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COMPARATIVE EFFICACY OF SAMORECIDE® PLUS AND TRYPAMIDIUM-SAMORIN® IN THE TREATMENT OF EXPERIMENTAL *TRYPANOSOMA BRUCEI BRUCEI* INFECTION IN ALBINO RATS

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ABSTRACT

The comparative efficacy of Samorecide® Plus and Trypamidium-Samorin® was investigated in Trypanosoma brucei brucei infected albino rats. Thirty adult albino rats divided into six groups of five rats each were used. All rats in groups II – VI were each infected with $8x10^5$ trypanosomes while those in group I served as uninfected controls. Groups III and IV were respectively treated singly with Samorecide® plus and Trypamidium-Samorin® on day 14 post infection. Group V was treated with Samorecide® plus first, and then with Trypamidium-samorin® seven days later, while group VI was treated with Trypamidium-samorin® first and then Samorecide® plus seven days later. Weekly temperature, weight changes, packed cell volume, haemoglobin concentrations, clinical signs, survivability and rate of parasite clearance from the blood were the parameters used to assess efficacy of the drugs. Aparasitaemia in the Samorecide® plus treated groups was noted 94 hours post treatment while this occurred in 124 hours post treatment with Trypamidium-Samorin®. All rats in the infected/untreated group died by 25 days post infection. Relapse parasitaemia was recorded in two of the rats treated only with Samorecide® plus. Significant reductions ($p<0.05$) in weight gain, packed cell volume, and haemoglobin concentration while the increase ($p<0.05$) in temperature following infection were reversed by the treatments. This reversal however lasted longer in the combined treatment groups than in the single treatment groups. The results obtained from this study suggest that Trypamidium-Samorin® (Isomethamidium Chloride) single and combined treatments produced better therapeutic effect than Samorecide® plus (diminazene diaceturate) only.

***Key words*:** Comparative efficacy; trypanocides; *Trypanosoma brucei brucei*; rats

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INTRODUCTION

Trypanosomosis is a parasitic disease of man and animals caused by haemoprotozoan parasites of the genus *Trypanosoma* [1]. The major species responsible for African Animal Trypanosomosis are *Trypanosoma vivax*, *T. brucei brucei* and *T. congolense*. The disease affects domestic animals including cattle, pigs, camels, goats, sheep and horses in which it causes enormous economic losses [2]. Several techniques are available for the detection of infected individuals but parasitological techniques are the most reliable with the detection of the parasites in peripheral blood as the most convenient method [3].

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No effective vaccine is presently available against trypanosomosis due to the phenomenon of antigenic variation exhibited by the parasites. However, the available means to protect and maintain livestock include tsetse control, chemoprophylaxis and chemotherapy, and the use of trypanotolerant livestock [4]. However, the control in Africa rely principally on chemotherapy and chemoprophylaxis using salts of three compounds namely diminazene (an aromatic dimidine derivative), homidium (a phenanthridine derivative) and Isomethamidium (a phenanthridine and aromatic amidine derivative) [5,6]. The most effective and widely used therapeutic agent among the drugs is diminazene aceturate while Isomethamidium chloride is the commonly used prophylactic agent [6]. However, drug treatment of trypanosomosis has not really been successful because of the presence of few trypanocides; wrong use of available trypanocides resulting to the emergence of drug resistant trypanosome strains; antigenic variation; toxicity of trypanocidal drugs and the inability of the readily available drugs to cross the blood-brain barrier [1,7,8]. These problems have led to a search for new and more effective drugs and the combination of two or more trypanocides. For instance, Samorecide plus is a combination of diminazene diaceturate, antipyrine and vitamin B12 whereas Trypamidium-samorin is an isomethamidium chloride powder.

Owing to the relatively limited market in Africa and the high cost of developing and licensing new drugs, international pharmaceutical companies have shown little interest in the development of new trypanocides for use in either animals or humans. Therefore, the current challenge is to achieve optimal use of existing drugs [9] through strategies like combination regimen [10], sanative pairs [11], high dose, repeated regimen [12] and adjuvantive therapy [13].
This study was therefore designed to compare the efficacy of single and combined use of Samorecide®plus and Trypamidium-samorin® in the treatment of experimental *Trypanosoma brucei brucei* infection of albino rats.

MATERIALS AND METHODS

Experimental animals

Thirty (30) adult albino rats (Sprague-Dawley strain) procured from the laboratory animal unit of the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka were used for the study. They were acclimatized to their new environment for two weeks during which feed and water were given *ad libitum*.

Trypanosome species

Trypanosoma brucei brucei (Federe strain) obtained from the National Institute for Trypanosomiasis Research (NITR), Vom, Nigeria was used in the study. The parasites were multiplied in donor mice that were later bled by cardiac puncture. The infected blood collected was serially diluted in normal saline to obtain a yield of $8x10^5$ trypanosomes that were delivered intraperitoneally in 0.2ml of saline per rat. The parasites were enumerated using the Rapid Matching method [14].

Experimental drugs

Samorecide®plus (Concept Pharmaceuticals Ltd. India) was administered at 7.0mg/kg body weight while Trypamidium-samorin® (Merial Pharmaceutical France) was given at 0.5mg/kg body weight. In all cases, the drugs were administered through the intramuscular route.

Experimental design

The 30 rats were divided into 6 groups of 5 rats each as follows:

Group I	uninfected/untreated (negative control)
Group II	infected/untreated (positive control)
Group III	infected and treated with Samorecide®plus only 14 days post infection
Group IV	infected and treated with Trypamidium-samorin® only, 14 days post infection
Group V	infected and treated first with samorecide®plus 14 days post infection and then with Trypamidium-samorin® 7days later
Group VI	infected and treated first with Trypamidium-samorin® 14 days post infection and then with Samorecide®plus 7 days later

The parameters used to assess therapeutic efficacy of the drugs were clinical signs, weekly temperature, weekly proportional weight changes, weekly packed cell volume, weekly haemoglobin concentration, survivability and daily parasitaemia.

Statistical Analysis

Data collected were subjected to descriptive statistics to obtain means and standard errors of means. The results were then statistically analysed using Analysis of Variance (ANOVA). Duncan's Multiple Range Test was used to separate the means. Values of $p < 0.05$ were considered significant.

RESULTS

All infected rats became parasitaemic 6 days post infection and were treated by day 14 (week 2) post infection. All untreated rats died between days 14 and 25 post infection (Table 1). The clinical signs of emaciation, starry hair coat, dullness, reluctance to move, weakness, depression, anorexia, paleness of visible mucous membranes and cuddling were observed. Following treatment, these signs gradually disappeared in the treated groups.

The parasitaemia recorded in the untreated control group II continued to increase until death of two rats on day 14, one on day 22 and the remaining two on day 25 post infection (Table 1). All the treated infected groups (III-VI) became aparasitaemic by one week following treatment. The rats in group III became aparasitaemic 94 hours post treatment although relapses occurred in two rats on days 56 and 70 respectively post treatment. In the other treated groups, the parasites were cleared from the blood within 124 hours in groups IV and VI and 94 hours in group V (Table 1). The infection caused a significant ($p < 0.05$) decrease in the rectal temperature of infected groups (II, III, IV, V, VI) when compared with that of uninfected/untreated group (I) by the second week post infection (Fig. 1). This decrease returned to normal levels seven days post treatment and remained comparable with the control group till the end of the experiment except for group III which recorded another slight decrease in rectal temperature two weeks post treatment.

Table 1: Parasitaemia and mortality pattern of rats infected with Trypanosoma brucei brucei and treated with Samorecide® Plus and Trypamidium-Samorin®

Number of infected rats in group/Number of rats surviving

Weeks	Group I	Group II	Group III	Group IV	Group V	Group VI
0	0/5	0/5	0/5	0/5	0/5	0/5
1PI	0/5	5/5	5/5	5/5	5/5	5/5
2	0/5	5/5	5/5	5/5	5/5	5/5
1PT	0/5	3/3	0/4	0/5	0/5	0/5
2	0/5	0	0/4	0/5	0/5	0/5
3	0/5	0	0/4	0/5	0/5	0/5
4	0/5	0	0/4	0/5	0/5	0/5
5	0/5	0	0/4	0/5	0/5	0/5
6	0/5	0	0/4	0/5	0/5	0/5
7	0/5	0	0/4	0/5	0/5	0/5
8	0/5	0	1/4	0/5	0/5	0/5
9	0/5	0	1/4	0/5	0/5	0/5
10	0/5	0	2/4	0/5	0/5	0/5
11	0/5	0	2/4	0/5	0/5	0/5

PI = post infection; PT = post treatment

There was significant ($p < 0.05$) reduction in weight gain two weeks post infection in all the infected groups when compared with the mean proportional weight changes of the uninfected/untreated group

(Fig. 2). By week 2 - 4 post treatment, all infected and treated groups experienced proportional appreciation in weight except group VI which showed a negative change in weight remaining slightly below their pre-infection value. This however gradually improved by the fifth week post treatment and remained similar ($p > 0.05$) in all treatment groups till the end of the experiment.

Fig. 1: Rectal temperature of albino rats infected with *T. brucei* and treated with samoricide® plus and trypamidium-samorin® and their controls

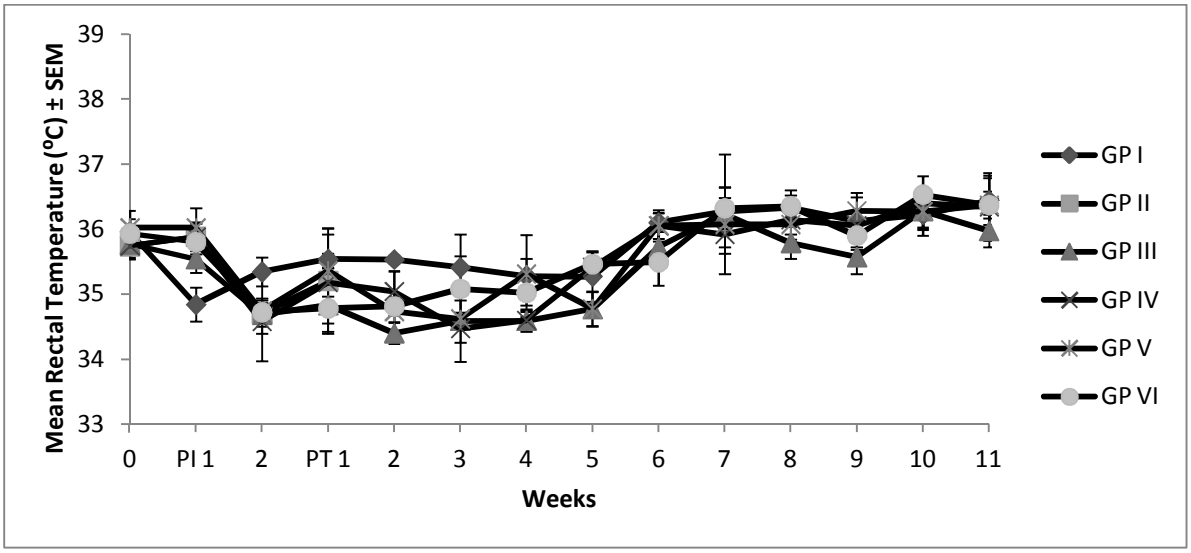
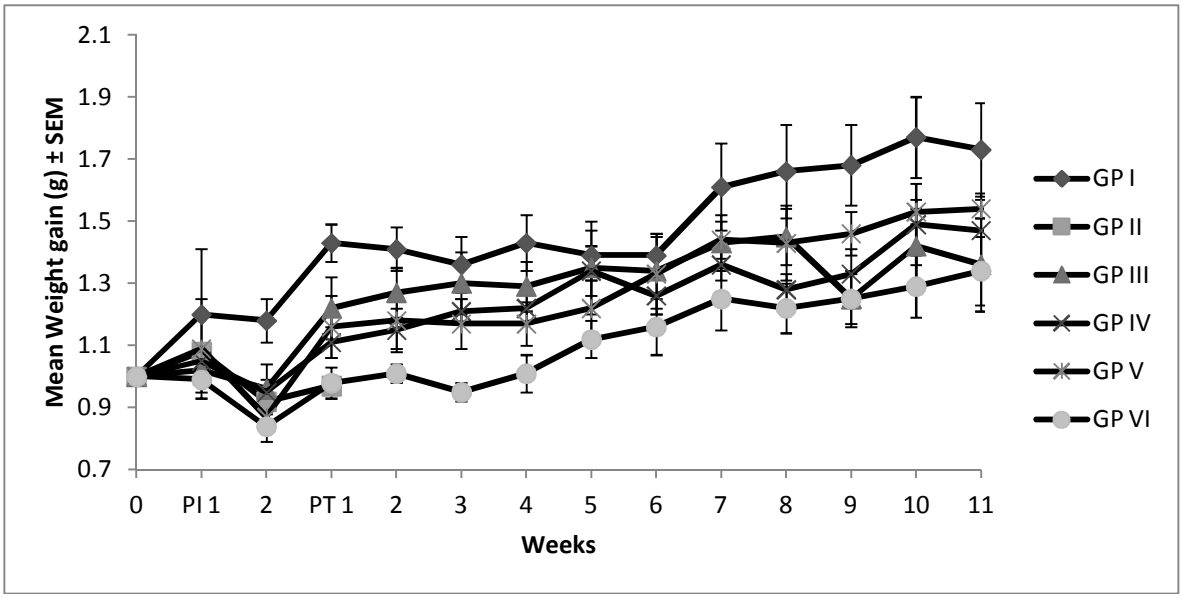


Fig. 2: Proportional weight differences of albino rats infected with *T. brucei* and treated with samoricide® plus and trypamidium-samorin® and their controls



By the second week post infection there was a marked reduction in the packed cell volume of all infected rats ($p<0.05$) compared with those of the uninfected control (Fig.3). At week one post treatment these values appreciated in all the treated groups. This appreciation did not last long in group III since the mean PCV values of the rats again began to fall. However, the PCV did not differ significantly ($p > 0.05$) with those of the uninfected controls except at week 6 post treatment. During

the remaining weeks the values improved slightly but steadily as with the rest of the treatment groups and the control.

The mean haemoglobin values of all the infected rats were significantly lower ($p < 0.05$) than the control by the first and second week post infection (Fig. 4). Following treatment however, all the groups had increased haemoglobin concentration values except group VI and the infected controls (group II). The haemoglobin values of group III declined gradually and was significantly lower ($p < 0.05$) than the other treated groups as well as the uninfected control between weeks 5 and 7 post treatment. Subsequently the various groups did not show significant differences ($p > 0.05$) in the mean levels of haemoglobin concentration.

Fig. 3: Packed cell volume of albino rats infected with *T. brucei* and treated with samoricide® plus and trypamidium-samorin® and their controls

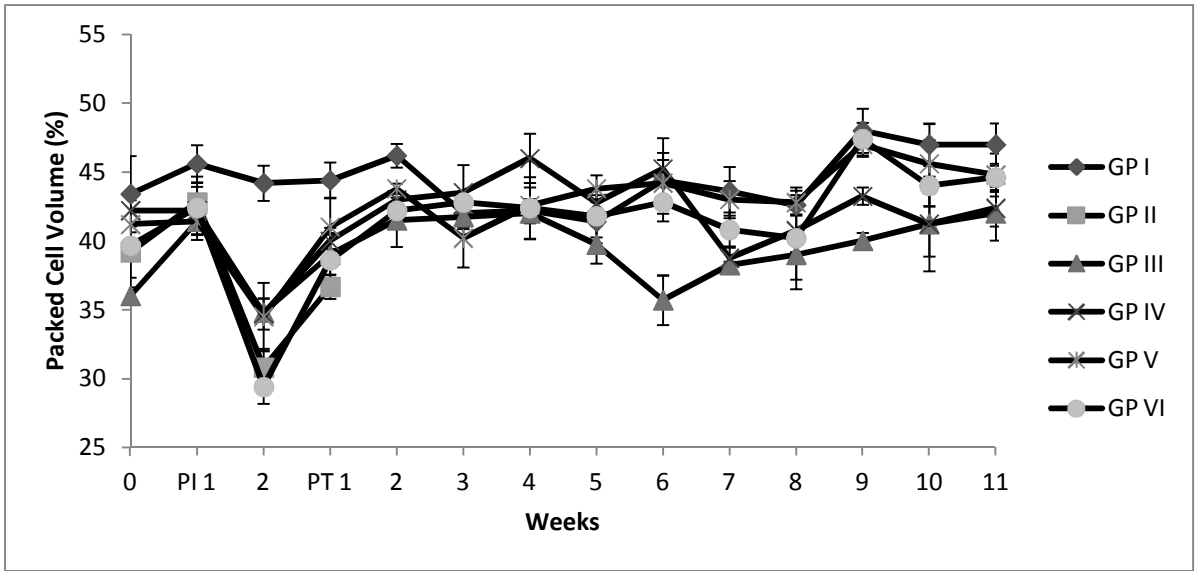
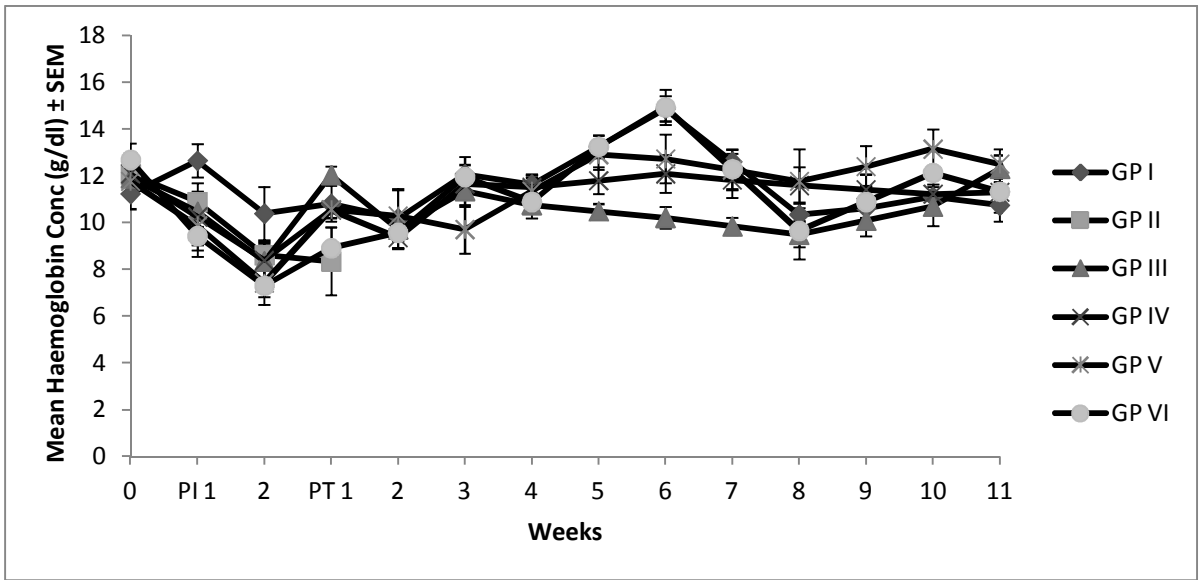


Fig. 4: Haemoglobin concentration of albino rats infected with *T. brucei* and treated with Samoricide® plus and trypamidium-samorin® and their controls



DISCUSSION

Trypanosoma brucei brucei parasitaemia was established in all infected rats within 7 days of infection. The infection produced an acute infection in the rats that resulted in the death of the infected/untreated rats between 14 and 25 days following infection.The clinical signs observed in the rats include anorexia, pale mucous membrane, rough hair coat, emaciation, dullness and depression and these were similar to those previously observed in rabbits infected with *Trypanosoma brucei brucei* [15] and cattle infected with *Trypanosoma congolense* [16]. These signs gradually disappeared with treatment in groups III to VI. The drugs used were therefore able to reverse the clinical signs observed after treatment. The ability of Samorecide® plus to clear the parasitaemia in the infected rats earlier (94 hours) than Trypamidium-Samorin® treated rats (124 hours) showed that Samorecide® plus (diminazene diaceturate) achieved a faster optimal therapeutic blood level and activity than Trypamidium-samorin® (Isomethamidium chloride).

Anaemia was evident in the infection, with a significant reduction ($p<0.05$) in the PCV and haemoglobin concentration values of all infected groups before treatment and in the untreated group before death. Anaemia is the most prominent feature of animal trypanosomosis [17,18] and the results obtained in this study agree with previous reports [19-21]. Treatment with the different combinations was able to reverse the anaemia which followed infection. However, this reversal was much pronounced in group V treated first with Samorecide® plus and then Trypamidium-samorin® seven days later. Furthermore, the PCV and Haemoglobin concentrations of the treated rats compared favourably with the uninfected control group ($p > 0.05$).

Increase in temperature during trypanosomosis has been attributed to the stimulation of the thermoregulatory centre of the hypothalamus by the parasites. Although decrease in temperature was observed in all infected groups two weeks following infection, these were reversed by treatment. Several reports disagree with this observation [13, 19, 20].

The observed decrease in weight gain following infection, a feature of trypanosomosis is believed to be associated with anorexia and dullness during infection. This has been reported in previous works [20, 22, 23]. Following treatment, however, a gradual gain in weight was observed in the treated groups (III-IV) and this was comparable with the uninfected control (Group I). The rapid weight gain in group III following treatment may be due to increased appetite of the rats. However, this was not sustained as relapse parasitaemia which occurred in two rats in this group may have negatively affected their weight. It is interesting to note that rats in groups IV (treated with Trypamidium-samorin® only) and VI (Trypamidium-samorin® first, then Samorecide® plus) respectively showed a marginal weight reduction which was marked in the combined treatment group throughout the experiment. This observation may be attributed to the effect of the drugs on the animals. It should also be noted that in routine clinical practice chemotherapy is combined with other patient management practices and care to help convalescent animals following treatment. The absence of this may have resulted in the observed effect on weight gain in the infected groups.

Relapse parasitaemia was recorded only in two of the rats treated with Samorecide® plus only (group III) from the 56th day post treatment. Relapse infections following treatment have been previously reported [24 – 27]. This could be attributed to re-entry of parasites sequestered in the brain into the blood circulation [28]. The molecules of diminazene aceturate have been shown to be too large to cross the blood brain barrier [29]. Thus, the treatment with this drug will not usually affect parasites that had sequestered in the brain. This may ultimately produce parasites that may become refractive to treatment with this drug and hence facilitate the development of drug resistance.

CONCLUSION

It is concluded from this study therefore that Trypamidium-samorin® only and in combination with Samorecide® plus produced a better therapeutic effect than Samorecide® plus alone. The results also suggest that combining the two drugs for treatment of experimental trypanosomosis gave a better result than their individual usage. This finding can be applied to larger animals and pets in view of the increasing incidence and reports of resistance to conventional trypanocides.

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