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Comparison of the Therapeutic Efficacy of Diminazene Aceturate and Isometamidium Chloride in Local Dogs Experimentally Infected with *Trypanosoma brucei*

[1]Akpa, P. O., [2*]Ukwueze, C. S., [3]Nnaji, T. O. and [1]Anene, B. M.

[1]Department of Veterinary Medicine, University of Nigeria, Nsukka
[2]Department of Veterinary Medicine, Micheal Okpara University of Agriculture, Umudike
[3]Department of Veterinary Surgery, University of Nigeria, Nsukka

[*] *Author for Correspondence: ukwueze.chigozie@mouau.edu.ng*

ABSTRACT

The chemotherapeutic efficacy of diminazene aceturate (DA) and isometamidium chloride (IMC) were compared in dogs experimentally infected with *Trypanosoma brucei*. Twenty Nigerian local breeds of dogs were used for the study. They were divided into four groups of five dogs each. Dogs in group I served as uninfected untreated control, group II was infected untreated, while groups III and IV were the infected treated with DA and IMC respectively. Administration of DA and IMC effectively cleared the parasites from the blood stream of the treated dogs. However, the infection subsequently relapsed at days 28 and 49 post treatment (pt) in DA and IMC treated groups, respectively. The red cell parameters (PCV, HB, and RBC) decreased significantly (P<0.05) following infection. They were significantly (P < 0.05) higher in IMC treated group than the DA treated group comparable with the control. The infected groups had elevated TWBC counts. However, the DA treated, and IMC treated groups did not show any significant (P> 0.05) difference when compared together with the control. The mean activities of SAP, AST, and ALT increased significantly (P<0.05) in the infected groups compared with the control. The mean BUN increased significantly (P<0.05) on days 21 and 28 pi in infected untreated group and IMC treated groups. The mean CRT was significantly (P<0.05) higher in all the infected groups on day 14 pi compared to the control. From day 35 pi the mean BUN and CRT levels returned to normal values. It was thus concluded from this study that IMC exhibited more chemotherapeutic therapeutic activity over DA, as evidenced from the result of relapsed infections post treatment and haematological changes. However, the serum biochemical parameters were significantly altered in both DA and IMC treated groups compared to the control.

Keywords: Chemotherapy; Diminazene aceturate; Dogs; Isometamidium chloride; *Trypanosoma brucei.*

INTRODUCTION

African animal trypanosomosis (AAT) caused by *Trypanosoma brucei, T. congolense*, and *T. evansi* is among the wasting and debilitating disease of man and animals (Barrett *et al.,* 2003; Nwoha *et al.,* 2013). The disease is widespread in sub-Saharan Africa countries, where it is cyclically transmitted by the tsetse fly of the *Glossina* species (Abenga *et al.,* 2002; Giordani*et al.,* 2016). AAT constitute a huge and devastating economic impact in Nigeria and most countries where the disease is endemic, causing impediments to sustainable livestock production, food security, morbidity and mortality of affected animals (Swallow, 2000). AAT has become a disease of global importance with the spread of the mechanically non-tse tse fly transmitted trypanosome infections due to *T. vivax* which is widespread many countries in south American and some parts of Asia continents (Jones and Davila, 2001; Abenga and Vuza, 2005).

The main focus on the control and prevention of trypanosomosis is Chemotherapy and chemoprophylaxis (Giordani*et al.,* 2016). Unfortunately, the available trypanocides are inadequate and far from satisfactory due to limitations such as severe toxicity, acquired resistance, poor efficacy, and relapse of infections. Other factors associated with decreased effectiveness of these chemotherapeutic agents are wrong route and schedule of administration which are not well adapted to the field conditions, antigenic variation and subsequent escape from immune clearance (Barrett *et al.,* 2004; Espuelas *et al.,* 2012). Among the currently licensed trypanocides, the two most commonly used compounds are diminazene aceturate and isometamidium chloride, either singly or as

a sanative pair for the treatment and control of trypanosomosis in Africa (Holmes *et al.* 2004). Of great worry is the increasing number of reports to resistance diminazene and isometamidium, which suggest that their future utility to be in doubt (Geerts *et al.* 2001; Delespaux and de Koning, 2007). Resistance to DA and IMC by *T brucei* isolated from Nigerian local dog breeds have been reported (Obi *et al.,* 2021). This study was therefore, designed with the objective, to compare the chemotherapeutic efficacy of diminazene aceturate (DA) and isometamidium chloride (IMC) in Nigerian local dogs experimentally infected with *Trypanosoma brucei* after treatment at the onset of parasitemia. Our null hypothesis therefore states that, there is no difference in the chemotherapeutic efficacy of DA and IMC in Nigerian local breeds of dogs experimentally infected with *T. brucei,* while the alternative hypothesis states that there is a difference in the chemotherapeutic efficacy of DA and IMC in Nigerian local breeds of dogs experimentally infected with *T. brucei,*

MATERIALS AND METHODS

Experimental Animals

Twenty local dogs of both sexes aged 6 -12 months with mean weight of 4 kg were used for this study. They were kept in metal cages in a fly- proof house. They were fed once daily with home-made and commercial dog food. Clean drinking water was provided *ad libitum*. The dogs were allowed to acclimatize for two weeks before the commencement of the study. The handling of the animals complied with guidelines of the ethical procedure of the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

Experimental Design

The dogs used were randomly assigned into four groups of five dogs each. Dogs in group I served as uninfected and untreated control, group II was infected and untreated, while groups III and IV were the infected and treated with DA and IMC respectively. The parasitaemia was monitored daily to determine the pre-patent period and then weekly thereafter. Group III and IV dogs were each treated intramuscularly with DA at 7 mg/kg and IMC at 0.5mg/kg on day 7 post infection (pi), at the onset of parasitemia.

Trypanosome Infections

The *Trypanosoma brucei* used in this work was obtained from Nigerian Institute for Trypanosomiasis Research (NITR) Vom Plateau state, Nigeria. The parasite was passaged in donor rats prior to infection of experimental dogs. Each dog was inoculated subcutaneously with 1.0 $x10^6$ trypanosomes from the rat blood. The number of trypanosomes in the innoculum was quantified by the method of Herbert and Lumsden, (1976).

Blood Collection and Determination of Parameters

Blood samples were collected from the cephalic vein of the dogs. 2 ml of blood for haematology was collected into vacutainer tubes containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. The sample bottles were rocked gently to prevent clotting. For biochemical analysis 3 ml of blood was collected from the cephalic vein of each dog into a vacutainer tube without anticoagulant and the tube was left undisturbed for 30 minutes to enhance serum production. The tubes were then centrifuged for 2 minutes at 30,000 revolutions per minute using a microhematocrit centrifuge. The serum supernatant was immediately aspirated into labelled sample bottles and stored in the refrigerator until use.

The packed cell volume was determined using microhaematocrit method (Coles, 1986). The haemoglobin concentration was determined using Cyanmethemoglobin method (Coles, 1986) The RBC and the total WBC counts were done by the use of the improved Neubauer counting chamber (Coles, 1986). While the serum aspartate amino transferase (AST), serum alanine aminotransferase (ALT) and serum alkaline phosphatase (ASP), blood urea nitrogen (BUN) and creatinine (CRT) levels were determined using commercial kits (Randox) according to the manufacturer's instruction.

Data Analysis

Data from the study were analysed using the SPSS 9.0 software package using an analysis of variance and Duncan's multiple range tests (Duncan, 1966). The level of significance was considered at $p< 0.05$.

RESULTS

Parasitaemia

The parasitaemia and survivability of *Trypanosoma brucei* infected dogs treated with either 7 mg/kg Diminazene aceturate or 0.5mg/kg Isometamidium chloride are presented in Table 1. The infected animals (Groups II, III and IV) were parasitaemic on day 6-7 post infection (pi). The treated groups became aparasitaemic following treatment on day 14 pi. The parasitaemia was sustained in the untreated group II till the death of all the animals.

After a period of aparasitaemia (days 28 and 49 post-treatment), relapse of infection occurred on days 35 and 56 pi in DA and IMC treated groups, respectively. The relapse occurred in 2/5 (40%) and 1/5 (20%) of the animals previously treated with DA and IMC, respectively. In DA treated group, mortality occurred in 1/5 out of 2/5 with relapse of infection on day 63 pi.

Haematological Parameters

The mean packed cell volume (PCV), haemoglobin (HB) and red blood cell (RBC) counts were significantly ($P< 0.05$) lower in all the infected groups compared with the control. However, the mean PCV, HB and RBC counts were significantly ($p < 0.05$) higher in IMC treated group than the DA treated group (Figures 1, 2, 3). The mean total white blood cell (TWBC) was significantly ($P< 0.05$) higher in the infected untreated group compared with the control and infected treated groups. However, the DA treated, and IMC treated groups did not show any significant ($P> 0.05$) difference when compared together and with the control (Figure 4).

Serum Biochemical Parameters

The mean serum alkaline phosphatase (SAP) of the infected groups were significantly ($P< 0.05$) increased, pi compared with the control (Figure 5). The mean serum alanine aminotransferase (ALT) was significantly ($P < 0.05$) increased in the infected untreated group compared with the control and infected treated groups. From day 42 pi the mean ALT of DA and IMC treated groups treated group significantly ($P< 0.05$) increased compared with the control (Figure 6). The mean serum aspartate amino transferase (AST) of the infected groups increased significantly ($P<0.05$) compared with the control. The increase was significantly ($P<0.05$) higher in DA treated group on day 49 pi than IMC treated group and higher in IMC treated group on day 63 compared to DA treated group (Figure 7). The mean blood urea nitrogen (BUN) increased significantly ($P<0.05$) on days 21 and 28 pi in infected untreated group and IMC treated group when compared with DA treated group and the control. The infected DA treated was similar and comparable with the control group (Figure 8). The mean serum creatinine (CRT) was significantly ($P<0.05$) higher in all the infected groups on day 14 pi compared to the control. Form day 35 pi the mean CR level returned to normal values comparable with the control (Figure 9).

Table 1: The parasitaemia and survivability of *Trypanosoma brucei* infected dogs treated with either 7 mg/kg Diminazene aceturate or 0.5mg/kg Isometamidium chloride.

Days post infection	Groups			
	Uninfected control	Infected untreated	Infected treated with DA	Infected treated with IMC
0	A 5/5	A 5/5	A 5/5	A 5/5
1	A 5/5	A 5/5	A 5/5	A 5/5
2	A 5/5	A 5/5	A 5/5	A5/5
3	A 5/5	A 5/5	A 5/5	A 5/5
4	A 5/5	A 5/5	A 5/5	A 5/5
5	A 5/5	A 5/5	A 5/5	A 5/5
6	A 5/5	P 4/5	P 3/5	P 4/5
7	A 5/5	P 5/5	*P 5/5	*P 5/5
14	A 5/5	P 5/5	A 5/5	A 5/5
21	A 5/5	P 5/5	A 5/5	A 5/5
28	A 5/5	M 4/5	A 5/5	A 5/5
35	A 5/5	M 4/5	R 2/5	A 5/5
42	A 5/5	M 4/5	R 2/5	A 5/5
49	A 5/5	M 4/5	R 2/5	A 5/5
56	A 5/5	M 5/5	R 2/5	R 1/5
63	A 5/5	M 5/5	M 1/5	R 1/5

P = Parasitaemic; A = Aparasitaemic; *= Day of treatment; R= Relapse; M= Mortality; Numerator = Number either aparasitaemic or parasitaemic; Denominator =Number of infected animals per group

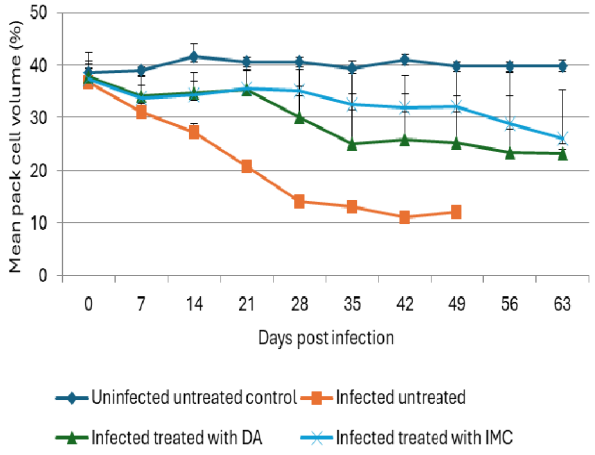

Figure 1: The mean packed cell volume (PCV) of *Trypanosoma brucei* infected dog groups treated with either 7mg/kg diminazene aceturate or 0.5mg/kg isometamidium chloride.

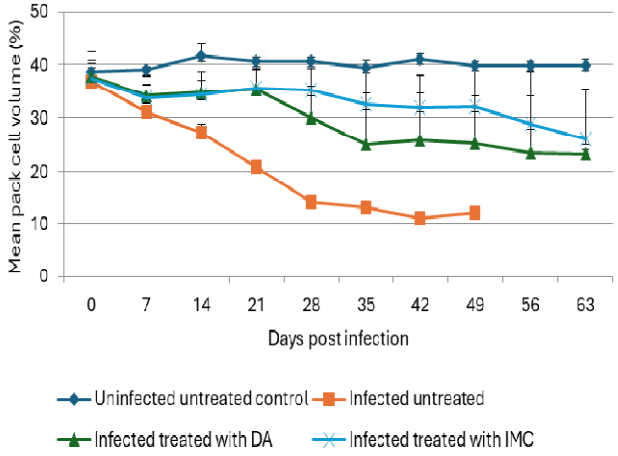

Figure 2: The haemoglobin (HB) concentration of *Trypanosoma brucei* infected dogs treated with either 7mg/kg diminazene aceturate or 0.5mg/kg isometamidium chloride.

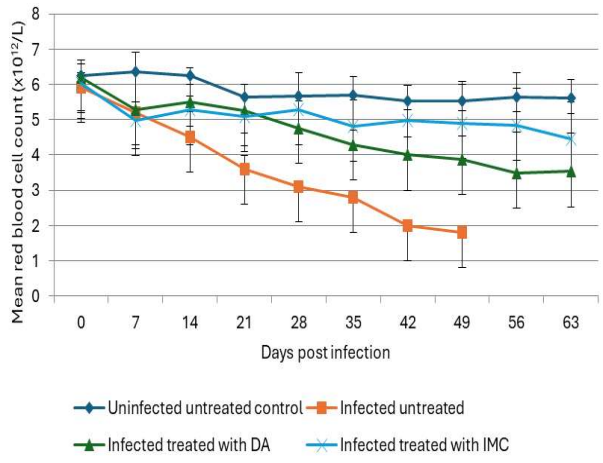

Figure 3: The red blood cell (RBC) counts of *Trypanosoma brucei* infected dogs treated with either 7mg/kg diminazene aceturate or 0.5mg/kg isometamidium chloride.

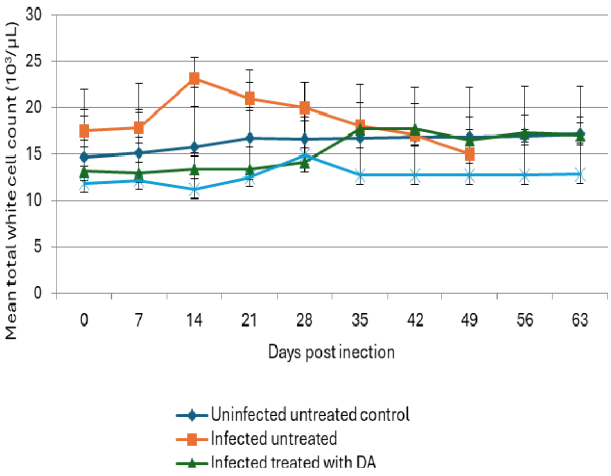

Figure 4: The mean total white blood cell (TWBC) counts of *Trypanosoma brucei* infected dogs treated with either 7mg/kg diminazene aceturate or 0.5mg/kg isometamidium chloride.

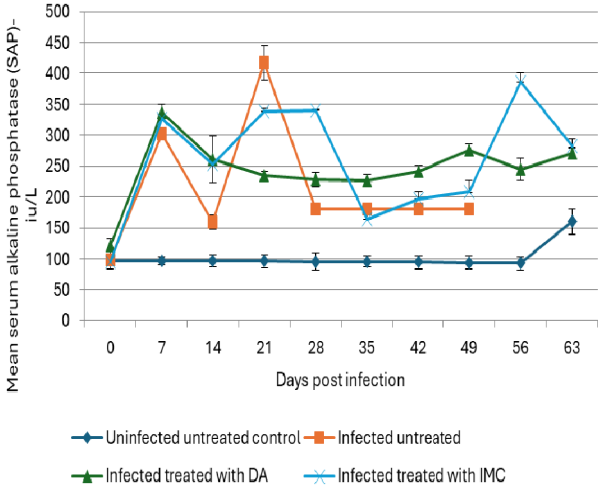

Figure 5: The mean serum alkaline phosphatase (SAP) of *Trypanosoma brucei* infected dogs treated with either 7mg/kg diminazene aceturate or 0.5mg/kg isometamidium chloride.

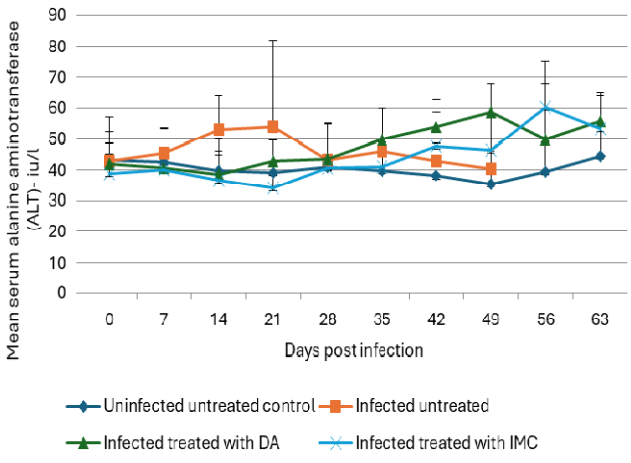

Figure 6: The mean serum alanine aminotransferase (ALT) of *Trypanosoma brucei* infected dogs treated with either 7mg/kg diminazene aceturate or 0.5mg/kg isometamidium chloride.

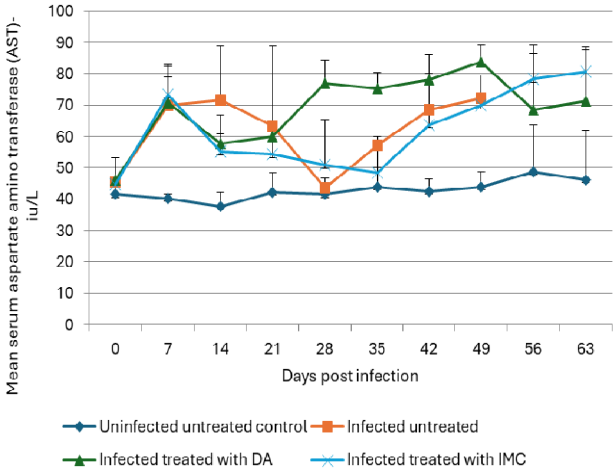

Figure 7: The mean serum aspartate amino transferase (AST) of *Trypanosoma brucei* infected dogs treated with either 7mg/kg diminazene aceturate or 0.5mg/kg isometamidium chloride.

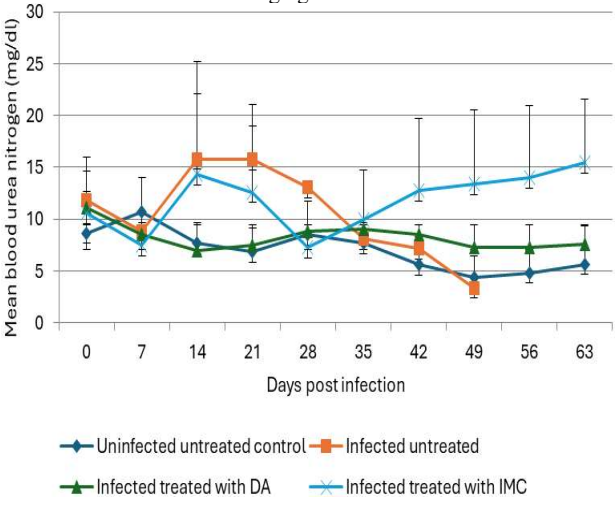

Figure 8: The mean blood urea nitrogen (BUN) of *Trypanosoma brucei* infected dogs treated with either 7mg/kg diminazene aceturate or 0.5mg/kg isometamidium chloride.

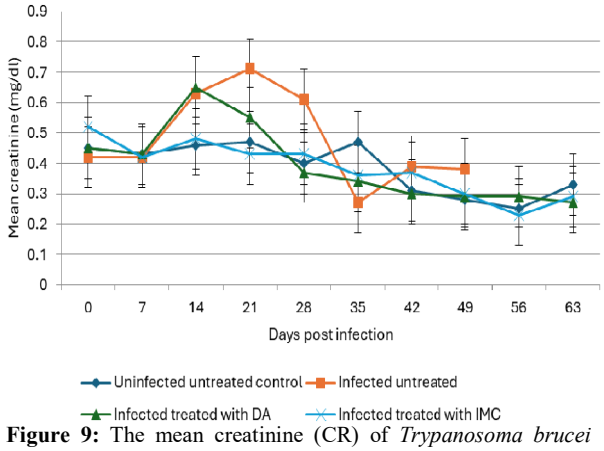

Figure 9: The mean creatinine (CR) of *Trypanosoma brucei* infected dogs treated with either 7mg/kg diminazene aceturate or 0.5mg/kg isometamidium chloride

DISCUSSION

The results of this study showed that experimental infection of dogs with *Trypanosoma brucei* through intraperitoneal route was established between six to seven days post infection (pi). The prepatent period of *T. brucei* is known to be variable. However, it agrees with the works of Anene *et al.* (1989) and CVBD (2010) who observed the incubation period for canine trypanosomosis caused by *T. brucei* to be between four to eight days. It contrasted with the work of Sewell and Brocelesby (1990) that recorded a prepatent period of 3-4 days in dogs. The variations in the prepatent period may be due to the strain of the *T. brucei* stock used or due to inherent quality of the infected dogs. In this experiment, one can deduce that canine trypanosomosis caused by *T. brucei* ran an acute course in dogs, as opposed to chronic disease caused by canine trypanosomosis due to *T. congolense, T. evansi, T. rangelli, T. cruzi* and *T. caninum* (Amole *et al.,* 1982; Mario *et al.,* 1997). The death of the dogs in the infected untreated control group within 28 days pi can be due to the fact that increased parasitaemia might have overwhelmed the immune response of the dogs thereby not allowing the dogs enough time to produce sufficient antibodies to fight the invading parasites. The leucopaenia observed in the early stages of the disease agrees with this fact.

The trypanocides cleared the parasitaemia following treatment by day 14 pi indicating that diminazene aceturate (DA) and isometamidium chloride (IMC) were efficacious in the treatment of *T. brucei* in dogs at the dose levels given. However, in this study relapse infection occurred in two members of DA treated by day (28 post treatment) 35 pi, and by day (49 post treatment) 56 pi in one member of the IMC treated dogs. By day 63 pi one member of the relapsing DA treated dogs died, while non from the IMC treated dogs died. Relapses after treatment have been suggested to be due to drug resistance and due to invasion of brain tissue by the parasites where the trypanocides' molecule cannot reach (Jennings *et al.,* 1980; Barrett, 2001). Jennings *et al.* (1980) established an inverse relationship between the duration of infection and occurrence of relapse, in other words the longer the infection lingered before treatment, the greater the chances of relapse. It was observed that treatment between 3-7 days after infection usually lead to permanent cure whereas treatment after 14 days or more pi usually relapsed as the parasite has entered the brain tissue. In the brain, the parasites are protected by the blood brain barrier which is impervious to the large molecules of the trypanocides subsequently, the parasites re-invade the vascular system when the effect of the drugs in the blood stream would have waned. It can therefore be inferred that the relapses in this study was because of parasite drug resistance as treatment commenced early (day 7 pi).

Similarly, relapses after treatment with IMC has been reported by some workers (Desquesnes *et al*., 2013). These workers therefore recommended alternate use of diminazene aceturate and isometamidium chloride, as these make a sanative pair, meaning that once resistance develops to one of the drugs, the other drug helps to control the infections. However, the results of this study have suggested that such strategy may not be effective as the parasites resisted both trypanocides.

The decrease in red blood cell values (PCV, RBC and Hb concentration) following infection indicates anaemia which is a cardinal feature in trypanosomosis (Franciscato *et al.,* 2007; Nwoha and Anene, 2011). This result agrees with the findings of Sivajothi*et al.* (2013) who observed a similar result in rabbits experimentally infected with *Trypanosoma evansi*. Anaemia is manifested clinically as pallor of the mucous membrane in the dogs. Many factors have been reported in the literature to be responsible for these reductions in the red cell parameters in trypanosomosis of livestocks. One of the factors includes a depression of erythropoiesis (Andrianarivo *et al.,* 1995). The reversal achieved with chemotherapy could only have been due to clearance of the parasites from the blood by the trypanocides.

The acute nature of the disease again can be an explanation for the leucopaenia observed toward the terminal stage of the experiment. This implies that *Trypanosoma brucei* had an immunosuppressive effect on the infected dogs leaving them with an impaired immune defensive mechanism hence the death of all the untreated dogs within 28-63 days of the experiment. The leucopaenia was not very marked in the treated groups. Perhaps, treatment with the trypanocides was responsible for this moderation in the reduction. Leucopaenia as observed in this work agrees with the findings of Anene and Omamegbe, (1987) and contrasted with the findings of Onyeyili and Anika (1989) who observed leucocytosis in dogs infected with *T. brucei*.

Liver enzymes like serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), and serum alkaline phosphatase (SAP) were all elevated following experimental infection of the dogs. Further increases following treatments could be attributable to relapses and for the fact that *T. brucei* accumulate in the liver and other tissues of the body following administration (Onyeyili and Anika, 1991). It has been reported that *T. brucei* sequestrates in several organs

including the liver during infection. This sequestration in the liver leads to the damage of the hepatoytes and the damaged hepatocytes in turn release the enzymes in large quantities leading to the elevation in the level of the enzymes in the serum (Omeke and Ugwu, 1991). The accumulation of the drugs in liver and other organs could lead to necrosis of their cells leading to further increases following treatment especially in the IMC treated group. However, in this experiment, the elevation in these enzymes was an indication of toxicity, which remained elevated throughout the duration of the experiment.

The increases of creatinine (CRT) and blood urea nitrogen (BUN) was an indication of renal impairment which agrees with the works of Aquinos (2002) and Nwoha *et al.* (2013) who recorded a similar result in canine trypanosomosis caused by *T. brucei brucei, T. congolense and T. evansi*. Similarly, Barr *et al.* (1991) also recorded elevated creatinine and blood urea nitrogen in acute phase of chagas disease caused by *T. cruzi*.

It can therefore be concluded from this study that diminazene aceturate and isometamidium chloride did not satisfactorily cure the trypanosomosis in dogs caused by *Trypanosoma brucei* at the dose rates employed as there were relapse of infections. However, isometamidium chloride appeared to have a better therapeutic effect than diminazene aceturate as the relapse interval was day 49 post treatment (day 56 pi), which was longer than in diminazene aceturate which relapsed day 28 post treatment (day 35 pi). We, therefore, recommend that further research be carried out using a different dose range and combination therapy of both trypanocides in Nigerian local breeds of dogs infected with *T. brucei.*

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Conflict of Interest

The authors have no conflict of interest to declare.

Authors' Contribution

POA conceptualized the study and designed the work along with BMA. TON carried out the literature search, while CSU prepared and wrote the manuscript. All the authors participated in the laboratory and technical work.

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