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ARTICLE

Invitro and in Vivo Trypanocidal Activitiy of *Combretumracemosum* Leaves

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SUMMARY

Combretumracemosum P. Beauv **(Combretaecea) is folklorically used as an antiulcer, trypanocidal, antihelminthic and antimicrobial agent. A study was conducted to determine the trypanocidal activity of crude methanolic extract of *Combretumracemosun* leaves against *Trypanosomabruceibrucei* both in vitro and in vivo. The extract exhibited in vitro activity against *T. brucei* by immobilizing the trypanosomes and rendering them uninfective to mice at concentrations ranging from 125 mg/ml – 0.2559 mg/ml. The extract also demonstrated in vivo trypanocidal effect by reducing parasitaemia and improving packed cell volume in *T brucei* infected mice at 50, 100 and 200 mg/kg body weight when administered intraperitoneally. Intraperitoneal administration of the extract to mice at 2,000 mg /kg body weight did not result in deaths during the acute toxicity study. This study provides evidence of the ethno pharmacological use of *C. racemosum* in trypanosomosis.**

KEY WORDS: ***Combretum racemosum,*** **leaves, methanolic extract, trypanocidal activity,** ***Trypanosoma brucei***

INTRODUCTION

Trypanosomosis is a severe often fatal disease widely spread in Africa where it affects the general health and wellbeing of human and livestock populations (Igwe *et al.*, 2002, Kamuanga, 2003). Since it has not been possible to develop an effective vaccine against the disease, due to the problem of antigenic variation, trypanocidal drugs play a major role in its management and control (Igwe *et al.*, 2002). The chemotherapy of African trypanosomosis still remains far from being satisfactory and there is growing resistance to the few drugs currently available (De Koning, 2001; Ogbadoyi *et al.*, 2007). Also, many of the drugs currently available for the treatment of trypanosomosis are highly toxic (Matovu *et al.*, 2001; Ogbadoyi *et al.*, 2007).

The serious problems encountered in the control of both human and animal trypanosomiasis, and the urgent need for new affordable trypanocidal drugs (Bizimana *et al.*, 2006) suggests herbal remedies as a reasonable alternative. Literature survey and field studies showed that plants are used in traditional medicine in Africa to treat trypanosomosis in humans and animals (Youan *et al.*, 1997, Bizimana *et al.*, 2006).

Combretum racemosum is a straggling

shrub widespread across Africa and bears a mass of crimson flowers which is very spectacular gaining it the local English name of Christmas rose in Southern Nigeria (Burkill, 1985). The leaf extract of *C. racemosum* has a folkloric reputation as an antiulcer (Okwuosa *et al.*, 2006), trypanocidal (Atindehou *et al.,* 2004), antihelminthic and antimicrobial (Onocha *et al*, 2008) agents. Previous phytochemical analysis of *C. racemosum* extracts revealed the presence of alkaloids, steroids, cardiac glycosides, saponins and tannins (Onocha *et al*, 2008)

The aim of this study was therefore .to evaluate the trypanocidal activity of crude 70% methanol extract of *C. racemosum* leaves, both in vitro and in vivo.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *C. racemosum* were collected in April 2009 from Ngwo in Enugu state, Nigeria and identified in the Department of Botany, University of Nigeria, Nsukka, where the voucher specimens are kept in their herbarium.

Preparation of extracts

The leaves were washed with water to remove dirt and dust and dried in the shade. The dried materials were ground into fine powder using a laboratory mill. Cold extraction was done with 70% methanol for 72 hours at room temperature with intermittent shaking. After filtration through Whatman's filter paper, solvent was removed using rotary evaporator and the extract stored at -4°C in sterile universal tubes until use.

Animals

Albino mice weighing between 30 - 39 g of either sex were used for the study. The animals were kept in clean wire meshed cages under standard animal house conditions in accordance with the recommendations in Guide for the Care and Use of Laboratory Animals (DHHS, NIH Publication No. 85-23, 1985). The mice were given standard pellet diet and water *adlibitum* during the entire period of experimentation.

Parasite

The *Trypanosomabruceibrucei* (Federe strain) was procured from the Nigerian Institute for Trypanosomiasis Research, Vom, Plateau State, Nigeria, where the initial stock was collected from a clinical case of bovine trypanosomiasis. The strain was maintained in the laboratory by successive passages in mice. Trypanosomes in whole blood of infected mice were used for in vitro studies. In the in vivo study, each mouse was inoculated intraperitoneally (IP) with a parasite inoculum of 10^6 trypanosomes in whole blood of infected mice. The quantitative estimation of trypanosomes was done by rapid matching method (Herbert and Lumsden, 1976).

Acute toxicity studies

Twelve mice were used for this study. The mice were divided into 4 groups (A - D) of 3 mice each. They were dosed IP once with 250, 500, 1000 and 2000 mg /kg body weight of the extract, respectively. The mice were observed for 24 hrs for signs of toxicity like changes in behaviour or death.

In-vitro evaluation of the extract

The methanolic extract of *C. racemosum* leaves was tested using phosphate buffered ringers glucose solution as supporting medium according to a modified method of Petana (1964). Briefly, test tubes each containing 1 ml of supporting medium were used to prepare 2- fold serial drug dilutions (of the extract) covering a range from 125 – 0.0640 mg/ml. A final tube (also with 1 ml supporting medium) did not

contain extract and served as control. 10^6 blood stream forms of *T. brucei* in 0.1ml of the supporting media were added to each one of the tubes and incubated for 3 hrs at 37°C. The contents of each tube were examined microscopically for motility assessment half hourly for the 3 hr incubation period. The infectivity of the trypanosomes after incubation with extract was checked by IP inoculation of 0.1ml of the contents of each tube into each of three mice. Tail blood from each mouse was checked daily for the presence of trypanosomes using the wet blood film and buffy coat methods (Woo, 1971). The abolition of infectivity of the parasite was concluded if no trypanosome was detectable for 60 days after inoculation (Geerts and Holmes, 1998).

In-vivo evaluation of the extract

Thirty mice randomly divided into 6 groups (I - VI) of 5 mice each were used for this study. Animals of groups I – V were inoculated with 10^6 trypanosomes IP while Group VI animals were not infected. After detection of parasitaemia, animals of groups I, II and III received test extract at 50, 100 and 200 mg/kg body weight respectively by IP route, daily for 5 consecutive days from day 12 post infection (PI). Animals of group IV were given standard trypanocidal drug (diminazene aceturate, Hoechst AG) at 7mg/kg body weight IP on day 12 PI. Animals of groups V and VI did not receive treatment and served as positive and negative controls respectively. The efficacy of the test extract was assessed on the basis of differences in parasitaemia and packed cell volume.

Statistical analysis

The data collected were subjected to analysis of variance (ANOVA). Means were compared using Duncan's multiple range test.

RESULTS

The extract of *Combretum racemosum* leaves was dark brown in colour and pasty in consistency. The percentage yield was 8.1 %(w/w).

There was no mortality recorded in the acute toxicity study. However, some of the mice that received the extract at 1,000 and 2,000 mg/kg were weak and depressed for about 20 min.

The methanolic extract of *C. racemosum* showed in vitro activity against the strain of trypanosome under investigation as evident from the ability of the extract to immobilize the parasites (Table I) and render them not infective to mice (Table II). The extract inhibited motility of parasites following incubation for 30 min at concentrations of 125 – 16.375 mg/ml but at 0.2559 mg/ml required 180 min to immobilize trypanosomes. Mice inoculated with tube contents with extract concentrations of 125 - 0.2559 mg/ml remained aparasitaemic for 60 days PI observation period while the control was parasitaemic 6 days PI.

The mean parasitaemia of mice infected with *T. brucei* and treated with extract or diminazene aceturate are presented in Fig. 1. Following detection of trypanosomes in the blood of mice at day 4 PI., there was a progressive increase in mean parasitaemia in all the infected groups of mice However, following treatment from day 12 PI., there was marked reduction in mean parasitaemia in all the treated groups (I - IV) with significant difference ($p < 0.05$) between the mean parasitaemia of the extract (I - III) and the infected untreated group. Diminazene aceturate (in group IV) was able to clear the parasites by day 16 PI. Resurgence in parasitaemia in the extract treated groups occurred by day 20 PI.

Following infection of mice, the PCV showed a similar course in extract and diminazene aceturate treated groups in which there were initial decline followed by improvements after treatment (Fig. 2). The PCV continued to improve in the diminazene aceturate treated group, but by day 24 PI depreciated appreciably in the extract treated groups. At day 16 PI there was no significant difference ($p<0.05$) in PCV between the extract and diminazene aceturate treated groups but by day 24 PI the PCV of the diminazene aceturate treated group was significantly higher than the extract treated groups.

DISCUSSION

During the acute toxicity test, there were no deaths recorded up to the maximum tested dose of 2,000 mg/kg. This shows that the extract was well tolerated. However, this result does not rule out the possible cumulative toxic effects of administering the extract for 5 consecutive days as was done in this in vivo test. Toxicity tests are employed in determining the possible dosages at which crude extracts can be administered to experimental animals. Arbitrary use of drugs/compounds in treatment without carrying out an acute toxicity teat could be fatal (Nweze and Obiwulu, 2009).

In the in vitro study, the concentration of extract which resulted in complete elimination of motility (minimum lethal concentration, MLC) was 0.2559mg/ml. In addition, inoculation of mice with trypanosomes exposed to varying extract concentrations (125 – 0.2559 mg/ml) following an incubation period of 3 hrs did not result in infection. This demonstrates the in vitro trypanocidal activity of *C. racemosum* leaves.

In the in vivo study, there was a reduction in parasitaemia following administration of extract in groups I-III mice. There was no significant difference in mean parasitaemia between the extract and diminazene aceturate treated groups at day 16 PI. This demonstrates the in vivo trypanocidal activity of the extract. Treatment of the group IV mice with diaminazene aceturate led to the clearance of the parasites from the blood by day 16 PI. Blood *et al.*, (1994) has reported the curative effects of diaminazene aceturate when used against *T. brucei* infection. The resurgence in mean parasitaemia in the extract treated groups by day 20 PI. may be due to the waning effect of the treatment. This resurgence in mean parasitaemia may also be due to release of trypanosomes from the tissues which can occur when treatment is delayed or the dose rate is inadequate (Blood *et al.,* 1994). Infection of mice with *T. brucei* resulted in a decrease in mean PCV in all the infected groups. Anaemia is a consistent finding in trypanosomosis (Blood *et al.*, 1994). Following administration of the extract or diminazene aceturate, there was an improvement in PCV in all the treated groups, demonstrating the antitrypanosomal activity of both the extract and diminazene aceturate.

CONCLUSION

It may be concluded from the result of this study that the crude extract of C. racemosum leaves possesses activity against *T. brucei*. This study also provides evidence of the ethno pharmacological use of *C. racemosum*.

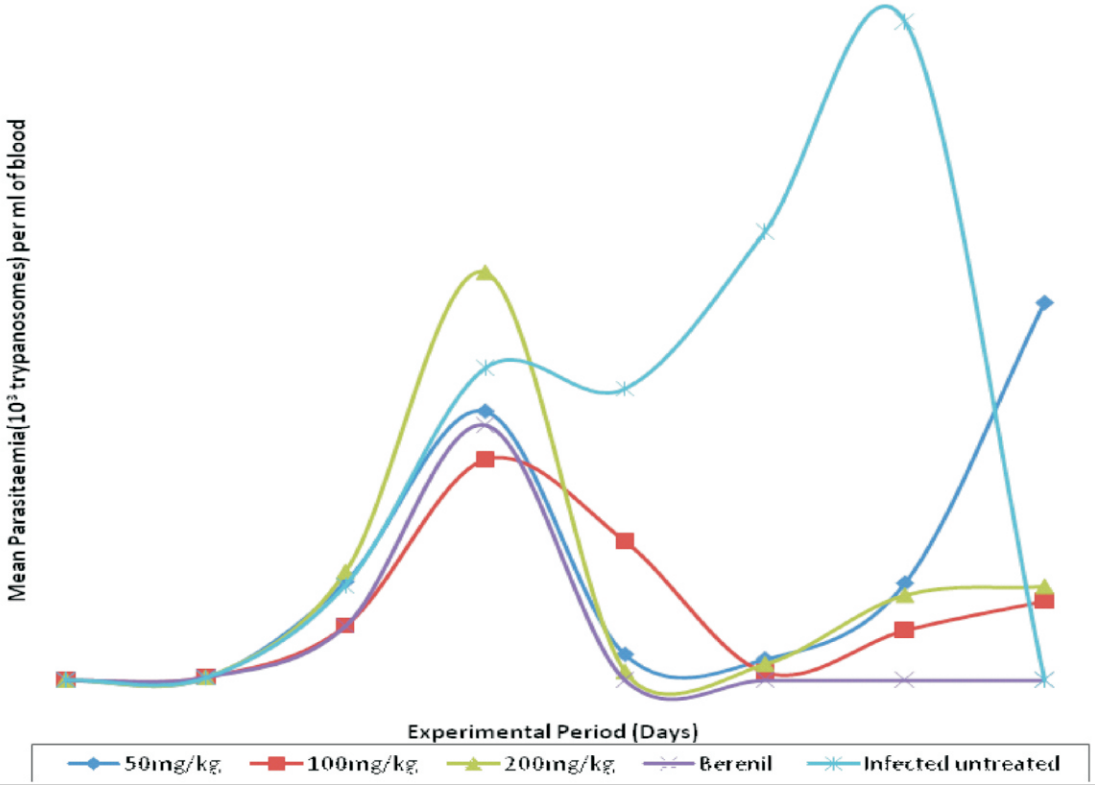

Fig I: Mean parasitaemia of mice infected with *Trypanosomabruceibrucei* and treated with v

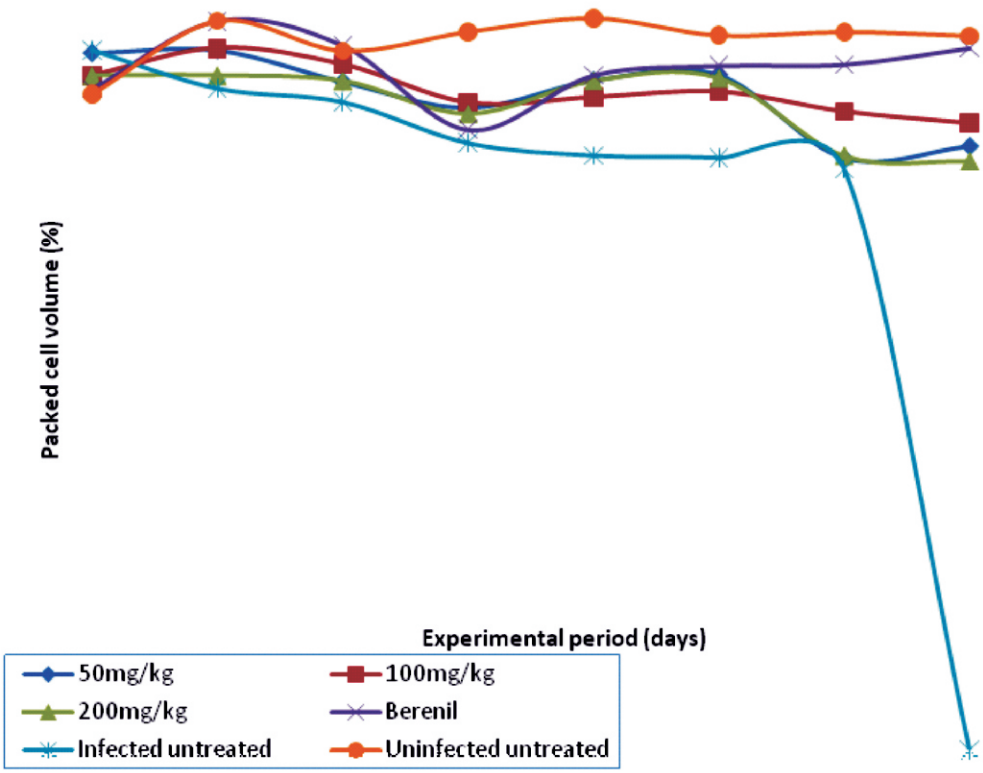

Fig II: Mean packed cell volume of *Trypanosomabruceibrucei* infected mice treated with varying doses of *Combretumracemosum* leaf extract and diminazene aceturate.

Table I:

Effect of *Combretum racemosum* leaf extract on motility of *Trypanosomabrucei*.

Extractconc.	**Minutes**						
Mg/ml	0	30	60	90	120	150	180
125	+ve	-ve	-ve	-ve	-ve	-ve	-ve
65.5	+ve	-ve	-ve	-ve	-ve	-ve	-ve
32.75	+ve	-ve	-ve	-ve	-ve	-ve	-ve
16.375	+ve	-ve	-ve	-ve	-ve	-ve	-ve
8.1875	+ve	+ve	-ve	-ve	-ve	-ve	-ve
4.0937	+ve	+ve	-ve	-ve	-ve	-ve	-ve
2.0467	+ve	+ve	+ve	-ve	-ve	-ve	-ve
1.0234	+ve	+ve	+ve	+ve	-ve	-ve	-ve
0.5117	+ve	+ve	+ve	+ve	+ve	-ve	-ve
0.2559	+ve	+ve	+ve	+ve	+ve	+ve	-ve
0.1280	+ve	+ve	+ve	+ve	+ve	+ve	+ve
0.0640	+ve	+ve	+ve	+ve	+ve	+ve	+ve
0.0 (control)	+ve	+ve	+ve	+ve	+ve	+ve	+ve

-ve = absence of motile trypanosomes. +ve = presence of motile trypanosomes

TABLE II:

Effect of *Combretumracemosum* leaf extract on infectivity of *Trypanosomabrucei* in mice.

S/No	Extract Conc. (mg/ml)	infection/ parasitaemia	Survival of mice
1	125.0	N	S
2	62.5	N	S
3	32.75	N	S
4	16.375	N	S
5	8.1875	N	S
6	4.0937	N	S
7	2.0467	N	S
8	1.0234	N	S
9	0.5117	N	S
10	0.2559	N	S
11	0.1280	P	NS
12	0.0640	P	NS
13	0.0 (control)	P	NS

N = No parasite detected **P** = Parasite seen, **S** = all mice survived the 60 days infectivity observation period. **NS** = none of the mice survived the 60 days infectivity observation period.

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