**(2): 24–32.

[9] Chitanga S, Marcotty T, Namangala B, Van den Bossche P, Van den Abeesche J, Delespaux V. High prevalence of drug resistance in animal trypanosomes without a history of drug exposure. *PLoS Negl Trop Dis* 2011; **5**(12): e1454.

[10] Jennings FW, Atouguia JM, Murray M. Topical chemotherapy for experimental murine African CNS-trypanosomiasis: the successful use of the arsenical, melasorprol, combined with the 5-nitroimidazoles, fexinidazole or MK-436. *Trop Med Int Health* 1996; **1**(5): 590–598.

[11] Anene BM, Anika SM, Chukwu CC. Effect of DFMO after

intravenous administration and its combination with Diminazene aceturate against *Trypanosoma brucei* experimentally infected Dog in Nigeria. *Rev Elev Med Vet pays Trop* 1997; 50: 221–225.

[12]Brown CGD, Hunter AG, Luckin AG. Disease caused by protozoa. In: Sewell MMH, Brocklesby DW(Eds). *Handbook of animal disease in the tropics*. 4th ed. London: Bailliere Tindall; 1990, p. 161–226.

[13]Greeta S, Holmes PH, Diall O, Eisler MC. African Bovine trypanosomosis. The problem of drug resistance. *Trends Parasitol* 2001; 17: 25–28.

[14]Logan LL, Goodwin JT, Tembely S, Craig TM. Maintaining Zebu maure cattle in a tsetse infested area of Mali. *Trop Anim Health Prod* 1984; 16(1): 1–12.

[15]Apted FIC. Treatment of human trypanosomiasis. In: Mulligan HW, Potts WH(Eds). *The African Trypanosomiasis*. London: George Allen and Unwin; 1970, p. 684–710.

[16]Wilkes JM, Peregrine AS, Zilbestein D. The accumulation and compartmentalisation of isometamidium chloride in *Trypanosoma congolense* monitored by its intrinsic fluorescence. *Biochem J* 1995; 312: 319–329.

[17]Soulsby EJL. *Helminths, arthropods and protozoa of domesticated animals*. 7th ed. London: Bailliere Tindall; 1982.

[18]Rickman LR, Robson J. The blood incubation infectivity test: a simple test which may distinguish *Trypanosma brucei* from *T. rhodesiense*. *Bull Wld Hlth Org* 1970; 42: 650–651.

[19]Coles EH. *Veterinary clinical pathology*. 3rd ed. Philadelphia: W.B. Saunders 1986; p.145–151.

[20]Schalm OW, Jain NC, Carol EJ. *Veterinary haematology*. 3rd Edition. Philadelphia: Hea and Fehiger; 1975.

[21]Duncan OD. Path analysis: sociological examples. *Am J Sociol* 1966; 72: 1–16.

[22]Taylor K, Authie EM. Pathogenesis of animal trypanosomosis. In: Maudlin I, Holmes PH, Miles MA.(Eds). *The Trypanosomiasis*. United Kingdon: CAB International; 2004, p. 331–353.

[23]Sewall MMH, Brocklesby DW. *Handbook of animal diseases in the tropics*. 4th Ed. London: Bailliere Tindall; 1990.

[24]Anene BM, Ezeokonkwo RC, Mmesirionye TI, Tettey JNA, Brock JM, Barret MP, et al. A diminazene-Resistant strain of *Trypanosoma brucei* isolated from a dog in cross resistant to Pentamidium in experimentally infected albino rats. *Parasitol* 2006; 132: 127–133.

[25]Ezeokonkwo RC, Agu WE. Experimental infection of domestic rabbits (Oryctologus cuniculus) with *Trypanosoma brucei* and *Trypanosoma congolense*: A comparative study. *Nig J Anim Prod* 2004; 31(1): 100–111.

[26]Seifert HSH. *Tropical animal health*. 2nd Ed. Netherlands: Kluwer Academic Publishers; 1996.

[27]Anene BM, Ogbuanya CE, Mbah ES, Ezeokonkwo RC. Preliminary efficacy trial of cymelarsen in dogs and mice artificially infected with *Trypanosoma brucei* isolated from dogs in Nigeria. *Rev Elev Med Vet Pays Trop* 1999; 52(2): 123–128.

[28]Rani NL, Suresh K. Canine trypanosomiasis. *Ind Vet J* 2007; 84: 186–187.

[29]Akpa PO, Ezeokonkwo RC, Eze CA, Anene BM. Comparative efficacy assessment of pentamidine isethionate and diminazene aceturate in the chemotherapy of *Trypanosoma brucei* infection in dogs. *Vet Parasitol* 2008; 151(2–4): 139–149.

[30]Abenga JN, Ezebuiro CO, David K, Fajinmi AO, Samdi S. Studies on anaemia in Nigerian local puppies infected with *Trypanosoma congolense*. *Vet Arhiv* 2005; 75(2) 165–174.

[31]Mbaya AW, Aliyu MM, Gamba B. The effects of single and combined chemotherapy of DL-a-difluoromethylornithine and diminazene aceturate in experimental *Trypanosoma brucei* gambiense infection in mice. *Nig Vet J* 2008; 29(1): 49–56.

[32]Biryomumaisho S, Eli Katunguka-Rwakishya, Rubaire-Akiiki CM. Serum biochemical changes in experimental *T. congolense* and *T. brucei* infection in small East African goats. *Vet Arhiv* 2003; 73(3): 167–180.

[33]Obidike IR, Aka L, Momah CV, Ezeokonkwo RC. Effect of *Trypanosoma brucei* infections and diminazene aceturate treatment on serum activities of certain enzymes. *Sahel J Vet Sc* 2005; 4(1): 17–23.

[34]Eze CA, Ezeokonkwo RC, Egbuji JO, Anene BM. Effect of castration on the establishment and treatment of trypanosomosis with diminazene aceturate in albino rats. *Nig J Exp Appl Biol* 2006; 7(1): 17–21.

[35]Stephen LE. *Trypanosomosis: A veterinary perspective*. United Kingdom: Pergamon Press; 1986.

[36]Ezeh IO, Agbo LI, Emchelu CO, Nweze EN, Ezeokonkwo RC, Onah DN. Berenil-resistant *Trypanosoma brucei brucei* infection in a dog in Nsukka area of Enugu State, Nigeria. *Nig Vet J* 2009; 29(4): 34–42.

[37]Dinarello CA. Infection, fever, and exogenous and endogenous pyrogen: some concepts have changed. *J Endotox Res* 2004; 10(4): 201–222.

[38]Holmes PH, Katunguka-Rwakishaya E, Bennison JJ, Wassink GJ, Parkins JJ. Impact of nutrition on the pathophysiology of bovine trypanosomiasis. *Parasitol* 2000; 120: 373–385.

[39]Anosa VO, Logan-Henfreg LL, Wells CW. The haematology of *Trypanosoma congolense* infection in cattle. 1. Sequential cytomorphological changes in the blood and bone marrow of Boran cattle. *Comp Haematol Int* 1997; 7: 14–22.

[40]Kagira JM, Thuita JK, Ngotho M, Mdachi R, Mwangangi DM, Ndung'u JM. Haematology of experimental *Trypanosoma brucei* rhodesiense infection in Vervet monkeys. *Afr J Health Sci* 2006; 13: 59–65.

[41]Murray M, Dexter TM. Anaemia in bovine African trypanosomosis. *Acta Trop* 1998; 45: 389–432.

[42]Mbaya AW, Aliyu MM, Nwosu CO, Egbe-Nwinyi T. The relationship between parasitaemia and anaemia in concurrent *Trypanosoma brucei* and *Haemonchus contortus* infection in red fronted gazelles (Gazella rufifrons). *Vet Arhiv* 2009; 79(5): 451–460.

[43]Kumar S, Dewey CE, Friendship RM, Bowland SL, Shewen PE. Improved weight gain in pigs using levamisole as an immunomodulator. *Swine Health Prod* 1999; 7(3): 103–107.

[44]Singla LD, Juyal PD. Blood cellular responses in *Trypanosoma evansi* infected and Levamisol treated bovine calves. *J Vet Parasitol* 2000; 14(2): 137–139.

[45]Abenga JN, David KM, Ezebuiro CO, Samdi S, Fajinmi AO. Leucocyte and thrombocyte changes in young dogs infected with *Trypanosoma congolense*. *J Protozool Res* 2004; 14(1–2): 8–15.