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Effect of ethanol root extract of *Gardenia sokotensis* on the level of parasitaemia in *Trypanosoma brucei brucei*-infected rabbits

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Abstract

A study was conducted to determine the effect of the ethanol roots extract of *Gardenia sokotensis* in rabbits infected with *Trypanosoma brucei brucei*. The animals were divided into five groups of 6 each. Group A was infected and treated with the extract of G. *sokotensis*; Group B infected and treated with Berenil®; Group C infected and untreated; Group D uninfected but treated with the extract; Group E was uninfected and untreated. The experimental animals were inoculated intraperitoneally with approximately 104 - 5 x 105 *Trypanosoma bruc*ei *brucei* parasites. Berenil® was administered once, intramuscularly, at the dose rate of 3.5 mg/kg body weight, while the extract of G. *sokotensis* was administered orally at the rate of 60mg/kg body weight daily for seven days. Administration was done at peak of parasitaemia (41 days post infection, p.i.). The animals were examined daily for parasitemia.

Key words: *Gardenia sokotensis*, *Trypanosoma brucei brucei*, Parasitaemia, Rabbits, Berenil®.

Introduction

Trypanosomiasis is a collective term for a group of diseases caused by one or more of the pathogenic trypanosome species. The disease is characterized by slow, progressive loss of condition accompanied by increasing anaemia (the main sign of the disease), weakness, extreme emaciation; coma and death, often due to heart failure (Murray, 1979; Monzon *et al.*, 2003; Ngaira *et al.*, 2003; FAO, 2005).

Despite advances in modern medicine, the treatment of African trypanosomiasis is still unsatisfactory. The only drug developed in the recent times is eflornithine, which is effective for late-stage of the Gambiense disease and its cost makes it unaffordable. According to analysis by WHO/TDR, 2004 relatively few drugs for tropical diseases reach the market, and even if they do there are huge inequities in access to drugs between rich and poor countries. Drugs are often inaccessible and unaffordable to the very people who need them (WHO/TDR, 2004).

Besides eflornithine, other drugs in use for the treatment of Sleeping Sickness include Pentamidine and Suramin, which are used for the treatment of early-stage of Gambiense and Rhodesiense diseases respectively. All the three drugs have adverse side effects. The ability of the trypanosomes to change their surface coat (antigenic variation) and thus evade the host's immune system makes it difficult for vaccines to be developed against the disease (Murray and Jennings, 1982; Donald, 1994; El Rayah *et al.*, 1999; Anene *et al.*, 2001), thus necessitating the need for research into new potentially trypanocidal agents.

Ancient man is known to have utilized plants for millennia. Based on current knowledge, at least in West Africa, we know that extracts of some of these plants are useful in a crude form. A large number of plants are used in traditional medical practices, and have been used for more than 3000 years, most of which probably exert therapeutic effects and would be proven as such if they were properly evaluated by western standards (Norman and Djaja, 1991; Dery and Osiyana, 2000).

In this study, the potential of using the plant, *G. sokotensis* as an alternative trypanocidal was assessed. The objective was to determine the effects of treatment with G. *sokotensis* on the pattern of parasitaemia in rabbits infected with *T. brucei brucei.*

Materials and Methods

Experimental Animals

Thirty healthy rabbits of both sexes between 3 – 4 months old weighing between 1.05 – 1.6kg were purchased from Sokoto Central market. The animals were screened for the presence of haemoprotozoan parasite using wet mount blood films and were confirmed negative. The initial weight before infection and final weight after infection were determined using a weighing balance.

All the selected animals were allowed to acclimatize for 7 days in fly proof animal house at the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto. They were fed fresh vegetables and grower's mash. Water was provided *ad libitum.*

The Parasite (Trypanosoma brucei brucei)

The *Trypanosoma brucei brucei* used in this study was obtained from the parasitology division of the Nigerian Institute for Trypanosomiasis Research (N. I. T. R), Vom, Jos, Plateau State, Nigeria. The parasite was transported to Sokoto in 4 donor rabbits, and was maintained in the laboratory by passage to another Rabbit. All rabbits were kept at room temperature in the animal house of the Usmanu Danfodiyo University Sokoto. A clean environmental condition was maintained through out the study period. They were fed on grower's mash (Pfizer Nigeria, Plc), fresh green vegetables and water *ad libitum*. The rabbits were grouped as shown in Table 1.

Table 1
Groups of rabbits infected with *T. brucei brucei* and treated with the extract of *G. sokotensis* and Berenil®

Group	*n*	Treatment
A	6	Infected and treated once daily with 60mg/kg of *G. sokotensis* for 7days (after the establishment of the parasitaemia)
B	6	Infected and treated once a day with 3.5mg/kg of Berenil® once (after the onset of parasitaemia)
C	6	Infected but not treated
D	6	Uninfected but treated with 60mg/kg of *G. sokotensis* for 7days.
E	6	Uninfected and untreated (control group)

Animal Inoculation

After the period of acclimatization of 7 days, the experimental rabbits were inoculated with *Trypanosoma brucei brucei.* 0.5ml of infected blood was taken from the donor rabbit with fulminating parasitaemia and was diluted with 5ml of normal saline. Then 1ml of the diluted blood containing approximately 5 parasites per field (104 to 5 x 105) per ml was intraperitoneally inoculated as described by Onyeyili (1994) and Akinwale *et al.* (1999) into each of the infected rabbits.

Preparation and Administration of Diminazene Aceturate (Berenil®)

After weighing each animal, Berenil® was administered intramuscularly at a dose rate of 3.5mg/kg using insulin syringe and needle and the required dose for each of the animal was determined as reported by Dina *et al.* (2002).

Estimation of Parasitaemia

Blood samples were collected from the ear (marginal ears vein) of the animals after disinfecting the surface with methylated spirit. Blood smear was then prepared from each sample and the parasitaemia was determined using rapid matching method of Herbert and Lumsden (1978). The first drop of blood that appears was collected directly on to the middle of the slide and covered with a cover slip. The fresh blood smear was then examined systematically under light microscope at x10 objective and x40 objective.

Packed Cell Volume (PCV) Determination

PCV or haematocrit value was obtained by the use of heparinized capillary tubes of about 75mm in length and having an internal diameter of about 1mm to collect blood sample. Blood was allowed into the tube (15mm unfilled) by capillary from the (marginal ears vein). The dry end of each of the tubes was sealed by heating the dry end of the tube rapidly with a fine flame. The tubes were then placed in a microhaematocrit centrifuge, covered spun at 3000 rpm for 5mins, after which the PCV was read using a microhaematocrit reader (Felidae world, 2005; Stanley, 1983 and Byrd *et al.*, 1966).

Erythrocyte Sedimentation Rate

Measurement of Erythrocyte sedimentation rate (ESR) was done by observing the time taken for the

red cells to settle. A rapid sedimentation of the cells shows the possibility of infection in the blood (Cheesbrough, 2006).

Results

Parasitological Findings

The result from this study showed that the non-infected (control) showed no signs of trypanosomiasis throughout the study period (Plate I). While the infected rabbit exhibited peak parasitaemia within 8 weeks, and the degree of parasitaemia in group C (infected untreated) remained high resulting in the death of all the rabbits with an average survival time of 9 weeks (Table 2; Figure 1).

The result observed in weight, PCV and ESR analysis showed that, as the parasitaemia increases the body weight decreases. All the infected groups had a significant drop in their PCV (%) values after infection and before treatment. Decrease in PCV values progress in Group C (infected/untreated), and increase in group A & B.

The erythrocyte sedimentation rate (ESR) value increase significantly as parasitaemia increase and it decrease when it decreases (Table 3).

Clinical Findings

All the experimental groups infected with *Trypanosoma brucei brucei* showed oedema of the face, encrustation of the lip, ocular discharges, emaciation, weakness, blindness and anaemia (Plate II). These signs were reversed or prevented in the group treated with Berenil® (Diminazine aceturate); and reduced in the group treated with G. *sokotensis*. Even though the infection relapsed in this later group, with the animals dying before the experiment was terminated, together with the animals in the untreated infected group.

Table 2
Mean levels (parasites per microscope field) of parasitaemia of *Trypanosoma brucei brucei* in rabbits before, during and after treatment with *Gardenia sokotensis*

Period	Group				
	A	B	C	D	E
BT (n=21)	11.26 ± 13.56	12.75 ± 12.50	11.33 ± 11.05	00 ± 00	00 ± 00
DT (n =8)	4.84 ± 2.01	0.33 ± 0.92	11.86 ± 6.50	00 ± 00	00 ± 00
AT (n=18)	30.28 ± 12.75	00 ± 00	30.09 ±10.62	00 ± 00	00 ± 00

Plate I
Uninfected control rabbit

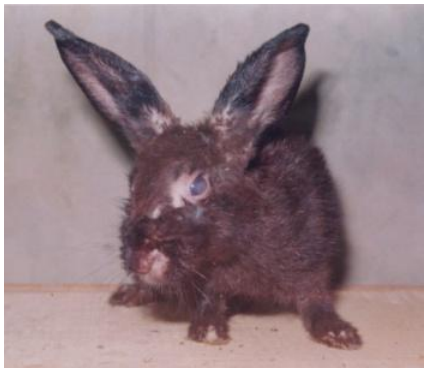

Plate II
Rabbit infected with *T. brucei brucei*.
(Note the ocular lesion)

Table 3
*Mean (±SD) body weight (BW), Packed cell volume (PCV) and Erythrocyte sedimentation rate in rabbits infected with *Trypanosoma brucei brucei* and treated with the extract of *Gardenia sokotensis* and Berenil®

Periods / Groups		Parameters		
		Body weight	Packed Cell Volume	Erythrocyte Sedimentation Rate
B.I.	A	1.342±0.180	30.833±3.371	2.167±0.753
	C	1.342±0.174	28.167±4.792	2.500±1.049
	D	1.058±0.132	36.333±2.066	1.833±0.408
	E	1.242±0.120	31.333±3.204	2.000±0.633
B.T.	A	1.190±0.108	20.200^{j}±2.950	4.000±1.000
	B	1.100±0.128	21.800±1.924	5.800±2.864
	C	1.025±0.113	20.833^{j}±3.601	5.667±2.503
	D	1.350±0.084	40.833±2.483	1.667±0.516
	E	1.433±0.117	39.167±2.137	2.000±0.000
A.T.	A	1.240±0.089	22.200±3.962	3.600±0.548
	B	1.190±0.129	27.000±3.742	4.600±2.702
	C	0.950±0.071	19.667±3.204	7.833±2.229
	D	1.400±0.071	41.833±2.041	2.000±0.000
	E	1.508±0.116	40.667^{j}±1.633	1.833±0.408

*Figures in a column with the same superscript differ significantly (P<0.05)
B.I = Before Infection
B.T = Before Treatment
A.T = After treatment

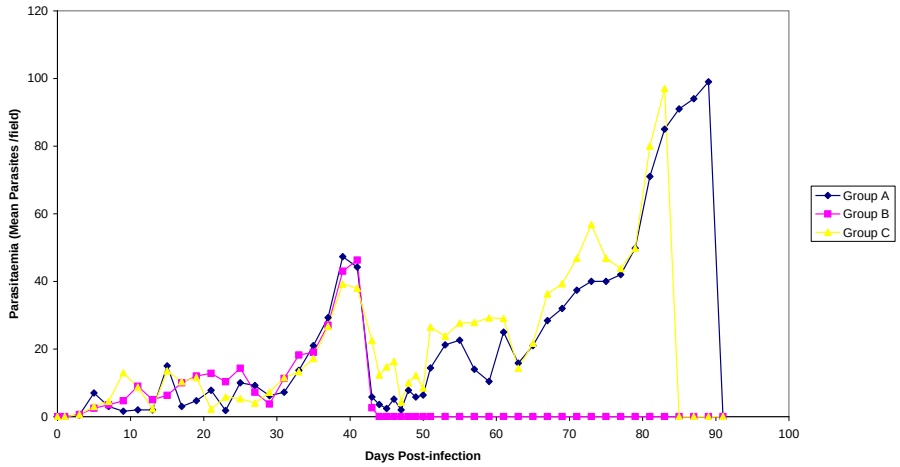

Figure 1
Parasitaemia levels before, during and after treatment in three groups of rabbits infected with *T. b. brucei*

Discussion and Conclusion

All the infected rabbits in the three groups developed parasitaemia, of variable intensity, 3 to 5 days post-infection. The parasitaemia continues to increase within the untreated group, which corresponds with the work of Akinwale *et al.* (1999) and Okochi *et al.*, 2003).The level of parasitaemia decrease significantly in group treated with Berenil® resulting in total cure for all the animals there was a slight increase in survival time within the group treated with the ethanol extract of

G. *sokotensis* which is statistically not significant. This finding suggest that, the plant G. *sokotensis* show some medicinal potential during treatment though not as effective as Berenil® there is therefore the need for further work on this plant such as its active ingredients and their quantity etc. Mansfield (1990) working with *T. b. brucei* in mice has shown that under normal conditions, the host is continually exposed to novel VATs and is never able to achieve parasite elimination. As infection progresses the host becomes immune suppressed, and the ability to deal with the infection declines even further. But immune clearance of parasitaemia is possible in artificial conditions, in which trypanosomes of a defined VAT are exposed to non – limiting quantities of VSG – specific antibody. IgM antibodies directed against VSG epitopes allow the uptake and destruction of antibody coated trypanosomes by Kupffer cells in the liver (Mansfield, 1990).

The clinical findings of edema of the face, encrustation of the lip, ocular discharge, emaciation, weakness, and anaemia agree with the earlier reported cases of *T. brucei* infection (Losos and Ikede 1972; Igbokwe *et. al*., 1998 and Onyeili *et. al*., 1994).

The responses of group A and B infected rabbits to treatment were variable. Group B fully responded to treatment, with a significant increase in survival time showing that the animals were cured while group A responded very slightly to treatment with a relapse and a slight increase in their survival time. This agrees with the earlier findings of Akinwale *et al.* (1999), showing that the plant can not be used alone; it needs further investigation and improvement, such as combining it with a conventional drug or by increasing the dose rate and by trying other routes of administration of medicine, like intramuscular or intravenous.

Anaemia and weight loss are a cardinal features of *T. brucei* infection in mammals, (Iliyasu *et. al.*, 1999; Igbokwe *et al.*, 1994 and 1998. Multiple factors have been proposed to account for trypanosome-induced disruption of the cell membrane (Anosa and Isoun, 1997). These factors are haemodilution (Anika *et al.*, 1990), increased red cell breakdown (Mama and Holmes, 1975) and reduced cell synthesis (Fiennes, 1970).

The increase or decrease in ESR is used to monitor the progress of the disease. It is also elevated in acute and chronic infections although the rate of red cell sedimentation is dependent upon the relative concentrations of plasma proteins it is also dependent on the number of red cells or PCV. Thus increased anaemic patients an increase ESR will be recorded because of the low PCV, and not necessarily because there is a change in the relative concentration of plasma protein (Baker and Silverton, 1985).

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