COMPARATIVE EFFICACY OF DIAMINAZINE ACETURATE AND ISOMETHAMIDIUM CHLORIDE IN RABBITS EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA BRUCEI BRUCEI*

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ABSTRACT

The comparative efficacy of diaminazine aceturate (DA) and isomethamedium chloride (IC) was investigated in Trypanosoma brucei brucei infected domestic rabbits (Oryctolagus cuniculus). A total of eighteen rabbits were used for the study. The rabbits were divided into six groups of three each. All the rabbits in groups B – F were infected with Trypanosoma brucei brucei, while those in group A served as the negative control (uninfected and untreated group). Group B contained the infected and untreated (positive control group), Group C rabbits were infected and treated with DA at 3.5 mg/kg, Group D were infected and treated with DA 7 mg/kg, Group E contained the infected and treated with 3.5 mg/kg of DA combined with 1.0 mg/kg of IC and Group F contained the infected and treated with 7 mg/kg of DA combined with 1.0 mg/kg of IC. The weekly rectal temperature, body weight gain, packed cell volume (PCV), total erythrocyte count, haemoglobin concentration (HbC), total leucocyte count (TLC), differential leucocyte count (DLC), clinical signs and survivability and rate of parasite clearance were used to assess the efficacy of the drugs and drug combinations. The parasites cleared from groups E and F 24 hours post treatment, while in group D, it was 48 hours post treatment. The rabbits in group C and group B died within 13 – 19 and 50 – 52 days post infection (PI), respectively. Relapse was recorded in all the rabbits treated only wit DA. There was significant ($p<0.05$) reductions in weight, PCV, erythrocyte count, HbC and TLC. The significant ($p<0.05$) increase in temperature following infection were reversed by the treatments, this reversal however, was faster and lasted longer in the combined treatment groups (E and especially F) than in the single treatment group. The results of this study suggest that the combined treatments of DA and IC produced better therapeutic effect than DA only.

Keywords: Parasitaemia, Trypanosomiasis, Rabbits, Diaminazine aceturate, Isomethamedium chloride

INTRODUCTION

The major species which are responsible for African animal trypanosomiasis, Nagana include *Trypanosoma congolense, Trypanosoma vivax, Trypanosoma brucei* and *Trypanosoma simiae* (AAT, 2009). The disease they cause is collectively referred to as tse-tsefly trypanosomiasis, while sura and dourine are caused by *Trypanosoma evansi* and *Trypanosoma equiperdum* species respectively in horse as they are transmitted by other biting flies (Aiello, 1998). They are extracellular parasites that cause persistent infection of the blood and induce immunosuppression (Tabel *et al.*, 2008).

The disease is most important in cattle where it causes a wasting disease but can cause serious losses in pig, camels, goats and sheep which pose a great constraint to development of livestock as a result low livestock productivity in Africa (Finelle, 1973). *Trypanosoma brucei* can also affect human where it causes trypanosomiasis also known as sleeping sickness (Barrett *et al*., 2003). The continual spread of the disease is accompanied with devastating economic impacts and this has made raising of animals in these areas problematic (Kabayo, 2002). There is no effective vaccine against trypanosomes due to their ability to regularly switch their surface coat and hence evade immune destruction; this is called antigenic variation (Verma *et al*., 1973). The control of trypanosomiasis in Africa is dependent on chemotherapy and chemoprophylaxis using salts of three compounds; diaminazine an aromatic diamidine, homidium a phenanthridine and isomethamidium a phenarthridine and aromatic amidine (Leach and Roberts, 1981). Diaminazine aceturate is the most commonly used therapeutic trypanocidal drug, while isometamidium chloride is used as both a therapeutic and prophylactic trypanocidal agent (Eisler, 1996). However, these trypanocides are expensive, toxic and have the tendency to elicit drug resistance (Legros *et al*., 2002; Wurochekke *et al.*, 2004). There is significant progress in the development of new antitrypanosomal drugs in recent decades (Nagle *et al.,* 2014). In this study, combinations DA and IC were used in rabbits to investigate their therapeutic efficacy and to know if their use can prevent or at least delay relapse of the infection.

MATERIALS AND METHODS

Animals: Eighteen adult Chinchilla and New Zealand white cross rabbits of both sexes and weighing between 0.80 kg and 2.30 kg were used for this study. The rabbits were procured from a breeder at Eha-Alumona, Nsukka. They were randomly divided into six groups of three rabbits each. They were marked and kept in clean cages in the laboratory animal house of the Department of Veterinary Medicine, University of Nigeria, Nsukka. The rabbits were provided with portable water, fed with commercial pelleted growers feed (Vital Feed) and left to acclimatize for 2 weeks before the commencement of the experiment. The ethical conditions governing the use and conduct of experiments with live animals were strictly observed and the experimental protocol was approved by the University of Nigeria, Nsukka Senate Committee on Medical and Research Ethics.

Design: The eighteen rabbits used for this experiment were divided into six (6) groups (A, B, C, D, E, F), replicated thrice with one rabbit per replicate. Group A contained the uninfected and untreated (negative control group), Group B contained the infected and untreated (positive control group), Group C contained the infected and treated with DA at 3.5 mg/kg, Group D infected and treated with DA 7mg/kg, Group E contained the infected and treated with 3.5 mg/kg of DA combined with 1 mg/kg of isomethamidium chloride and Group F contained the infected and treated with 7 mg/kg of DA combined with 1 mg/kg of IC.

Trypanosoma: *Trypanosoma brucei brucei* used for the study were obtained from the National Institute for Trypanosomiasis Research (NITR), Vom, Plateau State, Nigeria and identified based on morphological characteristics as described by Soulsby (1982) and by negative blood inhibition and infectivity test (BIIT) (Blejer *et al.,* 2008). The parasites were multiplied in donor mice according to the method of Trindade *et al.* (2016).

Inoculation: Infected blood from the donor mice were collected aseptically from the retrobulbar plexus of the medial canthus of the eye and the blood diluted using phosphate buffered saline (PBS) to obtain the delivery dose of 1×10^6 trypanosomes suspended in 0.5 ml of PBS. The experimental rabbits were then given 0.5 ml of diluted infected blood intraperitoneally.

Drugs: Diaminazine aceturate manufactured by Pantex Holland Veterinary Pharmaceutical

Products was administered at varied doses of 3.5 mg/kg and 7 mg/kg body weight intramuscularly, while Isomethamidium chloride manufactured by Viphavet, Vietnam was given at 0.5 mg/kg body weight intramuscularly.

Blood: Blood sample (2 ml) for haematological determination was collected through the marginal ear vein into EDTA sample bottles.

Parasitaemia: The levels of parasitaemia pre and post treatments were estimated using the standard procedure as described by Herbert and Lumsden (1976).

Body weight: The rabbits were weighed using a weighing balance, recorded in kilograms (kg) and this was done on a weekly basis.

Temperature: The rectal temperature of the rabbits was taken daily throughout the experimental period and the values were recorded in degree Celsius (^{0}C).

Haematology: The haematological indices were determined using routine laboratory methods. Packed cell volume (PCV) was determined using the method described by Coles (1986). Haemoglobin concentration estimation was done using the method described by Coles (1986). Leucocytes counts (WBC) and their differential counts were determined by method described by Schalm *et al*. (1975). The determinations of haematological parameters were done pre (immediately after infection at the peak of parasitaemia) and post treatments.

Survivability/Clinical signs: The rabbits were observed carefully throughout the experiment for clinical signs, morbidity and mortality. The survivability of the rabbits was determined by calculating the difference between the day of infection and the day the rabbits died. Mortality and morbidity rates were calculated.

Statistical Analysis: The values obtained were analysed using GENSTAT (Hemel Hempstead, Herts, UK). Data obtained were computed into means and analysed using Analysis of variance (ANOVA). Duncan's Multiple Range Test was used to separate the means of the different treatment groups. All results were expressed as mean ± standard error of mean (SEM) and values of $p<0.05$ were considered significant (Duncan, 1955).

RESULTS

Onset of Infection: All the infected rabbits exhibited parasitaemia 5 – 6 days post infection (PI) having a prepatent period of 5 days. The level of parasitaemia continued to increase and at the time of treatment which was done 16 days post infection, the level of parasitaemia has reached the references point in some of the infected groups B, C, D, E and F. Sixteen days PI groups C, D, E and F were treated. Two of the rabbits in group C died fourteen days PI, while the remaining one died seventeen days post infection. All the rabbits in group B died within 50 – 52 days post infection.

Clinical Signs: Clinical signs observed in the infected groups were anorexia, pale mucous membrane, depression and pyrexia. Following treatment, these signs gradually disappeared in the treated groups while it continued in the untreated group B with emaciation, ocular and nasal discharges and death.

Parasitaemia: All rabbits became parasitaemic on the 6th day PI and their levels continued to increase until day sixteen PI when the rabbits in groups C, D, E and F were treated (Table 1). The prepatent period for the infection in the experiment was 5 days. The parasites were cleared in all the infected and treated groups within 48 hours of treatment and remained aparasitaemic until 43 days after clearance when group D relapsed. Parasitaemia persisted in the rabbits in group B and this eventually led to their death between days 50 – 52 PI.

Temperature: There was no significant ($p<0.05$) difference in the rectal temperature of the rabbits at day 0. There were significant differences in the rectal temperatures of infected and treated groups (Group C, D, E and

Table 1: Parasitaemia and mortality pattern of rabbit infected with *Trypanosoma brucei brucei* and treated with varied doses of diaminazine aceturate and isomethamidium chloride

Day	Group A Uninfected/ untreated	Group B Infected / untreated	Group C 3.5 mg/kg DA	Group D 7.0 mg/kg DA	Group E 3.5 mg/kg DA and 1mg/kg IC	Group F 7.0 mg/kg DA and 1 mg/kg IC
0	0/3	0/3	0/3	0/3	0/3	0/3
7	0/3	3/3	3/3	3/3	3/3	3/3
14	0/3	3/3	1/1	3/3	3/3	3/3
21	0/3	3/3	1/1	3/3	3/3	3/3
28	0/3	3/3	0	0/3	0/3	0/3
35	0/3	3/3	0	0/3	0/3	0/3
42	0/3	3/3	0	0/3	0/3	0/3
49	0/3	2/2	0	0/3	0/3	0/3
56	0/3	1/1	0	0/3	0/3	0/3
63	0/3	0	0	0/3	0/3	0/3
70	0/3	0	0	3/3	0/3	0/3
77	0/3	0	0	3/3	0/3	0/3
84	0/3	0	0	3/3	0/3	0/3
91	0/3	0	0	3/3	0/3	0/3

Note: Number of infected rabbits in a group/Number of rabbits surviving

F) when compared with the infected untreated (Group B) and uninfected, untreated (Group A) on day 7 PI (Table 2). The rabbits in Group C and B had significant increases ($p<0.05$) in their rectal temperatures and this was indicative of parasitaemia. By day 14 PI there was no significant ($p<0.05$) difference in the rectal temperatures of rabbits in groups D, E and F and that of the uninfected and untreated group (Group A) (Table 2). These temperatures differed significantly ($p<0.05$) with those of groups B and C which had significant increases ($p<0.05$) in their rectal temperatures and continued until death of all the rabbits in these groups. Group D rabbits had significant increase ($p<0.05$) in the rectal temperature at day 84, when compared with other treated groups and the uninfected and untreated group (Table 2).

Body Weight: The infection in rabbits caused a significant decrease ($p<0.05$) in the mean body weights of the infected group (B, C, D, E and F) when compared with the uninfected and untreated group (A) at day 7 PI (Figure 1). By day 14 PI, there was significant decrease ($p<0.05$) in the mean body weight of rabbits in the infected untreated group (Group B). The mean body weight of group B and C continued decreasing until death. Finally by day 21 PI and 7 days post treatment, there was a significant increase ($p<0.05$) in the mean body weight of all the infected and treated groups (Group D, E and F) when compared with the uninfected and untreated group (Group A).

Packed Cell Volume (PCV): The mean PCV of the various groups indicated that by day 7 PI, there was reduction in the mean PCV of all the infected rabbits (Groups B, C, D, E and F) which differed significantly ($p<0.05$) from that of uninfected and untreated rabbits (Group A) (Table 3). The rabbits in Groups B and C showed a significant decrease ($p<0.05$) in mean PCV when compared with other treated groups (Group D, E and F). By day 14 PI, there was significant decrease ($p<0.05$) in the mean PCV of all infected groups (Group B, C, D, E and F). PCV of animals in Group B and C continued decreasing until death. By day 21(7 days post treatment), there was no significant difference ($p>0.05$) in the mean PCV of the treated groups (D, E and F), but showed a significant decrease ($p<0.05$) in the mean PCV when compared with

Table 2: Temperature (^{0}C) of rabbits infected with *Trypanosoma brucei* and treated with different doses of diaminazine aceturate and a combination of diaminazine aceturate and isomethamidium chloride

Day	Group A Uninfected/ untreated	Group B Infected / untreated	Group C 3.5 mg/kg DA	Group D 7.0 mg/kg DA	Group E 3.5 mg/kg DA and 1mg/kg IC	Group F 7.0 mg/kg DA and 1 mg/kg IC
0	39.00±0.06^a	39.47±0.09^a	39.30±0.12^a	38.83±0.09^a	39.17±0.20^a	39.20±0.20^a
7	39.40±0.12^a	40.27±0.07^b	41.10±0.17^a	39.97±0.12^{ab}	39.83±0.44^{ab}	39.97±0.29^{ab}
14	38.77±0.09^a	40.50±0.25^b	41.50±0.00^c	38.83±0.03^a	38.87±0.03^a	38.90±0.06^a
21	38.70±0.00^a	0.00±0.00	0.00±0.00	38.70±0.10^a	38.67±0.07^a	38.93±0.09^a
28	39.00±0.20^a	0.00±0.00	0.00±0.00	39.07±0.15^a	38.87±0.09^a	39.03±0.03^a
35	39.03±0.12^{ab}	0.00±0.00	0.00±0.00	39.17±0.09^{ab}	38.83±0.12^a	39.27±0.12^b
42	38.73±0.09^a	0.00±0.00	0.00±0.00	38.93±0.20^a	38.90±0.20^a	39.03±0.12^a
49	39.03±0.18^a	0.00±0.00	0.00±0.00	39.00±0.26^a	38.73±0.09^a	39.03±0.12^a
56	39.37±0.15^{bc}	0.00±0.00	0.00±0.00	38.97±0.12^{ab}	38.90±0.06^a	39.43±0.17^c
63	39.17±0.15^a	0.00±0.00	0.00±0.00	38.97±0.15^a	39.13±0.09^a	39.20±0.12^a
70	38.90±0.10^a	0.00±0.00	0.00±0.00	39.17±0.19^{ab}	39.33±0.12^a	39.33±0.03^b
77	39.23±0.18^a	0.00±0.00	0.00±0.00	39.30±0.15^a	39.10±0.10^a	39.47±0.03^a
84	39.23±0.12^b	0.00±0.00	0.00±0.00	39.60±0.06^c	38.93±0.03^a	39.17±0.07^a
91	39.07±0.09^a	0.00±0.00	0.00±0.00	39.37±0.12^b	39.23±0.18^{ab}	38.87±0.09^a

DA = diaminazine aceturate, IC = isomethamidium chloride, Different superscript in a row (a, b and c) indicates significant difference between the treatment means at p<0.05

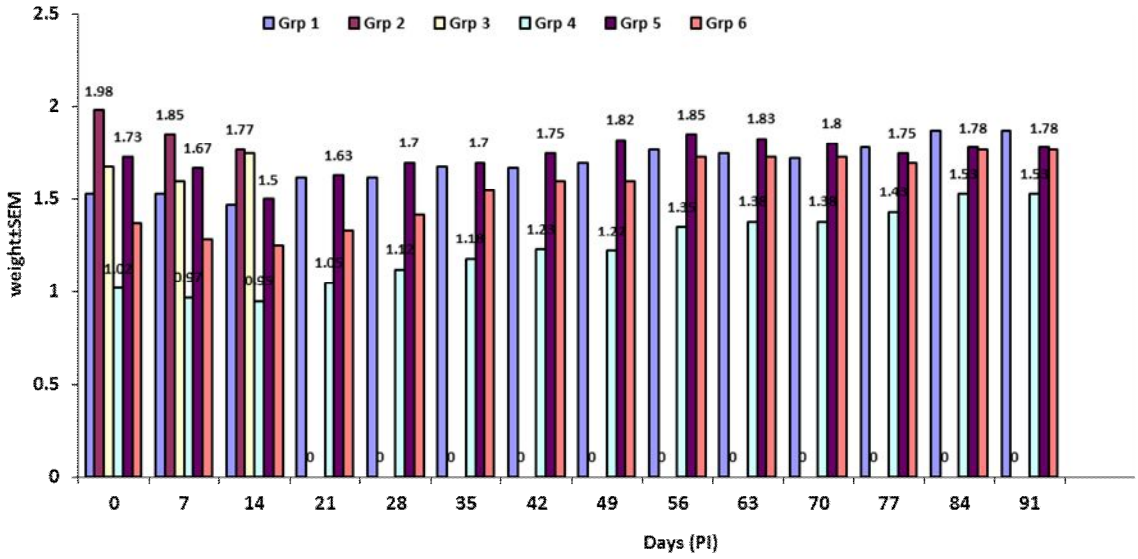

Figure 1: Weight (kg) of rabbits infected with *Trypanosoma brucei brucei* and treated with different doses of diaminazine aceturate and a combination of diaminazine aceturate and isomethamidium chloride

the uninfected and untreated (Group A). By day 28 (14 days post treatment), there was no significant difference (p>0.05) in the mean PCV of the treated groups (Group D, E and F) and that of the uninfected and untreated group (Group A).

By day 14 post treatment, Group D showed a significant decrease (p<0.05) in the mean PCV when compared to the other treated groups (Group E and F) and uninfected and untreated (Group A).

Table 3: Packed cell volume (%) of rabbits infected with *Trypanosoma brucei* and treated with different doses of diaminazine aceturate and a combination of diaminazine aceturate and isomethamidium chloride

Day	Group A Uninfected/ untreated	Group B Infected / untreated	Group C 3.5 mg/kg DA	Group D 7.0 mg/kg DA	Group E 3.5 mg/kg DA and 1mg/kg IC	Group F 7.0 mg/kg DA and 1 mg/kg IC
7	36.00±0.58^{c}	28.67±1.76^{bc}	29.33±0.88^{bc}	20.33±1.33^{a}	28.00±4.51^{ab}	25.33±2.85^{ab}
14	36.67±0.67^{d}	20.67±0.67^{ab}	26.00±0.00^{c}	17.33±0.88^{a}	22.33±1.76^{bc}	18.67±2.19^{ab}
21	36.33±0.88^{a}	0.00±0.00	0.00±0.00	25.00±1.53^{a}	25.67±0.67^{a}	28.33±1.20^{a}
28	36.67±0.88^{b}	0.00±0.00	0.00±0.00	34.33±0.88^{a}	33.67±2.19^{a}	33.33±1.20^{a}
35	36.33±0.67^{a}	0.00±0.00	0.00±0.00	37.67±0.88^{a}	37.00±1.53^{a}	35.33±0.33^{a}
42	37.00±0.58^{a}	0.00±0.00	0.00±0.00	40.33±1.45^{a}	37.67±3.84^{a}	38.33±0.67^{b}
49	36.67±0.67^{bc}	0.00±0.00	0.00±0.00	40.67±0.67^{c}	32.00±0.58^{a}	36.00±2.13^{ab}
56	42.67±1.20^{a}	0.00±0.00	0.00±0.00	43.33±1.45^{a}	40.33±2.19^{a}	44.00±1.73^{a}
63	36.00±0.58^{a}	0.00±0.00	0.00±0.00	34.67±3.28^{a}	32.67±1.45^{a}	35.67±1.76^{c}
70	35.67±0.33^{ab}	0.00±0.00	0.00±0.00	32.67±1.76^{a}	35.33±0.67^{ab}	38.67±2.19^{b}
77	36.67±0.33^{b}	0.00±0.00	0.00±0.00	31.33±1.33^{a}	35.67±0.67^{b}	39.67±2.03^{b}
84	37.67±0.67^{ab}	0.00±0.00	0.00±0.00	34.67±0.33^{a}	34.67±0.88^{a}	39.33±2.03^{a}
91	36.33±0.88^{b}	0.00±0.00	0.00±0.00	33.00±1.00^{ab}	31.33±0.88^{a}	34.41±1.53^{a}

DA = diaminazine aceturate, IC = isomethamidium chloride, Different superscript in a row (a, b and c) indicates significant difference between the treatment means at p<0.05

Table 4: Haemoglobin concentration (g/dl) of rabbits infected with *Trypanosoma brucei brucei* and treated with different doses of diaminazine aceturate and a combination of diaminazine aceturate and isomethamidium chloride

Day	Uninfected/ untreated	Infected / untreated	3.5 mg/kg DA	7.0 mg/kg DA	3.5 mg/kg DA and 1mg/kg IC	7.0 mg/kg DA and 1 mg/kg IC
7	11.48±4.76^{a}	9.23±0.35^{a}	9.10±0.76^{a}	9.13±0.09^{a}	9.57±0.26^{a}	8.67±0.69^{a}
14	16.17±0.26^{b}	8.83±0.27^{a}	9.00±0.00^{a}	8.63±0.23^{a}	8.30±0.55^{a}	8.23±0.58^{a}
21	11.48±4.76^{a}	0.00±0.00	0.00±0.00	9.13±0.09^{a}	9.57±0.26^{a}	8.67±0.69^{a}
28	16.17±0.26^{b}	0.00±0.00	0.00±0.00	8.63±0.23^{a}	8.30±0.55^{a}	8.22±0.58^{a}
35	14.73±1.11^{b}	0.00±0.00	0.00±0.00	9.40±0.09^{a}	9.57±0.19^{a}	9.23±0.23^{a}
42	15.60±0.47^{b}	0.00±0.00	0.00±0.00	10.64±0.38^{a}	9.97±0.55^{a}	10.50±0.50^{a}
49	16.30±0.36^{b}	0.00±0.00	0.00±0.00	13.40±0.49^{a}	12.13±1.13^{a}	11.80±0.17^{a}
56	14.87±0.54^{a}	0.00±0.00	0.00±0.00	15.33±0.39^{a}	12.36±1.96^{a}	13.37±0.43^{a}
63	14.87±0.55^{b}	0.00±0.00	0.00±0.00	14.83±0.77^{b}	10.40±0.06^{a}	11.70±0.86^{a}
70	16.43±0.07^{b}	0.00±0.00	0.00±0.00	15.37±0.79^{ab}	13.97±0.55^{a}	14.33±0.32^{a}
77	14.77±0.50^{a}	0.00±0.00	0.00±0.00	11.87±2.18^{a}	10.60±1.06^{a}	11.50±0.81^{a}
84	14.33±0.15^{b}	0.00±0.00	0.00±0.00	11.03±0.98^{a}	12.47±0.54^{ab}	12.80±0.25^{ab}
91	16.00±0.35^{c}	0.00±0.00	0.00±0.00	10.23±0.54^{a}	14.10±0.12^{a}	13.87±0.84^{b}

DA = diaminazine aceturate, IC = isomethamidium chloride, Different superscript in a row (a, b and c) indicates significant difference between the treatment means at p<0.05

Haemoglobin Concentration: The mean haemoglobin concentration value of the various groups indicated that there were no significant differences (p>0.05) in the mean Hb concentration value of the infected groups (Groups B, C, D, E and F) when compared with uninfected and untreated group (A) by day 7 PI (Table 4). There were significant differences (p<0.05) in the mean Hb concentration values of the infected groups (Group B, C, D, E and F) when compared to the uninfected and untreated group (A) by day 14 PI. The infected groups (B, C, D, E and F) showed a significant decrease (p<0.05) in the mean Hb concentration value

when compared with uninfected and untreated (Group A). The mean Hb concentration values of rabbits in Groups B and C continued decreasing until death. At day 21 PI there was no significant ($p>0.05$) difference in the mean concentration values when compared to the treated groups (Group E and F), and the uninfected and untreated (Group A).

Erythrocyte Count: There was a significant difference in the mean erythrocyte value of all the infected groups (Group B, C, D, E and F) when compared with uninfected and untreated group (A) by day 7 PI (Figure 2). All the infected groups showed a significant decrease ($p<0.05$) in the mean erythrocyte count when compared to the uninfected and untreated group (A) with the group D being significantly lower ($p<0.05$) than the entire infected group by day 14 PI. The erythrocyte count of rabbits in groups B and C continued to decrease until death. At day 21 and 28 there was significant increase ($p<0.05$) in the erythrocyte count of the infected and treated groups (Group D, E and F), but these values were significantly lower ($p<0.05$) than the erythrocyte value of the uninfected and untreated group (A). There was no significant difference ($p>0.05$) between treated groups (Group D, E and F) and the uninfected and untreated group (A) and by day 70 PI. Group D rabbits showed a significant ($p<0.05$) decrease in the mean erythrocyte count when compared to other treated groups (Group E and F) and the uninfected group (A), showing there was relapse in these treated groups. This decrease in erythrocyte count continued until day 91.

Leucocyte Count: The infection in the rabbit caused a significant ($p<0.05$) increase in the mean TLC value in Group A (uninfected and untreated) when compared to the other infected groups (Groups C, D, E and F) and the uninfected and untreated group (A) by day 7 PI (Table 5). There was significant ($p<0.05$) decrease in the mean TLC of all the infected groups (Group B, C, D, E and F) when compared with the uninfected and untreated group (A) by day 14 PI. There was significant difference ($p<0.05$) in the mean TLC value of the treated groups (Group D, E and F) when compared with the uninfected and untreated group (A) by day 21 PI. By day 70 PI there was no significant difference ($p>0.05$) in the mean TLC values of rabbits in groups D and E but had significant increase ($p<0.05$) in the mean TLC values when compared to groups F and A. Fourteen days later, at day 84 PI, group D rabbits had significant decrease ($p<0.05$) in mean TLC value when compared with the other treated groups (E and F), and uninfected and untreated group (A).

Lymphocyte Count: The mean absolute lymphocyte count (ALC) value for the various groups indicated that the infection in the rabbits produced a significant increase ($p<0.05$) in ALC value in group B (infected and untreated group) when compared with other infected groups (C, D, E and F) and uninfected and untreated group (A) by day 7 PI (Table 6). Furthermore, there was a significant ($p<0.05$) decrease in the mean ALC value of the infected groups (Groups B, C, D, E and F) compared to the uninfected and untreated group (A) by day 14 PI. This decrease continued in groups B and C until death. By day 21 PI, there was no significant difference ($p>0.05$) in the mean ALC values of the treated groups (D, E and F) compared to uninfected and untreated group (A), and by day 70 PI, groups D and E had significant increase ($p<0.05$) in the mean ALC values when compared to the other treated group (F) and the uninfected and untreated group (A).

Neutrophil Count: The mean absolute neutrophil count (ANC) values of the various groups indicated that the infection in the rabbits produced significant increase ($p<0.05$) in the mean ANC value of group B rabbits compared to other infected groups (Group B, C and E) (Table 7). There was also a significant decrease ($p<0.05$) in the mean ANC value of the infected group (Group B, C, D, E and F) when compared with the uninfected and untreated group (A). This decrease leads to the death of the rabbits in group B and C. By day 21 PI there was no significant difference ($p<0.05$) between the treated groups (Group D, E and F) and the uninfected and untreated group (A).

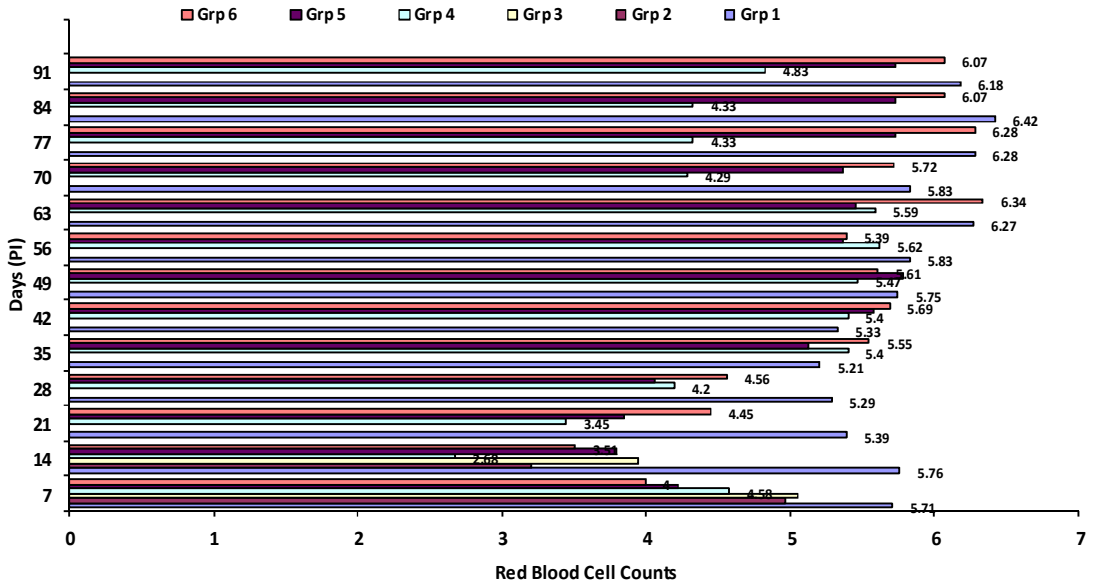

Figure 2: Erythrocyte counts of rabbits infected with *Trypanosoma brucei brucei* and treated with different doses of diaminazine aceturate and a combination of diaminazine aceturate and isomethamidium chloride

Table 5: Total leucocyte count (10^3 cells/mm^3) of rabbits infected with *Trypanosoma brucei brucei* and treated with different doses of diaminazine aceturate and a combination of diaminazine aceturate and isomethamidium chloride

Day	Group A Uninfected/ untreated	Group B Infected / untreated	Group C 3.5 mg/kg DA	Group D 7.0 mg/kg DA	Group E 3.5 mg/kg DA and 1mg/kg IC	Group F 7.0 mg/kg DA and 1 mg/kg IC
7	10.01±0.96^a	18.11±2.54^b	10.81±0.24^a	12.50±1.44^a	12.11±1.03^a	8.11±1.42^a
14	9.60±0.90^b	5.93±0.93^a	6.80±0.00^{ab}	1.33±0.77^a	2.94±1.70^{ab}	1.31±0.76^a
21	9.41±0.28^a	0.00±0.00	0.00±0.00	11.13±3.97^a	11.60±1.06^a	12.53±1.70^a
28	11.01±0.20^a	0.00±0.00	0.00±0.00	11.66±2.53^a	11.06±0.45^a	10.80±2.40^a
35	8.60±0.72^a	0.00±0.00	0.00±0.00	8.90±1.30^a	7.41±1.24^a	7.58±0.85^a
42	9.13±0.42^b	0.00±0.00	0.00±0.00	7.60±1.06^a	7.53±0.50^a	6.93±0.42^a
49	7.13±1.70^a	0.00±0.00	0.00±0.00	7.26±1.40^a	8.26±2.72^a	7.93±1.33^a
56	7.40±1.40^a	0.00±0.00	0.00±0.00	6.80±1.56^a	7.06±0.99^a	8.20±0.92^a
63	9.20±0.40^a	0.00±0.00	0.00±0.00	8.06±1.76^a	8.18±0.51^a	9.05±0.61^a
70	8.20±0.92^a	0.00±0.00	0.00±0.00	13.10±0.26^b	11.93±1.10^a	7.96±1.00^a
77	9.13±0.70^a	0.00±0.00	0.00±0.00	8.93±0.70^a	7.90±0.44^a	8.93±0.81^a
84	9.24±0.13^b	0.00±0.00	0.00±0.00	8.06±0.01^a	9.16±0.51^{ab}	9.30±0.43^b
91	9.46±0.42^b	0.00±0.00	0.00±0.00	7.76±0.90^a	9.03±0.43^b	8.91±0.64^a

DA = diaminazine aceturate, IC = isomethamidium chloride, Different superscript in a row (a, b and c) indicates significant difference between the treatment means at p<0.05

By day 70 PI group D rabbits had significant increase ($p<0.05$) in the mean ANC value when compared with other treated groups (Group E and F) and the uninfected and untreated group (A). By day 77 and 84 PI, there was no significant difference ($p>0.05$) in the mean ANC value between the treated groups (D, E and F) and the uninfected and untreated group (A) but on day 91 PI, group D rabbits had significant decrease ($p<0.05$) in the mean ANC value compared to other treated group (group E and F) and the uninfected and untreated group (A).

Table 6: Lymphocyte counts (10^3 cells/mm^3) of rabbits infected with *Trypanosoma brucei brucei* and treated with different doses of diaminazine aceturate and a combination of diaminazine aceturate and Isomethamidium chloride

Day	Group A Uninfected/ untreated	Group B Infected / untreated	Group C 3.5 mg/kg DA	Group D 7.0 mg/kg DA	Group E 3.5 mg/kg DA and 1mg/kg IC	Group F 7.0 mg/kg DA and 1 mg/kg IC
7	141.90±15.96^{a}	245.04±52.01^{b}	158.63±3.20^{a}	176.25±22.62^{a}	165.46±18.07^{ab}	118.00±28.52^{a}
14	133.29±8.74^{b}	83.25±12.47^{a}	96.56±0.00^{ab}	84.75±13.08^{a}	109.00±20.95^{ab}	75.85±10.74^{a}
21	125.25±8.57^{a}	0.00±0.00	0.00±0.00	161.51±32.47^{a}	175.23±6.09^{a}	189.09±16.32^{a}
28	151.25±12.99^{a}	0.00±0.00	0.00±0.00	180.49±28.91^{a}	165.40±12.39^{a}	189.09±21.41^{a}
35	118.73±13.24^{a}	0.00±0.00	0.00±0.00	135.09±13.93^{a}	114.36±6.45^{a}	110.89±5.48^{a}
42	119.97±3.60^{b}	0.00±0.00	0.00±0.00	107.33±12.25^{ab}	101.64±0.66^{ab}	97.20±1.65^{a}
49	82.40±11.73^{a}	0.00±0.00	0.00±0.00	87.31±6.20^{a}	96.76±18.09^{a}	90.51±4.95^{a}
56	85.29±5.42^{a}	0.00±0.00	0.00±0.00	83.40±7.33^{a}	84.47±4.64^{a}	94.64±2.68^{a}
63	97.38±0.33^{a}	0.00±0.00	0.00±0.00	90.47±8.42^{a}	97.52±2.32^{a}	97.37±1.19^{a}
70	92.71±4.30^{a}	0.00±0.00	0.00±0.00	165.01±3.45^{b}	153.28±6.84^{b}	92.92±2.74^{a}
77	98.27±0.57^{a}	0.00±0.00	0.00±0.00	97.48±2.39^{a}	92.09±2.40^{a}	97.75±2.18^{a}
84	116.17±4.23^{b}	0.00±0.00	0.00±0.00	92.03±3.48^{a}	97.56±2.40^{a}	102.96±2.18^{a}
91	113.75±6.99^{a}	0.00±0.00	0.00±0.00	99.17±4.87^{a}	116.16±2.72^{a}	114.31±7.14^{a}

DA = diaminazine aceturate, IC = isomethamidium chloride, Different superscript in a row (a, b and c) indicates significant difference between the treatment means at p<0.05

Table 7: Neutrophil counts (10^3 cells/mm^3) of rabbits infected with *Trypanosoma brucei brucei* and treated with different doses of diaminazine aceturate and a combination of diaminazine aceturate and isomethamidium chloride

Day	Group A Uninfected/ untreated	Group B Infected / untreated	Group C 3.5 mg/kg DA	Group D 7.0 mg/kg DA	Group E 3.5 mg/kg DA and 1mg/kg IC	Group F 7.0 mg/kg DA and 1 mg/kg IC
7	50.57±13.47^{a}	97.27±2.88^{b}	49.00±1.21^{a}	62.69±11.06^{a}	57.95±13.47^{a}	36.78±8.70^{a}
14	54.53±10.18^{b}	32.12±6.77^{ab}	38.08±0.00^{ab}	25.88±3.49^{a}	41.08±10.27^{ab}	24.76±50.55^{a}
21	59.14±4.99^{a}	0.00±0.00	0.00±0.00	51.60±11.78^{a}	48.56±9.04^{a}	50.55±3.77^{a}
28	56.61±11.81^{a}	0.00±0.00	0.00±0.00	38.65±3.08^{a}	43.76±12.98^{a}	52.24±5.82^{a}
35	37.49±6.79^{a}	0.00±0.00	0.00±0.00	33.87±10.98^{a}	26.99±6.88^{a}	33.21±4.87^{a}
42	54.12±0.88^{b}	0.00±0.00	0.00±0.00	38.08±3.42^{a}	40.95±4.82^{ab}	36.35±5.60^{a}
49	52.31±7.41^{a}	0.00±0.00	0.00±0.00	59.25±4.82^{a}	61.75±11.68^{a}	60.65±13.17^{a}
56	61.29±3.69^{a}	0.00±0.00	0.00±0.00	54.13±7.59^{a}	50.12±5.28^{a}	62.92±8.64^{a}
63	79.92±5.84^{a}	0.00±0.00	0.00±0.00	63.41±11.91^{a}	59.60±4.19^{a}	77.01±7.78^{a}
70	63.63±5.87^{ab}	0.00±0.00	0.00±0.00	84.72±3.37^{b}	74.17±6.37^{b}	59.53±8.54^{a}
77	75.83±7.03^{a}	0.00±0.00	0.00±0.00	72.91±6.18^{a}	59.04±3.33^{a}	71.92±8.33^{a}
84	59.89±4.59^{a}	0.00±0.00	0.00±0.00	61.89±8.62^{a}	77.87±6.39	75.08±5.97^{a}
91	64.21±3.35^{b}	0.00±0.00	0.00±0.00	48.22±3.72^{a}	57.33±3.95^{ab}	58.04±1.77^{ab}

DA = diaminazine aceturate, IC = isomethamidium chloride, Different superscript in a row (a, b and c) indicates significant difference between the treatment means at p<0.05

DISCUSSION

The findings obtained from this study which showed that *Trypanosoma brucei brucei* is pathogenic in rabbits was in agreement with the reports of Kuzovkov *et al*. (1980) and Ezema *et al*. (2009). *Trypanosoma brucei brucei* produced parasitaemia in all infected rabbits between days 5 – 6 PI, this was in agreement with the reported incubation period of 5 – 6 days by Rovid Spickler *et al.* (2010) but, did not agree

with the findings of Sewell and Brocklesby (1990) and Ezeokonkwo and Agu (2004) who recorded an incubation period of 3 – 4 days in domestic rabbits and Ezebuiro *et al*. (2012) who recorded the same period for rabbits. This maybe particular to the strain of the isolate used in the experiment. Parasitaemia in a susceptible animal could be influenced by some factors which may include the number of parasites inoculated, stressors such as starvation, age, presence or absence of concurrent infections, immune response of the host and the pathogenicity of the strain of *Trypanosoma brucei brucei* (Taylor and Authie, 2004). The results of this study showed variations in parasitaemia in the groups, this maybe as a result of age differences as the animals in group B which were older than those in group C. Following treatment, all the treated group with the exception of group C, became aparasitaemic by 48 hours post treatment, though the groups (E and F) treated with the combination became aparasitaemic 24 hours post treatment, they remained aparasitaemic until day 56 post treatment when there was relapse in group D. Relapse following treatment has been severally reported (Sutherland *et al.,* 1992; Egbe-Nwiyi and Antia, 1996; Gall *et al*., 2004; Anene *et al*., 2006; Ezeh *et al*., 2011). This may be attributed to the fact that the very large molecules of diaminazine aceturate could not pass the blood brain barrier; therefore treatment with this drug will not affect parasites sequestrated in the brain (Jennings *et al*., 1980; Barrett, 2001; Geert *et al.,* 2001). Treatment in this study was done late on day 14 PI and thus could be the reason for the relapse and death in group C as suggested by Akpa *et al*. (2008) that relapse after early treatment could be due to drug resistance but relapse after late treatment could be due to pre-treatment invasion of the brain tissue by the parasite.

The clinical signs observed in the rabbits such as anorexia, pale mucous membrane, pyrexia, rough hair coats, emaciation, depression, ocular and nasal discharges were similar to those previously reported in mice, rats and dogs infected with *Trypanosoma brucei brucei* (Onyeyili and Anika, 1990; Anene *et al*., 1999; Ezeokonkwo and Agu, 2004; Obidike *et al*., 2005) and cattle infected with *Trypanosoma congolense* (Valli *et al*., 1978). However, following treatment, these signs gradually disappeared in groups D, E and F, showing that the drugs used were able to reverse the signs. The ability of the combination therapy to clear the parasitaemia in infected rabbits earlier (24 hours) than DA treated rabbits (48 hours) showed that combination therapy achieved a faster optimal therapeutic blood level and activity than single DA therapy.

Anaemia which was evident in this work corroborated previous reports that established anaemia as a cardinal feature in animal trypanosomiasis (Igbokwe and Mohammed, 1992; Omotainse and Anosa, 1995; Akinbamijo *et al*., 1998; Dina *et al*., 2002). Although *T. brucei brucei* is a tissue trypanosome, it has a high pathological effect on haematological parameters of the host (Van den Bossche and Rowlands, 2001), which is capable of inducing severe anaemia and death if untreated or treated late. Anaemia being the most important clinicopathological finding in animal trypanosomiasis occurs due to immune-mediated haemolysis (Chaudhary and Iqbal, 2000) which accounted for the reduction in PCV post infection and this is consistent with the findings of Ezeokonkwo and Agu (2004), Obidike *et al*. (2005) and Anene *et al*. (2006). Other factors that can cause anaemia include; depression of erythropoiesis (Andrinarivo *et al*., 1995), disorders of coagulation (Murray and Dexter, 1998) and increased plasma volume and haemodilution (Taylor and Authie, 2004). However this reversal was much pronounced in group F that received 7 mg/kg and 1 mg/kg of DA and isomethamidium, respectively. Furthermore, PCV and red blood count values of the treated rabbits compared favourably with the uninfected control group (A) but this was not sustained in group d because relapse parasitaemia occurred by day 56 post treatment. Leucocytosis was observed 7 days post infection in this study, this agreed with the findings of Onyeyili and Anika (1989) in dogs infected with *Trypanosoma brucei brucei,* Omer *et al*. (2007) and Adeyemi *et al*. (2010) in rats infected with *Trypanosoma evansi* and *Trypanosoma brucei brucei,* respectively.

Leucocytosis which may be due to lymphocytosis has been implicated in trypanosomiasis and these conditions are usually as a result of wax and wear syndrome on the animal's immune system (Abubakar *et al.,* 2005). Leucopenia was observed in all the infected groups by day 14 post infection and this was in agreement with the findings of Abubakar *et al*. (2005) in *Trypanosoma brucei* infected rats. The neutropenia recorded in the infected groups agreed with the findings of Kagira *et al*. (2006) and Allam *et al*. (2011) but disagreed with that Chaudhary and Igbal (2000), who observed an increase in neutrophil counts in camels infected with *Trypanosoma evansi.* The decrease in neutrophil counts observed in the present study may be as a result of overwhelming secondary bacterial infection due to immunosuppression in the infected groups (Kagira *et al*., 2006; Allam *et al*., 2011). The lymphopenia and neutropenia noticed returned to normal 7 days post treatment when they showed a significant increase ($p<0.05$) when compared with the uninfected and untreated with the exception of group B and C. however, this was not sustained in group D as relapse occurred by 56 days post treatment resulting in leucocytosis and lymphocytosis.

Conclusion: The clinical signs and haematological parameters were reversed and restored faster in groups treated with the combination of Diaminazine aceturate and Isomethamidium chloride thus producing a better therapeutic effect than DA alone. More so, the group treated with higher dose of DA in combination with IC produced the best therapeutic effect. This indicates that the synergy of the drug pair had not yet been overwhelmed by prevalent resistance to trypanocides. From the above finding, it was concluded that early treatment of Trypanosomiasis using agents singly or their combination may be necessary to prevent relapse or death. We recommend that combination therapy of DA and IC should be adopted in the treatment of animals with trypanosomiasis which will elongate their period of protection from this disease since relapse was not recorded in the aforementioned group that produced the best therapeutic effect throughout the period of this work.

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