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Full Length Research Paper

Therapeutic Efficacy Of Cotecxin^(R) alone and Its Combination with Diminazene aceturate (Berenil^(B)) in the Treatment of Experimental *Trypanosoma brucei brucei* Infection of Rats

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ABSTRACT

The therapeutic efficacy of Cotecxin^(R) (Dihydroartemisinin) alone and its combination with diminazene aceturate (Berenil^(R)) was studied in rats infected with Federe strain of *Trypanosoma brucei brucei*. Fifty healthy adult albino rats of both sexes weighing between 100-180g used were divided into five groups (A-E) of 10 rats each. Rats in groups A-D were each infected with *T.b. brucei* (1×10^6 trypanosomes). Rats in groups A were treated with 7.0mg/Kg of diminazene aceturate (Berenil^(R)) as a single dose, group B with 1.0mg/Kg of Cotecxin^(R) and group C with a combination of diminazene aceturate (3.5mg/Kg) and Cotecxin^(R) (1.0mg/Kg). Rats in group D remained untreated while group E animals served as the uninfected and untreated control. The pre-patent periods in groups A, B, C and D were 3.5 ± 0.9 , 3.1 ± 0.9 , 3.2 ± 0.6 and 3.5 ± 0.9 days respectively. There was no significant ($P > 0.05$) difference in pre-patent periods among the infected groups. The infected rats were treated on day 6 post-infection (Pi). Survival time was 26.6 ± 0.5 and 15.8 ± 1.6 days in groups B and D respectively and shorter ($P < 0.05$) in group D. Cotecxin^(R) treatment alone suppressed level of parasitaemia, prolonged survival time but could not completely clear the parasites from the blood. Complete clearance of the parasites was achieved in groups A and C rats as there was no relapse. In conclusion, Cotecxin^(R) alone was not effective but its combination with diminazene aceturate (Berenil^(R)) was effective in *Trypanosoma brucei brucei* clearance from the blood.

Key words: Cotecxin^(R), diminazene aceturate, *Trypanosoma brucei brucei*, rats.

INTRODUCTION

Trypanosomosis is a disease caused by protozoan organism under the family Trypanosomatidae. The

organism is capable of multiplying in lymphatic vessels, tissues, blood and central nervous system (CNS) (Losos, 1986; Radostits *et al.*, 1994; Sweetman, 2002). The disease affects man and animals (Losos, 1986). Resistance of trypanosomes to trypanocides has been reported (Losos, 1986, Onyeyili and Egwu, 1995) and diminazene aceturate (Berenil^(R)) is a drug of choice for the treatment of animal trypanosomosis. The drug is efficacious, expensive and not easily available to the farmers. Chloroquine, Metakelfin^(R) and Camoquine^(R) are anti-malarial agents reported to be efficacious in the treatment of plasmodiosis but less effective in the treatment of trypanosomosis (Onyeyili and Egwu, 1995; Egbe-Nwiyi, *et al.*, 2005; 2011). Both plasmodiosis and trypanosomosis are caused by protozoan organisms (Radostits *et al.*, 1994). A new anti-malarial agent Cotecxin^(R) (Dihydroartemisinin) has been introduced into the Nigerian market and proved to be effective in treating chloroquine and piperazine –resistant *Plasmodium falciparum* which causes malaria in man (Sweetman, 2002). There is little or no information on the effect of Cotecxin^(R) on

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trypanosomes in animals or man. In view of this, the present study was undertaken to evaluate the therapeutic efficacy of Cotecxin^(R) administered alone and in combination with diminazene aceturate (Berenil^(R)) in the treatment of experimentally induced *Trypanosoma brucei brucei* infection of albino rats.

MATERIALS AND METHODS

Experimental Animals:

Fifty healthy adult albino rats of both sexes weighing between 100-180g were obtained from the National Trypanosomosis Research Institute (NITR), Vom, Plateau State, Nigeria. They were maintained on a standard laboratory diet (Vital Feeds Ltd, Nigeria) and housed in clean plastic cages in a fly-proof house at ambient temperature (30-35°C). Water was provided *ad-libitum*. The rats were conditioned for 10 days, screened and certified free of haemoparasites (Jain, 1986) before the commencement of the experiment. Animal welfare was observed in accordance with International guidelines for the use of laboratory animals for biomedical research (EC, 1996).

Trypanosomes:

Trypanosoma brucei brucei (Federe strain) obtained from NITR, Vom, was used and the parasites were maintained by serial passages in rats. One millilitre of donor rat blood diluted in phosphate glucose buffered saline solution (pH 7.4), containing an estimated 1×10^6 trypanosomes was intraperitoneally injected into each rat in groups A-D. This strain was initially isolated from Ndama/Muturu cattle in Federe Local Government Area of Plateau State, Nigeria in 2004.

Test Drugs

Cotecxin^(R) (Dihydroartemisinin) an anti-malarial drug (Jiaxing Nauhu, China) and diminazene aceturate (Berenil^(R), Hoechst Germany) a trypanocide were prepared for administration according to the manufacturers' specifications.

Experimental Protocol:

The rats were divided into five groups (A-E) of 10 rats each. Rats in groups A-D were each infected with *T. b. brucei*. Rats in group A were treated with 7.0mg/Kg of diminazene aceturate (Berenil^(R)) as a single dose, group B with 1.0mg/Kg of Cotecxin and group C with a combination of diminazene aceturate (3.5mg/Kg) and Cotecxin^(R) (1.0mg/Kg). Rats in group D remained untreated while group E served as the uninfected and untreated control. Cotecxin^(R) was administered by stomach tube (Waynforth and Flecknell, 1992) while diminazene aceturate was given intramuscularly. The

infected rats were treated on day 6 post-infection (Pi) when parasitaemia was established in all the infected groups (A-D) but the Cotecxin^(R) treatments continued on days 7-11 Pi in groups B and C rats. To assess the therapeutic efficacy of the treatments, tail blood was collected from rats pre- and post-parasite inoculation. Packed Cell Volume (PCV) was determined every 4 days by the microhaematocrit method to assess haematological response while parasitaemia level was measured every 2 days by haemocytometry (Jain, 1986).

Statistics:

The data obtained were summarized as means \pm Standard deviations and compared between groups by the analysis of variance (ANOVA) (Chatfield, 1983).

RESULTS

The infected rats became parasitaemic within 2-4 days post-infection (Pi) and the mean pre-patent periods in the infected groups A,B,C and D were 3.5 ± 0.9 , 3.1 ± 0.9 , 3.2 ± 0.6 and 3.5 ± 0.9 days respectively. There was no variation ($P > 0.05$) in pre-patent periods among the infected groups (A-D). The level of parasitaemia increased gradually and became established in all the infected groups by day 6 Pi (Fig. 1). The infected rats were treated on day 6 (Pi) and the trypanosomes were cleared in groups A and C treated with diminazene aceturate (Berenil^(R)) alone and its combination with Cotecxin^(R) respectively by day 4 post-treatment (PT). Survival time was 26.6 ± 0.5 and 15.8 ± 1.6 days respectively in groups B and D. Cotecxin^(R) treatment alone in group B suppressed the level of parasitaemia and prolonged survival time. The latter was shorter in group D. (infected and untreated) ($P < 0.05$) when compared with that of group B (infected and treated rats). The level of parasitaemia in that group B decreased remarkably ($P < 0.05$) from day 2-8 PT and later increased from day 10-14 PT. All the rats in group B died by day 16 PT. In the infected untreated rats (group D), 6 and 5 rats each were left on days 14 and 16 Pi respectively (Fig. 1) and the remaining rats died by day 18 Pi.

The PCV decreased in all the infected groups A-D when compared with the value of the uninfected untreated control (Fig. 2). Following treatment, the PCV gradually returned to pre-infection values in groups A and C but significantly ($P < 0.05$) decreased in groups B and D until all the rats in these groups (B and D) died. The PCV value was lower ($P < 0.05$) in the infected untreated rats (group D) when compared with the value recorded in the rats infected and treated with 1.0mg/Kg of Cotecxin^(R) (group B) by day 6 PT.

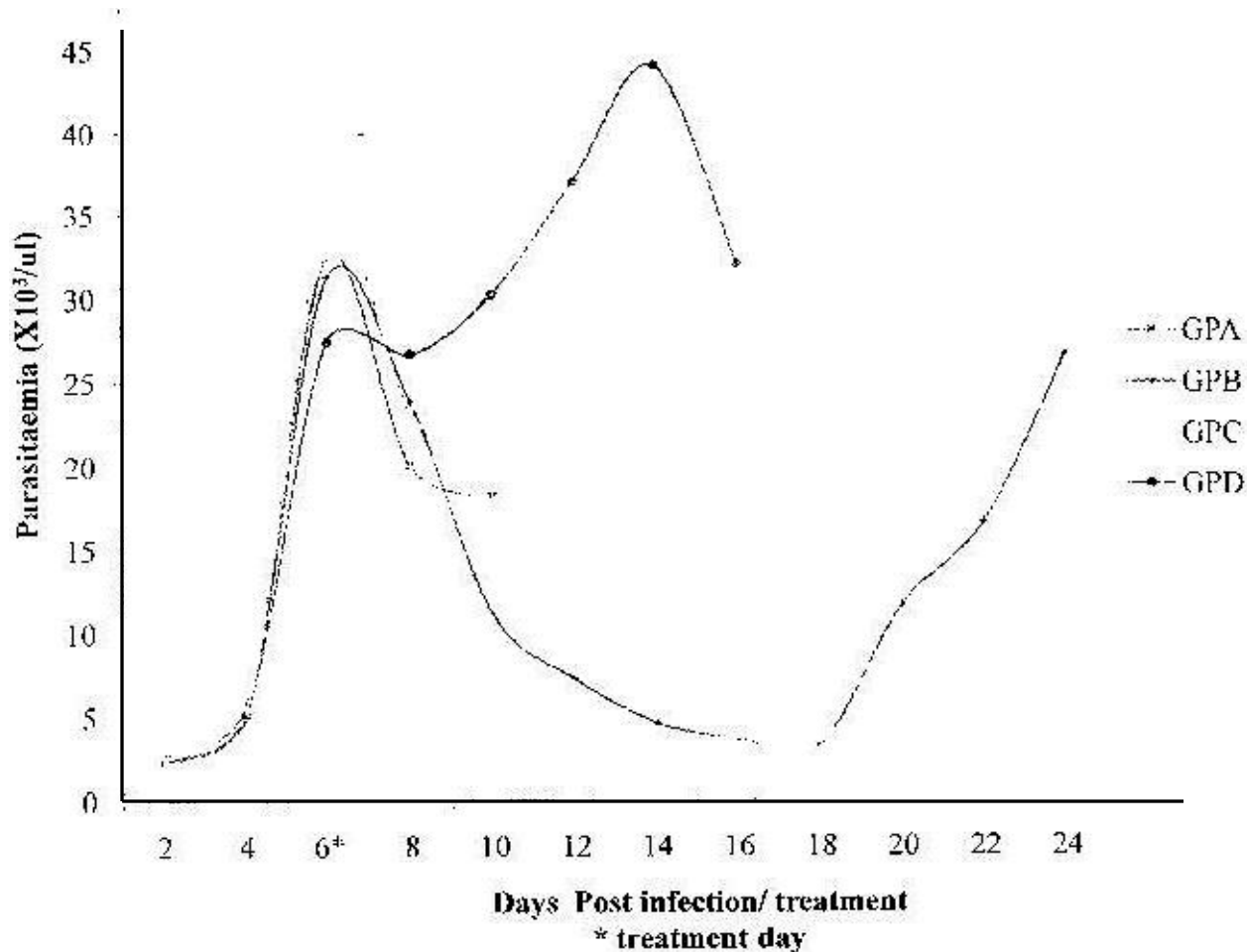


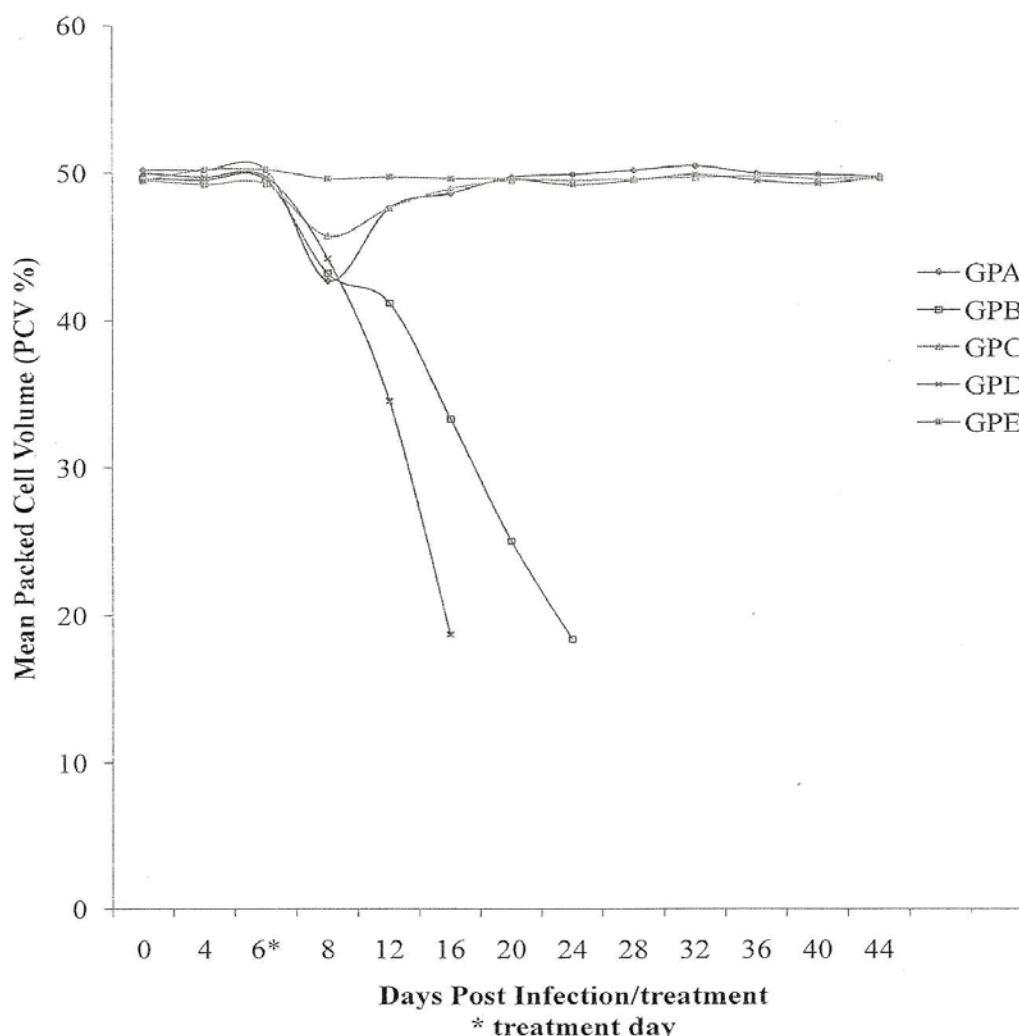
Fig 1:

Parasitemia in rats infected with *T. brucei* and treated with diminazene aceturate alone (group A), cotecxin alone (group B), a combination of diminazene aceturate and cotecxin (group C), untreated (group D) and uninfected untreated control rats (group E).

DISCUSSION

This study has shown that Cotecxin^(R) a strong anti-malarial agent is less effective in the treatment of trypanosomosis at 1.0mg/Kg. The drug slightly suppressed the level of parasitaemia resulting from *T.b. brucei* in rats and failed to clear the trypanosomes completely from the peripheral blood. However, the drug succeeded in prolonging the survival time of the rats in group B when compared with that of the infected untreated rats (group D) but its combination with diminazene aceturate (Berenil^(R)) appeared to be effective. Diminazene aceturate is a conventional trypanocide and the administration of 7.0mg/Kg of diminazene aceturate led to the elimination of the parasites from the peripheral blood in group A. The absence of relapse in group A might suggest that the

Federe strain of *T. b. brucei* was amenable to treatment and the drug at 7.0mg/Kg could have resulted in sufficient therapeutic dose crossing the blood brain barrier to cause complete elimination of the trypanosomes within the central nervous system. It is important to note that the recommended dose 3.5mg/Kg, was doubled in the present study, however, relapses have been reported in rats infected with different strains of trypanosome species and treated with diminazene aceturate at 3.5-10.5 mg/Kg doses (Losos, 1986; Radostits *et al.*, 1994; Onyeyili and Egwu, 1995). Similarly, there was no relapse in group C rats treated with lower doses of diminazene aceturate (3.5mg/Kg) and Cotecxin^(R) (1.0mg/Kg) and this combination might have precipitated synergism between the two agents.

**Fig 2:**

Mean PCV (%) of rats infected with *T. brucei* and treated with diminazene aceturate alone (group A), cotecxin alone (group B), a combination of diminazene aceturate and cotecxin (group C), untreated (group D) and uninfected untreated control rats (group E).

This observation is in consonance with reports of Egbe-Nwiyi *et al.*, (2005) where lower doses of diminazene aceturate (3.5mg/Kg) and Metakelfin^(R) (10.0mg/Kg) cleared the parasites completely from the blood. Although, the mechanism of action of Metakelfin^(R) may be different from that of Cotecxin^(R). The suppression of parasitaemia in the Cotecxin^(R) alone treated rats did not differ from the findings of Onyeyili *et al.*, (1994) and Egbe-Nwiyi *et al* (2005; 2011) where chloroquine alone and Metakelfin^(R) alone as well as Camoquine^(R) alone could not clear trypanosomes totally from the peripheral blood of infected rats. Cotecxin^(R) is a strong plasmodicide and its inability to clear the parasites from the blood of infected rats may possibly suggest that the agent lacks some basic anti-parasitic actions of the aromatic diamidines which diminazene aceturate belongs. Diminazene aceturate was reported to interfere with trypanosome DNA

synthesis and aerobic glycolysis with resultant dyskinetoplasty (Jones *et al.*, 1977; Onyeyili and Egwu, 1995). Maxie and Losos, (1977) also suggested that the drug does not kill trypanosomes directly but makes them accessible to other body defense mechanisms such as the macrophage system.

The PCV decreased in all the infected groups and the decrease in PCV manifested anaemia. The latter is a common observation in trypanosomosis and it signifies severity of disease (Losos, 1986; Anosa, 1988; Murray and Dexter, 1988; Radostits *et al.*, 1994). The level of anaemia which was more severe in the infected untreated rats (group D) when compared with that of group B (infected and treated with Cotecxin^(R) alone) might among other factors be attributed to the effect of the drug in that group B which suppressed the level of parasitaemia and prolonged the survival time of the rats. The degree of parasitaemia has been reported to

correlate with the severity of some trypanosome infections (Losos, 1986; Murray and Dexter, 1988) although, this depends on the virulence of the strain of the trypanosome, dose of inoculum, immunity and nutritional status of the host. Diminazene aceturate at 7.0mg/Kg alone and a combination of 3.5mg/Kg of diminazene aceturate and Cotecxin^(R) at 1.0mg/Kg treatments caused recovery of declined PCV of the infected rats and this is in agreement with previous reports (Onyeyili *et al*; 1994; Egbe-Nwiyi *et al*; 2005; 2011;).

In conclusion, diminazene aceturate alone at 7.0mg/Kg cleared the parasites from the blood, but Cotecxin^(R) alone at 1.0mg/Kg could not. A combination of diminazene aceturate at 3.5mg/Kg and Cotecxin^(R) at 1.0mg/Kg cleared the trypanosomes from the peripheral blood of the infected rats. Cotecxin^(R) seems to potentiate the chemotherapeutic activity of diminazene aceturate (Berenil^(R)). There is need to evaluate the efficacy of several different combinations regime of Cotecxin^(R) and diminazene aceturate in late stage trypanosomosis caused by *T. b. brucei*.

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