



Sahel J. Vet. Sci. Vol. 17, No. 2, pp. 7-12 (2020)
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Article History

Received: 1st March, 2020
Revised: 25th April, 2020
Accepted: 13th May, 2020
Published: 30th June, 2020

Evaluation of the Efficacy of a Combination of Artemether and Diminazene Aceturate Therapy in Experimental *Trypanosoma brucei* Infection in Rats

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ABSTRACT

This study evaluated the efficacy of a combination of artemether and diminazene aceturate therapy in experimental *Trypanosoma brucei* infection in rats. Thirty five male albino rats used for the study were randomly assigned to seven groups of five rats each as follows: Group A- infected and treated with diminazene aceturate (DA) at 7.0 mg/kg body weight (bw), intramuscular (IM) once on day 7 post-infection (pi); Group B – infected and treated with artemether (ART) at 3.2 mg/kg bw IM on day 7pi and 1.6 mg/kg bw IM on days 8,9,10 and 11pi; Groups C,D and E, – infected and treated with DA at 7.0 mg/kg bw IM, 3.5 mg/kg bw IM, and 1.75 mg/kg bw IM respectively, once on day 7pi and ART at 3.2 mg/kg bw IM on day 7pi plus 1.6 mg/kg bw on days 8,9,10 and 11pi; Group F –infected, untreated and Group G – uninfected, untreated. Onset of parasitaemia (OP), level of parasitaemia (LOP), clearance of parasites post treatment, mortality post infection, relapse of parasitaemia post clearance, rectal temperature, and body weight, were determined at specified intervals during the 70-day experimental period. Results showed that there were no significant ($p > 0.05$) variations in the OP and LOP between the infected groups. Trypanosomes were cleared from the blood of rats in group A, C, D, and E after treatment. All the rats in groups B and F were dead by day 14pi. The infection relapsed in groups C and E. It was concluded that a combination of DA (3.5 mg/kg bw once) and ART (3.2 mg/kg bw on day 1 of treatment and 1.6 mg/kg bw for 4 consecutive days) exhibited efficacy comparable to the standard dose of DA at 7 mg/kg in the treatment of *trypanosome brucei* in rats and could thus possibly constitute an effective treatment regimen to reduce the dose of DA and avoid toxicity.

Keywords: Efficacy; Combination therapy; Artemether; Diminazene aceturate; *Trypanosoma brucei*

INTRODUCTION

Trypanosomiasis is a parasitic disease of man and animal caused by infection with one or more of the blood parasites belonging to the genus *Trypanosoma* (Anene *et al.*, 2001). It is transmitted mainly through the bite of an insect vector, commonly Glossina species, when the infective stage of the trypanosome is inoculated with the saliva (Adam *et al.*, 2012).

Chemotherapy and chemophylaxis remain the most widely used method of trypanosomiasis control in Africa (Sulaiman and Adeyemi, 2010) but despite all efforts aimed at its control, the disease remains a limiting factor to livestock production in sub-saharan Africa. (Swallow, 2000).

Recently emphasis on the control and eradication of the disease has shifted to renewed search for safe, effective and affordable drugs (Ekanem and Yusuf, 2008; Abimbola *et al.*, 2013). In the face of increasing resistance, combination therapy has been suggested as the next level in the effort to overcome drug resistance (Apted, 1980).

Diminazene aceturate (DA), a diamidine compound has been used successfully in animal trypanosomiasis (Holmes *et al.*,

2004). Its use is however commonly associated with toxicity and even death (Losos and Crocket, 1969, Muller, 1988).

Artemether (ART) is a derivative of artemisinin, an extract from *Artemisia annua* which has been used against malaria in many parts of the world (Woodrow *et al.*, 2005). It has high schizontocidal action against blood forms of plasmodium through inhibition of nucleic acid and protein synthesis in the parasite (Classen *et al.*, 1999). Artemether has been reported to reduce motility of trypanosomes *in vitro* and on *in vivo* administration in mice infected with trypanosomes produced days of aparasitaemia (Akande and Fagbemi, 2011). Mbaya *et al.* (2009) and Adeyemi *et al.* (2009), reported the anti-trypanocidal effect of a combination of artemether and lumefantrin leading to reduction in level of parasitaemia and changes in the course of the disease. Mishina *et al.* (2007), investigated the effect of artemisinins on *Trypanosoma cruzi* and *Trypanosoma brucei rhodiense* and reported inhibition of parasitic development *in vitro*. Egbe-Nwiyi and Yakubu (2015), reported clearance of parasitaemia by a combination of diminazene aceturate and a Dihydroartemisinin in *Trypanosoma brucei brucei* infected rats.

There is little information in available literature on the efficacy of a combination therapy of diminazene aceturate and artemether, hence the present study which evaluated the efficacy of a combination of diminazene aceturate and artemether in experimental *Trypanosoma brucei* infection in albino rats.

MATERIALS AND METHODS

Ethical Statement

This study complied with global ethical standard for animal use and was approved by the ethical committee of the Faculty of Veterinary Medicine, UNN.

Experimental Animals and Groups

Thirty-five adult male albino rats weighing between 150 and 250 grams were procured from the Zoology Department of the University of Nigeria, Nsukka. The rats were randomly assigned to seven group (A-G) of 5 rats each, housed in spacious galvanized metallic cages and allowed to acclimatize for 7 days before the commencement of the study. They were fed on commercial feed pellets (Grand Cereals Limited, Jos, Nigeria) throughout the period of the experiment and clean drinking water was also provided *ad libitum*.

Trypanosome Infection of Animals

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 (Soulsby, 1986). The trypanosome count was estimated in a wet mount of the tail blood from a donor rat using the rapid matching method of Herbert and Lumsden (1976). Infected blood containing trypanosomes was diluted with normal saline and an inoculum containing 1.25×10^5 trypanosomes was used to infect each experimental rat through the intra-peritoneal route. The infected rats were in groups A-E and rats in group G were uninfected.

Treatment of Infected Animals

Injectable artemether (ATR) (Paucor^R; Jin Ling Pharmaceutical, China), and diminazene aceturate (Diminaze^R; Pantex, Holland) were used in this experiment to treat the trypanosome infections around the first peak of parasitaemia (day 7 post-infection) as single or combination therapy. The ART treatment regime was intramuscular injection at the thigh muscle for 5 days at 3.2 mg/kg body weight on day 7 post-infection followed by intramuscular injections at 1.6 mg/kg body weight on days 8,9,10 and 11 post-infection. The DA treatment regime was an intramuscular injection of a single dose on day 7 post-infection at either an increased (7.0 mg/kg, IDA) recommended (3.5 mg/kg, RDA) or decreased (1.75 mg/kg, DDA) dose rates. The treatments of infected groups were as follows: Group A with IDA, Group B with ART, Group C with IDA + ART, Group D with RDA + ART, Group E with DDA + ART and Group F with no treatment (NT)

Parasitological Techniques

Wet mount of blood was examined using a light microscope ($\times 400$ objective). Trypanosome parasites when present were detected and estimated by matching the density of parasites observed in a microscopic field with a standard (Herbert and Lunsden, 1976).

Rectal Temperature (RT) and Body Weight (BW)

Rectal temperature of rats was determined by insertion of aseptic clinical thermometer manually into the rectum of the animals and the reading taken after 2 minutes. The body weight of the rats was determined by placing each rat on a sensitive weighing scale and the value recorded.

Statistical Analyses

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. Values of $p < 0.05$ were considered as statistically significant.

RESULTS

Onset of Parasitaemia

Trypanosome parasites appeared in the blood of some rats in Groups A, C, D and E three days post infection. On day four, post infection, all the infected rats (Groups A, B, C, D, E and F) were parasitaemic. There were no significant variations ($p > 0.05$) in the mean onset of parasitaemia (OP) of rats in the infected groups (Table 1).

Level of Parasitaemia

The mean level of parasitaemia (LOP) of rats in the infected groups did not vary significantly ($p > 0.05$) starting from onset of parasitaemia (day 3) to day 8 post-infection. On days 9 and 10 post infection the mean LOP of rats in Group B was significantly ($p < 0.05$) higher than that of Group F while no parasite were seen in Groups A, C, B, and E (Table 2).

Clearance of Parasitaemia Post Treatment

There was complete clearance of parasites from the blood of rats in Groups A, C, D and E by day 2 post treatment (Table 1). There was no clearance of parasitaemia in rats in Groups B and F. The mean time taken to clear the parasites after treatment was 1.40 ± 0.25 , 1.60 ± 0.29 , 1.80 ± 0.25 and 2.00 ± 0.00 days, respectively, among Groups A, C, D and E and did not vary significantly ($p > 0.05$).

The Rectal Temperature (RT)

The mean RTs of the groups are presented in Figure 1. The mean RT of rats in Groups A, B, C, D, E and F did not vary significantly ($p > 0.05$) from one another but were significantly higher ($p < 0.05$) than that of Group G on day 7 post infection. There were no significant variations ($p > 0.05$) between the mean RT of rats in Groups A, C, D, E and G between days 14 and 42 post infection. The mean RT of rats in Groups A, C, D and G were significantly lower ($p < 0.05$) than that of Group E rats on day 56 post-infection.

Table 1: 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	G
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 (7.0 mg/kg), B – infected, treated with ART, C – infected, treated with ART and DA (7.0 mg/kg) D – infected, treated with ART and DA (3.5 mg/kg) E. Infected, treated with ART and DA (1.75 mg/kg), F – infected, untreated, G – uninfected, untreated.

Table 2: The Mean (\pm SEM) Level of Parasitaemia (10^5 parasites/mL) 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	G
3	0.35 (0.09)	0	0.41 (0.08)	0.36 (0.09)	0.44 (0.05)	0	0
4	4.34 (1.09)	3.72 (1.48)	3.82 (1.48)	4.23 (1.43)	3.93 (1.43)	3.88 (1.43)	0
5	4.54 (1.19)	3.92 (1.05)	3.78 (1.08)	4.03 (1.13)	3.81 (0.84)	4.43 (1.43)	0
6	4.34 (1.49)	3.72 (1.48)	3.72 (1.48)	4.03 (1.43)	3.51 (0.84)	4.53 (1.43)	0
7	4.54 (1.49)	3.92 (1.48)	3.72 (1.48)	4.03 (1.43)	4.23 (1.43)	4.23 (1.43)	0
8	3.54 (1.49)	2.95 (1.48)	2.76 (1.48)	2.09 (1.43)	2.28 (1.43)	3.28 (1.43)	0
9	0	8.68 ^a (1.33)	0	0	0	4.42 ^b (1.25)	0
10	0	9.82 (1.43)	0	0	0	7.68 (1.33)	0

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

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

The Mean RT of rats in Groups A, D and G did not vary significantly from one another but they were significantly lower ($p < 0.05$) than that of Group C rats on day 70 post infection (Figure 1).

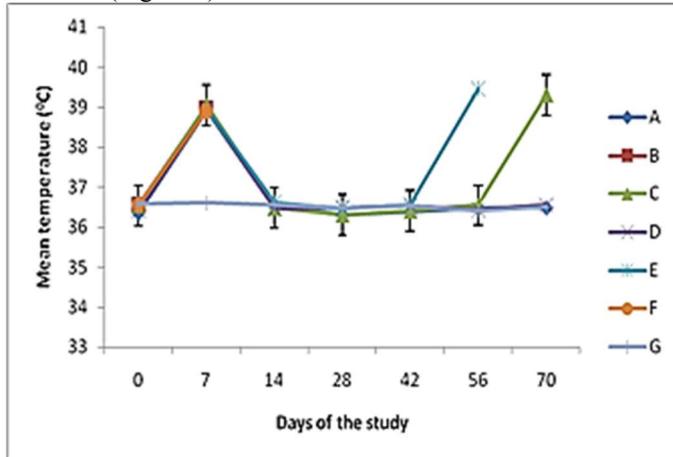


Figure 1: The mean (\pm SEM) rectal temperature ($^{\circ}$ C) in rats infected with *Trypanosoma brucei* and treated with a combination of artemether and diminazene aceturate.

The Body Weight (BW)

The mean BWs of the rat groups are presented in Figure 2. The mean BW of rats in Groups A, B, C, D, E, and F were significantly lower ($p < 0.05$) than that of Group G rats on day 7 post-infection. The mean BW of rats in Group G was significantly higher ($p < 0.05$) than that of Group A, and that of Group A was significantly higher ($p < 0.05$) than that of Groups C, D, and E on day 14 post infection. The mean BW of rats in Group C was significantly higher ($p < 0.05$) than Group E rats on day 14 post infection. The mean BW of rats in Group G was significantly higher ($p < 0.05$) than those of groups C D and E, on day 28 post-infection. The mean BW of rats in Group D was significantly higher ($p < 0.05$) than that of Group E rats on day 42 post infection. Similarly, that of rats in Group A was higher ($p < 0.05$) than that of Group C rats. The mean BW of rats in Group G rats was significantly higher ($p < 0.05$) than that of Group D rats on day 42 post infection. The mean BW of rats in Group G was significantly higher ($p < 0.05$) than those of Groups A, C, D, and E on day 56 post infection. The mean BW of rats in Groups A, C and D were significantly higher than that of Group E rats on day 56 post infection. The mean BW of rats in Groups G and D were significantly higher than those of Groups A and C on day 70 post infection (Figure 2).

Survival Time Post-Infection

Results of the survival time post-infection are presented on Figure 3. There was no death observed in rats in Groups A, C and D and thus their survival time exceeded 70 days study period. All the rats in Group B, F, and E died with mean survival time of 10 ± 2.0 , 12 ± 2.0 , and 56 ± 2.5 days, respectively. The mean post infection survival time of rats in Groups B and F were comparable but were significantly ($p < 0.05$) shorter than that of Group E (Figure 3).

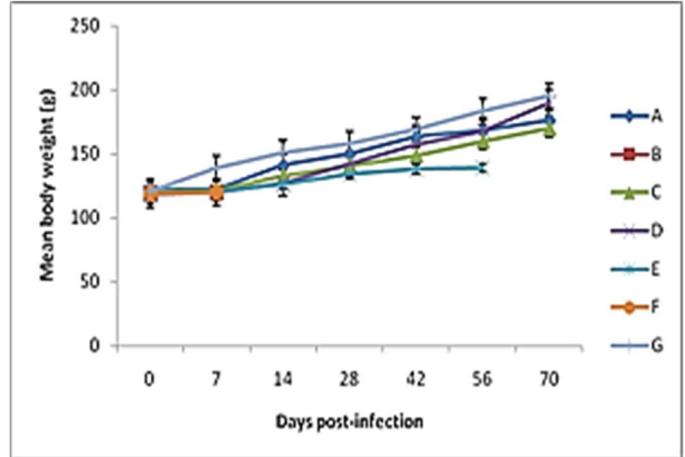


Figure 2: The mean (\pm SEM) body weight (g) in rats infected with *Trypanosoma brucei* and treated with a combination of artemether and Diminazene aceturate.

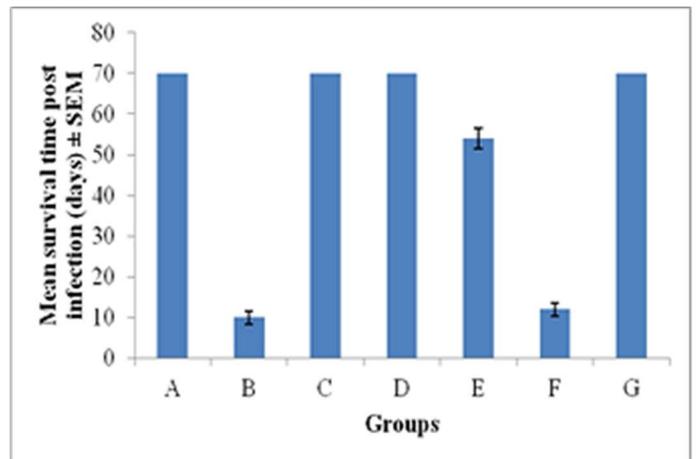


Figure 3: The mean (\pm SEM) survival time post-infection in rats infected with *Trypanosoma brucei* and treated with a combination of artemether and diminazene aceturate.

Relapse of Parasitaemia Post Clearance

Results of the relapse of parasitaemia post clearance are presented in Figure 4. There was no relapse of parasitaemia in rats in Groups A and D after clearance. There was relapse of parasitaemia in rats in Groups C and E. Rats in Groups A and D showed longer period of aparasitaemia (more than 60 days).

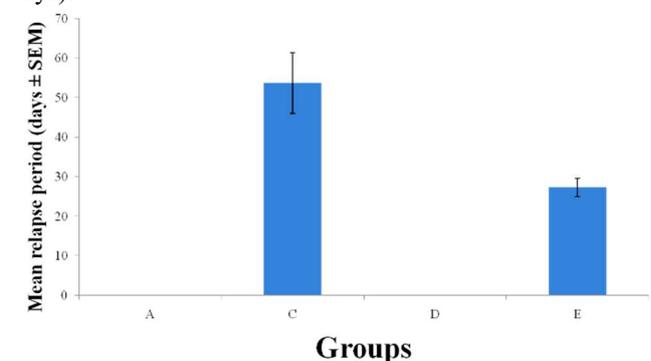


Figure 4: The mean (\pm SEM) relapse period in rats infected with *Trypanosoma brucei* and treated with a combination of artemether and Diminazene aceturate.

DISCUSSION

In this study, high temperature was observed in all the infected groups 7 days post infection. This is consistent with earlier studies on trypanosomosis infection (Anosa 1988a). The temperature returned to normal in all the treated groups except the group treated with ART alone by the 14th day of study. Only the group treated with diminazene aceturate (7.0 mg/kg) alone and the group treated with diminazene aceturate (3.5 mg/kg) in combination with ART, were able to maintain normal temperature range till the end of the experimental period.

There was significant variation in the level of weight gain between the infected and uninfected groups. The uninfected group showed greater degree of weight gain throughout the study period. This may be linked to reduced feed intake and poor conversion efficiency due to trypanosomosis (Ilemobade and Balogun, 1981). The group treated with diminazene aceturate (3.5 mg/kg) in combination with ART was able to show growth efficiency comparable to the uninfected group.

Parasitaemia was observed in all the infected groups before treatment. This period of parasitaemia corresponds with the period of high temperature signifying an acute crisis (Anosa, 1988a). Treatment with diminazene aceturate alone and in combination with ART led to clearance of parasitaemia. There was no clearance of parasitaemia in the group treated with ART alone. This is consistent with the findings of Omotainse *et al.* (2011), who reported non-reversal of parasitaemia in rabbits experimentally infected with *Trypanosoma brucei* and treated with ART and inconsistent with the findings of Akande and Fagbemi. (2011), who reported days of aparasitaemia in mice infected with trypanosomes and treated with artemether.

Relapse of infection was observed in the group treated with a combination of arthemether and diaminazene aceturate (at 1.75 mg/kg) on day 28 post infection but the group treated with artemether and diminazene aceturate (7 mg/kg) developed relapse at 42 days post infection.

The group treated with diminazene aceturate at 7.0mg/kg and the group treated with a combination of Artemether (3.2 mg/kg on day 1 and 1.6 mg/kg body weight for the following 4 days) and diminazene aceturate (at 3.5 mg/kg once) did not show any relapse for 70 days. Apparently, there was a degree of synergy between Artemether and diminazene leading to clearance of parasitaemia and prevention of relapse in this combination therapy group. This finding is comparable with the findings of Bacchi *et al.* (1994), which demonstrated strong synergism (100% cure rate) against CNS trypanosomosis following administration of diflouromethyl- ornithine (DFMO) in combination with Suramin even at doses which were without effect when used singly.

Relapse of infection in the group treated with a combination of diminazene aceturate (7.0 mg/kg) and artemether (3.2 mg/kg on day 1 and 1.6 mg/kg for the following 4 days) in contrast with the group treated with diminazene aceturate (7.0 mg/kg) alone, which did not relapse points to the possibility that artemether depresses the effectiveness of diminazene aceturate (at high doses) in treating trypanosomosis and preventing relapse. Apparently the synergy between

diminazene aceturate and artemether in the treatment of trypanosomosis is dose related and diminishes as the dose of diminazene aceturate increases.

Conclusion

Results of this study showed that a combination of diminazene aceturate (3.5 mg/kg bw once) and arthemeter (3.2 mg/kg bw on day 1 and 1.6 mg/kg body weight for the following 4 days) effectively cleared trypanosomes from the blood of infected rats and prevented relapse all through the 70 days experimental period. It is recommended that further study on this combination be undertaken to enable its prospective future use in the therapy of trypanosomosis.

Author Contribution

Okonkwo E.W., Anene B. M. and Ihedioha J. I. designed the work. Okonkwo E. W. undertook the laboratory analysis under the supervision of Anene B. M. and Ihedioha J. I. and drafted the initial manuscript. Okonkwo E. W. conducted the statistical analysis while Anene B.M. and Ihedioha J.I. proof-read the manuscript.

Conflict of Interest

The authors declare that they do not any conflict of interest regarding the publication of this work.

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