

Full Length Research Paper

Antitrypanosomal activity of *Aristolochia ringens* against *Trypanosoma congolense* infection in mice

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The methanolic extract of *Aristolochia ringens* whole plant, commonly used in the traditional treatment of various diseases in humans and animal by some phytotherapist in Nigeria, was evaluated for anti-trypanosomal efficacy in mice infected with *Trypanosoma congolense*. Following three days dose intra-peritoneal administration, the extract produced anti-trypanosomal effect at the three dosage levels of the four tested that is, 433.2, 288.8 and 144.8 mg/kg body weight through the complete suppression or delay in parasite establishment. There was a reduction in the level of parasitaemia as well as enhanced survival of the infected mice, although the plant extract did not significantly ($P < 0.05$) increase the survival period of the mice compared to the negative control (infected untreated). The results suggest that the use of the extracts traditionally has a pharmacological basis.

Key words: *Aristolochia ringens*, *Trypanosoma congolense*, anti-trypanosomal.

INTRODUCTION

Animal trypanosomosis is one the major constraints to livestock production, particularly in the subhumid and to a lesser extent in the wetter parts of the semiarid zone of Africa (Osho, 2005). Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity (Ebana et al., 1991). Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects (Shariff, 2001).

Aristolochia ringens is a plant that belongs to the family Aristolochiaceae (Watson and Dallwitz, 1992). The plant is commonly known in the south-western part of Nigeria as Akogun (Yoruba). It is an aromatic lianes, scramblers, climbing shrub or rhizomes. All species of *Aristolochia* have broad primary medullary rays. The herbal plant was known to contain alkaloids and aristolochic acids,

(Mabberley, 1993). In the present investigation the *in vivo* activity of the medicinal plant (*A. ringens*) which have some medicinal values among local herds men for the treatment of both human and animal diseases is therefore evaluated.

MATERIALS AND METHODS

Drying of samples and extraction of plant specimens

The plant was purchased from the herbal sellers in an Akure market, Ondo state, and was identified in the Department of Forestry and Wood Technology, Federal University of Technology, Akure. The plant was identified by the chief technologist of the forestry and wood technology department in The Federal University of Technology Akure, Dr ogunnika. The whole plant was air dried for two weeks and crushed into smaller particles using mortar and pestle and later pulverized into fine powder in a pulverizing machine (Thomas-Willey milling machine). Thereafter, 100 g of the pulverized sample was measured and soaked in 300 to 500 ml of methanol

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and left for 72 h after which it was filtered into a clean flask using muslin cloth and then filtered through Whatman No 42 (125 mm) filter paper. The extract obtained was then concentrated *in vacuo* using rotary evaporator until the entire methanol was removed. The methanolic extracts preparations were freeze-dried after concentrated and stored in a freezer.

Inoculated parasite into mice

The *Trypanosoma congolense* inoculated was obtained from the National Institute of Trypanosomiasis Research (NITR), Jos, Plateau State, Nigeria. The parasite was multiplied through animal passage.

Experimental animals

Blood was taken from the parasitised animals (the donor animal with parasite level at log 8.1 using "Rapid matching" method of Hebert and Lumsden (1976)). The donated blood was diluted in saline water to give log 6.9 before the mice weighing between 18.7 and 28.3 g were inoculated intraperitoneally.

Experimental design

Thirty mice were weighed and grouped randomly into six experimental units of each unit, with five experimental animals per group. Therapy were given of the plant extract based on calculation in milligram per kilogram of body weight for three days.

Group 1: Infected untreated animals in this group did not receive any drug and served as (negative control).

Group 2: Animals of group 6 were given the standard antitrypanosomal drug that is, Diminal® at 10 mg/kg intraperitoneally at manufacturer recommendation dosage for only one day and this served as the positive control.

Group 3: Infected and treated with 433.22 mg/kg/day.

Group 4: Infected and treated with 288.8 mg/kg/day.

Group 5: Infected and treated with 144.8 mg/kg/day.

Group 6: Infected and treated with 69.7 mg/kg.

These doses were chosen for administration based on the calculated values of 75 to 12.5% of the obtained LD₅₀ for the plant in a separate preliminary toxicity experiment.

Stages of the experiments

In vivo evaluation of antitrypanosomal activity

The inoculated mice were left for two days for the parasite to get established, and after the appearance of parasitaemia in 48 h post inoculation attaining log value of 5.4, treatment commenced with the extracts at different dose levels.

Reconstitution and administration of extract

All extracts were freshly reconstituted in saline solution prepared with distilled water and administered at the various doses. Animals of group 2, 3, 4, 5 and 6 were administered the test extract consecutively for three days on the basis of calculated dose range levels derived from LD₅₀ previously determined by intraperitoneal route. Mice were checked daily during and after the treatment to estimate the number of trypanosomes from blood at their tail in a wet blood film preparation.

Determination of parasitaemia

After 24 h of administration of the extract, a drop of blood from tail of each mouse was taken for examination under microscope for determination of level of parasitaemia as described by Hebert and Lumsden (1976) to check for effect of the extract on the parasite. The absolute number of parasites per millilitre of blood was calculated as log using the rapid matching method for estimating the host's parasitaemia according to Herbert and Lumsden (1976). Briefly, higher levels of infection, parasite levels was measured by matching microscopic fields of a wet blood film against charts and when fewer parasites were present, by counting the number of trypanosomes in 5, 10 or 20 such microscope fields.

Assesement of extract efficacy

For the assessment of the antitrypanosomal activity of the extracts, the level of parasitaemia (expressed as log of the absolute number of parasites per millilitre of blood) in treated animals was compared with that in the untreated control animals.

Statistical analysis

The therapeutic effects were assessed by subjecting the parasitological data of treated and control animals to one way analysis of variance (ANOVA) using statistical package for social sciences (SPSS) version 17 with Duncan multiple range test, and the P-values of < 0.05 considered as significant and those > 0.05 as insignificant.

RESULTS

Effects of therapy of methalonic stem of *A. ringens*

The effect of the efficacy of *A. ringens* on the level of parasitemia in mice infected with *T. congolense* are shown on Table 1. The response on the first day post therapy showed that there was no significant difference ($P > 0.05$) in the effect observed on the mean level of parasitemia in all the extract levels of concentration and both positive and negative control. Visible effects were seen on the second day, where the positive control reduced ($P < 0.05$) the parasites in the mice. There was no significant difference ($P > 0.05$) in the reduction level of parasitemia of all levels of the extract concentration on the same day. However, on the third day post therapy the extract of concentrations of 433.2, 288.8 and 144.8 mg/kg were able to reduce the level of parasitemia and the pronounced effect of the cumulative therapy was seen. On the 4th day, when all grades of extract concentration further reduced, level of parasitemia in the infected mice showed no significant difference ($P > 0.05$) between the positive control and all three higher levels of the concentration of the extract used. A more noticeable effect was seen in the highest level of concentration of extract (433.2 mg/kg) as the cumulative plasma level completely reduced the parasites and appeared; the whole parasites were totally eliminated on the 4th day, however on the 5th day there was a recrudescence of

Table 1. Effect of therapy of methanolic extract of stem bark of *A. ringens* in mice infected with *T. congolense*.

Treatment (mg/kg)	Level of parasitemia post therapy with extract (days)→						
	1	2	3	4	5	6	7
Negative control	5.40±0.00	5.58±0.07 ^b	6.78±0.15 ^c	6.96±0.11 ^b	7.98±0.12 ^c	8.04±0.17 ^c	7.92±0.12 ^c
Positive control	5.40±0.03	3.42±1.40 ^a	1.14±1.14 ^a	0.00±0.01 ^a	0.00±0.01 ^a	0.00±0.01 ^a	0.00±0.01 ^a
433.22	5.40±0.02	5.52±0.07 ^b	2.22±1.36 ^a	0.00±0.01 ^a	1.08±1.08 ^{a b}	1.14±1.14 ^a	1.26±1.26 ^a
288.8	5.40±0.01	5.52±0.07 ^b	3.24±1.32 ^{a b}	1.20±1.20 ^a	2.16 ^b ±1.32 ^a	4.44±1.11 ^b	4.44±1.12 ^b
144.8	5.40±0.01	5.46±0.06 ^b	3.42±1.40 ^{a b}	1.38±1.38 ^a	3.24±1.32 ^b	4.44±1.12 ^b	4.68±1.18 ^b
69.7	5.40±0.01	5.58±0.07 ^b	5.70±0.30 ^{b c}	5.70±0.23 ^b	6.01±0.21 ^c	6.96±0.06 ^c	6.96±0.26 ^{b c}

Values are log Mean ± standard error of mean (SEM) of 5 replicate determinations; Means with different superscript on the same vertical column are significantly different (p<0.05). * Level of parasitemia is expressed as log of the absolute number of parasite per millilitre of blood.

Table 2. Post treatment survival period (days) of *T. congolense* infected mice treated graded doses of methanolic stem bark extract of *A. ringens*.

Extract concentration (mg/kg)	Mean±S.D	Minimum	Maximum	Range
69.70	13.40±1.75	8.00	18.00	10.00
144.80	12.00±0.86	9.00	14.00	5.00
288.80	14.20±2.65	8.00	24.00	16.00
433.22	16.40±2.04	9.00	21.00	12.00
Negative control	11.20±0.86	9.00	14.00	5.00

Mean Values ± standard Error of Mean (SEM) of 5 replicate determinations. Means with different superscripts on the same vertical column are significantly different (p<0.05).

parasitemia in some of the mice. The plant extract did not significantly increase the survival period of the mice compared to the negative control (Table 2). However some were able to stay longer than the negative control group. The maximum days obtained for the doses of 288.80 and 433.22 mg/kg were higher than the negative control.

DISCUSSION

The demonstrable effect of the *A. ringens* on the level of parasitemia in the mice showed evidently from the results that the extract had antitrypanosomal activity *in vivo* at 433.288.8 and 144.8 (the dosage levels tested). Even at its crude status, the exhibited trypanocidal effect on the fourth day is an indication of high antitrypanosomal principle. This effect may be associated with level of some bioactive molecules (alkaloid) revealed as one the component. Hoet et al. (2003) reviewed that the quinoline alkaloids were from *Cinchona* bark (Rubiaceae). The observed action could also be attributed to some other phytochemical components present in the plant probably working in synergy. The relapse observed on day 5 for the highest dose which earlier caused the total clearance of the parasite might be due to reduced level of the extract in the plasma. The plant extract may also possess some valued antitrypanosomal principles which were responsible in prolonging some survival period of infected

mice.

Conclusion

A. ringens demonstrated antitrypanosomal action and requires further elucidation and exploratory investigation.

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