

Full Length Research Paper

## ***In vivo* trypanocidal effect of aqueous root extracts of *securidaca longepedunculata* and its phytochemical analysis**

Haruna, Y.<sup>1\*</sup>, Elinge, C. M.<sup>1</sup>, Peni, I. J.<sup>2</sup>, Dauda, D.<sup>3</sup> and Aiki, F.<sup>3</sup>

<sup>1</sup>Department of Medicinal Chemistry, Kebbi State University of Science and Technology, Aliero.

<sup>2</sup>Department of Animal Science, Kebbi State University of Science and Technology, Aliero.

<sup>3</sup>Department of Animal Health and Production College of Agriculture, Zuru. Kebbi State.

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The aqueous root extracts of *Securidaca longepedunculata* (Fresen, polygalaceae) were used to treat trypanosomiasis in this experiment. 25 Wistar albino rats were inoculated with *Trypanosoma brucei*. Its trypanocidal activity was assessed through daily examination of blood samples, clinical and haematological changes at intervals, and possible deaths were among the parameters which were carefully monitored. The treatment involved a therapeutic dose of diminazene aceturate (3.5 mg/kg), a combination of sub-therapeutic dose of diminazene (1.75 mg/kg) and sub-therapeutic dose of the extract, oral infusion of 200 and 100 mg/kg of the extract, respectively for 7 days. In all rats treated with diminazene and the extract, there was a significant decrease ( $p < 0.05$ ) in parasitemia even though those that received the extract alone relapsed. And there was a significant increase ( $p < 0.05$ ) in haematological values as well. Hence, these findings provide a possible, cheap and available alternative to the existing but costly trypanocides additionally, due to phytochemical data revealed.

**Key words:** *Securidaca longepedunculata*, trypanosomiasis, diminazene aceturate, parasitemia, *Trypanosoma brucei*, Human African trypanosomiasis.

### INTRODUCTION

African trypanosomiasis causes sleeping sickness in people and "nagana" (depressed and low in spirits) in cattle. Two sub-species of trypanosomes infect humans: (1) *Trypanosoma brucei gambiense*, which causes the more chronic form of the disease; and (2) *Trypanosoma brucei rhodesiense*, which is responsible for the more acute form. Accurate statistics for Human African trypanosomiasis (HAT) are not available, but it is estimated that there are currently 300,000 to 500,000 cases with 50,000 deaths annually (Fairlamb, 2003). HAT or sleeping sickness is a major public health problem in 36 Sub-saharan African countries and is caused by *T. b. gambiense* and *T. b. rhodesiense*. About 25,000 new cases

of the disease are being reported annually, and around 50 million people are classed as at risk of contracting the disease.

Until now, the only effective drug for treatment of advanced HAT is the trypanocidal melarsoprol. The mortality rate of melarsoprol treated patients is reported to be 1 to 5% (Bouteille et al., 1998; World Health Organization (WHO), 2007). The search for alternative treatment against African trypanosomiasis remains elusive and effective treatment is beset with problems of drug resistance and toxicity (Onyeyili and Egwu, 1995; Guttridge, 1985; Aldhous, 1994). The four drugs (suramin, pentamidine, melarsoprol and eflornithine) are

\*Corresponding author. E-mail: harufna@yahoo.com.

currently available to treat trypanosomiasis (Kuzoe, 1993), with only melarsoprol and eflornithine being effective against the meningoencephalitis that develops in the late stages of the disease. In addition to emerging cases of drug resistance, all four drugs require lengthy, paraneural administration and all but eflornithine have severe toxic side effects (Onyeyili and Egwu, 1995; Gutteridge, 1985) thus, underscoring the urgent need to develop more effective and safer trypanocidal drugs.

Atawodi et al. (2002, 2003) recently, investigated *in vitro* trypanocidal activity of some plants that are commonly used traditionally to treat trypanosomiasis in Nigeria. One of such plants is *Securidaca longepedunculata* which was reported to have *in vitro* activity against *T. congolens* and *T. brucei* organisms. This *in vitro* claim was however confirmed by Aderbauer et al. (2008). "The root of *S. longepedunculata* Fresen (Polygalaceae) and the extract of *Guiera senegalensis* J. F. Gmel (Combretaceae) were able to reduce parasitemia in mice, experimentally infected with *T. brucei brucei* by 48 and 42% at the dose of 150 mg/kg b.w. intra peritoneal, two times daily for three days". Still confirmed in animals injected with *T. evansi* and *T. brucei*, and also giving an excellent result, were studies carried out by Ameh et al. (2007) and Yusuf et al. (2008), respectively. All the more, further work needs to be done in order to ascertain/establish the efficacy, phytochemistry and possibly fractionate the structures of *Securidaca longepedunculata* plant extract. In addition to these works is presented in this publication, a report on systematic *in vivo* assessment of aqueous root extracts of *S. longepedunculata* trypanocidal activity using *T. brucei* in Wister albino rats.

## MATERIALS AND METHODS

### Plant

*S. longepedunculata* roots were collected from Zuru town, in Kebbi State, North-western Nigeria. The plant was confirmed by a botanist in Usmanu Danfodio University Sokoto, (UDUS), Northern Nigeria, where a voucher specimen was deposited for reference purposes. The roots of *S. longepedunculata* plant were harvested and carefully air-dried at room temperature (to prevent fungal or bacterial growth) in a laboratory to a constant weight. The dried materials were pounded with a pestle and mortar and sieved to fine powder. This work was carried out at the Department of Veterinary Pharmacology and Physiology, UDUS between September and October, 2007.

### Preparation of plant extract

*S. longepedunculata* powder (500 g) was weighed and macerated with 1,500 ml of distilled water and heated to boiling point. The mixture was filtered using Whitman filter paper. The filtrate obtained was further concentrated in an oven (Gallenkamp oven BS size three) at 50°C. The concentrate then was preserved in a refrigerator pending further experiments. The percentage yield was

calculated using this formula:

$$\text{Percentage yield} = \frac{\text{Weight of oven dried crude extract}}{\text{Weight of powdered extract}} \times 100$$

### Experimental animals

Wister albino rats of both sexes were purchased for this research work and housed in metallic cages in groups of fives, and were fed with animal feed and water. They were clinically examined and confirmed to be free of trypanosomes and other micro protozoa organisms.

### Trypanosome stock

The *T. brucei* organisms (Basa strains) used for this studies were obtained from an experimentally infected rat previously inoculated with the parasite from the Department of Biochemistry, Ahmadu Bello University (ABU), Zaria, Northern Nigeria. The organisms were maintained by sub-passaging into healthy wister albino rats every 5 to 7 days through intra peritoneal injection of 0.2 ml/kg blood solution made in phosphate buffered solution (PBS) to contain approximately  $10^6$  to  $10^7$  infected red cells (David et al., 2004; Peter and Anatoli, 1998). Parasitemia was confirmed in the infected rats after 48 h of infection with *T. brucei*. All the experimental rats were inoculated through intra peritoneal (i.p) routes. The parasitemia was checked daily with an electronic microscope (model no.0602279) of 400 magnification using wet blot film method from the blood collected through the tails of the animals.

### Acute toxicity test

A limit dose of 3000 mg/kg b.w. of *S. longepedunculata* extract (SLE) was used. Animals were dosed one at a time and observed at least once during the first 30 min after dosing, periodically during the first 4 h and thereafter for a total of 14 days. At the expiration of the initial 48 h, four additional animals were sequentially dosed and observed just as described earlier. This is in accordance with Organisation for Economic Co-operation and Development (OECD) guidelines 425 (2000) and interagency research animal committee (IRAC) (2004) recommendations.

### Administration of the plant extract

A standard protocol was drawn up in accordance with the Good Laboratory Practice (GLP) regulations of the World Health Organization (WHO Document, 1998). Thirty healthy albino rats were randomly selected for this study and divided into 6 groups of 5 rats each, and treated as follows after confirming parasitemia:

1. Group A: Infected with *T. brucei* and treated with diminazene aceturate once 3.5 mg/kg b.w. therapeutic dose (i.p.)
2. Group B: Infected with *T. brucei* and treated with sub-therapeutic dose of diminazene aceturate (1.75 mg/kg b.w.) once and 100 mg/kg b.w. of SLE once daily for 7 days orally.
3. Group C: Infected with *T. brucei* and treated with 200 mg/kg b.w. of SLE once daily for 7 days orally.
3. Group D: Infected with *T. brucei* also treated with 100 mg/kg b.w. of the root extract (SLE) once daily for 7 days orally.
4. Group E: Is a negative control group that is infected with *T. brucei* but no treatment.

5. Group F: Is a positive control group, not infected and no treatment.

#### Phytochemical screening of the experimental plant

The phytochemical tests that were carried out included; qualitative screening to identify saponins, (including saponin glycosides), volatile oils, triterpenoids, steroids, alkaloids, tannins, glycosides, flavonoids and anthraquinones in the test material using extract residue. Also, the quantitative test to estimate the quantity of saponins, alkaloids and volatile oils in a known weight of the powdered form of the test material using standard procedures as described by Trease and Evans (1989), El-Olemmy et al. (1994) and Harbone (1993) were carried out.

#### Assessment of therapeutic activity

The criteria used in the assessment of the trypanocidal effect of the various agents included the examination of blood specimens daily for degree of parasites, clinical changes at daily intervals following treatment, possible death, and also haematological changes. In this case, blood was collected from the heart by cardiac puncture of the animals using ethyldiaminetetraacetic acid (EDTA) as an anticoagulant. Packed cell volume (PCV), red blood cell count (RBC), white blood cell (WBC) and haemoglobin (Hb) count was measured in all cases at the pre-inoculation stage, at the peak of parasitemia and at post treatment stage. PCV was determined by microhaematocrit method. WBC and RBC were done using the improved Neubauer haemocytometer, respectively (Ajagbonna and Adebayo, 2002).

#### Statistical analysis

Results are presented as mean  $\pm$  standard deviation (SD) and test of significance between the mean parameters is done using analysis of variance (ANOVA), and significance is considered as  $p < 0.05$  (google.com).

## RESULTS

#### Trypanocidal efficacy

In response to both the therapeutic and sub therapeutic doses to the drugs, test shows that parasitemia decreased significantly ( $p < 0.05$ ) after one to two days in rats (groups A and B) treated with therapeutic dose of diminazene (3.5 mg/kg) alone as well as in combination regimen of SLE (100 and 1.75 mg/kg of diminazene, no trypanosomes were detected in the blood samples of these rats (groups A and B). Interestingly, from the second day post treatment, the animals remained parasite free for the next forty days of observation. The result also shows that in all animals treated with *securidaca* alone (200 and 100 mg/kg) groups C and D parasitemia decreased gradually until the 7th day of post treatment observation when no parasites were detectable in their blood samples.

Microscopy after the second free day however, revealed

trypanosomes in the blood of the first two of the rats and then later on the others. Thereafter, parasitemia re-established in these rats and by the 27th day all rats in groups C and D were dead, apparently from re-emergent parasitemia. There was no significant decrease ( $p < 0.01$ ) in the response to treatment with either 200 or 100 mg/kg of SLE but relapse parasitemia set faster in group D than in group C.

#### Toxicity results

No death records or any sign of toxic effect were observed in the rats given 3000 mg/kg of SLE and this means that the median lethal dose ( $LD_{50}$ ) for the oral administration of the extract was therefore greater 3000 mg/kg. Table 2 shows that PCV (%) and mean WBC count ( $10^3/mm^3$ ) increased from  $31.0 \pm 0.6$  and  $6.3 \pm 0.22$  at parasitemia to  $40.0 \pm 0.30$  and  $10.7 \pm 0.1$ , respectively in animals treated with the combination of diminazene and SLE. The RBC and Hb count followed the same patterns of improvement.

#### Phytochemical screening

Table 3 revealed some chemical constituents of the plant including alkaloids (0.30), volatile oils (1.25), flavonoids (0.34), while terpenoids, tannins and steroids were in lesser amounts.

## DISCUSSION

The clinical signs of pale mucous membrane, anorexia, weakness and emaciation in this study are characteristics and typical of trypanosomiasis in animals. These findings are in agreement with Ezeokonkwo and Agu (2003, 2004) and Anene (2006). The results of this work show that all the fractions exhibited mild to moderate trypanocidal activity *in vivo* but did not clear the parasitaemia completely. Furthermore, all the fractions of *S. longepedunculata* dose dependently showed no significant changes in the liver parameters (Table 1) but enhanced a quicker recovery from haematological depression caused by parasitaemia (Table 3), which is in agreement with the findings of Ameh et al. (2007), Ajagbonna et al. (2005) and Asuzu and Chineme (1990). That the result shows development of parasitemia within 4 to 5 days (Table 1) is not surprising since earlier reports (Anene et al., 2006., Ameh et al., 2007; Ajagbonna et al., 2005; Onyeyili and Onwualu, 1999) are in agreement with it.

The treatment in all groups commenced on day 4 (at peak parasitemia) and a corresponding significant decrease in parasitemia was observed ( $p < 0.05$ ). The parasites totally disappeared from the blood stream two

**Table 1.** Parasitemia per day of observation in different groups of rats with *Trypanosoma brucei* infection.

Treatment	1	2	3	4	5	6	7	8	9	10
Groups (rats)	13	15	17	19	21	25	30	35	40	45
A	0/5 0/5	1/5 0/5	3/5 0/5	5/5 0/5	5/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5
B	0/5 0/5	3/5 0/5	4/5 0/5	5/5 0/5	5/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5
C	0/5 0/5	3/5 3/5	4/5 4/5	5/5 5/5	5/5 5/5	5/5 5/5	3/5 3/3	2/5 2/2	1/5 1/1	1/5 0
D	0/5 0/5	2/5 1/5	4/5 2/5	5/5 4/5	5/5 5/5	5/5 5/5	3/5 5/5	3/5 5/5	2/5 0	1/5 0
E	0/5 2/2	3/5 1/1	4/5 0	5/5 0	5/5 0	5/5 0	5/5 0	4/4 0	4/4 0	3/3 0
F	0/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5	0/5 0/5

0/5 = No parasitemia. 0 = Dead rats. SLE = *Securidaca longepedunculata* extract.

**Table 2.** Comparison of changes in the haematological indices in the different treatment groups of rats with *Trypanosoma brucei*.

Haematological indices	A	B	C	D	E	F
PCV (%)	*39.0±0.2	*40.0±0.3	36.3±0.2	35.0±0.3	*31.0±0.6	46.8±1
RBC (10 <sup>6</sup> /mm <sup>3</sup> )	*5.10±0.1	*5.9±0.1	4.8±0.1	4.8±0.1	*4.1±1.5	6.7±0.0
WBC (10 <sup>3</sup> /mm <sup>3</sup> )	*9.9±0.3	*10.7±0.1	8.6±0.1	8.0±0.4	*6.3±0.22	12.1±0.6
Hb (gm/100ml)	*15.8±0.3	*16.4±0.2	13.9±0.1	13.7±0.3	*10.9±0.1	18.8±0.8

Results are presented as mean ± SD, \*P<0.01. \* = Significant haematological recovery (p< 0.05) when compared to control E and F.

**Table 3.** Phytochemical results.

Constituent	Percentage yield
Triterpenoids	+++
Steroids	++
Volatile oils	(1.25)
Alkaloids	+++ (0.30)
Tannins	+++
Glycosides	-
Flavonoids	++ (0.34)

++ = Constituents in moderate concentrations, +++ = Constituents in high concentrations.

days post treatment in groups A and B, and six days post treatment in groups C and D. This treatment regimen result is in agreement with Ameh et al. (2007), Ajagbonna et al. (2005) and Asuzu and Chineme (1990). Meanwhile, there was progressive increase in parasitemia in the infected control up to 11 days post inoculation when they eventually died (Table 1). It is noteworthy that all groups treated with extract alone (C and D) developed relapse parasitemia two days after clearance and eventually died after days 25 and 30, respectively. This could be associated to the crude nature of the plant material, thus responsible for the low content of the active ingredient (Barakat et al., 2013).

Table 2 presents that combination therapy offered the best result in terms of enhancing a quicker recovery from haematological depression caused by parasitemia. The PCV recovery was 40.0 ± 0.3 from 31.0 ± 0.6, the RBC

decrease in parasitemia was observed (p < 0.05). The parasites totally disappeared from the blood stream two

also recovered as  $5.9 \pm 0.1$  from  $4.1 \pm 1.5$ , as well as WBC  $10.7 \pm 0.1$  from  $6.3 \pm 0.22$  and Hb  $16.4 \pm 0.2$  from  $10.9 \pm 0.1$ , respectively, in addition to the good result of its treatment regimen (Table 1). This is also in agreement with Sammy et al. (2013), Ameh et al. (2007), Ajagbonna et al. (2005) and Asuzu and Chineme (1990).

In the present study, *S. longepedunculata* a recently discovered plant in the treatment of trypanosomiasis has been shown to possess an *in vivo* trypanosomal activity. Other plants have also been reported to possess *in vivo* activities against *T. brucei* (Asuzu and Chineme, 1990; Nok et al., 1993). Although, the mechanism for the *in vivo* anti-trypanosomal activity observed is not known, it is suggestive that since the phytochemical results of SLE indicate the presence of volatile oils, alkaloids, flavonoids, terpenoids and steroids; these substances may be attributed to the trypanocidal activity observed in this study (El-olemy et al., 1994; Steenkamp et al., 2013). Thus, this plant promises to be a readily available, affordable and effective alternative trypanocide. However, more work is required to establish the structures of the active compound in *S. longepedunculata* so as to ascertain if this compound is the same as other trypanocidal compounds found in other members of the genus.

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