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Full Length Research Paper

Efficacy of *Dissotis rotundifolia* on *Trypanosoma brucei brucei* infection in rats

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The therapeutic potential of the crude extract of *Dissotis rotundifolia* was investigated in rats infected with *Trypanosoma b. brucei*. Animals were treated orally or intraperitoneally at 200, 600 and 800 mg/kg body weight. At 800 mg/kg, parasitemia was reduced by 66.7 and 78.4% after oral and intraperitoneal administration respectively. *In vitro* exposure of blood forms to high concentration (800 mg/kg) crude extract test resulted in complete paralysis or killing within 45 s of exposure. It is concluded that *D. rotundifolia* may contain antitrypanosomal constituents.

Key words: Efficacy, trypanosoma, parasitemia, *Dissotis rotundifolia*, antitrypanosomal activity.

INTRODUCTION

African trypanosomiasis has continued to threaten human health and economical development (Kuzoe, 1993; WHO, 2000). African trypanosomes cause human sleeping sickness and livestock trypanosomiasis in sub-Saharan Africa. *Trypanosome*, the causative parasite, is most prevalent in Africa and has been responsible to a great extent for the under development, poverty and suffering in many parts of Africa (Holmes, 2000) . The common parasites are *Trypanosoma brucei brucei*, *T. congolense*, *T. brucei gambiense* and *T. brucei rhodesiense*; they are unicellular and transmitted by the bite of tsetse fly as the vector of sleeping sickness in humans and related diseases in animals (Warren, 1988; Kuzoe, 1993).

Up to 80% of the Nigerian land mass is infested by the vector of the parasite, tsetse fly (genus, Glossina). Presently, the disease in cattle has been on the increase due to the menace of the vector, drug resistance and the presence of other haematophagous flies (Holmes, 2000).

In the chemotherapy of African trypanosomiasis, each of the drugs in use has its drawbacks (Kuzoe, 1993; Onyeyili and Egwu, 1995). The search for vaccination against African trypanosomiasis remains elusive and effective treatment is beset with problems of drug resistance and toxicity (Aldhous, 1994; Gutterridge, 1985; Onyeyili and Egwu, 1995). Therefore, there is still an urgent need for the development of new, cheap, safe, and easy-to-administer drugs for the treatment of African trypanosomiasis for both human and animal and drugs of natural origin may be more effective and safer. Moreover, among the indigenes of trypanosome prevalent areas, there are several claims of medicinal plants with possible therapeutic activities, which have not been proved scientifically (Atawodi et al., 2002; Mann et al., 2003; Oliver-Bever, 1986; Onyeyili and Egwu, 1995). However, a number of African medicinal plants were evaluated for their *in vitro* trypanocidal activity (Abegaz et al., 2002; Asres et al., 2001; Atawodi et al., 2003; Freiburghaus et al., 1997; Hoet et al., 2004a, 2004b; 2007; Igweh and Onabanjo, 1989; Kamanzi et al., 2004; Ogbunugafor et al., 2007; Owolabi et al., 1990; Nok et al., 1993; Wosu and Ibe, 1989; Wurochekke and Nok, 2004). Furthermore, several plant extracts or plant derivatives were investigated *in vivo* for the antitrypanosomal efficacies in mice (Asuzu and Chineme, 1990; Asuzu and Ugwuja, 1989; Youan et al., 1997).

Dissotis rotundifolia is a medicinal plant which is widely used in Nupe ethnomedicine for treatment of trypanosomiasis (Mann et al., 2003). Gills (1992) in his collection of medicinal plants of Nigeria reported that the active constituents of *D. rotundifolia* include: insulin, saponin and tannins. It is against this background that the present investigation is conducted to primarily justify the acclaimed effi-

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Table 1. Mean Relative weight of experimental mice infected with *Trypanosoma brucei* and treated with crude extracts of *D. rotundifolia*.

Day-post infection	Dose (mg/kg body wt)	Weight of mice (g)			
		A	B	C	D
0	200	182±2.9a	180±2.4d	173±2.6f	162±2.9h
1	200	182±2.9a	179±2.4c	173±2.6f	162±3.0h
2	600	180±1.8a	178±2.3c	171±2.5f	163±2.9h
3	600	179±2.3b	174±2.5c	168±2.6g	163±3.0h
4	800	187±1.6a	192±2.0e	166±2.7g	164±3.0h
5	800	189±1.5a	196±2.0e	162±3.2g	165±2.9

Mean = SD followed by different letters in a column are significantly different at $p < 0.05$.

cacy of *D. rotundifolia* used in curing human trypanosomal infections among the Nupe tribe in Northern Nigeria.

MATERIAL AND METHODS

Uninfected adult whistar rats were obtained from the National Institute of Trypanosomiasis Research (NITR) Jos, Nigeria, while *T. b. brucei* was obtained from the Department of Biochemistry, University of Ilorin, Kwara State, Nigeria. *D. rotundifolia* were collected from the bushes around Bida, Niger state, Nigeria and were identified at the herbarium section of the biology unit of the Federal Polytechnic Bida, Niger State, Nigeria. All the reagents used were of the BDH grades

Extraction procedure

D. rotundifolia leaves were cut into smaller pieces and dried under the laboratory conditions for 2 weeks and pulverized to powder using a hand blender. The leaf powder was extracted with ethanol by cold maceration for 48 h to obtain the ethanol extract. The extract was concentrated in a rotary evaporator under reduced pressure.

Animal handling and infection

The adult whistar rats of both sexes were allowed to acclimatize for two weeks in the animal house of the Biochemistry unit of the Department of Science Laboratory Technology of the Federal Polytechnic, Bida, Niger State, Nigeria. After acclimatizing, the rats were randomly distributed into four groups. Food and water was given without restriction. Rats in groups A, B and C were infected intraperitoneally with 0.2 ml of blood collected from an infected donor rat. Group D was not infected and served as positive control (non-infected, non-treated).

Treatment and evaluation

The crude extract of *D. rotundifolia* was reconstituted in physiological saline to treat the animals. Groups A and B was dosed with increasing concentration of extract: 200, 600 and 800 mg /kg body weight. For group A and B, treatments were conducted in triplicate per concentration that is using three rats per dosage of the extract (nine rats per group). Rats in group A received oral administration while group B received intraperitoneal administration. Rats in group C (oral) received an equivalent volume of normal saline only and served at negative control (infected, non- treated). The rats were treated 3 times daily while blood parasitemia collected through the tail blood were counted under the light microscope and body weight were determined on daily basis.

Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA) at ($p < 0.05$) confidence level. There is significant increase in weight of the animals in groups A and B after 3 days.

Percentage inhibition of parasitemia was calculated using following formula $Y = 100(X_1 – X_0)/ X_1$, where Y = Percentage inhibition of parasitemia, X_1 = highest blood parasitemia, X_0 = lowest blood parasitemia after commencement of treatment. The % inhibition of parasitemia values were compared using ANOVA at ($p < 0.05$) confidence level.

In vitro test

Blood samples from tails of infected rats were collected in different EDTA bottles. 1 ml of infected blood was sampled on a glass slide and exposed to 0.1 ml of the different crude extract concentrations. This was then replicated per concentration. The slides were observed under the light microscope at 15 s interval and the number of motile parasites was recorded.

RESULTS AND DISCUSSION

Table 1 shows the weight of the rats up to day–5–post infection. Rats in group D (non-infected and non-treated) showed consistent gain in weight, groups A and B showed weight loss up to day 3 and picked up there after.

Group C showed consistent loss in weight. Death among group C was recorded from day 4 and continued till the end of the experiment while no death was recorded in groups A, B and D. This result suggests that the crude extract of *D. rotundifolia* may contain antitrypa-nosomal constituents.

Table 2 shows parasitemia count in blood of treated infected rat at 200 and 600 mg/kg, no reduction in blood parasitemia was observed.

However, at 800 mg/kg body weight, blood parasitemia was reduced from 12.6×10^4 to 4.2×10^4 after oral dosing and from 9.7×10^4 to 2.1×10^4 after intraperitoneal dosing

Table 2. Blood parasitemia from mice infected with T. *brucei* and treated with crude extract of *D. rotundifoli*a at day post infection.

Day-post infection	Dose mg/kg	Parasitemia in blood			
		A	B	C	D
1	-	Nil	Nil	Nil	Nil
2	200	$2.50x10^{4}$	$3.0x10^{4}$	$2.2x10^{4}$	Nil
3	600	$4.20x10^{4}$	$4.0x10^{4}$	$4.0x10^{4}$	Nil
4	600	$9.02x10^{4}$	$7.3x10^{4}$	$6.0x10^{4}$	Nil
5	800	$12.60x10^{4}$	$9.7x10^{4}$	$10.0x10^{4}$	Nil
6	800	$6.30x10^{4}$	$5.2x10^{4}$	$12.0x10^{4}$	Nil
7	800	$4.20x10^{4}$	$2.1x10^{4}$	Death	
% inhibition		66.7	78.4	0	-

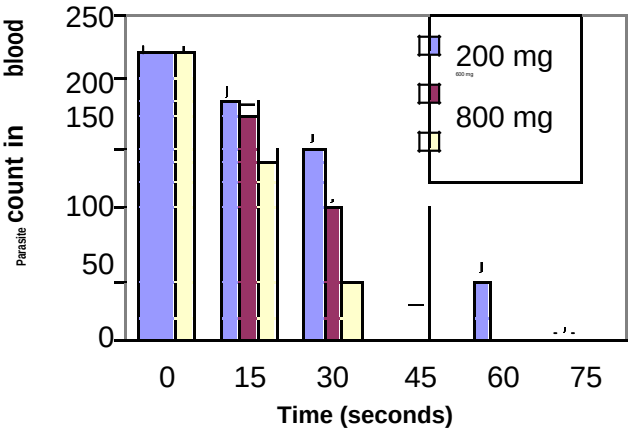

Figure 1. Exposure of blood parasite to crude extract of *D. rotundifolia*.

compared the parasitemia in group C which continued to increase. The present result confirms that the crude extract of *D. rotundifolia* contains active compounds against *T. brucei*. Oral and intraperitoneal administration showed 66.7 and 78.4% inhibition of parasitemia respectively, which can be considered as a positive result. According to Cartier (1973) who reported that 50% deparasitation is an indication of significant activity. The intraperitoneal route is more effective which can be expected because the active constituents may reach the blood stream faster and to a larger extent. The lower activity after oral administration may be due to enzymatic inactivation of active compounds in the gut or reduced absorption from the gut or a combination of both. The crude extract of *D. rotundifolia* could not eliminate blood parasitemia completely even at high concentration of 80 mg/100 g- body weight up to day 7 at which the animals in control group C were all dead.

The present observation suggests a possible treatment failure as observed by Legros et al. (1999). Legros et al. (1999) have shown that treatment failure is possible in cases of massive parasitemia at the time of the therapeutic intervention. It is possible that the early treatments with lower concentrations of the crude extract may have favoured treatment failure. It may be that blood parasitemia could be completely eliminated if the treatment is initiated at high dose of crude extract of *D. rotundifolia* which is illustrated by the *in vitro* results (Figure 1).

The *in vitro* test showed that the crude extract at high concentration (800 mg/kg) was able to paralyze or kill the parasite completely within 45 s.

Further investigations should include purification of the crude extract in order to identify and isolate the active ingredient(s) before recommending the crude extract for treatment and management of trypanosomal infection.

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