



Comparative Study of Diminazene Aceturate in Trypanosomiasis Therapy in Nigerian Local Dogs Using Intramuscular and Intravenous Routes of Administration

Olumuyiwa A. Adejumobi¹, Afusat J. Jubril², Jeremiah M. Afolabi¹, Temidayo O. Omobowale¹, James K. Olopade³

¹Department of Veterinary Medicine, University of Ibadan, Nigeria.

²Department of Veterinary Pathology, University of Ibadan, Nigeria.

³Department of Veterinary Anatomy, University of Ibadan, Nigeria.

ABSTRACT

Trypanosoma brucei infection is an important protozoan disease of dogs in tsetse fly infested areas of Africa. It is associated with great economic losses in dogs. Diminazine aceturate (DA) is commonly used in Nigeria for the management of protozoan diseases including trypanosomiasis. DA has however been found to have potential harmful effect on the liver, heart, kidney and brain. The need to evaluate the efficacy of DA administered intramuscularly and intravenously in acute infection and evaluate the biochemical and cardiovascular effect warranted this study.

In this study, we compared the treatment of single doses of DA intramuscular (i/m), and slow intravenous (i/v) administration. Sixteen local dogs (4-5 months) were used and grouped as Control (C), Trypanosome infested (untreated, T1), Trypanosome infested (i/m treated, T2), and Trypanosome infested (i/v treated, T3).

DA was administered to T2 and T3 eight days after inoculation and trypanosome parasitaemia had been established. Daily body weight and body temperature, haematological tests, serological tests, parasitaemia and electrocardiograms of the dogs were evaluated in each group.

Results show a steady rise in temperature after parasitaemia which resolved after treatment. There were significant increases in packed cell volume, mean corpuscular haemoglobin concentration, and creatinine, in the treated groups when compared with the control and untreated groups. There was significant increase in lymphocyte count in T2 compared to the control as well. There was a reduction in blood pressure of the infected untreated group. The electrocardiogram data showed significant increase in PR interval and QT interval in T1 and QTc intervals in T1, T2, and T3 across groups.

We conclude that, though both routes cleared the parasitaemia and the intravenous route was well tolerated, no summative comparative advantage was seen over the intramuscular routes in the parameters observed in this study. Further work is needed to examine if advantage can be observed in long term prevention of relapse by the intravenous route.

Key words:

words: *Trypanosoma brucei*,
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*Correspondence to:
mayenkola@yahoo.com

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1. INTRODUCTION

Trypanosomiasis, a disease of several vertebrates is caused by a monophyletic group of unicellular parasitic flagellate protozoa of the genus *Trypanosoma*

(Hamilton, 2004). It affects both humans and domestic animals and is transmitted mainly by blood feeding insects called tsetse flies (Kabayo, 2004).

The clinical signs in natural infection include progressive anaemia, leucocytosis, lethargy, anorexia, oedema and weight loss (Mwangi *et al.*, 1995). Other signs seen in *T. brucei* related infections reported by other workers are progressive weights loss, apathy, debility, cheek oedema, fever (41°C), urticarial patches all over the body, increased palpable lymph node and abdominal insensitivity (Souza *et al.*, 2008), conjunctivitis, oedema of the limbs and lower parts, progressive weakness, and gradual paresis of the hind limbs (Herrera, 2005), enlarged submandibular lymph nodes and signs of renal compromise (Franciscato *et al.*, 2007).

Studies on serum enzymatic activity in experimentally infected animal showed an increased AST and the increase was reported to be due to hepatic or muscle tissue lesions (Franciscato *et al.*, 2007). Lymphadenopathy and splenomegaly are features of this infection. CNS lesions are infrequently observed; however, manifestation of neurological symptom has been attributed to the more advanced stages of the disease (Chirimwami *et al.*, 1988).

Myocarditis with intense diffuse mononuclear cell infiltrate affect the right ventricle and atrium with cardiac fibre degeneration similar to that of chagasic myocarditis has been reported in dogs (Schijman *et al.*, 2004).

Trypanosomosis can simply be diagnosed by demonstration of the presence of trypomastigote forms of the protozoa in the blood by a blood smear stained by the Giemsa method or by a thick smear (Amora, 2004) and viewed under microscope. Parasites can also be viewed in the buffy coat of centrifuged blood among other sophisticated methods.

Cases of relapse have been widely reported in human and animals (Pepin and Mbia, 2005, Whitelaw *et al.*, 1985). In the study by Cross *et al.*, (2006), it was discovered that majority of the relapses were recorded within 12 months of discharge from the hospital in humans.

Balasegaram *et al.*, (2006) in their work found a relapse rate of 5% (n=33) in humans treated for human Africa trypanosomosis caused by *Trypanosoma brucei gambiense* and the only identified risk factor for relapse was a CSF white cell count of 6-10 cells/mm³ rather than 0-5 cells/mm³. Relapse has been observed in canine trypanosomosis clinical management (Greene, 2012). Relapse is often experienced after treatment with diminazine aceturate (DA) and it mostly results in fatality if not properly managed.

This research work was therefore designed therefore to evaluate the efficacy and safety of DA given intramuscularly and intravenously in the management of canine trypanosomosis by using serum biochemistry, haematological, cardiac and electrocardiographical indices.

2. MATERIALS AND METHODS

2.1. Animals

Sixteen local dogs with an estimated age of 4-5 months were sourced from local dog breeders in Ibadan. The animals were housed in expanded dog cages at the Veterinary Teaching Hospital, University of Ibadan and dewormed 24 hours after arrival. The animals were allowed to acclimatize for four days and were screened for trypanosomosis, constantly observed for signs for ill-health and fed *ad libitum*.

2.2. Parasites

T. brucei organisms were obtained from Nigerian Institute of Trypanosomosis Research (NITR), Vom, Plateau State, Nigeria in live rats and maintained through passage (every 3-4 days) in rats. Parasitaemia in rats were confirmed from blood smears and observed using a laboratory microscope (Paris, *et al.*, 1982).

2.3. Inoculation of Dogs and Animal Groupings

The dogs were divided into four groups of four dogs each. One group served as control (C) while the other three groups (T1, T2, and T3) were infected with trypanosomes. Parasitaemia was quantified using the haemocytometer and desired infective dose established (Schalm *et al.*, 1975).

A total of 12 dogs were inoculated intraperitoneally with approximately 1×10^4 *T. brucei* (Day 1) while parasitaemia monitored on Days 8, 13, 29 and 36. On Day 8, dogs in T2 and T3 were treated with diminazene aceturate (DA) at the dosage of 3.5 mg/kg by intramuscular (i/m) (T2) and slow intravenous (i/v) (T3) routes.

2.4. Clinical Parameters

2.4.1. Body Temperature

The rectal temperature was taken daily using a digital thermometer at the time of acclimatization, after inoculation and during the experiment and measured in degree centigrade (°C).

2.4.2 Collection of Blood for Haematology and Serology.

Blood was collected by ear pricks on slides as wet mount to check for parasitaemia on days 8, 13, 29 and 36 post infection.

Once weekly, about 2mls of blood was collected from the cephalic veins of all dogs in anti-coagulant tubes

for standard haematological tests (haematocrit (PCV), Hemoglobin level (Hb), platelets, red and white blood cells counts (Plat., RBC and WBC,), mean corpuscular hemoglobin concentration (MCHC), and lymphocytes count.

In addition, another 2ml of blood was collected and separated for sera, used for serological parameters (Sodium (Na), Potassium (K), Chloride (Cl), Bicarbonate (HCO_3), Urea, Creatinine (CR),

2.4.3 Blood Pressure

The blood pressure (mmHg) of each dog, was taken twice (at the onset and end of the experiment) using the KRUTECH VET 400A automatic oscillometric veterinary blood pressure monitor with the appropriate sized cuff.(Bodey *et al.*, 1996)

2.4.4. Electrocardiography

The electrocardiography measurements of each dog were taken at the onset of the experiment, days 14 and 19 using a 6/7 lead computer ECG machine (EDAN VE-1010) as described by Miller *et al.*, (1999).

Electrocardiographic parameters taken were the P duration (P-dur), P-R interval, QRS duration and QT corrected Bazett (QTc B) and QT segment [all in milliseconds (ms)] and the R-amplitude in millivolts (mv).

2.5 Statistical Analysis

All data were analysed using the SPSS software and graph prism 5.0. The data was obtained as mean and standard deviation and compared using student T-test and ANOVA as appropriate.

3. RESULTS

The mean body weights of dogs used in this experiment, as shown in figure 1, varied from 3.88 – 4.28 kg in control group (control), 3.49-4.15 kg in Trypanosoma infected but untreated group (T1), 4.38-5.21 kg in Trypanosoma infected, intramuscular diminazene aceturate treated group (T2) and 3.1-3.44 kg in infected, intravenous diminazene aceturate treated (T3) group.

Also, the mean body temperature readings (Figure 2) for control group from day one to day 12 varied from 38.5 to 38.7°C. The average body temperature of group T1 was 38.5 °C on day 0. The temperature rose on day 2 post infection to 39.4°C and 39.8 °C on day 3. The average temperature measurement from day 5 to day 12 ranged from 39.7°C - 40.1°C. More so, the average rectal temperature readings for T2 rose from 38.3°C on day 0 to 39.9°C on

day 3, ranged between 39.9 and 40.1°C from days 3 to 8 after infection. The mean temperate, however, reduced to 38.5 °C on day 9 after intramuscular injection of diminazene aceturate on day 8 and it further reduced to 37.9°C on day 11. The rectal average temperature reading of group T3 was 38.63°C on day 0, it increased to 31.98°C on day 3 post infection, and gradually increased to 40.2°C on days 7 and 8. The average rectal temperature measurements in days 9, 10 & 11, after it intravenous treatment with DA were 38 °C, 38.5 °C and 38.1 °C respectively.

Parasitaemia was seen on the blood of dogs in the 3 infected groups when observed microscopically on day 8. The control group did not show parasitaemia. On days 13, and 29 only T1 showed parasitaemia while the control, T2 and T3 did not show parasitaemia on the wet mount microscopic examination.

The mean Hb was observed to be significantly ($P<0.05$) higher in T2 when compared to T1 (Figure 3) The PCV was significantly ($P<0.05$) higher in groups T2 and T3 when compared to T1 (Figure 4). The MCHC was significantly ($P<0.05$) lower in T1 when compared to the control group. It was however significantly ($P<0.05$) higher in groups T2 and T3 when compared respectively to the control and T1 groups (Figure 5).

There was a non-significant ($P>0.05$) increase in WBC counts in group T1 when compared to the control, T2 and T3 groups (Figure 6). The lymphocyte count however, showed a significantly ($P<0.05$) higher count in group T1 when compared to the control group and significantly lower counts in groups T2 and T3 when compared to the control and T1 groups (Figure 7)

The serum biochemistry (Table 1) revealed no significant deviation in the electrolytes and metabolites analysed, however a rise in the level of blood urea nitrogen (BUN) and creatinine was observed in groups T2 and T3.

The mean heart rate measurement across the group as shown in Figure 11 ranged from 120-140 beats per minutes between days 0 and 14. A significantly ($P<0.05$) higher reading was recorded in group T1 when compared to the control group and a significant ($P<0.05$) lower readings in T2 and T3 when compared to that of group T1 on day 11 post infection.

The electrocardiogram revealed no significant ($P>0.05$) difference in mean P wave readings across the four groups on days 0, 14 and 19 post infection with Trypanosoma (Figure 12).

The mean PR interval (Figure 13) was significantly ($P<0.05$) higher in group T2 when

compared with group T₁ when measured in day 14 post infection. The QRS readings on day 0, 14 and 19 revealed no significant ($P>0.05$) differences across the four groups when measured on days 0, 14 and 19 (Figure 14). The mean QTc segment (Figure 15) and QTc (bazette) (Figure 16) were significantly ($P<0.05$)

higher in groups T₁, T₂ and T₃ when compared with the control group on day 14. However, there was no significant ($P>0.05$) difference in QTc segment across the groups on days 0 and 19. The R-wave amplitude showed no significant ($p>0.05$) differences across the groups when measures on days 0, 14 and 19 (Figure 17)

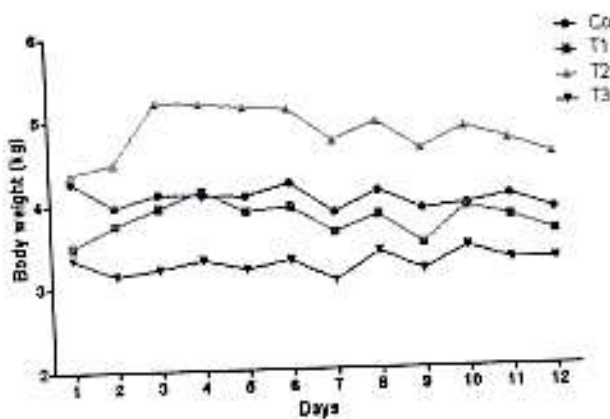


Figure 1: The comparative effect of diminazene aceturate treatment on the mean body weight in *Trypanosoma brucei* infected dogs. (Control: uninfected, T₁: *Trypanosoma*-infected and untreated, T₂: *Trypanosoma*-infected and I.M.-treated and T₃: *Trypanosoma*-infected and slow I.V.-treated dogs)

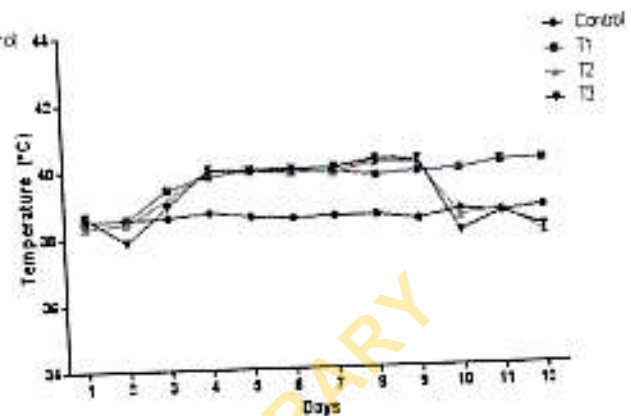


Figure 2: The comparative effect of diminazene aceturate treatment on the mean rectal temperature in *Trypanosoma brucei* infected dogs. (Control: uninfected, T₁: *Trypanosoma*-infected and untreated, T₂: *Trypanosoma*-infected and I.M.-treated and T₃: *Trypanosoma*-infected and slow I.V.-treated dogs)

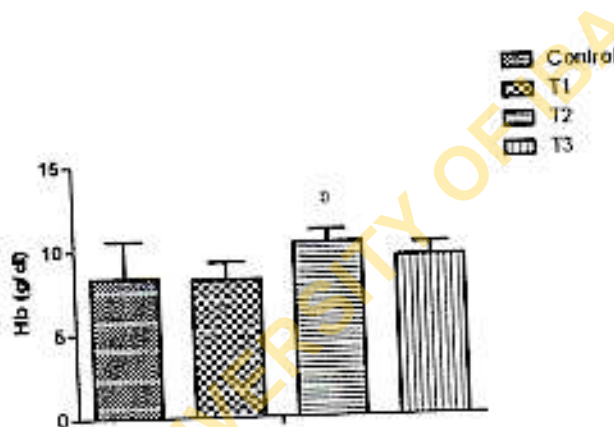


Figure 3: The comparative effect of diminazene aceturate treatment on the mean haemoglobin concentration in *Trypanosoma brucei* infected dogs. *b denotes significantly higher Hb in T₂ when compared to T₁ at ($p<0.05$). (Control: uninfected, T₁: *Trypanosoma*-infected and untreated, T₂: *Trypanosoma*-infected and I.M.-treated and T₃: *Trypanosoma*-infected and slow I.V.-treated dogs)

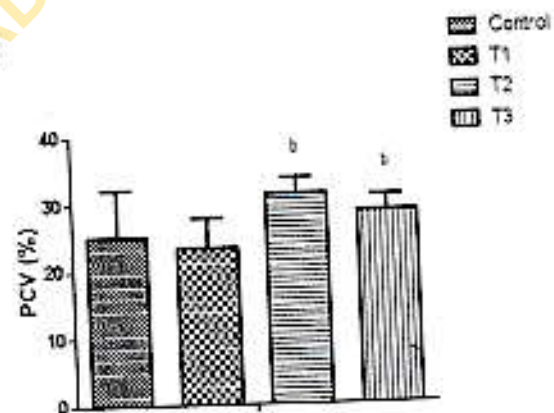


Figure 4: The comparative effect of diminazene aceturate treatment on the packed cell volume (PCV) in *Trypanosoma brucei* infected dogs. (*b denotes that PCV of T₂ and T₃ were significantly higher than that of T₁ at ($p<0.05$). Control: uninfected, T₁: *Trypanosoma*-infected and untreated (T₁), I.M.-treated (T₂) and slow I.V.-treated (T₃) dogs)

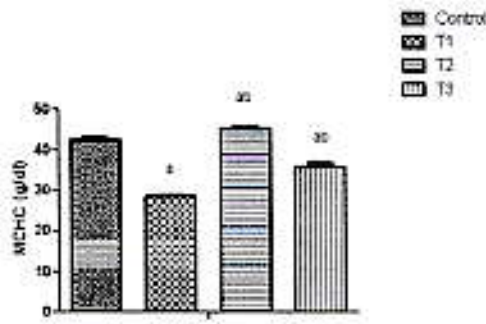


Figure 5: The comparative effect of diminazene aceturate treatment on the mean corpuscular hemoglobin concentration (MCHC) in *Trypanosoma brucei* infected dogs. (*a denotes significant differences in MCHC of T1, T2 and T3 when compared with the control at (P<0.05), *b denotes significantly higher MCHC of T2 and T3 compared to T1 at (p<0.05); (Control: uninfected, T1: Trypanosoma-infected and untreated, T2: Trypanosoma-infected and I.M.-treated and T3: Trypanosoma-infected and slow I.V.-treated dogs)

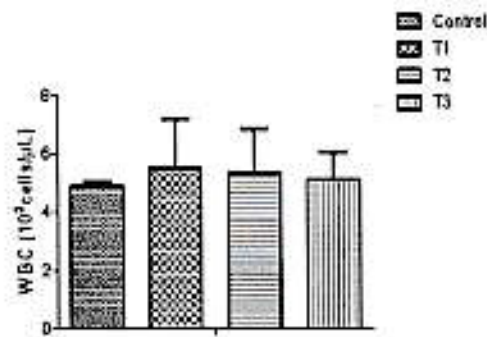


Figure 6: The comparative effect of diminazene aceturate treatment on the white blood cells (WBC) in *Trypanosoma brucei* infected dogs (Control: uninfected, T1: Trypanosoma-infected and untreated, T2: Trypanosoma-infected and I.M.-treated and T3: Trypanosoma-infected and slow I.V.-treated dogs)

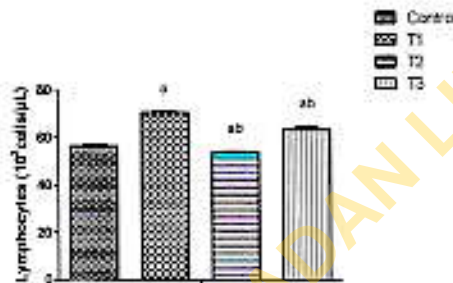


Figure 7: The comparative effect of diminazene aceturate treatment on the blood lymphocytes in *Trypanosoma brucei* infected dogs. (*a denotes significant differences in lymphocyte counts compared with that of control at (P<0.05), *b denotes significant differences when compared with T1 at (p<0.05), (Control: uninfected, T1: Trypanosoma-infected and untreated, T2: Trypanosoma-infected and I.M.-treated and T3: Trypanosoma-infected and slow I.V.-treated dogs)

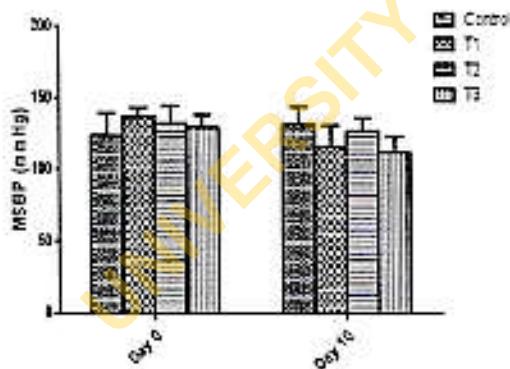


Figure 8: The comparative effect of diminazene aceturate treatment on the mean systolic blood (MSBP) pressure in *Trypanosoma brucei* infected dogs. (Control: uninfected, T1: Trypanosoma-infected and untreated, T2: Trypanosoma-infected and I.M.-treated and T3: Trypanosoma-infected and slow I.V.-treated dogs)

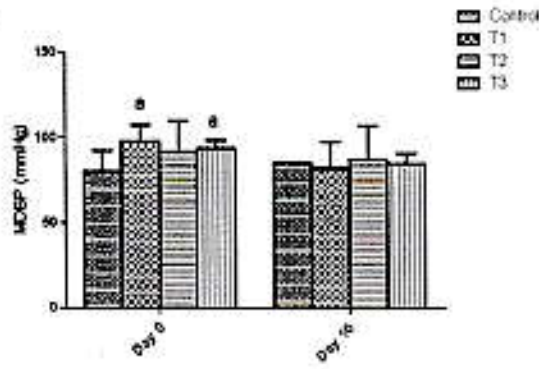


Figure 9: The comparative effect of diminazene aceturate treatment on the mean diastolic blood (MDBP) pressure in *Trypanosoma brucei* infected dogs. (*a denotes significant differences when compared with control at P<0.05), (Control: uninfected, T1: Trypanosoma-infected and untreated, T2: Trypanosoma-infected and I.M.-treated and T3: Trypanosoma-infected and slow I.V.-treated dogs)

Table 1: The comparative effect of diminazene aceturate treatment on serum biochemistry analytes and electrolytes in *Trypanosoma brucei* infected dogs.

Variables	Descriptive Statistics			
	Treatment groups	Mean	Std. Error	Standard range (Hines 2014)
Na (mmol/L)	Control	135.00	1.37	139-154
	T1	134.25	1.24	
	T2	136.67	2.53	
	T3	132.63	1.34	
K (mmol/L)	Control	3.62	0.13	3.6-5.5
	T1	3.56	0.10	
	T2	3.75	0.15	
	T3	3.39	0.13	
Cl (mmol/L)	Control	105.00	1.83	102-120
	T1	103.75	1.25	
	T2	106.67	1.67	
	T3	103.13	1.32	
HCO ₃ (mmol/L)	Control	23.17	0.79	18-25
	T1	23.30	0.65	
	T2	22.35	0.99	
	T3	23.25	0.75	
Urea (BUN) (mg/dL)	Control	17.17	1.54	8.7-30.5
	T1	16.50	1.48	
	T2	23.83	1.96	
	T3	20.13	2.36	
Creatinine	Control	0.42	0.05	0.3-1.6
	T1	0.40	0.04	
	T2	0.55	0.04	
	T3	0.58	0.06	
Total Bilirubin	Control	0.37	0.03	
	T1	0.41	0.04	
	T2	0.43	0.04	
	T3	0.54	0.05	

(Control: uninfected, T1: *Trypanosoma*-infected and untreated, T2: *Trypanosoma*-infected and I.M.-treated and T3: *Trypanosoma*-infected and slow I.V.-treated dogs)

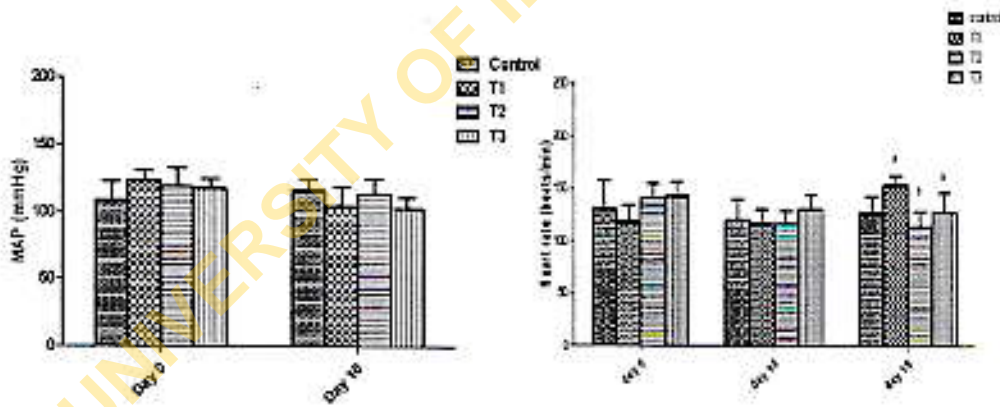


Figure 10: The comparative effect of diminazene aceturate treatment on the mean arterial pressure (MAP) in *Trypanosoma brucei* infected dogs. (Control: Uninfected, T1: *Trypanosoma*-infected and untreated, T2: *Trypanosoma*-infected and I.M.-treated and T3: *Trypanosoma*-infected and slow I.V.-treated dogs)

Figure 11: The comparative effect of diminazene aceturate treatment on the heart rate in *Trypanosoma brucei* infected dogs. (*a denotes significantly difference in heart rate of T1 when compared to the control at (P<0.05). (*b denotes significantly difference in heart rate of T2 and T3 when compared to T1 at (P<0.05). (Control: uninfected, T1: *Trypanosoma*-infected and untreated, T2: *Trypanosoma*-infected and I.M.-treated and T3: *Trypanosoma*-infected and slow I.V.-treated dogs)

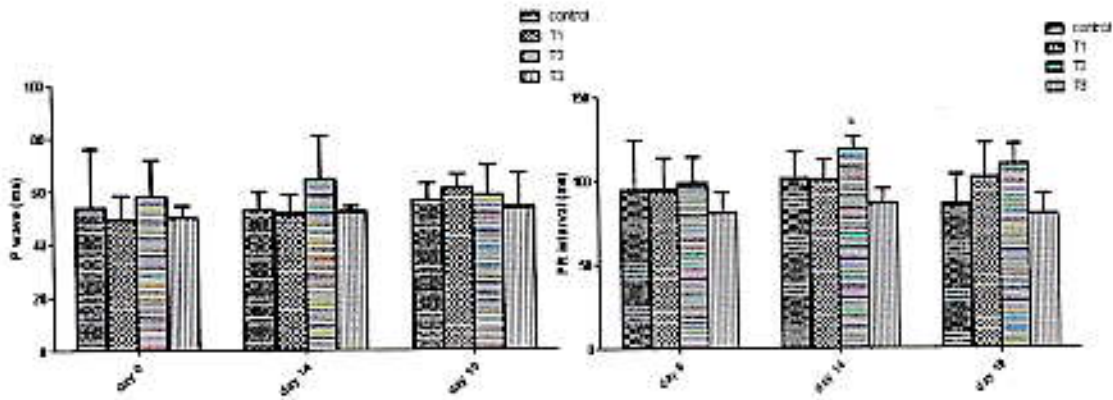


Figure 12: The comparative effect of diminazene aceturate treatment on the P wave duration in *Trypanosoma brucei* infected dogs (Control: uninfected, T1: *Trypanosoma*-infected and untreated, T2: *Trypanosoma*-infected and LM-treated and T3: *Trypanosoma*-infected and slow I.V.-treated dogs)

Figure 13: The comparative effect of diminazene aceturate treatment on the PR interval in *Trypanosoma brucei* infected dogs. (*b denotes significant difference in PR intervals of T2 when compared with that of T1 at $P < 0.05$). (Control: uninfected, T1: *Trypanosoma*-infected and untreated, T2: *Trypanosoma*-infected and LM-treated and T3: *Trypanosoma*-infected and slow I.V.-treated dogs)

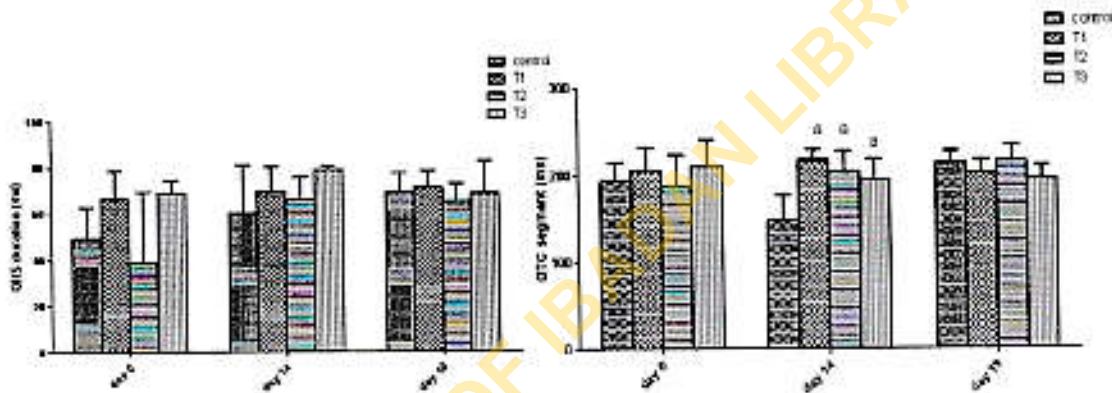


Figure 14: The comparative effect of diminazene aceturate treatment on the QRS duration in *Trypanosoma brucei* infected dogs (Control: uninfected, T1: *Trypanosoma*-infected and untreated, T2: *Trypanosoma*-infected and LM-treated and T3: *Trypanosoma*-infected and slow I.V.-treated dogs)

Figure 15: The comparative effect of diminazene aceturate treatment on the QTc segment in *Trypanosoma brucei* infected dogs (*a denotes significant differences in QTc of T1, T2 and T3 compared to the control at $P < 0.05$). (Control: uninfected, T1: *Trypanosoma*-infected and untreated, T2: *Trypanosoma*-infected and LM-treated and T3: *Trypanosoma*-infected and slow I.V.-treated dogs)

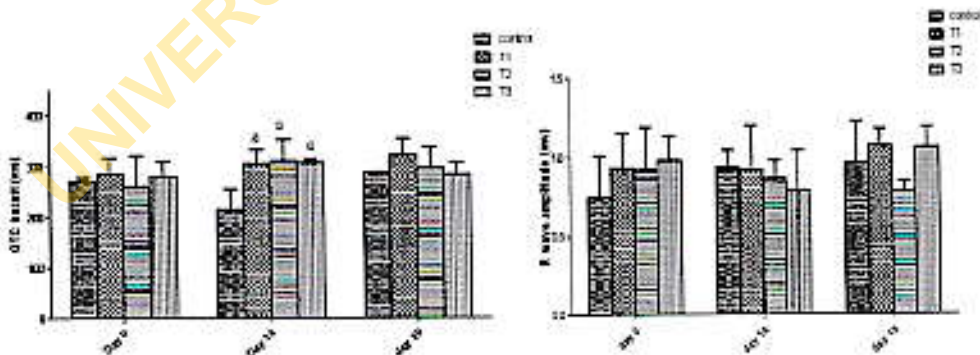


Figure 16: The comparative effect of diminazene aceturate treatment on the QTc base in *Trypanosoma brucei* infected dogs. (*a denotes significant differences in QTc base of T1, T2 and T3 when compared with the control at $P < 0.05$). (Control: uninfected, T1: *Trypanosoma*-infected and untreated, T2: *Trypanosoma*-infected and LM-treated and T3: *Trypanosoma*-infected and slow I.V.-treated dogs)

Figure 17: The comparative effect of diminazene aceturate treatment on the R wave amplitude in *Trypanosoma brucei* infected dogs. (Control: uninfected, T1: *Trypanosoma*-infected and untreated, T2: *Trypanosoma*-infected and LM-treated and T3: *Trypanosoma*-infected and slow I.V.-treated dogs)

4. DISCUSSION

The experiments showed a steady rise in body fever after inoculation of trypanosomes in the dogs which persisted in the untreated group. The rectal temperature however returned to normal after treatment with Diminazene aceturate through both routes (intramuscular and intravenous). This rise in temperature showed that there was fever and evidence of establishment of Trypanosomal infection which was also confirmed by the positive parasitemia on wet mount microscopic examination. This finding was in agreement with the reports from many workers who have associated trypanosomosis with fever (Morrison *et al.*, 1981; Kagawa *et al.*, 1983; Souza, 2008), Greene (2012) also reported that fever and anorexia are clinical features of acute trypanosomosis in dogs.

The observed decrease in the haemoglobin concentration of T_1 dogs revealed anaemia which was not seen in the treated groups and control. This is consistent with the reports of Harrus *et al.* (1985) and Ezeokonkwo *et al.* (2010) who reported that the clinical features of trypanosomosis include persistent fever, pallor, lethargy, anorexia, weight loss, mucopurulent oculonasal discharge, lymphadenopathy, hepatosplenomegaly, variable peripheral oedema, abdominal distention due to ascites, pericarditis, and ocular signs such as unilateral or bilateral uveitis,

corneal oedema, and / or keratitis. Greene (2012) also reported anaemia as a feature of trypanosomosis. The mean corpuscular haemoglobin concentration was also observed to decrease in the T_1 group which also indicated anaemia. This finding was consistent with the results of Kagira *et al.* (2006) who reported a decreased MCHC in velvet monkeys inoculated with *T. brucei rhodensiense* which started at the onset of the disease and persistent throughout the period. The reduced MCHC is a measure of reduced oxygen carrying capacity of each individual erythrocyte and the persistence throughout the disease process may account for the dizziness, weakness and mortality observed in the untreated group. Habila *et al.* (2012) attributed causes of anaemia to haemolysis, erythrophagocytosis, oxidative stress and lipid peroxidation of erythrocytes. The treatment with DA through both routes led to the recovery of the MCHC indicating that one major recovery path of this drug is to increase cellular oxygen availability to the animals.

The increased lymphocyte count seen after trypanosome inoculation is consistent with the findings of Shoda *et al.* (2001) who found out that *T. brucei* is mitogenic for B lymphocytes and stimulated their proliferation. Allam *et al.* (2011) also reported increased lymphocyte counts in gilts experimentally infected with *T. brucei*.

The level of creatinine in local dogs was relatively low when compared to data in the literature and this is likely related to the relatively low muscle mass of local dogs. Surprisingly, the level of creatinine did not increase after trypanosome inoculation unlike what was reported by Allam *et al.*, (2011) in gilts; rather creatinine level increased after treatment with DA in both routes suggesting either a renal impairment or muscular degeneration. Further work is needed to affirm if this is however transient in nature.

The electrocardiogram of the dogs revealed significant prolongation of PR in T_2 and prolongation of QT and QTc (Bazette) in T_1 , T_2 and T_3 intervals on day two weeks after inoculation of *T. brucei* suggesting that trypanosomosis can cause cardiac arrhythmia which was not corrected immediately by i/v and i/m DA treatment. This agrees with the report of Cobucci *et al.* (2006) who found sinus arrhythmia, low voltage QRS complex and QT interval prolongation in acute trypanosomosis.

A decrease in blood pressure was seen in dogs after inoculation of *T. brucei* which was even lower after intravenous administration of DA although all figures were within reference range for dogs. This agrees with the reports of Steinman, *et al.* (1987) who reported the low blood pressure after intravenous administration of DA in rats and Vogler and Rottecher, (1982) who found transient hypotension after intravenous injection of DA in camel.

5. Conclusion

This study showed that DA administered through both intramuscular and intravenous routes was able to reverse the fever, anaemia and cardiac arrhythmia caused by *T. brucei* infection in dogs at the recommended dose without damaging the heart and kidney. Although the slow intravenous route was well tolerated, no summative comparative advantage was seen over the intramuscular routes in the parameters observed in this study. Further work is needed to examine the effect of the routes of administration of DA treatment in chronic trypanosomosis and to

evaluate the long term factors of relapse following treatment.

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