



Efficacy of *Terminalia avicennoides* and its combination with diminazene aceturate (Berenil®) in rats experimentally infected with *Trypanosoma brucei brucei*

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Abstract

A comparative study was carried out to investigate the efficacy of the aqueous extract of *Terminalia avicennoides* alone and its combination with diminazene aceturate. Thirty Wistar albino rats of both sexes weighing between 250-260gms were distributed into six groups (A-F) of five rats each. All the rats in groups A-E were infected with 0.5ml of blood containing 1×10^6 *Trypanosoma brucei brucei*. Rats in group F were uninfected and untreated (control). Rats in groups A-D were treated 5 days post-inoculation orally, with 3.5mg/kg Berenil® alone, 80mg/kg plant extract alone, 80mg/kg extract plus 1.75 mg/kg Berenil® and 1.75 mg/kg Berenil® respectively were infected Group E, not treated. Treatment with extract alone resulted in reduction in parasitaemia, but was not curative. However, Berenil® treatment alone and its combination with the extract resulted in the clearance of the parasite. The result obtained suggests that *T. avicennoides* alone and its combination with Berenil® offer great potentials as alternative products for the treatment of trypanosomiasis.

Keywords: diminazene aceturate, efficacy, rats, *Terminalia avicennoides*, trypanosomiasis

Introduction

Trypanosomiasis is a potentially fatal disease of humans and domestic animals in tropical Africa and South America (Smith *et al.*, 1998). This disease has undergone a dramatic and devastating resurgence in recent years (Welburn *et al.*, 2001).

In Nigeria, it occupies latitude 4° N to 13° N (Onyiah, 1997). This covers all the six geo-ecological zones of the country and the disease has an overall prevalence in cattle, sheep and goats of 10%, 8.6% and 8.1% respectively (Onyiah, 1997). Similarly, horses and camels are affected. The asymptomatic to acute, sub-acute and chronic forms have been reported (Onyeyili & Egwu, 1997). The disease is characterized by fever, weight loss, abortion and reduced production (Ikede *et al.*, 1988), leading to loss in revenue in the livestock industry in many parts of Africa (Onyeyili & Egwu, 1997)

Chemotherapy seems to be the only readily available measure for both curative and prophylactic management because no vaccine is available yet for the disease. Unfortunately, the existing drugs for trypanosomiasis are toxic and/ or expensive (Ajagbonna *et al.*, 1995; Atougwa & Costal, 1999). In addition to the

unavailability of these drugs in rural areas, incidence of therapeutic failure and relapses from treatments with these drugs are known to occur (Onyeyili & Egwu, 1997; Atougwa & Costal, 1999).

This study investigated efficacy of *T. avicennoides* in the treatment of trypanosomiasis. *T. avicennoides* is a tropical tree abundant in the Savannah region of West Africa. In Nigeria, the plant is commonly found in the North Central region where most of the country's cattle are reared. It is locally known as *Baushe* among the Hausas, (Keay *et al.*, 1960).

The plant is a durable wood, hard and yellowish brown in color. The extract from various parts of the tree (leaves, stem bark and roots) are claimed to be useful in treating dental caries (Gill & Akinwumi, 1986), skin infections and malaria. It is also claimed to be used locally against trypanosomiasis (Bulus *et al.*, 2008) in animals but this is yet to be ascertained.

Therefore, the aim of this study is to comparatively study the efficacy of *T. avicennoides* alone, and its combination with diminazene aceturate (Berenil®) an existing conventional synthetic trypanocide.

Materials and methods

Plant Material

Fresh plant materials (stem bark) were collected from users of this plant, *T. avicinoides*, in Sokoto metropolis with the aid of traditional practitioner. The botanical identity and authentication of this plant were carried out at the Botany Department, Usmanu Danfodiyo University, Sokoto. Samples were subsequently deposited in the herbarium of the Department of Veterinary Physiology and Pharmacology of the University.

Methods of Extraction

Hot extraction method was used in this study as described by Ajagbonna & Onyeyili (2003). The stem-bark collected, was sun-dried and pulverized into powder using mechanical grinder. Two hundred grams of the pulverized materials was boiled in 500ml of distilled water for 30 minutes. This was filtered through pieces of (Muslin) cloth and the filtrate was again filtered using a piece of cotton wool placed in a funnel into a beaker and the filtrate collected and evaporated to dryness in an oven at 60°C for 12 hours. The dark brown extract obtained subsequently was used for this experiment.

Experimental Rats

Wister albino rats of both sexes weighing between 250g and 260g were used for this study. The rats were obtained from Nigeria Institute for Trypanosomiasis and Onchocerciasis Research (NITOR), Vom, Jos, Plateau State, Nigeria. They were housed in metallic cages, allowed to stabilize for a week and fed with standard commercial rat feed and provided with clean water *ad libitum*.

Trypanosome Stock

Trypanosoma b. brucei used in this experiment was obtained from NITOR. Four rats were initially inoculated with the organism from NITOR. These rats served as donors from where other rats were inoculated intraperitoneally using 1.0ml blood containing 1×10^6 *T. b. brucei* from donor rats.

Experimental Procedure

The rats were separated into six groups of five rats each in a cage and were treated as follows;-

Group A - Infected and treated with 3.5mg/ kg IM of diminazine aceturate once, at the peak of parasitaemia.

Group B - Infected and treated with 80mg/kg *T. avicinoides* orally for 6 days at peak parasitaemia.

Group C - Infected and treated with 80mg/kg *T. avicinoides* (orally) + 1.75mg/ kg diminazine aceturate IM at the peak of parasitaemia.

Group D - Infected and treated with 1.75mg/kg of diminazine aceturate alone IM at the peak of parasitaemia

Group E- Infected and untreated rats (positive control)

Group F - Uninfected and untreated rats (negative control).

Inoculation of Animals

One millilitre of *T. b. brucei* infected blood was taken from the donor rat and diluted with phosphate buffered saline. The diluted blood containing approximately 1×10^6 parasites was inoculated via intraperitoneal route using "23G" needle (Onyeyili *et al.*, 1994; Dina *et al.*, 2002) into the rats in groups A, B, C, D and E.

Administration of the Drug

At the peak of parasitaemia, diminazine aceturate (3.5mg/Kg B.W) was administered intraperitoneally to group A using 23 gauge needle (Onyeyili *et al.*, 1994; Dina *et al.*, 2002). At the peak of parasitaemia, 80mg/Kg of *T. avicinoides* was administered to rats in Groups B and C. A concentration of 1 gm *T. avicinoides* was prepared from powdered crude extract after weighing using Metler digital balance. This was dissolved in 100ml of water to obtain one percent solution. The dose was then calculated using the standard dosage of the plant. Standard dosages of the plant is 80mg/Kg (Gills & Akinwumi, 1986; Akinside & Olukoya, 1995).

Parasite Detection

Parasite was detected by wet film method from the tail blood collected from each rats in each of the test groups, the wriggling movement of trypanosomes was observed between the blood cells (Boyt, 1984) when fresh infected blood from each rat was placed on microscopic slide, cover slip and examined under the light microscopic field using x40 magnification. This method was also used to assess the efficacy of the extract based on the motility of the trypanosomes under the light microscope.

The Erythrocytic Changes

The packed cell volume was determined by the microhaematocrit method as described by Dacie & Lewis (1999). Briefly; this method involved filling capillary tubes with blood to about three quarter of the length of the tube with one end sealed with crista seal. The capillary tubes were then spun in a Hawksley microhaematocrit centrifuge for 5 minutes at 10,000rpm and PCV values were read using microhaematocrit reader. Red blood cell (RBC) was estimated using Neubauer haemocytometer (Schalm *et al.*, 1975).

Estimation of Parasitaemia

This was carried out using the microscopic field of wet preparations. About 1.0 ml of fresh blood was obtained from the tail, pre-sterilized with 70% alcohol. The number of parasites was determined microscopically using the 'Rapid Matching' method of Herbert & Lumsden (1976).

Statistical Analysis

Packed cell volume (PCV) was presented as mean \pm SE. Test of significance between the mean parameters were done using ANOVA and significance was considered when $p < 0.05$.

Results

The summary of the trypanocidal efficacy of the extract (*T. avicennoides*), diminazine aceturate (Berenil[®]) and their combination in *T. brucei* infected rats is shown in Table 1.

Table 1: Total number of deaths, cured animals and average survival periods in the berenil[®] and *T. avicennoides* treated and untreated groups

Indices determination	Uninfected untreated (Control)	Infected not treated	Infected + (treated) with 3.5mg/kg berenil [®]	Infected + 1.75mg/kg berenil [®]	Infected treated with 80mg plants	Infected treated berenil [®] 1.75mg/kg + plants.
Total No. of death	0	5	0	5	5	0
No. of animal cured	5	0	5	0	0	5
Average survival in days	28	13	28	19	18	28

Parasitological examination of the blood samples obtained 5 days post-inoculation with *T. brucei* showed that all rats exhibited peak parasitaemia within 5 days. The group treated with 3.5mg /Kg berenil became completely free of the parasites 3 days post-treatment while those treated with the plant extract and sub-therapeutic dose of berenil[®] became free 5 days post-treatment. The rats in the two groups remained parasite free throughout the 25 days of observation. However, in animals treated with 1.75mg/Kg berenil[®] alone, the rats were parasite inactivation for 2 days only, before relapse occurred which eventually resulted into the death of all the rats before the end of the experiment.

Rats treated with the plants extract alone (80mg/Kg) survived for 18 days, with reduced parasitaemia was (about $\frac{3}{4}$ of its peak value). The extract alone did not completely clear the rats of the parasite. In the infected not treated group, the infected rats exhibited progressive parasitaemia that resulted in early deaths on or before day 13 post-inoculation.

Effects of various treatment regiments on erythrocyte values

Results of haematological study showed that there was a significant decrease ($p < 0.05$) in PCV and RBC of untreated parasitaemic rats within five days of inoculation when compared with the values of uninfected (control group) or in the infected but treated group (Table 2).

Table 2: The Effects of *Terminalia avicennoides*, diminazine aceturate (Berenil[®]) and its combination on some haematological values.

Parameters	Uninfected untreated (Control)	Infected not treated	Infected + 3.5mg/kg berenil [®]	Infected + 1.75mg/kg berenil [®]	Infected Treated 80mg/kg plants	Infected treated 1.75mg/kg berenil + plants
PCV	48 \pm 2.3 ^x	32 \pm 1.8 ^{xx}	45 \pm 1.7 ^x	35 \pm 1.2 ^{xx}	34 \pm 2.5 ^x	43 \pm 2.2 ^x
RBC	8.5 \pm 0.3	4.3 \pm 0.2	8.2 \pm 0.6	5.2 \pm 0.3	5.0 \pm 0.4	8.0 \pm 0.2

xx represent $p < 0.05$ compared to control

x represent $p > 0.05$ compared to infected not treated group

There were significant increases in PCV and RBC in the control group, berenil[®] treated group and its combination with the plant extract when compare with the infected untreated group. In the group treated with the extract alone and those treated with sub-therapeutic dose of berenil[®], there was an insignificant increase ($p > 0.05$) in both the PCV and RBC values compared with the infected untreated group.

Discussion

The parasitological findings of this study show that rats treated with 3.5mg/Kg berenil[®] alone and its combination with plant extracts were cured and cleared of the parasites three days post-treatment till the end of the experiments. Similarly, in rats treated with the plant extract alone, there was a significant increase in the average survival time (18 days) before they all died of parasitaemia. Although the plant extract alone was not able to clear the rats of the parasites, however in the group, where the rats were treated with a combination of the plant extract and half- therapeutic dose of berenil[®], there appears to be some synergism that resulted in complete clearance of the parasites, a result

that was not seen when the plant extract alone and half-therapeutic dose of berenil[®] was used separately. This is in line with the work of Onyeyili *et al.* (1994).

In the infected, untreated group there was progressive parasitaemia, which resulted in early death of the animals in this group, showing the lethality of this infection without the plant extract or berenil[®] or their combinations. Thus confirming therapeutic effectiveness of the agents employed in this study.

Following treatment, the haematological results obtained from this study showed that PCV and RBC increased almost at the same rate and later fell, but not to the pre-inoculation level as in the berenil[®] treated group, this is similar to that reported by Akinwale *et al.* (1999). This is also in agreement with the established fact that trypanosomiasis is usually accompanied by haematological depression (Anosa, 1988; Ajagbonna *et al.*, 2003; Ajagbonna *et al.*, 2005) and this also supports the suggestion that PCV and RBC values could be used as an index of the severity of the disease (Midachi *et al.*, 1995; Van den & Rowland, 2001). In view of these, the combination of plant extract and berenil[®] had better efficacy when compared with the individual agents studied (berenil[®] or the extract).

Drug combination is one of the few optimum suggestions by some workers (Ajagbonna & Olaniyi, 1999; Atougwa & Costa, 1999) to minimize current drug failure due to drug resistance and or toxicity on the face of lack of new trypanocides (Ajagbonna *et al.*, 2005).

The mechanism by which the extract alone or in combination with berenil[®] exert their action is not determined in this study. However, it has been suggested that many natural products exhibit their trypanocidal activity by virtue of their interference with the redox balance of the parasite acting either on the respiratory

chain or on the cellular defence against oxidative stress (Pinto & Castro, 2009). The aromatic diamidines including berenil[®] are known to cause hypoglycaemia in the treated animals (Laha *et al.*, 1991). Trypanosomes as blood parasites depend on the host glucose for aerobic glycolysis (Marshall, 1948) and so the use of berenil[®] during infection deprives them of this necessary glucose and this is responsible for their trypanocidal action. It is therefore suggested that the synergistic action observed in this study between the berenil[®] and the plant extract of *T. vecinniodes* when used in combination might be through these mechanisms.

In conclusion, *T. vecinniodes* alone can serve as trypanocidal to some extent, but not completely as the berenil group, but prolong the life span of the animals. This study has highlighted the potential trypanocidal effect of *T. vecinniodes* bark extract, even though it could not eliminate the parasite completely, but it reduces the level of parasitaemia and improved the status of anaemia. Therefore, *T. vecinniodes* and its combination with conventional drug (Berenil[®]) can completely clear the parasite. In this case, *T. vecinniodes* extracts serve as a potential, alternative and cheap in cost as natural product for the treatment of trypanosomiasis by the local people.

The findings in this study showed that if the plant extract alone is given at higher dose than 80mg/Kg as used in this study, an appreciable result may be achieved. Thus it is recommended that further work should be carried out to determine the toxicity of the drug at a dose higher than 80mg/Kg and determination of LD₅₀ will be of importance and enhances the acceptability of the *T. vecinniodes* extract as a trypanocide scientifically.

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