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Haematological and Biochemical Changes Associated with A Combination Therapy of Artemether and Diminazene Aceturate on Experimental Trypanosoma Brucei Infection in Rats

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ABSTRACT

This study investigated the haematological and biochemical changes associated with a combination therapy of artemether and diminazene aceturate on experimental Trypanosoma brucei infection in albino rats. Thirty five male albino rats were used for the study. The groupings and their specific treatments were as follows: Group A- infected and treated with diminazene aceturate (DA) at the dose of 7.0 mg/kg body weight (bw) once on day 7 post-infection (pi); Group B – infected and treated with artemether (AM) at the dose of 3.2 mg/kg bw on day 7pi and 1.6mg/kg bw on days 8,9,10 and 11pi; Group C – infected and treated with DA at the dose of 7.0 mg/kg bw once on day 7pi and AM at the doses of 3.2 mg/kg bw on day 7pi plus 1.6mg/kg bw on days 8,9,10 and 11pi; Group D – infected and treated with DA at the dose of 3.5mg/kg bw once on day 7pi and AM at the doses of 3.2 mg/kg bw on day 7pi and 1.6mg/kg bw on days 8,9,10 and 11pi; Group E –infected and treated with DA at the dose of 1.75mg/kg bw once on day 7pi and AM at 3.2mg/kg bw on day 7pi and 1.6mg/kg bw on days 8,9,10 and 11pi; Group F – infected, untreated and Group G – uninfected, untreated. Red blood cell (RBC) counts, haemoglobin concentration (HBC) packed cell volume (PVC) total white blood cell (TWBC) counts, serum alanine amino-transferase (ALT) aspartate amino-transferase (AST), alkaline phosphate (ALP), urea and creatinine were assayed at specified intervals during the 70-day experimental period. Infection with trypanosomes led to decrease in erythrocytic parameters and TWBC counts, and elevations in serum ALT, AST, urea and creatinine which were reversed by treatment in rats in groups A and D but not in groups B, C and E. It was concluded that a combination of DA (3.5mg/kg bw once) and AM (3.2 mg/kg bw on day 1 of treatment and 1.6 mg/kg bw for 4 consecutive days) was able to reverse all haematologic and biochemical changes in trypanosomes-infected rats at rate comparable to the standard diminazene aceturate dose of 7mg/kg. It was concluded that the combination of 3.5 mg/kg of diminazene aceturate with artemether as used in group D may constitute an effective treatment regimen to reduce the occurrence of toxicity and resistance.

Keywords: *Combination therapy, Artemether, Diminazene aceturate, Trypanosoma brucei infection, rats*

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INTRODUCTION

Trypanosomosis is a haemoparasitic infection of man and animals, caused by trypanosome parasites belonging to the genus Trypanosoma [1]. Its transmission can be mechanical, when the parasite is transferred directly from an infected host to another without undergoing further development in the vector [2] or cyclical, when the parasite develops in the digestive tract of the vector [3].

A lot of pathological changes are associated with *trypanosomosis* in man and animals but, the severity of these changes, are related to the strain of the invading parasites and response of the host animal [4, 5]. Among the changes associated with trypanosomosis are alterations in the physiology of the host animal clinically manifesting as haematological and biochemical changes in the body system of the infected animal [6]. Reversal of these parameters to normal values are part of the signs of recovery by the infected animal [7].

Diminazene aceturate is a diamidine compound recommended for use as a therapeutic agent against animal trypanosomosis. It has been used successfully in the treatment of trypanosomosis of dogs and livestock [8]. However, several cases of toxicity have been associated with its administration in animals [9] and the parasite is also fast developing resistance to the drug [10]. Combination chemotherapy appears the only way out of the present challenge in the absence of new drugs against the disease [11].

Artemether, a derivative of artemisinin is an isolate from *Artemisia anua* which has been used effectively against malaria parasites the world over [12]. It inhibits protein and nucleic acid synthesis in plasmodium parasites resulting in high schizontocidal action against the blood forms of the parasite [13]. Artemether has been reported to reduce the motility of trypanosomes in-vitro, as well as days of aparasitaemia after in-vivo administration in trypanosomes infected mice [14].

Presently, there is little or no information on a combination therapy of artemether and diminazene in animal trypanosomosis hence this study which investigated the haematological and biochemical changes associated with a combination therapy of artemether and diminazene aceturate on experimental *Trypanosoma brucei* infection in rats.

MATERIALS AND METHODS

Experimental Animals

Thirty-five adult male albino rats weighing between 150 and 250 grams were procured from the Zoology Department of the University of Nigeria, Nsukka and used for the study. The rats were housed in the Animal House of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka and allowed to acclimatize for 7 days before the commencement of the study. They were fed on pelleted commercial feed (Grand Cereals Limited Jos, Nigeria) from Grand Cereals Limited throughout the period of the experiment and provided with clean drinking water,ad libitum.

Trypanosome parasites

Trypanosoma brucei used in the experiment was obtained from the Veterinary Parasitology Department of the University of Nigeria, Nsukka. The parasite was identified using its morphological characteristics on a Giemsa stained thin blood film preparation [15].

Drugs

Artemether injection (Pauco^{R}) (Jin Ling Pharmaceutical, China), and diminazene aceturate (Diminaze^{R}) (PANTEX HOLLAND) were used in this experiment.

Infection of Experimental Animal

Trypanosomes for infection of the experimental animals were suspended in normal saline and 1.25 x 10^{5} trypanosomes were used to infect each experimental rat in the infected groups through the intra-peritoneal route. The trypanosomes were quantified using the rapid matching method of Herbert and Lumsden [16].

Experimental Design

The 35 male adult albino rats were weighed and randomly assigned to seven groups (A – G) of five rats each, as follows;

Group A: Infected and treated with Diminazene aceturate at dose of 7.0mg/kg body weight once on day 7 post infection.

Group B:- Infected and treated with Artemether at the dose of 3.2 mg/kg on day 7 post-infection and at the dose of 1.6mg/kg body weight on days 8, 9, 10 and 11 post infection.

Group C:- Infected and treated with a combination of Diminazene aceturate at the dose of 7.0mg / kg body weight once on day 7 post- infection, and Artemether at the dose of 3.2mg/kg body weight on day 7 post-infection and 1.6 mg /kg daily for the following 4 days.

Group D:- Infected and treated with a combination of Diminazene aceturate at the dose of 3.5mg/kg body weight once on day 7 post-infection and Artemether at a dose of 3.2mg per kg body weight on day 7 post-infection and 1.6mg/kg body weight for the following 4 days.

Group E:- Infected and treated with a combination of Diminazene aceturate at the dose of 1.75mg/kg body weight on day 7 post-infection and Artemether at 3.2mg/kg body weight on day 7 post-infection and at a dose of 1.6mg/kg body weight for the following 4 days.

Group F: Infected and Untreated
Group G: Uninfected; Untreated

Blood Collection

Blood for haematological and biochemical tests were collected from the retro-bulblar plexus of the medial canthus of the eye into a sample bottle containing ethylene diamine tetra-acetic acid (EDTA).

Blood was also collected from the tail vein of the rats for determination of onset, level and clearance of parasitaemia.

Parasitaemia* in rats infected with Trypanosoma brucei and treated with a combination of artemether and diminazene aceturate

Days post infection	GROUPS						
	A	B	C	D	E	F	
1	0/5	0/5	0/5	0/5	0/5	0/5	0/5
2	0/5	0/5	0/5	0/5	0/5	0/5	0/5
3	3/5	0/5	3/5	4/5	2/5	0/5	0/5
4	5/5	5/5	5/5	5/5	5/5	5/5	0/5
5	5/5	5/5	5/5	5/5	5/5	5/5	0/5
6	5/5	5/5	5/5	5/5	5/5	5/5	0/5
7+	5/5	5/5	5/5	5/5	5/5	5/5	0/5
8	4/5	5/5	5/5	4/5	5/5	5/5	0/5
9	0/5	5/5	0/5	0/5	0/5	5/5	0/5
10	0/5	5/5	0/5	0/5	0/5	5/5	0/5
11	0/5	All Dead	0/5	0/5	0/5	4/4	0/5
12	0/5	All Dead	0/5	0/5	0/5	4/4	0/5
13	0/5	All Dead	0/5	0/5	0/5	3/3	0/5
14	0/5	All Dead	0/5	0/5	0/5	All Dead	0/5
28	0/5	All Dead	0/5	0/5	3/5	All Dead	0/5
42	0/5	All Dead	1/5	0/5	5/5	All Dead	0/5
56	0/5	All Dead	3/5	0/5	4/4	All Dead	0/5
70	0/5	All Dead	3/5	0/5	All Dead	All Dead	0/5

+ Commencement of treatment

* Number of parasitaemic animal in the group/total number of animals in the group

A – Infected, treated with DA (7.0mg/kg), B – infected, treated with AM , C – infected, treated with AM and DA (7.0mg/kg) D – infected, treated with AM and DA (3.5mg/kg) E. Infected, treated with AM and DA (1.75mg/kg), F – infected, untreated, G – uninfected, untreated.

Parameters Monitored

Haematological tests were conducted on samples to determine RBC count, haemoglobin concentration, PCV, total white blood cell count, at fortnightly.

Finally, serum from the blood was used to determine the levels of alanine aminotransferase (ALT), aspertate aminotransferase (AST), alkaline phosphatase (ALP), urea and creatinine.

Haematological Techniques

The total RBC and WBC count were done by the haemocytometer method, using the improved Neubauer Counting Chamber [17]. The PCV was determined by microhaematocrit method [18].

Haemoglobin concentration was determined by the cyanomethaemoglobin spectrophotometric method as described by Coles [17].

Biochemical Techniques

Serum harvested from blood was used to determine the following biochemical parameters:

a. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activitieswere determined by the Reitman – Frankel colorimetric method [19].

b. Serum alkaline phosphatase activity was determined following the phenolphthalein monophosphate method [20].

c. Serum urea levels were determined following the modified Berthelot- Searcy method [21].

d. Serum creatinine levels were determined following the modified Jaffe method [22]

Statistical Analysis

One way analysis of variance (ANOVA) and Duncan's post hoc were applied in the analysis of data. Results were presented as means and standard errors of the parameters, in tables, graphs. Values of $P<0.05$ were considered as statistically significant.

RESULTS

The Red Blood Cell Counts

The results of the red blood cell (RBC) counts are presented in Table1. Values of red blood cell count decreased in rats in groups A,B,C,D,E and F after day zero. There was significant ($p<0.05$) reduction in RBC counts of groups A,B,C,D,E and F when compared to that of rats in Group G on day 7 post-infection. There was no significant difference among the RBC counts of rats in Groups G,A,C,D and E from day 14 to day 42 post infection. The mean RBC counts of rats in Group E was significantly lower ($p<0.05$) than those of Groups G,A,C and D on day 56 post infection. The RBC counts of rats in Groups G, A and D were significantly higher than those of Group C rats on day 70 post-infection. All the rats in groups B and F died before day 14 while all the rats in group E died before day 70.

The Haemoglobin Concentration

Results of the haemoglobin (HB) concentration are presented in Table 2. Values of haemoglobin concentration decreased in rats in groups A,B,C,D,E, and F. The mean HB of rats in Groups A,B,C,D,E and F on day 7 post-infection were significantly lower than that of Group G rats. There was no significant difference in the HB of rats in Groups A,C,D,E and G between day 14 and 42 of the experiment. The mean HB of rats in Groups C and E did not vary significantly on day 56 post infection but were significantly lower than that of Groups G, A, and D. The mean HB of rats in Groups A,D and G on day 70 post infection were significantly higher than that of Group C rats. All the rats in groups B and F died before day 14 while all the rats in group E died before day 70.

Packed Cell Volume

The results of the packed cell volume (PVC) are presented in Table 3. Values of the packed cell volume decreased in rats in groups A,B,C,D,E and F. The mean PCV of rats in Groups A,B,C,D,E, and F were significantly lower than that of Group G rats on day 7 post-infection. There was no significant difference among the PCV of rats in Groups A,C,D and E on day 14 post infection. On day 28, the mean PCV of Groups G, A, D, and C rats were significantly higher ($p<0.05$) than that of Group E rats. On day 42 post infection the mean PCV of rats in Groups G, A, and D were significantly higher ($p<0.05$) than those of Groups C and E. On day 56 post infection, the mean PCV of rats in Group G was significantly higher ($p<0.05$) than those of Groups A,C,D and E. There were no significant differences ($p>0.05$) between Groups C and E as well as between Groups A and D on day 56 post infection. The mean PCV of rats in Groups A and D were significantly higher ($p<0.05$) than those of Groups C and E on day 56 post infection. On day 70 post-

infection, the mean PCV of rats in Group G was significantly higher ($p<0.05$) than that of Groups A, C and D. All the rats in groups B and F died before day 14 while all the rats in group E died before day 70.

The White Blood Cell Counts

The results of the white blood cell (WBC) counts are presented in Table 4. Values of WBC count decreased in rats in groups A,B,C,D,E, and F after day zero. The mean WBC counts of rats in Groups A, B, C, D, E and F were significantly lower than that of Group G rats on day 7 post-infection. The mean WBC counts of rats did not differ significantly ($p>0.05$) between day 14 and 28 post infection among Groups A, B, C, D, E, and G. The mean WBC counts of rats in Groups A, D, and G were significantly higher ($p<0.05$) than those of Groups E and C on day 42 post infection. The mean WBC counts of rats in Groups A,D, and G did not vary significantly from one another but were significantly higher ($p<0.05$) than that of Group C rats. The mean WBC counts of Group C rats were higher than that of Group E on day 56 post infection but significantly lower than those of Groups G, A, and D. The mean WBC counts of rats in Groups G and D on day 70 post infection were significantly higher than that of Group A rats, but the mean WBC of Group A rats was significantly higher than that of Group C. All the rats in group B and F died before day 14 while all the rats in group E died before day 70.

Alanine Aminotransferase

The results of the mean alanine aminotransferase (ALT) activity of rats are presented in Table 5. Values of Alanine Aminotrasferase increased in rats in groups A,B,C,D,E, and F after day zero. The mean ALT of rats in Groups A,B,C,D,E and F on day 7 post infection did not vary significantly but were significantly higher ($P<0.05$) than that of Group G. On day 56 post infection, the mean ALT of rats in Group E was significantly higher ($P<0.05$) than those of rats in Groups G,A,D and C. On day 70, the mean ALT of rats in Groups A, G and C were significantly lower than that of Group D. All the rats in groups B and F died before day 14 while all the rats in group E died before day 70.

Alkaline Phosphotase

The results of the mean alkaline phosphotase (ALP) of rats are presented in Table 6. There was no significant variation ($P>0.05$) in the mean ALP activity of rats on days zero and 7 of the experiment. By day 14 all the rats in Groups B and F were dead but there was no significant difference between the ALP of rats in Groups A, C, D, E and G. By day 70 all the rats in Group E were dead and there was no significant difference between the mean ALP of rats in Groups A, C, D and G.

Aspartate Aminotransferase

The results of the mean Aspartate Aminotransferase (AST) activity of rats are shown in Table 7. Values of Aspartate Aminotransferase increased after day zero in rats in groups A,B,C,D,E and F. The mean AST of rats in Group G was significantly lower ($p<0.05$) than those of the infected groups on day seven post infection. On day 14 post infection, the mean AST of rats in Group C was significantly higher ($p<0.05$) than those of Groups D, E and G. The mean AST of rats in Group E was significantly higher than those of Groups A,C,D and G between day 28 and 56 post infection. On day 70 post infection, the mean AST of rats in Group G was significantly lower ($p<0.05$) than that of Groups A, D and C. All the rats in groups B and F died before day 14 while all the rats in group E died before day 70.

Blood urea nitrogen

Results of the blood urea nitrogen (BUN) levels are presented in Table 8. Values of blood urea nitrogen increased in rats in groups A,B,C,D,E and F after day zero.The mean BUN of rats in Groups A,B,C,D,E, and F were significantly higher than those of Group G on day 7 post infection. There was no significant difference

(P>0.05) among the mean BUN of rats in Groups A, C, D and E on day 7 post infection. There was no significant difference (P>0.05) among the mean BUN of rats in Groups A, C,D and E between day 14 and 42 post- infection. The mean BUN of rats in Groups A, C, D, E and G did not significantly vary (P>0.05) on day 56 post infection. The mean BUN of rats in Groups G, A and D were significantly lower (P<0.05) than that of Group C rats on day 70 post infection. All the rats in groups B and F died before day 14 while all the rats in group E died before day 70.

Creatinine

Results of the creatinine (CRT) levels in rats are presented in Table 9. Values of creatinine increased in rats in groups A,B,C,D,E, and F after day zero. The mean CRT of rats of Groups A,B,C,D,E,F were significantly higher (p<0.05) than that of Group G on day 7 post infection. There was no significant difference (p>0.05) among the mean CRT of rats in Groups A,G,C,D and E between day 14 and 28 post infection. The mean CRT of rats in Group E and C was significantly higher (p<0.05) than that of Groups A, D and G between day 42 and 56 post infection. There was no significant difference among the mean CRT of rats in Groups A, D and G between day 42 and 70 post infection. The mean CRT of rats in Groups G, A and D were significantly lower than that of Group C rats on day 70 post infection. All the rats in groups B and F died before day 14 while all the rats in group E died before day 70.

Table 1: The mean (±SEM) RBC (x $10^6/\pi l$) in rats infected with *Trypanosoma brucei* and treated with a combination of artemether and diminazene aceturate.

Days of Study	Groups						
	A	B	C	D	E	F	G
0	7.33 ±0.54	7.58 ±0.46	7.30 ±0.42	7.32 ±0.42	7.45 ±0.27	7.34 ±0.56	7.45 ±0.50
7	5.41[a] ±0.38	4.89[a] ±0.24	5.06[a] ±0.18	5.31[a] ±0.38	5.30[a] ±0.53	5.20[a] ±0.14	8.24[b] ±0.54
14	7.80 ±0.74	All Dead	7.65 ±0.5	7.65 ±0.56	8.48 ±0.31	All Dead	7.49 ±0.87
28	7.19 ±0.29	All Dead	7.03 ±0.31	7.18 ±0.43	7.60 ±0.63	All Dead	7.61 ±0.60
42	7.26 ±0.30	All Dead	7.32 ±0.63	6.70 ±0.15	6.92 ±0.37	All Dead	7.24 ±0.42
56	7.00[a] ±0.27	All Dead	7.10[a] ±0.32	7.05[a] ±0.18	4.89[b] ±0.42	All Dead	7.32[a] ±0.25
70	7.20[b] ±0.36	All Dead	5.41[a] ±0.16	7.31[b] ±0.22	All Dead	All Dead	7.42[b] ±0.31

a b – different superscripts in a row indicate statistically significant difference at $P \leq 0.05$.

A – Infected, treated with DA (7.0mg/kg), B – infected, treated with AM, C – infected, treated with AM and DA (7.0mg/kg) D – infected, treated with AM and DA (3.5mg/kg) E. Infected, treated with AM and DA (1.75mg/kg), F – infected, untreated, G – uninfected, untreated.

Table 2: The mean (±SEM) Haemoglobin concentration (mg/dl) in rats infected with *Trypanosoma brucei* and treated with a combination of artemether and diminazene aceturate.

Days of Study	GROUPS						
	A	B	C	D	E	F	G
0	12.49 ±0.38	13.45 ±0.78	12.79 ±0.94	13.06 ±0.69	14.12 ±0.31	13.87 ±1.08	13.66 ±0.35
7	11.88[a] ±0.25	11.55[a] ±0.69	11.58[a] ±0.21	11.78[a] ±1.48	11.38[a] ±0.47	11.52[a] ±0.21	14.85[b] ±0.39
14	15.12 ±1.32	All Dead	14.16 ±1.47	14.62 ±0.60	15.77 ±0.87	All Dead	15.62 ±0.29
21	15.52 ±0.87	All Dead	14.72 ±0.70	13.95 ±2.15	13.28 ±0.66	All Dead	14.35 ±0.59
42	15.65 ±0.81	All Dead	14.57 ±0.59	13.83 ±2.30	14.20 ±0.47	All Dead	14.50 ±1.02
56	15.28[b] ±0.70	All Dead	11.60[a] ±0.57	14.67[b] ±1.80	11.21[a] ±0.59	All Dead	14.56[b] ±1.02
70	15.36[b] ±0.80	All Dead	11.41[a] ±0.59	15.00[b] ±1.90	All Dead	All Dead	14.89[b] ±1.02

a b – different superscripts in a row indicate statistically significant difference at $P \leq 0.05$.

A – Infected, treated with DA (7.0mg/kg), B – infected, treated with AM, C – infected, treated with AM and DA (7.0mg/kg) D – infected, treated with AM and DA (3.5mg/kg) E. Infected, treated with AM and DA (1.75mg/kg), F – infected, untreated, G – uninfected, untreated.

Table 3: The mean (± SEM) PCV (%) in rats infected with *Trypanosoma brucei* and treated with a combination of artemether and diminazene aceturate.

Days of Study	GROUPS						
	A	B	C	D	E	F	G
0	41.25 ±3.71	39.75 ±1.44	39.00 ±2.04	39.00 ±0.71	40.75 ±2.29	39.00 ±1.63	40.00 ±1.91
7	30.50^{a} ±1.26	30.25^{a} ±0.85	29.50^{a} ±2.87	29.50^{a} ±2.0	31.00^{a} ±0.71	31.75^{d} ±0.63	40.25^{b} ±1.38
14	34.50 ±0.50	All Dead	35.75 ±0.25	36.25 ±1.18	34.25 ±1.11	All Dead	39.25 ±1.25
28	36.50^{a} ±0.29	All Dead	36.75^{a} ±1.25	38.25^{a} ±1.44	33.75^{b} ±2.17	All Dead	39.50^{a} ±1.32
42	37.50^{a} ±1.04	All Dead	36.25^{b} ±0.85	38.25^{a} ±1.25	35.00^{b} ±0.58	All Dead	40.00^{a} ±1.08
56	37.50^{a} ±1.06	All Dead	34.17^{b} ±1.80	37.50^{a} ±0.80	34.08^{b} ±0.62	All Dead	40.00^{c} ±1.5
70	37.62^{a}	All Dead	36.50^{a} ±0.84	38.62^{a} ±1.20	All Dead	All Dead	40.52^{b} ±0.62

a b c – different superscripts in a row indicate statistically significant difference at $P \leq 0.05$.

A – Infected, treated with DA (7.0mg/kg), B – infected, treated with AM, C – infected, treated with AM and DA (7.0mg/kg) D – infected, treated with AM and DA (3.5mg/kg) E. Infected, treated with AM and DA (1.75mg/kg), F – infected, untreated, G – uninfected, untreated.

Table 4: The mean (± SEM) WBC (x10 $^{3}/\pi l$) in rats infected with *Trypanosoma brucei* and treated with a combination of artemether and diminazene aceturate.

Days of Study	GROUPS						
	A	B	C	D	E	F	G
0	12.68 ±0.35	13.08 ±0.53	13.35 ±0.25	13.22 ±0.46	12.80 ±0.46	13.25 ±0.39	12.95 ±0.39
7	8.65^{a} ±0.67	8.33^{a} ±0.84	8.25^{a} ±0.27	8.57^{a} ±0.63	8.45^{a} ±0.23	8.13^{a} ±0.22	13.63^{b} ±0.40
14	12.55 ±0.53	All Dead	12.25 ±0.60	11.93 ±0.19	12.23 ±0.89	All Dead	13.00 ±0.67
28	13.50 ±0.49	All Dead	12.65 ±0.79	12.85 ±0.77	12.70 ±0.64	All Dead	13.08 ±0.33
42	13.45^{a} ±0.53	All Dead	11.98^{b} ±0.44	13.28^{a} ±0.54	10.75^{b} ±0.70	All Dead	12.80^{a} ±0.30
56	13.48^{a} ±0.32	All Dead	11.41^{b} ±0.21	13.30^{a} ±0.23	9.25^{c} ±0.18	All Dead	13.24^{a} ±0.32
70	12.68^{a} ±0.22	All Dead	10.16^{b} ±0.16	13.40^{c} ±0.13	All Dead	All Dead	13.48^{c} ±0.24

a b c – different superscripts in a row indicate statistically significant difference at $P \leq 0.05$.

A – Infected, treated with DA (7.0mg/kg), B – infected, treated with AM, C – infected, treated with AM and DA (7.0mg/kg) D – infected, treated with AM and DA (3.5mg/kg) E. Infected, treated with AM and DA (1.75mg/kg), F – infected, untreated, G – uninfected, untreated.

Table 5: The mean (± SEM) ALT activity (u/l) in rats infected with *Trypanosoma brucei* and treated with a combination of artemether and diminazene aceturate.

Days of Study	GROUPS						
	A	B	C	D	E	F	G
0	40.83 ±6.33	30.06 ±5.54	30.83 ±6.31	28.93 ±0.62	26.32 ±5.68	26.05 ±4.89	28.55 ±1.48
7	122.97 [a] ±3.81	125.82 [a] ±3.46	117.99 [a] ±5.95	109.30 [a] ±6.04	121.95 [a] ±3.42	117.30 [a] ±6.56	31.32 [b] ±10.02
14	46.14 ±1.30	All Dead	55.69 ±4.94	42.60 ±7.32	37.70 ±5.46	All Dead	30.90 ±6.48
28	22.01 ±3.30	All Dead	24.52 ±1.85	31.23 ±9.78	43.49 ±9.93	All Dead	24.11 ±2.23
42	21.75 ±3.10	All Dead	24.02 ±1.59	30.73 ±9.86	43.55 ±9.96	All Dead	23.67 ±2.23
56	29.45[a] ±3.68	All Dead	37.75[a] ±1.90	24.00[a] ±1.03	64.11[b] ±6.24	All Dead	22.82[a] ±2.57
70	41.05[a] ±4.22	All Dead	42.60[a] ±7.32	117.99[b] ±5.95	All Dead	All Dead	30.90[a] ±6.48

a b – different superscripts in a row indicate statistically significant difference at $P \leq 0.05$.

A – Infected, treated with DA (7.0mg/kg), B – infected, treated with AM, C – infected, treated with AM and DA (7.0mg/kg) D – infected, treated with AM and DA (3.5mg/kg) E. Infected, treated with AM and DA (1.75mg/kg), F – infected, untreated, G – uninfected, untreated.

Table 6: The mean (± SEM)ALP activity (u/l)in rats infected with *Trypanosoma brucei* and treated with a combination of artemether and diminazene aceturate.

Days of Study	GROUPS						
	A	B	C	D	E	F	G
0	89.70 ±13.21	58.79 ±8.23	64.90 ±17.20	55.25 ±11.25	62.04 ±13.11	58.75 ±18.02	48.05 ±13.07
7	62.05 ±17.24	59.25 ±8.30	38.55 ±6.74	70.52 ±19.02	64.40 ±24.22	66.15 ±18.75	55.05 ±11.39
14	40.95 ±13.68	All Dead	40.90 ±13.70	41.45 ±13.78	34.35 ±6.95	All Dead	34.35 ±6.95
28	55.30 ±18.96	All Dead	41.42 ±7.93	42.25 ±13.26	42.25 ±7.43	All Dead	53.18 ±16.94
42	66.04 ±15.05	All Dead	51.15 ±8.20	75.90 ±13.21	58.79 ±8.23	All Dead	55.25 ±11.25
56	59.08 ±8.17	All Dead	44.35 ±12.45	63.70 ±18.72	49.50 ±3.29	All Dead	42.60 ±5.68
70	66.04 ±15.05	All Dead	51.15 ±8.20	75.90 ±13.21	All Dead	All Dead	55.25 ±11.25

A – Infected, treated with DA (7.0mg/kg), B – infected, treated with AM, C – infected, treated with AM and DA (7.0mg/kg) D – infected, treated with AM and DA (3.5mg/kg) E. Infected, treated with AM and DA (1.75mg/kg), F – infected, untreated, G – uninfected, untreated.

Table 7: The mean (± SEM) AST activity (u/l) in rats infected with *Trypanosoma brucei* and treated with a combination ofartemether and diminazene aceturate.

Days of Study	GROUPS						
	A	B	C	D	E	F	G
0	95.56 ±12.67	91.29 ±5.17	79.33 ±7.23	69.35 ±6.29	75.60 ±14.45	86.62 ±7.34	71.47 ±5.10
7	307.88[a] ±10.26	296.56[a] ±1.60	303.62[a] ± 9.85	294.69[a] ±6.00	306.72[a] ±11.61	310.37[a] ±5.60	63.79[b] ±8.86
14	77.72[a][b] ±1.33	All Dead	81.04[a] ±5.89	60.09[b] ±7.45	60.32[b] ±10.19	All Dead	67.30[b] ±8.86
28	82.64[a] ±7.41	All Dead	77.95[a] ±5.91	77.86[a] ±5.86	122.85[b] ±5.00	All Dead	69.30[a] ±8.21
42	86.20[a] ±5.43	All Dead	77.60[a] ±5.05	84.20[a] ±3.03	121.35[b] ±7.64	All Dead	67.49[a] ±6.53
56	95.64[a] ±6.41	All Dead	80.95[a] ±5.91	77.86[a] ±5.86	171.35[b] ±17.64	All Dead	71.31[a] ±7.64
70	88.20[a] ±7.43	All Dead	87.60[a] ±5.05	84.20[a] ±3.03	All Dead	All Dead	63.32[b] 7.64

a b – different superscripts in a row indicate statistically significant difference at $P \leq 0.05$.

A – Infected, treated with DA (7.0mg/kg), B – infected, treated with AM, C – infected, treated with AM and DA (7.0mg/kg) D – infected, treated with AM and DA (3.5mg/kg) E. Infected, treated with AM and DA (1.75mg/kg), F – infected, untreated, G – uninfected, untreated.

Table 8: The Mean (± SEM) BUN levels (mg/dl) inrats infected with *Trypanosoma brucei* and treated with a combination of artemether and diminazene aceturate.

Days of Study	GROUPS						
	A	B	C	D	E	F	G
0	17.93 ±0.66	17.59 ±1.30	19.04 ±1.44	19.13 ±1.37	19.54 ±0.89	19.42 ±1.71	17.78 ±1.86
7	23.67^{b} ±1.12	24.05^{b} ±1.23	22.98^{b} ±1.20	24.05^{b} ±0.43	24.82^{b} ±0.38	23.88^{b} ±0.45	19.52^{a} ±0.89
14	19.55 ±1.00	All Dead	18.75 ±0.89	19.38 ±0.50	19.15 ±0.97	All Dead	17.53 ±1.20
28	19.55 ±0.90	All Dead	18.75 ±0.87	19.38 ±0.78	19.15 ±0.67	All Dead	17.53 ±0.90
42	20.18 ±0.48	All Dead	20.07 ±1.06	19.55 ±0.98	19.92 ±0.49	All Dead	19.67 ±1.58
56	19.13 ±0.98	All Dead	19.48 ±1.26	19.68 ±0.67	19.70 ±0.76	All Dead	19.80 ±0.90
70	19.13^{a} ±0.42	All Dead	29.48^{b} ±1.59	19.68^{a} ±0.24	All Dead	All Dead	19.80^{a} ±1.39

a b – different superscripts in a row indicate statistically significant difference at $P \leq 0.05$.

A – Infected, treated with DA (7.0mg/kg), B – infected, treated with AM, C – infected, treated with AM and DA (7.0mg/kg) D – infected, treated with AM and DA (3.5mg/kg) E. Infected, treated with AM and DA (1.75mg/kg), F – infected, untreated, G – uninfected, untreated.

Table 9: The Mean (±SEM) Creatinine levels (mg/dl) in rats infected with *Trypanosoma brucei* and treated w ith a combination of artemether and diminazene aceturate.

Days of Study	Groups						
	A	B	C	D	E	F	G
0	0.27 ±0.16	0.44 ±0.06	0.35 ±0.09	0.16 ±0.02	0.17 ±0.01	0.26 ±0.09	0.32 ±0.12
7	0.78^a ±0.06	0.78^a ±0.05	0.72^a ±0.08	0.82^a ±0.05	0.80^a ±0.07	0.82^a ±0.05	0.33^b ±0.13
14	0.32 ±0.07	All Dead	0.39 ±0.21	0.27 ±0.13	0.42 ±0.21	All Dead	0.24 ±0.05
28	0.37 ±0.12	All Dead	0.32 ±0.12	0.33 ±0.09	0.33 ±0.09	All Dead	0.32 ±0.13
42	0.28^a ±0.03	All Dead	0.89^a ±0.07	0.30^a ±0.03	0.92^b ±0.12	All Dead	0.28^a ±0.13
56	0.24^a ±0.12	All Dead	0.89^b ±0.03	0.30^a ±0.07	0.90^b ±0.07	All Dead	0.25^a ±0.05
70	0.28^a ±0.03	All Dead	0.92^b ±0.02	0.29^a ±0.04	All Dead	All Dead	0.24^a ±0.03

a b – different superscripts in a row indicate statistically significant difference at $P \leq 0.05$.

A – Infected, treated with DA (7.0mg/kg), B – infected, treated with AM, C – infected, treated with AM and DA (7.0mg/kg) D – infected, treated with AM and DA (3.5mg/kg) E. Infected, treated with AM and DA (1.75mg/kg), F – infected, untreated, G – uninfected, untreated.

DISCUSSION

Decrease in erythrocytic parameters of infected animals is a consistent feature in animal African trypanosomosis [23]. These parameters significantly decreased in all the infected groups by the 7th day post-infection. This period apparently corresponds with the first wave of parasitaemia in the infected groups. This decrease has severally been linked to destruction of red blood cells (RBC) by the trypanosome parasites [24, 25] and was reversed significantly ($p<0.05$) after treatment with diminazene aceturate (7.0 mg/kg body weight) and a combination of diminazene aceturate (3.5 mg) and Artemether (Group D). The restoration of erythrocytic values possibly corresponds with clearance of parasites after treatment [26] and activation of erythropoiesis [27]. The group treated with Artemeter alone did not show reversal in erythrocytic values and succumbed to the disease less than two weeks after infection leading to total mortality.

Leucopenia is one of the pathological changes associated with trypanosomosis in animals [28]. This may be associated with immusuppressive action of trypanosomes, [29, 24]. In the present study, a combination of artemether and diminazene aceturate (at 3.5mg/kg body weight) was able to restore leucocyte level to normal just like Diminazene aceturate alone (at 7.0mg/kg body weight).

Treatment with a combination of artemether and Diminazene aceturate (at 3.5mg/kg body weight) also reversed biochemical changes associated with trypanosomosis including elevated levels of AST, ALT , Creatinine and blood urea Nitrogen.

Conclusion

A combination of Artemeter and Diminazene aceturate (at 3.5mg/kg body weight) was able to reverse all haematological and biochemical changes associated with *trypanosomosis*in rats at a rate comparable to the standard Diminazene aceturate dose (7.0mg/kg body weight) and thus can possibly constitute an effective treatment regimen to reduce the occurrence of Diminazene aceturate toxicity and resistance in animals.

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