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# Efficacy of Isometamidium in Combination with Verapamil, Chlorpromazine or Sodium-ethylenediaminetetra-acetic Acid in Treatment of Experimental Diminazene Aceturate-resistant Strain of *Trypanosoma brucei brucei* Infection in Rats

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**ABSTRACT**

This study investigated the efficacy of different chemotherapeutic regimes in the treatment of rats experimentally infected with diminazene aceturate-resistant strain *Trypanosoma brucei brucei.* Thirty Sprague Dawley male rats used for the study were randomly assigned to six groups of five rats each as follows: group A-uninfected untreated (negative control), group B-infected and untreated (positive control), groups C-F were infected and treated with 1.0 mg/kg isometamidum chloride, administered intramuscularly on day 11 post-infection. However, rats in groups D, E and F received further treatments with 700 mg/kg sodium-ethylenediamine tetra-acetic acid, 0.4 mg/kg verapamil and 3 mg/kg chlorpromazine, respectively, administered orally for four days. Clearance of parasite post-treatment (PT), mortality PT, relapse parasitaemia post-clearance, body weight change, rectal temperature, packed cell volume (PCV), haemoglobin (HB) concentration and red blood cell count (RBC) were determined during the experiment. Result showed parasite clearance PT of 100% in groups D and E, 80% in group F and 20% in group C by 24 hours PT. The infection relapsed on day 35 PT in 40% of rats in group C, on day 37 PT in 20% of rats in group F and lastly 20% of rats in groups D and E on day 39 PT. Rats that received drug combination showed marginal improvement in erythrocytic parameters analysed when compared with those treatment with isometamidium alone. Combination therapy showed faster clearance of parasite from the blood and also prolonged relapse post-clearance, thus had a better promising efficacy when compared to using isometamiduim chloride alone.

**Keywords**: Chlorpromazine, Diminazene aceturate-resistance; Isometamidium chloride; Sodium-EDTA salt*; Trypanosoma brucei brucei*; Verapermil

## INTRODUCTION

African trypanosomosis is a debilitating disease caused by a group of haemoflagellate protozoan parasites of the genus Trypanosoma and has continued to cause a major setback to livestock production in Nigeria despite attempts at its control. Trypanosomosis control in Africa relies mainly on chemotherapy and chemoprophylaxis using diminazene aceturate, homidium and isometamidium chloride (Sonibare *et al.*, 2016). Diminazene resistance was reported to be widespread, which frustrated efforts to the control of the disease in livestock (Delespaux and de Koning, 2007). With the current challenges posed by existence of drug resistant strains of trypanosomes and increased incidence of toxicity, development of new antitrypanosomal drugs and or compound becomes a necessity (Onyeyili and Aliyoo, 2015; Sonibare *et al.*, 2016). Effort towards this would however be limited by the expensive nature of developing and patenting new drugs, which discouraged pharmaceutical companies from developing new trypanocides for use in animals or humans (Geerts *et al.,* 2001). There was therefore the need to explore ways of achieving the optimum results from use of the existing trypanocides (Abimbola *et al.,* 2013).

Isometamidium chloride/hydrochloride has been used for both chemotherapy and chemoprophylaxis in livestock for over so many decades (Giordani *et al.*, 2016). There were reports of development of multiple drug resistance to isometamidium and diminazene aceturate and this was a setback to the use of sanative pair in overcoming problem of drug resistance to a single drug (Mamoudou *et al.,* 2008). It has been documented that drug resistance emerges when a genetic change, a mutation, deletion or amplification alters drug uptake, drug metabolism, drug-target interaction or drug efflux (Baker *et al.*, 2013).

Sodium-ethylenediamine tetra-acetic acid (Na-EDTA) increases membrane permeability and enhances absorption of substances, such as molecules of isometamidium, into various cells through cell membranes, thereby potentiating their actions (Haque and Russell, 1974). Verapamil is a calcium channel blocker and has been used as inhibitor of

drug efflux pump protein, such as P-glycoproteins in cell biology. Thus, verapamil limits the efflux of drugs from their sites of activity (Anene *et al.*, 1996). Verapamil also has a resistance-reversing effect and was shown to inhibit chloroquine-resistance-transporter (CRT) thereby abolishing chloroquine resistance of *Plasmodium falciparum* (Malaria parasite) (Mier *et al.*, 2018). Verapamil and chlorpromazine were effective in reversing resistance in neoplastic cells (Gottesman and Pasten, 1998) and malaria parasites (Oduola *et al.*, 1998; Mier *et al.*, 2018). In humans, treatment of malaria parasite infection was carried out using verapamil, in combination with other antimalaria drugs, where it limits the parasite cell's ability to expel the drugs outside its digestive vacuole (Ramirez and Villafuerte, 2004). Chlorpromazine is a tricyclic drug and was reported to have a reversal effect on resistant malaria parasite (Oduola *et al.*, 1998).

Against the background on these reports, this study evaluated the efficacy of combinations of these agents with isometamidium in the treatment of experimental diminazene aceturate-resistant (DA-resistant) strain of *Trypanosoma brucei brucei* infection.

## MATERIALS AND METHODS

### Ethical Statement

All rats were handled according to the international guiding principles (NASA, 2008) and was approved by the Animal Ethics committee of the Faculty of Veterinary Medicine, University of Nigeria.

### Experimental Animals

Thirty Sprague Dawley adult male albino rats weighing 140-200 g used for this study were procured from the breeding colony of the Department of Zoology, University of Nigeria, Nsukka. They were randomly assigned into six groups of 5 rats each and kept in their respective standard rat cages in the Laboratory Animal Unit of the Department of Veterinary Medicine. They were fed proprietary rat feed (Vital feeds®), clean water was provided *ad libitium*; the rats were allowed to get acclimatized for two weeks. During this period, they were screened and confirmed to be free of blood parasites and were also dewormed with albendazole (Zolat®) at 7.5 mg/kg body weight.

### Trypanosomes (Diminazene Aceturate-resistant Strain)

The *Trypanosoma brucei brucei* (*T.b. brucei*) used in this study was originally isolated from cattle presented to the abattoir in Nsukka, Enugu State. The parasites were identified morphologically (Soulsby, 1986) and by the blood incubation infectivity test (BII'I') (Rickman and Robson, 1970). The parasite was confirmed and established to be a DA- resistant strain using the standardized test described by Eisler *et al.* (2001). The trypanosomal organisms were subsequently maintained in mice in the Department of Veterinary Entomology and Parasitology, UNN from which donor rats were first infected.

### Infection of Experimental Rats

Trypanosome infection of the donor rats was by intra-peritoneal inoculation and pre-patent period of 3-5 days was recorded and the parasitaemia level on the day the experimental rats were infected was 5.0 x $10^8$ *T. b. brucei*/ml. Infected blood from the donor rats was obtained from the retrobulbar plexus via the median canthus of the eye into an EDTA sample bottles. This infected blood was diluted with normal saline. The experimental rats were then infected with an inoculum containing 1 x $10^6$ trypanosomes suspended in 0.25ml normal saline via intra-peritoneal route.

### Experimental Drugs

Three drugs and a compound used for this experiment were: isometamidiun chloride (MERIAL, France), verapamil (TEVA UK Ltd) (Eagle Company Korea), chlorpromazine (Wuhan Grand Pharmacy, Wuhan China) and Sodium-EDTA (BDH Chemicals Company ltd, Poole England). Working solutions of these drugs (except isometamidium chloride) were prepared immediately before use, by appropriate dilution in distilled deionized water (DDW) and sterilized by filtration through a 0.22 μm pore-size filter.

### Experimental Design

The thirty Sprague Dawley albino rats were randomly assigned to six groups (A-F) of five rats each. All the rats in groups B-F were each, infected with 1 x $10^6$ *T. b. brucei* organisms via intra-peritoneal route, while rats in group A were left uninfected and thus served as negative control. All the rats in groups C-F were treated with 1.0 mg/kg isometamidium chloride intramuscularly 11 days post-infection (PI). However, rats in groups D, E and F received additional treatments with Sodium-EDTA at 700 mg/kg, verapamil at 0.4 mg/kg and chlorpromazine at 3 mg/kg body weight, respectively, orally for 4 days which commenced on day 11 PI. The rats in group B were left untreated as positive control.

### Parameters for Assessing Efficacy of Treatments

The efficacy of treatments was assessed using the following: parasite detection (clearance of parasite post-treatment and relapse parasitaemia post-clearance) by wet mount method using a light microscope (x400 objective) to observe the wriggling movement of the trypanosomes between the blood cells (Boyt, 1984), body weight changes by placing rats on sensitive weighing scale, rectal temperature by insertion of aseptic clinical thermometer manually into the rectum of the rats, packed cell volume (Dacie and Lewis, 1999), red blood cell count and haemoglobin concentration (Schalm *et al.,* 1975). The rats were also observed throughout the experiment for clinical signs of trypanosomosis.

### Statistical Analyses

Data obtained was computed into means and analysed using one-way analysis of variance (ANOVA). The means were separated at post hoc using Duncan's Multiple Range test (Duncan, 1996) at 95% confidence interval.

## RESULTS

**Onset of Parasitaemia:** Trypanosome parasites were seen in the blood of all the infected rats 5 days post-infection (PI) and the level of parasitaemia progressively increased until rats in groups C-F were treated on day 11 PI (Table 1).

**Clearance of Parasitaemia Post-treatment (PT):** There was complete clearance of parasites from the blood of 20% of group C rats; 80% of group F rats and 100% of group D and E rats by 24 hours PT. By 72 hours PT, the blood of all treated rats of the infected groups had become 100% aparasitaemic, while the positive control (group B) rats remained parasitaemic and were euthanized on day 42 PI (Table 1).

**Relapse Parasitaemia Post-clearance:** Relapse infection occurred in 40% of group C rats on day 35 PT, and then in 20% of groups F rats on day 37 PT. By day 39 PT, 20%, each, of groups D and E rats had relapse infections, while 60% and 40% of group C and F rats, respectively, had the relapse infections. By the end of the experiment, relapse infection was recorded in at least 40% of the treated groups C, D, E and F rats, irrespective of the combinations, with a range of 40-100% in the various groups (Table 1).

**Survival Post-infection:** No mortality was recorded among the treated groups. Mortality was recorded in 40 % of rats in group B by day 35 PI which led to the euthanasia of the remaining rats by day 42 PI (Table 1).

**Clinical Signs**: The following clinical signs were observed: weakness, cuddling, depression, decreased feed intake and pale mucous membrane. These clinical signs gradually disappeared following treatment of the rats in groups C- F, while the clinical signs remained in the positive control (group B) in addition to enlarged abdomen and death. These clinical signs gradually returned with relapse infection in the treated groups.

**Table 1:** Parasitaemia, Clearance and Relapse Infection Post-treament in Rats Infected with DA-resistant *T. b. brucei* and Treated with Isometamidium in Combination with either Na- EDTA, Verapamil or Chlorpromazine

| Days Post-Infection | Group B | Group C | Group D | Group E | Group F |
|---|---|---|---|---|---|
| **0** | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| **3*** | 0/5 | 1/5 | 2/5 | 0/5 | 1/5 |
| **5** | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| **7** | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| **9** | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| **11**** | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| **12** | 5/5 | 4/5 | 0/5 | 0/5 | 1/5 |
| **14** | 5/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| **21** | 5/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| **35** | 3/3 | 0/5 | 0/5 | 0/5 | 0/5 |
| **46***** | | 2/5 | 0/5 | 0/5 | 0/5 |
| **48** | | 2/5 | 0/5 | 0/5 | 1/5 |
| **50** | | 3/5 | 1/5 | 1/5 | 2/5 |
| **52** | | 3/5 | 1/5 | 1/5 | 2/5 |
| **54** | | 3/5 | 2/5 | 2/5 | 2/5 |
| **60** | | 4/5 | 2/5 | 2/5 | 2/5 |
| **67** | | 5/5 | 2/5 | 2/5 | 2/5 |
| **74** | | 5/5 | 2/5 | 2/5 | 3/5 |
| **85** | | 5/5 | 2/5 | 2/5 | 3/5 |

*Commencement of parasitaemia; **Treatment and parasite clearance, ***Commencement of relapse infection
Numerator: Number of parasitaemic animal in the group; Denominator: total number of rats surviving in the group.
Group B- infected, untreated (positive control); C- Infected, treated with 1mg/kg ISM; D- Infected, treated with 1mg/kg ISM + 700 mg/kg Na-EDTA; E- Infected, treated with 1mg/kg ISM + 0.4mg/kg Verapamil; F- Infected, treated with 1mg/kg ISM + 3mg/kg Chlorpromazine.

**Proportional Body Weight Change:** There was a significant decrease ($P < 0.05$) in the mean proportional weight change in rats of group B when compared with corresponding values in other infected groups and the negative control group from day 21 PI until they were euthanized (Figure 1).

**Rectal Temperature (RT):** The mean RT of all infected rats (groups B, C, D, E and F) were significantly higher ($P < 0.05$) on day seven PI (Figure 2). However, by day 21 (10 days PT), the mean RTs the treated groups (C, D, E and F) were not statistically different from the negative control (group A) up to day 42 PI, while the positive control (group B) continued to show a significant increase ($P<0.05$). There mean RT ($P < 0.05$) was significantly higher in rats of groups C and F (day 49 P1) and in groups C, D, E and F (day 63 PI) than in the negative control.

**Packed Cell Volume (PCV):** The mean PCV of rats in the infected groups was significantly lower ($P < 0.05$) than the corresponding value in the negative control (group A) by day 7 PI (Figure 3). However, by day 28 PI (17 days PT) up to day 49 PI (day 38 PT), the mean PCV values of the treated groups (C, D, E and F) were not statistically

different from the corresponding value in negative control, while the mean PCV in the positive control (group B) continued to show significant decrease ($P < 0.05$) up to when they were euthanized. There was a significantly lower ($P < 0.05$) mean PCV in rats of groups C, E and F, on day 63, and groups C, D and F, on day 77, when compared with the negative control and other treated groups, and a significantly higher ($P < 0.05$) mean PCV of rats in groups D, E and F on day 84 when compared to group C (treated with ISM alone) and group A (negative control).

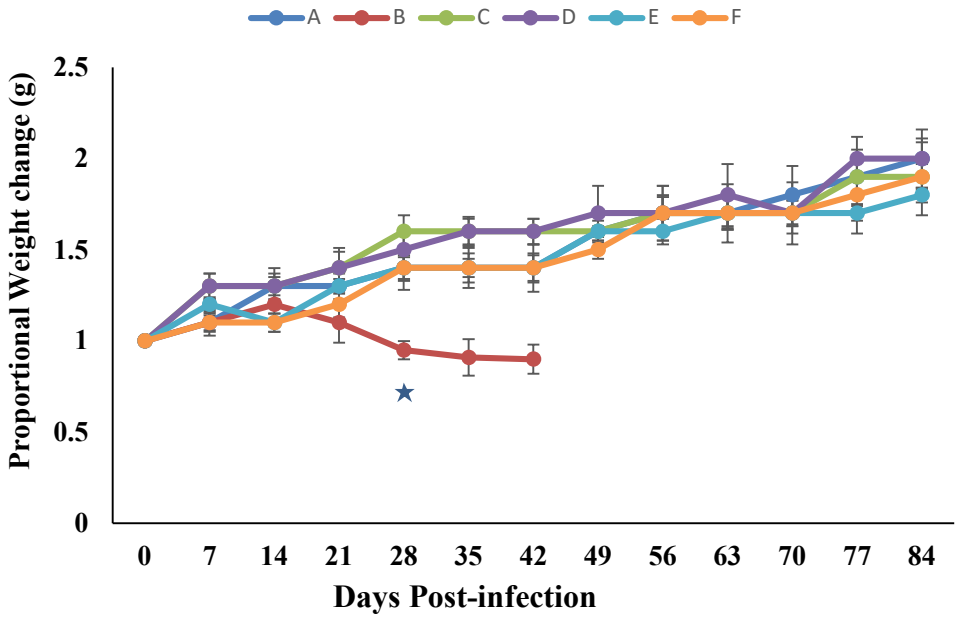


**Figure 1:** The mean (±SEM) proportional body weight change (g) of rats infected with DA-resistant *T. b. brucei* and treated with ISM alone and in combination with either Na-EDTA, verapamil or chlorpromazine. * significant at p>0.05
A-uninfected, untreated (negative control); B-positive control; C-infected, treated with 1mg/kg ISM; D-infected, treated with 1mg/kg ISM+ 700mg/kg Na-EDTA; E-infected, treated with 1mg/kg ISM + 0.4mg/kg verapamil; F-infected, treated with 1mg/kg ISM + 3mg/kg chlorpromazine

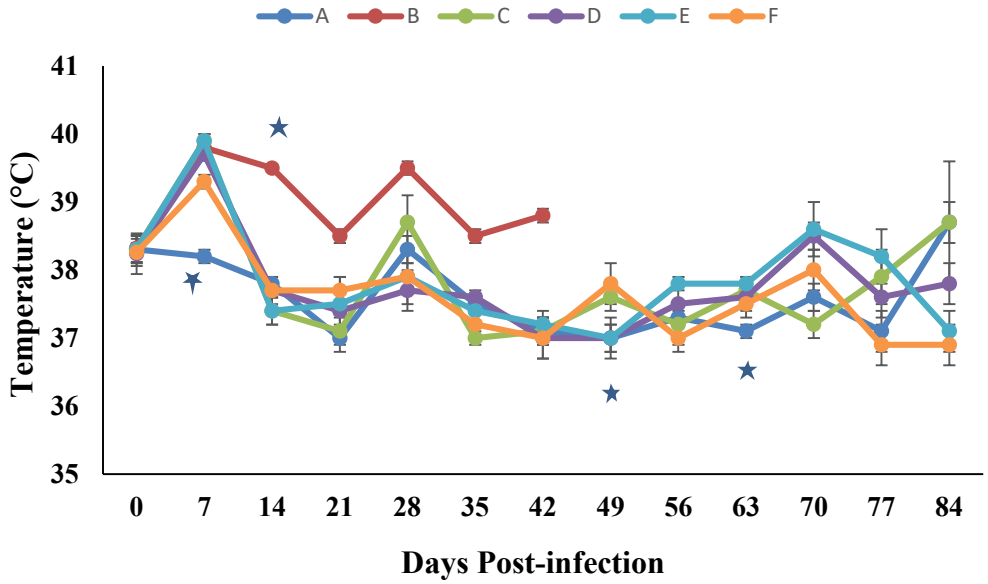


**Figure 2:** The mean (±SEM) rectal temperature (°C) of rats infected with DA-resistant strain *T. b. brucei* and treated with ISM alone and in combination with Na-EDTA, verapamil and chlorpromazine. * significant at p>0.05
A-uninfected, untreated (negative control); B-positive control; C-infected, treated with 1mg/kg ISM; D-infected, treated with 1mg/kg ISM + 700mg/kg Na-EDTA; E-infected, treated with 1mg/kg ISM + 0.4mg/kg verapamil; F-infected, treated with 1mg/kg ISM + 3mg/kg chlorpromazine

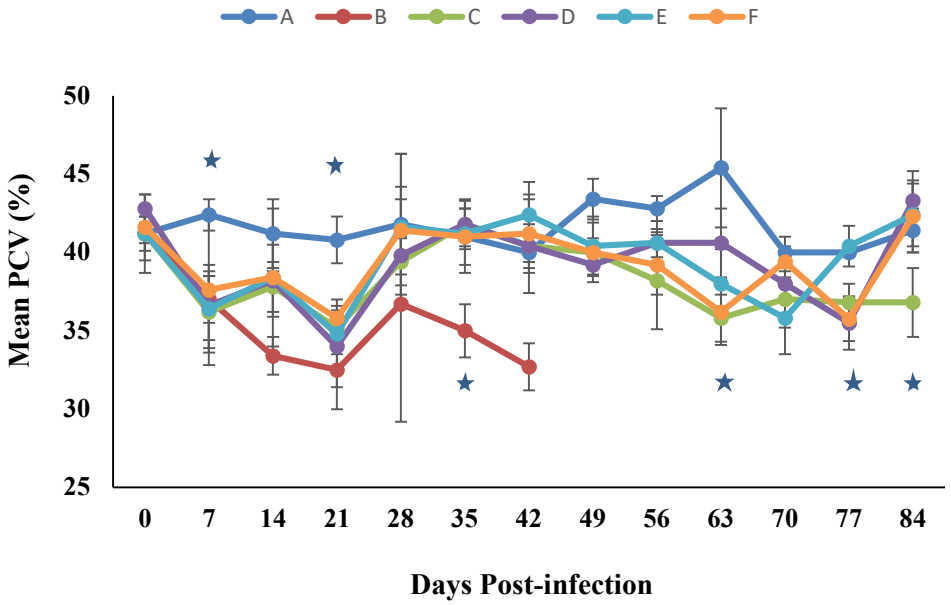


**Figure 3:** The mean (±SEM) packed cell volume (%) of rats infected with DA-resistant strain *T. b. brucei* and treated with ISM alone and in combination with Na-EDTA, verapamil and chlorpromazine. * significant at p>0.05
A-uninfected, untreated (negative control); B-positive control; C-infected, treated with 1mg/kg ISM; D-infected, treated with 1mg/kg ISM + 700mg/kg Na-EDTA; E-infected, treated with 1mg/kg ISM + 0.4mg/kg verapamil; F-infected, treated with 1mg/kg ISM + 3mg/kg chlorpromazine

**Red blood cell count (RBC):** The mean RBC counts of rats in the infected groups B, C, D, E and F were significantly lower ($P < 0.05$) by day 7 PI, when compared to the corresponding value in negative control group A (Figure 4). However, by day 14 PI (i.e. 3 day PT), the values of mean RBC count of rats in groups D, E and F (groups treated with drug combination) were not statistically different from that of the negative control while they were significantly higher ($P < 0.05$) than those of group C (treated with isometamidium only) and group B (positive control). By day 28 PI, the mean RBC count of rats in group C was not statistically different from rats of other treated groups (D, E and F) and those of negative control. By day 35, rats in groups D, E and F showed a significantly lower ($P < 0.05$) mean RBC count when compared to group C and the negative control (group A), while those of positive control (group B) continued showing a significant decrease ($P < 0.05$) up to when they were euthanized. By day 42, rats in groups C, D, E and F showed a significant lower ($P < 0.05$) mean RBC count when compared to the negative control (group A).

**Haemoglobin concentration (Hb):** The mean Hb values of rats in the infected groups (B, C, D, E and F) were significantly lower ($P < 0.05$) by day 7 PI than that of the negative control (group A) (Figure 5). However, by day 14 PI (i.e. 3 days PT), the mean Hb values of rats in groups D, E and F (groups treated with drug combination) were not significantly ($P > 0.05$) different from that of the negative control, while the mean Hb values in group C (treated with isometamidium only) and group B (positive control) remained significantly lower ($P < 0.05$) when compared with that of the negative control. By day 42 PI, rats in group C showed a significantly higher ($P < 0.05$) mean Hb when compared to the corresponding values in rats of groups D, E and F and the negative control, while the mean Hb in positive control rats (group B) continued to be lower significantly ($P < 0.05$) up to when they were euthanized. There was a significantly lower ($P < 0.05$) mean Hb of rats in groups C and F (days 56) and group D (day 77) and a significantly higher ($P < 0.05$) mean Hb of rats in group C (day 77) when compared with the negative control and other treated groups.

## DISCUSSION

The pre-patent period of 5 days observed in this study falls within the range, 4-5 days, reported by Anene *et al.* (2006) in rats infected with DA-resistant strain of *T. brucei*. The level of parasitaemia, which increased slowly but progressively, led to the death of some of the rats on day 35 PI that necessitated euthanasia of the infected untreated rats on day 42 PI. Factors that influence parasitaemia in susceptible animals were documented, which include the number of parasites inoculated (which influences not only the prepatent period but also the height and duration of parasitaemia), inter-current infections, host immune competence, nutritional status, and the pathogenicity of the strain of *T. brucei brucei* (Taylor and Authie, 2004).

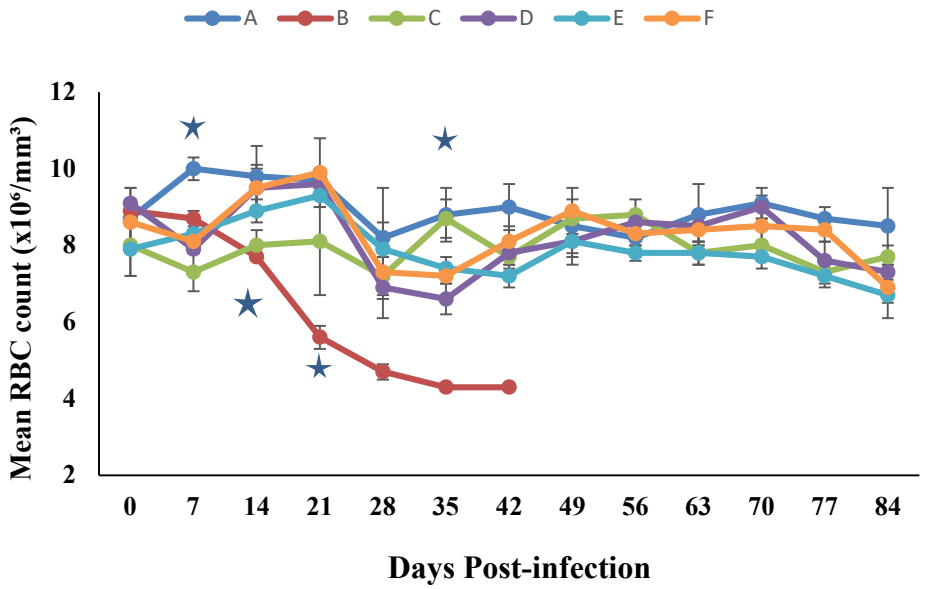


**Figure 4:** The mean (±SEM) red blood cell count (x $10^6$/$mm^3$) of rats infected with DA-resistant strain *T. b. brucei* and treated with ISM alone and in combination with Na-EDTA verapamil and chlorpromazine. * significant at p>0.05
A-uninfected, untreated (negative control); B-positive control; C-infected, treated with 1mg/kg ISM; D-infected, treated with 1mg/kg ISM + 700mg/kg Na-EDTA; E-infected, treated with 1mg/kg ISM + 0.4mg/kg verapamil; F-infected, treated with 1mg/kg ISM + 3mg/kg chlorpromazine

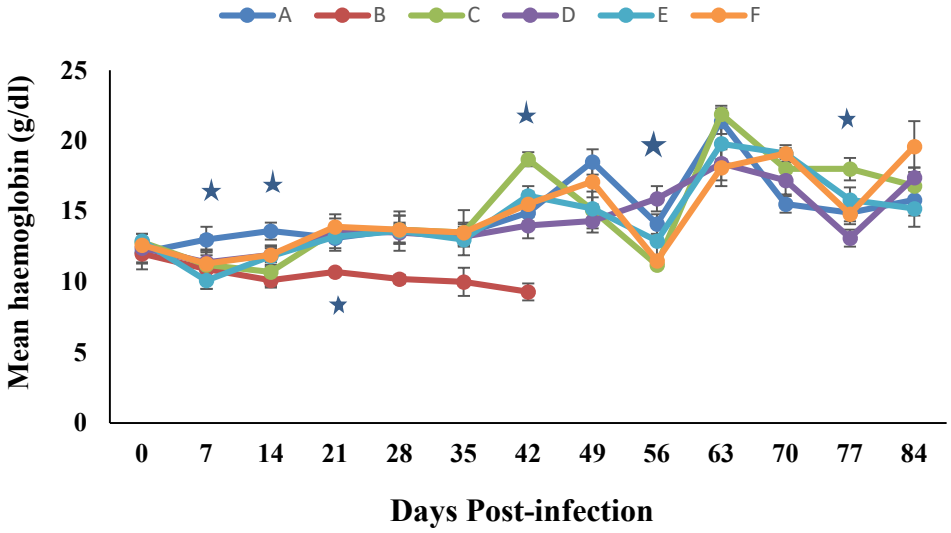


**Figure 5:** The mean (±SEM) haemoglobin concentration (g/dl) of rats infected with DA-resistant strain *T. b. brucei* and treated with ISM alone and in combination with Na-EDTA, verapamil and chlorpromazine. * significant at p>0.05
A-uninfected, untreated (negative control); B-positive control; C-infected, treated with 1mg/kg ISM; D-infected, treated with 1mg/kg ISM + 700mg/kg Na-EDTA; E-infected, treated with 1mg/kg ISM + 0.4mg/kg verapamil; F-infected, treated with 1mg/kg ISM + 3mg/kg chlorpromazine

The clinical signs observed such as, rough hair coat, pale mucous membrane dullness, decreased feed intake in the infected groups were typical of those reported of the trypanosomosis. The high temperature observed, which corresponded with the period of parasitaemia (Taylor and Authie, 2004; Adieme *et al.*, 2014). Significant funding in this study is that clearance of parasites PT varied among the treatment combinations. Groups treated with Sodium-EDTA and with verapamil combinations had 100% clearance rate by 24 hours PT. This parasitological evaluation is a measure, which could be used as an index for assessing efficacy of treatment of trypanosomosis in addition to the return of haematological values to pre-infection values (Dina *et al.*, 2002). Thus, verapamil and sodium-EDTA combination with isometamidium could be said to have a better effect when compared with other treatment regimes.

EDTA has been reported to disrupt the lipopolysacharide structure in the outer membrane of cells resulting in the membranes becoming more permeable to more isometamidium molecules and hence potentiating the action of isometamidium (Haque and Russell, 1974). Ihedioha *et al.* (2007) also reported from his study, a slight potentiation of diminazene aceturate (in the treatment of mice infected with DA-resistant *T. brucei*) when co-administered with Sodium-EDTA and that the administration of Sodium-EDTA alone significantly enhanced the resistance of the infected mice. Similarly, Anene *et al.* (1996) demonstrated in an *in vitro* study the anti-trypanosmal activity of verapamil and chlopromazine. From our observation also, group treated with chlorpromazine combination had 80% clearance by 24 hours PT. It has also been reported that many tricyclic compounds have anti-trypanosomal and anti-leishmanial activity of which chlorpromazine was found to be the most potent (Benson *et al.*, 1992). The activity of chlorpromazine is said to arise from its selective inhibition of trypanothione reductase interference with micro-tubule assembly and proton based electrochemical gradient membrane disruption (Hammond *et al.,* 1985).

The significant body weight change observed in the infected untreated rats is consistent with other reports that trypanosomosis cause loss of weight (Adieme *et al.*, 2014). This may be attributed to anorexia and dullness, and thus decreased feed intake observed. However, a continuous but gradual increase in the mean proportional weight changes was observed in the treated groups despite the relapse of parasitaemia post-clearance when compared with the uninfected untreated control. It has also been found that weight changes in trypanosomosis are markedly influenced by the levels of protein intake, and high intake allows infected animals to grow at same rate as uninfected controls provided energy intake is adequate whilst low energy levels can exacerbate the adverse effects of trypanosomosis on body weight (Holmes *et al.*, 2000; Chukwudi *et al.,* 2016). Thus, the gradual increase in proportional weight change seen may be attributed to the chronic nature of the infection at this stage and, also may be due to the fact that the rats were on good nutrition with high quality protein in the diet which helped in overcoming the weight loss associated with trypanosomosis.

Anaemia observed as decreased in the erythrocyte parameters (PCV, haemoglobin concentration and RBC values) is the prominent feature of animal trypanosomosis (Taylor and Authie*,* 2004) and has been reported by so many authors (Anene *et al.*, 2006; Adieme *et al.*, 2014). Following treatment, the anaemia was reversed in all the treated groups. But following relapse of parasitaemia which was low (average log 6.55), the erythrocytic parameters did not differ from the negative control. This may be attributed to the chronic nature of the infection at this stage. Chronic phase of trypanosomosis has been reported to be characterised by low level of parasitaemia, which persists with minor fluctuation in the erythrocytic value (Mbaya *et al*., 2012).

Relapse of infection observed in all treated groups may be attributed to the parasite return to the vascular system after the effect of the drug had waned. Relapse infection has been explained to occur due to drug resistance and also due to migration of the parasites into the brain tissue where drug could not affect them (Anene *et al.*, 2001; Geerts *et al.*, 2001). Jennings *et al.* (1977) established an inverse relationship between the duration of infection and the occurrence of relapse. Relapse infection observed in early treatment (between day 3-7 PI) could be due to drug resistance while in late treatment (from day 14 PI) could be due to the reappearance of parasites that had migrated into the brain tissue where they were inaccessible to the drugs (Akpa *et al.*, 2008). Treatment in this study was done on day 11 PI, and also DA-resistant strain of *T.b brucei* was the infecting parasite. Thus, relapse infection could be attributed to both the reappearance of parasite, which may have been sequestered in the brain and the DA-resistant strain of parasite. A study by Eisler *et al.* (1993) showed that isometamidium serum concentration has a half-life of approximately 25 days while another study Eisler (1996) indicated an elimination half-life 9-19 days. Groups treated with Sodium- EDTA and verapamil were the last to relapse on day 39 PT (i.e. day 50 post infection) while groups treated with chlorpromazine combination relapsed on day 37 PT. This agrees with the reports of earlier studies that verapamil did not reverse resistance of *T. brucei* to diminazene or isometamidium*in vitro* or *in vivo* (Kaminsky and Zweygarth, 1991) or of *T. evansi* to suramin, berenil or melCy (Anene *et al.*, 1996). It is in contrast with the findings of Neal *et al.* (1989) and Dey *et al.* (1994), who reported a reversal of drug resistance in *T. cruzi* and *Leishmania spp* by verapamil and also Sutherland *et al.* (1991) who reported that co-administration of verapamil effectively blocked isometamidium chloride efflux from resistant *T. congolense* infection. Although it has been reported that mutation in an important mitochondrial protein can strongly reduce mitochondrial membrane electrical potential and this could result in isometamidium resistance thus leading to high levels of resistance and even cross-resistance to various drugs (Eze *et al.*, 2016). Active extrusion of drugs leading to reduced accumulation of drugs in resistant parasites when compared with drug sensitive organisms has been reported to be one mechanism associated with trypanocidal resistance (Yarlett *et al.*, 1991; Sutherland and Holmes, 1993).

In conclusion, treatment of diminazene aceturate-resistant strain of *Trypanosoma brucei brucei* infection using isometamidium in combination with EDTA or verapamil were able to effectively facilitate the clearance of the parasites from the blood and, also were able to delay relapse of parasitaemia post-clearance.

**Conflict of Interest**

The authors declare that they do not have any conflict of interest.

**Authors Contribution**

CIC and ABM contributed to the study conception and design. Material preparation, data collection, and analysis were performed by all authors. All authors read and approved the final manuscript.

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