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A comparative study of the chemotherapeutic effects of diminazene aceturate and Ivermectin on *Trypanosoma brucei brucei* infected rats

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ABSTRACT

Objective: To investigate the comparative effect of diminazene aceturate (DA) or ivermectin in albino rats experimentally infected with *Trypanosoma brucei brucei*.

Methods: A total of 21 adult male albino rats were divided into three groups consisting 7 albino rats each and all the members of the groups were infected intraperitoneally with 6.3×10^6 trypanosomes in infected mouse blood diluted with normal saline. By 7 days, post-infection when parasitaemia was fully established and Groups A and C were treated with DA and ivermectin respectively, while Group B served as the control (untreated). Parameters assessed included rectal temperature, body weight changes, packed cell volume, total leucocyte counts, differential leucocyte counts and parasitaemia.

Results: The results showed that following the treatment with DA and ivermectin at the peak of parasitaemia, the ivermectin in treated group remained parasitaemic till the end of the experiment. The survivability of ivermectin treated group was longer than those of the control group. DA on the other hand was able to effect a complete plasma clearance of the parasites within 48 h post-treatment at a dose of 3.5 mg/kg body weight. In the untreated control group, parasitaemia peaked on Day 7 post-infection, dropped transiently on Day 28 post-infection and peaked again with the second wave of parasitaemia showing no remission until the end of the experiment.

Conclusions: It was concluded from the results of this present study that DA has a better efficacy than ivermectin which has no chemotherapeutic effect against *Trypanosoma brucei brucei* infection. The efficacy of DA is on the decline because of drug resistance and incidence of relapse, therefore a search for effective alternative chemotherapy or drug combinations should be encouraged.

1. Introduction

High incidence of infectious diseases constitutes a major constraint to livestock production in most developing countries[1]. Parasitic infections are of great worldwide significance[2]. African trypanosomiasis which is also called nagana disease is an infectious disease of humans and animals of similar aetiology and epidemiology[3].

The etiologic agents of the disease are protozoan parasites of the genus *Trypanosoma* that live and multiply extracellularly in blood and tissue fluids of their mammalian hosts and are transmitted by the bite of infected tsetse flies of the *Glossina* species[3]. It produces the following clinical signs of pyrexia, apathy, anaemia

and corneal opacity[4].

African trypanosomes are protozoan parasites responsible for both animal and human trypanosomiasis. The disease is fatal if left untreated and chemotherapy which is the major means of control in Africa is faced with problems of toxicity and the ever increasing incidence of resistance[5-7]. Animal trypanosomiasis continues to constitute a major threat to food security in several parts of sub-Saharan Africa including Nigeria where it is a menace in the livestock industry despite the length of years puts in an attempt to control the disease[8-11]. Diminazene aceturate (DA) commonly known as berenil, isometamidium chloride commonly known as trypamidium, homidium salt (ethidium), cymelarsan and suramin are the drugs commonly used for the treatment of African animal trypanosomiasis. Of these drugs, DA is the most commonly used therapeutic agent[12,13]. While ivermectin, a macrolide antibiotic produced from a fungus *Streptomyces avermitilis* and as a broad spectrum antiparasitic agent has also been previously reported, and its potent effects

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has been clearly demonstrated using different hosts, doses and methods of administration[14,15].

Despite increases in the incidence of many parasitic infections in recent years, the number of studies designed to improve the treatment of these infections have not been able to address the situation[2]. The burgeoning problem of resistance to effective antiparasitic agents which are in use in the last decade has added urgency to the need to discover new antiparasitic agents and to make better use of existing ones[2]. In the light of the above problem of undue resistance by trypanosomes to trypanocides coupled with the mechanism of relapse, this study was undertaken to compare the chemotherapeutic effect of DA and ivermectin on *Trypanosoma brucei brucei* (*T. brucei brucei*) infection.

2. Materials and methods

2.1. Experimental animals

A total of 21 male albino rats of uncharacterized sexes were used in this study. The rats which weighed from 45 g to 105 g, were purchased from the breeding stock of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were fed with commercially prepared, standard rat feed (Vital feeds®) and given water *ad-libitum* throughout the duration of the experiment. The animals were allowed to acclimatize for 7 days pre-infection.

2.2. *T. brucei brucei*

The strain of trypanosome used for this study was initially isolated from a naturally infected dog presented at the Veterinary Teaching Hospital, University of Nigeria, Nsukka. It was identified as *T. brucei brucei* based on morphological characteristics as described by Soulsby[16].

2.3. Research methods

A drop of blood was aseptically collected from the tail vein of the albino rats and used for the preparation of a wet mount using methods described by Herbert and Lumsden[17].

2.4. Experimental design

2.4.1. Infection of experimental animals

The male albino rats were divided randomly into 3 groups of 7 each. They were then infected intraperitoneally with 6.3×10^6 trypanosomes in normal saline diluted infected blood of mice. By 7 days, post-infection (PI) when parasitaemia was fully established and they were divided into three groups (A, B and C) consisting of 7 albino rats each. Groups A and C were treated with DA and ivermectin respectively while Group B served as the control (untreated). The blood collected from these albino rats was examined daily PI and post-treatment to establish the onset of parasitaemia and parasite clearance time.

2.4.2. Estimation and monitoring of parasitaemia

The degree of parasitaemia in the infected blood of mice was estimated using standard procedures used in performing wet mount technique as described by Herbert and Lumsden[17] and microhematocrit buffy coat microscopy as described by Murray et al.[18].

2.4.3. Administration of DA and ivermectin

All animals in Group A were treated with DA at a dose of 3.5 mg/kg body weight as a single intraperitoneal injection. Also, all animals in Group C were also treated with ivermectin at the approved dose rate of 0.2 mg/kg body weight intraperitoneally as a single injection.

2.4.4. Haematological studies

Blood samples were collected from each albino rat through the tail vein. This was done by gradually massaging the snipped tails of the rats into 21 different sterile vials containing anticoagulant, ethylene diamine tetraacetic acid for haematological analysis. The following indices were determined using routine laboratory methods. Packed cell volume (PCV) was used as described by Schalm et al.[19-21]. Leucocytes counts were determined by the method described by Jain[22], while differential count was determined as described by Schalm et al.[19]. The determination of haematological parameters was done pre-infection, at the peak of parasitaemia and post-treatment.

2.5. Statistical analysis

The collected data were subjected to descriptive statistical analysis to obtain mean and mean \pm SD. Differences between treatment group's changes overtime were determined using the Student's *t*-test. The *P* values less than 0.05 and 0.01 were considered statistically significant.

3. Results

3.1. Parasitaemia

Results of daily estimation of parasitaemia were presented in Figure 1. All infected animals showed detectable parasitaemia within 5 days PI. Parasitaemia increased rapidly in all groups and the groups attained the first peak of parasitaemia on 7 days post-infection. Barring drug effects, parasitaemia was sustained in Groups B and C till the death of all the animals in these two groups.

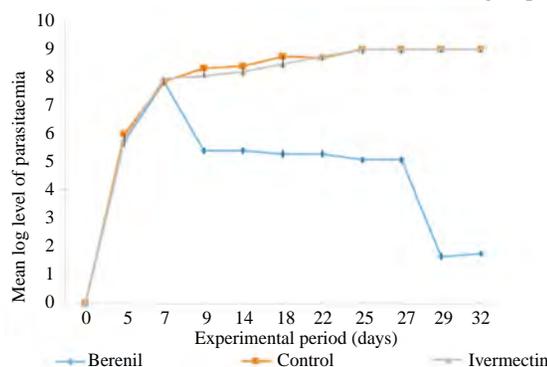


Figure 1. The level of the mean log of parasitaemia in rats experimentally infected with *T. brucei brucei* and treated with berenil or ivermectin.

3.2. Effects of drug treatments

Following the treatment of DA and ivermectin at the peak of parasitaemia as shown in Figure 1, the ivermectin in treated group remained parasitaemic till the end of the experiment. The ivermectin was able to depress the level of parasitaemia though it was an insignificant extent ($P > 0.05$). DA on the other hand was able to clear the infection within 48 h post-treatment at a dose of 3.5 mg/kg body weight. However, relapse was observed in two of the

treated groups on about 15 days post-treatment and the parasitaemia increased progressively till the end of the experiment. In the untreated control group, parasitaemia peaked on Day 7 PI, dropped transiently on Day 28 PI and peaked again with the second wave of parasitaemia showing no remission until the end of the experiment.

3.3. Effects on the PCV

A progressive fall in PCV was observed in all the groups on Day 7 PI and this was shown in Figure 2. The general trend in the PCV of the untreated group was that of progressive fall till the death of the rats except in albino rat labelled 5 where there was an increase on Day 18 and a decrease on Day 21 before the death of the animal. On Day 14 and 18 PI, the mean PCV values of DA in treated group, untreated control group and ivermectin treated group showed that there was significant difference ($P < 0.01$) and the same was observed on Day 21 PI, which was significant difference ($P < 0.05$).

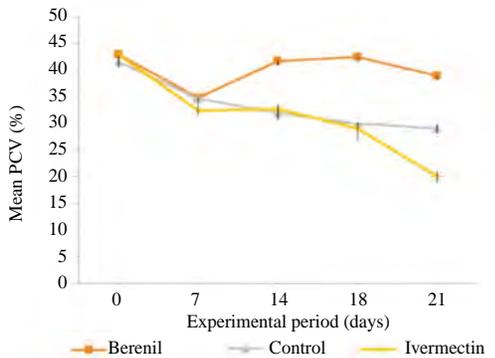


Figure 2. The changes in mean PCV of albino rats experimentally infected with *T. brucei brucei* and treated with berenil or ivermectin.

3.4. Effects on body weight

All treated animals in Group A showed a significant increase ($P < 0.05$) in overall body weight throughout the period of the experiment. The mean \pm SD of the body weight of the albino rats in Group A was increased continually, though a transient decrease in the SD was noticed on Day 14, which later peaked. The trend was also at the same for the ivermectin-treated group (Figure 3). The mean \pm SD of the body weight of the untreated group was significantly ($P < 0.05$) lower than those of the treated Groups A and B respectively (Figure 3).

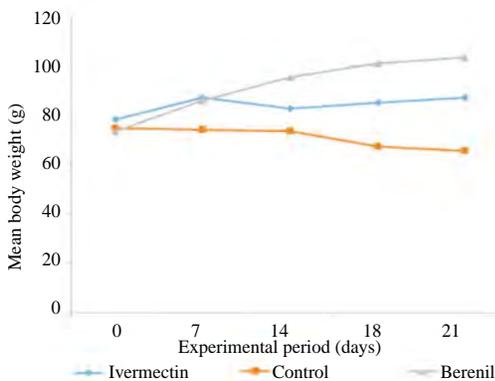


Figure 3. The changes in the mean body weight of albino rats experimentally infected with *T. brucei brucei* and treated with berenil or ivermectin.

3.5. Effects on body temperature

A progressive increase in temperature was observed in the

ivermectin treated group and in the untreated control group on Day 7 PI. The increase in temperature for these two groups was statistically significant ($P < 0.05$). But with the DA (berenil) treated group, there was an initial increase in temperature, pre-treatment but this was resolved back to normalcy following treatment with berenil (Figure 4). The decrease in temperature to normalcy in the berenil treated group was statistically significant ($P < 0.01$). Also the worthy of note was the fall in temperature in some animals below the normal value of 38 °C on Day 21 PI.

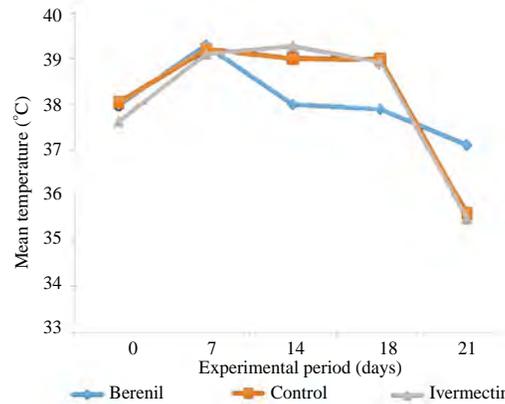


Figure 4. The changes in the mean rectal temperature of albino rats experimentally infected with *T. brucei brucei* and treated with berenil or ivermectin.

3.6. Effects on total white blood cell count

Following the establishment of parasitaemia, there was a general increase in the total leucocyte counts in all the groups. However, after treatment, there was a reversal back to normal for the DA treated group but this was not same for the ivermectin treated group and the control group (Table 1). The reversal to normalcy of the total leucocyte counts was statistically significant ($P < 0.01$).

Table 1

Changes in the total leucocyte counts of albino rats experimentally infected with *T. brucei brucei* and treated with Berenil® or ivermectin.

Experimental period (days)	Mean total leucocyte counts $\times 10^3$ cells/ μ L of blood with SDs		
	Berenil®	Control	Ivermectin
0	7.32 \pm 0.94	7.39 \pm 0.96	7.11 \pm 0.90
7	9.69 \pm 0.65	8.48 \pm 1.25	9.21 \pm 1.63
14	5.99 \pm 0.75	10.46 \pm 1.09	9.98 \pm 0.95
18	4.91 \pm 1.02	10.02 \pm 1.10	10.49 \pm 1.78
21	7.32 \pm 0.46	10.50 \pm 0.00	8.75 \pm 3.54

3.7. Effects on differential white blood cell counts

Changes in lymphocytes, neutrophils, monocytes, eosinophils and basophils were shown in Tables 2, 3, 4, 5 and 6 respectively. *T. brucei brucei* infection resulted in significant increase in the proportion of lymphocyte in all the groups until treatment began and these changes in leucocyte counts were statistically significant for both ($P < 0.01$) and ($P < 0.05$) for berenil and ivermectin groups respectively.

In the DA treated group, 71% of animals in this group showed reversal in lymphocyte number. In the ivermectin treated group, the treatment had little effect on the lymphocyte counts as only 14.1% showed a change in lymphocyte number. The increase in

lymphocyte number of the control group increased continually till all the animals died (Table 2).

Table 2

Changes in the differential percentage of lymphocyte in the blood of albino rats experimentally infected with *T. brucei brucei* and treated with Berenil® or ivermectin.

Experimental period (days)	Mean percentage of lymphocyte counts with SDs		
	Berenil®	Control	Ivermectin
0	62.14 ± 4.45	60.86 ± 3.13	59.00 ± 5.54
7	74.86 ± 6.96	71.29 ± 5.35	71.57 ± 9.62
14	53.00 ^a ± 16.15	76.00 ^b ± 11.33	74.67 ^b ± 6.12
18	61.71 ^a ± 8.12	74.67 ^c ± 3.51	72.75 ^c ± 3.77
21	65.00 ± 13.11	68.00 ± 0.00	65.00 ± 7.07

^{a,b,c}: Different superscripts in a row indicated significant difference between the designated means.

Drug treatment for the infection with *T. brucei brucei* resulted in proportional decrease and increase in neutrophils which reflected the increase and decrease observed in the percentage values of lymphocytes (Table 3). The percentage number of monocytes varied prior to infection and at the peak of parasitaemia, and the differences in monocytes numbers observed was minimal but following treatment with DA, the monocyte numbers returned back to normal. The level of variation between the three groups was statistically significant ($P < 0.05$) and ($P < 0.01$) respectively and as shown in Table 4. The number of monocytes following the treatment with ivermectin, the initial level was maintained on Day 14 PI but it began to increase till Day 18 before it started coming down. The control group had an initial increase in monocyte number continually till Day 14 before it started coming down on Day 18 PI. This increase was statistically significant ($P < 0.01$) and ($P < 0.05$) respectively.

Table 3

Changes in the differential percentage of neutrophils in the blood of albino rats experimentally infected with *T. brucei brucei* and treated with Berenil® or ivermectin.

Experimental period (days)	Mean percentage of neutrophils with SDs		
	Berenil®	Control	Ivermectin
0	32.43 ^{ab} ± 4.31	30.00 ^a ± 1.83	36.00 ^b ± 5.54
7	20.83 ± 5.74	22.29 ± 6.16	25.71 ± 7.54
14	30.14 ± 10.75	18.43 ± 10.72	18.83 ± 5.73
18	23.14 ± 9.14	20.33 ± 4.51	21.25 ± 3.69
21	25.57 ± 14.01	24.00 ± 0.00	24.00 ± 1.41

^{a,b}: Different superscripts in a row indicated significant difference between the designated means; ^{ab}: $P < 0.01$.

Table 4

Changes in the differential percentage of monocytes in the blood of albino rats experimentally infected with *T. brucei brucei* and treated with Berenil® or ivermectin.

Experimental period (days)	Mean percentage of monocytes with SDs		
	Berenil®	Control	Ivermectin
0	7.00 ± 1.91	5.86 ± 1.95	5.14 ± 2.12
7	4.00 ^a ± 1.29	2.29 ^a ± 1.11	3.43 ^{ab} ± 1.72
14	9.71 ^a ± 4.46	2.14 ^c ± 1.35	4.00 ^c ± 2.00
18	8.71 ^a ± 2.56	3.67 ^c ± 1.53	3.25 ^c ± 1.50
21	4.86 ± 1.95	4.00 ± 0.00	4.50 ± 0.71

^{a,b,c}: Different superscripts in a row indicated significant difference between the designated means; ^{ab}: $P < 0.05$.

T. brucei brucei infection also resulted in an increase in the number of circulating eosinophils, but following drug treatment, there was a reduction in the number of circulating eosinophils

with ivermectin treatment than with DA treatment. The pattern of eosinopenia in the control group was same with the ivermectin treated group. The increase before treatment and after treatment (Table 5) was statistically significant ($P < 0.01$) and the proportion of basophilia was constant in all the groups and only increased at the peak of parasitaemia. Following treatment with DA, it increased tremendously on Day 14 PI and started returning to normal on Day 18 PI and the normal value was achieved on Day 21 PI (Table 6). In summary, the clinical signs observed were depression, anaemia, pyrexia, anorexia and weight loss.

Table 5

Changes in the differential percentage of eosinophils in the blood of albino rats experimentally infected with *T. brucei brucei* and treated with Berenil® or ivermectin.

Experimental period (days)	Mean percentage of eosinophils with SDs		
	Berenil®	Control	Ivermectin
0	0.43 ^a ± 0.79	1.86 ^b ± 0.69	1.00 ^{ab} ± 0.82
7	4.00 ^a ± 1.29	2.29 ^a ± 1.11	3.43 ^{ab} ± 1.72
14	2.86 ± 1.86	1.71 ± 1.50	1.50 ± 1.64
18	2.43 ± 0.98	0.33 ^b ± 0.58	0.25 ^b ± 0.50
21	1.53 ± 1.13	0.00 ± 0.00	0.00 ± 0.00

^{a,b}: Different superscripts in a row indicated significant difference between the designated means; ^{ab}: $P < 0.01$.

Table 6

Changes in the differential percentage of basophils in the blood of albino rats experimentally infected with *T. brucei brucei* and treated with Berenil® or ivermectin.

Experimental period (days)	Mean percentage of basophils with SDs		
	Berenil®	Control	Ivermectin
0	0.14 ± 0.38	0.43 ± 0.53	0.42 ± 0.53
7	0.71 ± 0.76	1.29 ± 1.38	0.42 ± 0.53
14	4.29 ^a ± 2.75	2.00 ^{ab} ± 1.15	0.67 ^b ± 0.82
18	4.14 ^a ± 1.68	1.33 ^c ± 1.15	2.25 ^{ac} ± 0.50
21	0.86 ± 1.21	1.00 ± 0.00	1.50 ± 0.71

^{a,b,c}: Different superscripts in a row indicated significant difference between the designated means; ^{ab}: $P < 0.01$; ^{ac}: $P < 0.05$.

4. Discussion

Experimental infection of albino rats with *T. brucei brucei* was successful and the parasitaemia was observed from the 4th day PI, which agrees with the findings of Chekwube *et al.*[23]. The parasitaemia, which increased progressively, leads to death of all rats in the infected untreated group between 24 and 31 days PI.

This study showed that ivermectin, a versatile macrolide antibiotic had little or no effect in clearing the trypanosomes completely from peripheral blood, which disagrees with the findings of Udensi and Fagbenro-Beyioku[24]. DA was also not able to effect a permanent cure at a dose of 3.5 mg/kg body weight, which agrees with the report of Desquesnes and Gutiérrez[25].

The achievement of a parasitaemia, 48 h post-treatment of the infected rats with DA shows that DA achieves a comparatively very fast optimal therapeutic blood level activity within 24 h, which agrees with previous work by Brander and Pugh[26] and Chekwube *et al.*[23]. It has also been shown that the *T. brucei brucei* infection in albino rats, the trypanocidal activity of DA is due to the blockage of carbohydrate metabolism by inhibiting the glycolytic reaction in the trypanosomes and DNA intercalation which leads to the inactivation and subsequent removal of the trypanosomes from the blood stream[27]. This is done by immunological clearance which was largely accompanied by antibody mediated hepatic phagocytosis[28].

The occurrence of relapse on 15 days post-treatment as observed in the DA treated group agrees with similar findings by Chukwu *et al.*[29] and Anene *et al.*[30]. This was attributed to the fact that DA does not cross the blood brain barrier just like 95% of drugs used in chemotherapeutics, though in the work by Van den Berg *et al.*, they considered that the occurrence of relapse was not necessary as a result of presence of resistant strains[31-33]. The transient and insignificant drop of parasitaemia in the ivermectin treated group may not be attributed to the effect of drug treatment because a similar wave pattern was observed in the control untreated group. This agrees with the pattern of parasitaemia in the study by Bakhiet *et al.*[34] who considered the drop to be as a result of host immune response to the parasites. Losos and Ikede[35], and Anika *et al.*[36] also attributed this drop to be due to the movement of trypanosomes from peripheral blood into tissues and fluid of body cavities.

In the ivermectin treated group (Group C), there was insignificant change in the level of parasitaemia. However, a significant drop in mean PCV compared to the DA treated group ($P < 0.01$) was observed. This is an indication of severe anaemia which may have also been contributed to the death of all the animals in this group, which agrees with previous findings by Stephen[37].

Anaemia exhibited by the members of Groups B and C resulted from the deposition of immune complexes that interfered with or prevented the normal functioning of organ-system. Anaemia, reportedly the most prominent feature of animal trypanosomiasis was seen as a significant reduction ($P < 0.05$) in the mean PCV of all infected groups by days 7 and 14 PI respectively[38,39]. The fall in PCV as observed in this study was consistent with the findings of Rashid *et al.*[4], Ezeokunkwo and Agu[40], Obidike *et al.*[41], Amadi *et al.*[42], and Anosa[43]. Factors involved in the pathogenesis of anaemia in African trypanosomiasis include haemolysis, haemodilution, haemorrhages and dyshaematopoiesis[44]. The continuous increase in main body weight observed in all DA treated group agrees with the reports of Losos and Ikede[35] and Chekwube *et al.*[23], but Allam *et al.*[45] reported an increase in body weight of sheep and goats infected with *Trypanosoma vivax*, especially in cases of acute trypanosomiasis.

In this present study, there was an initial increase in the percentage of lymphocyte PI in all the groups followed by a sharp drop observed only in the DA treated group on Day 14 PI which also coincides with the mean neutrophils and PCV values dropped at the same time, which agrees with the findings of Sternberg and McGuigan[46]. By Day 14 post infection, there was significant ($P < 0.05$) increase in the percentage number of monocytes. The significant increase in the mean monocyte values agrees with the study of Ogunsanmi *et al.*[47] but was in contrast to the findings of Sternberg and McGuigan[46]. The latter recorded no significant changes in the mean monocyte count. According to the sharp fall in mean lymphocyte counts, *T. brucei brucei* infection also resulted in an increase in the number of circulating eosinophils, which agrees with the findings of Sternberg and McGuigan[46]. In the previous study by Ogunsanmi *et al.*, they attributed the increase in lymphocyte number to be due to the inhibitors of lymphocyte proliferation by the release of high level of nitric oxide produced by activated macrophages from *T. brucei brucei* infected albino rats[47].

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 sheep mice, dogs and rabbits infected with *T. brucei brucei*. It is

also consistent with the reports of Rashid *et al.*[4], Obidike *et al.*[41], Amadi *et al.*[42], Ezeh *et al.*[48] and, Rani and Suresh[49]. Following the treatment, the clinical signs gradually disappeared showing that DA was effective in reversing these clinical signs, which agrees with the findings of Rashid *et al.*[4] and Chekwube *et al.*[23].

There was pyrexia on Day 7 PI in all the groups and this increase in temperature was maintained in Groups B and C till the end of the experiment, which agrees with the findings of Chekwube *et al.*[23], Ezeokunkwo and Agu[40] and, Rani and Suresh[49]. This was due to the positive stimulation of the thermoregulatory center of the hypothalamus by pyrogens and this is in line with previous work done by Losos and Ikede[35].

It is evident from the results of this present study that DA has a better efficacy than ivermectin on treating *T. brucei brucei* infection, though this efficacy is on the decline because of drug resistance and relapse. Therefore, further search for alternative therapeutic drug or drug combinations that can cross the blood brain barrier to deal with the sequestration of *T. brucei brucei* in the brain that later lead to relapse and resurgence of *T. brucei brucei* parasitaemia should continue to provide such drugs that do not exert adverse toxicological effects.

Conflict of interest statement

We declare that we have no conflict of interest.

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