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Effects of *Sterculia tomentosa* stem bark extract on haematological parameters and *Trypanosoma brucei* infection in rats

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Abstract
Evaluation of antitrypanosomal efficacy of stem bark water extract of *Sterculia tomentosa* using rats as a model revealed significant suppressive effect on parasitaemia with extended survival time in group D rats treated with extract subcutaneously. The extract did not affect packed cell volume of treated rats. There was no significant difference ($p<0.05$) in packed cell volume (PCV) in groups A, B and C rats on days 3, 6 and 9. Except for group D rats which maintained PCV close to control group from days 3 to 9 post-infection (pi). In group D the PCV continued to decline from 38±0.84% to minimum value of 30.0±0.11% on day 24 pi. Rats in group B and C all died of severe trypanosome infection on days 9 and 11 pi respectively. The maximum survival time reached was 18.2±2.74 days and the minimum was 6.0±10.0 days in rats treated subcutaneously and in rat that did not receive treatment at all. The results also showed that the route of extract administration played an important role on the efficacy of extract used, as evidenced by profound effects observed in rats treated with subcutaneous injection. While further toxicological studies are progressing, supportive treatment with the extract in areas of scarce conventional trypanocide is encouraged for now.

Keywords: Sterculia tomentosa, bark extract, haematological parameters, Trypanosoma brucei, rats.

Introduction

Serious economic losses resulting from animal diseases have long been acknowledged as one of the constraints to the productivity of livestock in Nigeria (Okon and Fabiyi, 1990). Traditional healers and herdsmen have made various attempts to control these diseases through the use of medicinal plants. Only few of these plants have been properly identified and documented (Ibrahim and Nwude.1984). It is important that experiences of our people in the traditional treatment of animal diseases be recorded and that studies be carried out to establish the toxicity and efficacy of the plants used.

Trypanosomiasis is an important protozoan disease that has been a major constraint to livestock production and human health in Africa decades after the discovery of the etiologic agent (Rabo *et al.*, 1994). The problem of trypanosomes as parasites is, to a large extent, due to their ability to undergo

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antigenic variation, which enables the parasites to evade host's immune response resulting in persistent infection (Shapiro and Pearson, 1986). However, lack of drug development coupled with the development of resistance by trypanosomes and relapse parasitaemia, have led to an impasse in the chemotherapy of trypanosomiasis. There is the need to integrate traditional animal health care with modern veterinary practice because to some pastoralists modern veterinary inputs and services are often not readily available. They are either too difficult to obtain or too expensive for the poor marginal farmers and pastoralists. Thus, this will provide easily accessible, low cost alternative to farmers.

Recent studies (Muhammad and Abubakar, 1999; Kaikabo *et al.*, 2005) have shown that greater number of pastoralists in arid zone of Nigeria have relied in part on traditional veterinary care for the treatment of their livestock diseases. This postulate has led to the current investigation on the efficacy of *Sterculia tomentosa* (vernacular: Kukkuki) used in the treatment of animal trypanosomiasis in arid zone of northern Nigeria.

Experimental

Plant material. The plant (*Sterculia tomentosa*) was identified by Mr. Abdulhameed Isyaku Mohammed, a botanist in arid zone ecology unit of the Ministry of Environment, Damaturu, Nigeria. Stem bark of the plant was collected during the dry season (April 2003) for the study. Some parts have been deposited at the herbarium of the Ministry for future reference.

Extraction. The stem bark of *Sterculia tomentosa* (kukkuki) was air dried in the laboratory for three weeks and ground into powder with the aid of wooden mortar and pestle. One hundred grams of the powdered plant material were exhaustively extracted in water (700 ml) for 8 hours using Soxhlet extractor (Quickfit, England). The extract was concentrated in a vacuum rotary evaporator. Then the extract was stored at 4°C until required.

Pilot toxicity studies. Before commencement of chemotherapeutic trials with the extract, rats were randomly divided into four groups of 4 rats each (n=4) and treated orally with a single dose of 100, 200, 400, 800 mg/kg water extract of the stem bark of Sterculia *tomentosa* respectively. The experimental animals were observed over a period of 24 hours for clinical signs of intoxication and mortality. The signs were scored according to severity. The median lethal dose (LD_{50}) of the extract was calculated using modified method of Aliu and Nwude (1982).The dose of the extract that produced 100% mortality was 400mg/kg, while the dose that produced no mortality was 100mg/kg. The estimated LD_{50} was 200mg/kg, which was calculated as follows: The sum of the product of the mean dead and the dose difference (x) divided by number of animals in each group (y).The result is subtracted from the least dose that kills all animals (z).

Thus, $LD_{50} = z - (x / y)$
$= 400 - 800/4 = 200mg/kg$

Experimental animals and trypanosome infection. Thirty albino rats weighing between 180 – 220g were obtained from the livestock unit of National Institute of Trypanosomiasis Research (NITR), Vom. Plateau State, Nigeria and used for the study. The animals were randomly divided into five groups of 6 rats each (n=6), kept in fly-proof plastic cages and maintained on commercial rat pellets (Saunders, Nig. Ltd) and watered *ad libitum.* Sawdust was used as bedding and changed every three days. *Trypanosoma brucei* (Federe strain) was obtained from NITR, Vom, Plateau State, Nigeria. The parasite was originally isolated from Muturu cattle in 1995 in Federe area of Plateau State, Nigeria. This parasite was used to inoculate the experimental animals, following serial

passage from the donor mice. The test animals were inoculated intraperitoneally with an infected tail blood containing 1×10^6 trypanosomes standardized in phosphate glucose buffered saline solution. The rats were examined daily for the presence of trypanosomes.

Chemotherapeutic trials. Experimental animals were treated with a safe dose of 100mg/kg using an 18G needle fitted with a plastic canula and or insulin syringe as follows:

Groups A - Treated with extract per os concurrently with infection
Group B - Treated with extract per os 3 days following prepatent
Group C - No treatment
Group D - Treated with extract subcutaneously for three days after prepatent period
Group E - Control (uninfected)

Haematological analysis. Packed Cell Volume (PCV) was determined before parasite inoculation and administration of extract and each three day after using the microhaematocrit method (Coles, 1974). Parasitaemia was monitored using wet mount and Buffy coat microhaematocrit technique(s) as described by Murray *et al* (1983) and the parasitaemia scores were estimated as described by Woo (1969).

Statistical analysis. The t-test was used to analyze the difference between mean values of PCV, Parasitaemia score and Survival time and one way ANOVA was used to analyse the extent of variations between groups, and probability values equal to or less than 0.05 were considered significant as described by Armitage (1980).

Results

Parasitaemia score of Trypanosoma brucei infected rats. The parasitaemia score of rats infected with *T. brucei* and treated with stem bark extract of *Sterculia tomentosa* is presented in Table 2. Following a prepatent period of 3 days the parasitaemia of rats in group A (those infected and treated with extract concurrently) rose steadily from $5.5\times10^3/\mu l$ to $27.5\times10^3/\mu l$ by day 6 pi. There was a progressive increase of up to $50.0\times10^3/\mu l$ by day 9 pi when they died. Group B rats (which received extract for 3 days after prepatent period) had a rather gradual rise in parasitaemia value from $5.5\times10^3/\mu l$ on day 4 pi to $27.5\times10^3/\mu l$ on day 8 pi and maintained the parasitaemia at that level for 3 days before it went down to $5.5\times10^3/\mu l$ by day 11 pi. The fluctuation continued with a rise in the level of parasitaemia to $25.5\times10^3/\mu l$ by day 13 pi and day 17 pi when the rats died, the value was as high as $500\times10^3/\mu l$. On days 6,7,8 and 9 the parasitaemia were significantly ($p<0.05$) lower than those of group A rats. Group C rats which never had the extract, had a significant ($p<0.05$), rapid and progressive rise in their parasitaemia from $5.5\times10^3/\mu l$ by day 4 pi to $25.5\times10^3/\mu l$ by day 6 pi. By day 8 pi when they died the parasitaemia score was $50.0\times10^3/\mu l$. The parasitaemia score of group D rats was maintained at $5.5\times10^3/\mu l$ up to day 8 pi. The mean parasitaemia score was significantly ($p<0.05$) lower in this group than in group A rats on days 6, 7, 8 and 9 pi. The treatment significantly suppressed the parasitaemia of rats and the most effective route was the subcutaneous injection. The protocol of administering the extract for 3 consecutive days after prepatent period was more effective than concurrent infection and treatment with the extract.

Packed cell volume. The mean PCV of rats infected with *T. brucei* and treated with extract is presented in Table 1. Following infection and concurrent treatment with extract, the mean PCV value of the rats was observed to drop from the pre-infection value of 40% to 36% by day 6 post infection (pi) and was maintained at this level until day 9 pi when they died. This decrease in mean PCV value to 36% was statistically significant

($p<0.05$) when compared to the uninfected control level. The mean PCV of rats given extract for 3 days after prepatent period did not change during the course of the treatment as the preinfection value of 39% was maintained up to 3 day pi when the treatment with extract was stopped. However, when the treatment was terminated, the average PCV dropped significantly ($p<0.05$) to 36% by day 6 pi and there was progressive decline in the level to 35% by day 15 pi when they died. The mean PCV value of rat not given extract at all fell sharply from preinfection value of 42% to 32% by day 6 pi and the rats died 9 days pi. The decrease in PCV in this group was significantly ($p<0.05$) lower than those of the treated groups and the control (uninfected) group. The rats that were infected and given extract subcutaneously had a drop in PCV on day 6 pi, but the drop was gradual. As in the other groups, there was no change in their PCV during the first 3 days of infection. However, by day 6 pi the mean PCV had dropped slightly from preinfection value of 40% to 39%. The drop in PCV by day 6 pi was significant ($p<0.05$). The mean PCV of this group decreased to 38% by day 9 pi. By day 12 pi the mean PCV had dropped to 36% and it maintained that value up to day 15 pi before it dropped further to 34% by day 18 pi. By day 24 pi when all the rats died the mean PCV value was as low as 30%. The drop in PCV was statistically significant ($p<0.05$) by day 12 pi up to the time they died when compared to the preinfection value and the PCV of control rats. The PCV of control rats remained the same.

Table 1: Effect of *Sterculia tomentosa* stem bark extract on packed cell volume (%) of rats infected with *Trypanosoma brucei* (federe strain)

Group	Preinfection	Days of post-infection							
		3	6	9	12	15	18	21	24
A[a]	40±1.2	40±1.4	36±1.4	36±1.3	*				
B[b]	39±0.12	39±0.31	38±0.52	36±0.3	35±0.41	35±0.11			
C[c]	42±0.20	42±1.0	32±0.20	*					
D[d]	40±0.70	40±0.7	40±0.84	38±0.84	36±0.61	36±0.51	34±0.82	34±0.21	30±0.11
E[e]	40±0.21	40±0.21	41±0.35	40±0.5	40±0.2	40±0.8	41±0.2	41±0.20	40±0.25

* = Dead before that day; [a] = Treated concurrently with infection; [b] = Treated for 3 days after prepatent period
[c] = Not treated at all; [d] = Treated subcutaneously after prepatent period; [e] = Control (uninfected).

Table 2: Parasitaemia score of rats infected with *Trypanosoma brucei* ($\times 10^3$) and treated with stem bark extract of *Sterculia tomentosa*

Group	Days post-infection												
	4	5	6	7	8	9	10	11	12	13	14	15	16
A	5.5	5.5	27.5*	25.5	50.0								
B	5.5	5.5	5.5	5.5*	27.5*	27.5*	27.5	5.5	25.5	25.5	25.5	25.5	50.0
C	5.5	27.5*	25.5	25.5	50.0[a]								
D	5.5	5.5	5.5	5.5	27.5	25.5	25.5	5.5	0.5*	5.5*	27.5*	25.5	5.5*

[a] Rats died by that day; * Values in the same column vary significantly ($p<0.05$)

Table 2 contd.

Group	Days post-infection							
	17	18	19	20	21	22	23	24
A								
B								
C								
D	5.5	27.5	25.5	27.5	27.5	5.5	5.5	25.5

Table 3: Effect of *Sterculia tomentosa* stem bark on mean survival time of rats infected with *Trypanosoma brucei*

Groups	Treatment	Survival time (days ± SEM)
A	Given extract per os concurrently with infection (100mg/kg body weight)	8.60 ± 0.50*
B	Given extract per os for 3 days (100mg/kg body weight) after prepatent period	11.20 ± 3.30*
C	Not given extract at all	6.00 ± 1.10*
D	Given extract subcutaneous for 3 days After prepetent period	18.20 ± 2.74*
E	Control (uninfected)	25

* values vary significantly ($p<0.05$)

Discussion

The results of the study showed significant trypanosuppressive effects in rats treated with *Sterculia tomentosa* extract through subcutaneous injection. The extract led to progressive decline in parasitaemia and extended survival time in rats up to 24 days post infection. Experimental rats in groups A, B and C have died of severe trypanosomiasis infection as judged by the peak parasitaemia level, which was recorded on days 8, 16 and 8 respectively. The trypanocidal study shows that stem bark extract of *Sterculia tomentosa* can significantly modify the course of *Trypanosoma brucei* infection in rats. The extract suppressed the level of parasitaemia and prolonged survival time in rats. The rats that lived longest were those treated with extract by subcutaneous route. This shows that oral administration of the extract may result in the degradation of bioactive components by digestive enzymes or that absorption of the bioactive components is poor or a combination of both factors. Suppression of parasitaemia has also been reported in *T. brucei* infected mice treated with ethanol extract of root bark of *Nauclea latifolia* (Madubunyi, 1995).

The administration of water extract of *Sterculia tomentosa* for a period of 3 days did not adversely affect haematological parameters. There was mild haematological concentration, which might have been due to dehydration observed in the study. The fact that the extract did not adversely affect haematological parameters might be beneficial in the management of trypanosomiasis, which is usually characterised by marked reduction in haematological parameters (Anosa, 1998).

In this study, trypanosomiasis was characterised by anaemia with parlor of mucous membrane, weakness, fluctuating parasitaemia and mortality. Anaemia is a consistent feature of trypanosomiasis and is mainly haemolysis (Anosa, 1988). The intermittent parasitaemia has been previously explained in relation to changes in surface coat antigens of trypanosomes, such that antibodies produced against the parasites with particular surface coat antigens do not eliminate all of them in the animals. The surviving parasites change their coat antigens and multiply until new antibodies against them are produced (Shapiro and Pearson, 1986). Body weakness was observed in the rats exposed to trypanosomes and was probably due to hypoglycaemia associated with trypanosomiasis as the infection can destroy blood glucose (Igbokwe, 1995).

In conclusion, water extract of *Sterculia tomentosa* used traditionally for the treatment of trypanosome infections can significantly suppress parasitaemia as it was observed to have prolonged survival time of *T. brucei* infected rats. The treatment was more effective when given at the dose rate of 100mg/kg for three days through subcutaneous route. Further research is necessary to establish toxicity, photochemistry and plasma concentration of this plant.

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