SAFETY ASSESSMENT, *IN-VIVO* ANTI-TRPANOSOMAL ACTIVITY OF METHANOL ROOT EXTRACT OF *SECURIDACA LONGEPEDUNCULATA* IN MICE INFECTED WITH *TRYPANOSOMA BRUCEI BRUCEI*

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Abstract

Securidaca longepedunculata is a savannah shrub commonly used by traditional medicine practitioners in Nigeria; the plant is reputed to have over one hundred medicinal indications. The study aims at assessing the safety of the plant which is 2.8 mg/kg, and its trypanocidal activity using Swiss albino mice of both sexes: The animals were randomly selected and divided weight dependently into groups of 5 mice each, consisting of three methanol extracts groups of 5%, 10%, and 20% of the extract's LD_{50} which is equivalent to (0.14, 0.28, & 0.56 mg/kg) respectively, and also a standard control drug (diminazene aceturate 3.5 mg/kg), infected and not treated group and no infection no treatment group. Except the no infection no treatment group, all other groups were infected with T. brucei. Invariably, each animal received inoculums of about 1.0 x 10^7 parasites per gramme body weight through needle passage and produced parasitaemia in the mice. On commencement of the medications the methanol root extract of S. longepedunculata was given to the three groups in divided doses for seven days and the diminazene aceturate was given at a therapeutic dose of 3.5 mg/kg just once. All the drugs were given through intra-peritoneal routes after confirming parasitaemia.

Keywords: *Drug, mice, Securidaca longepedunculata, Parasitaemia, Treatment,*

Introduction

Human African trypanosomiasis or sleeping sickness is a widespread tropical disease in 36 sub-Saharan African countries that can be fatal if not treated. It is spread by the bite of an infected tsetse fly (*Glossina Genus*) and caused by *Trypanosoma brucei gambiense* and *T. b. rhodesiense*. It threatens over 60 Million lives on daily basis (Steverding *et al*, 2005). In advanced stages, the disease attacks the central nervous

system, causing changes in personality, alteration of the biological clock (the circadian rhythm), confusion, slurred speech, seizures, and difficulty in walking and talking. These problems can develop over many years in the *Gambiense* form and some months in the *Rhodesiense* form; if not treated, the person will die. Until now, the current chemotherapy of human trypanosomiasis relies on six drugs; Suramin,Pentamidine, Melarsoprol, Eflorinithine, Arsobal and Mel B. Five of which were developed more than30 years ago (Steverding *etal*, 2005). These drugs display undesirable toxic side effects and drug resistance. (Steverding *etal*, 2005 and Perez-Morga, 2007). And the mortality rate of melarsoprol treated patients is 1-5% (Bouteille *et al*, 1995 and W.H.O, 2007). These problems together with the associated expensive nature of most drugs in developing nations with poor economic status make it pertinent to search for new, better and cheaper trypanocides.

However, it has also been observed that natural products derived from plants offer possibilities to obtain new drugs that are active against trypanosomes (Hoet *et al*, 2004). Many investigators targeted finding new anti-trypansomal agents to combat the trypanosomiasis by screening indigenous plants from Africa (Atawodi *et al.* 2002., Adewumi *et al,* 2001). Nigeria is naturally endowed with both Savannah and Tropical rainforest vegetations. These divers flora offer a wide spectrum of unique medicinal plants. In Nigeria, a variety of plants are being exploited for effective curing of various ailments (Burkill, 1985); and several ethnobotanical studies of indigenous plants used in the management of trypanosomiasis indicated both significant *in-vitro* / in-*vivo* antitrypanosomal activity (Abubakar *et al*,2005 and Atawodi *et al* 2002). Notably among these plants studied within the Nigerian biosphere, are extracts of *Securidaca longepedunculata*, *Annona senegalensis*, *Allium sativum*, *Aformosia laxiflora*, *Anogeissus leiocarpus*, *Khaya senegalensis* etc distinctively exhibited the trypanocidal activity (Atawodi *et al*, 2002). Furthermore, it is reported that almost all parts of *S. longepedunculata* (leaves, twigs, stem, bark, root and seeds) are used by man for different purposes; the root and the bark are taken orally either powdered or as infusion for treating chest complaints, inflammation, abortion, ritual suicide, tuberculosis, infertility, venereal diseases and for constipation. Tooth ache can also be relieved by chewing the roots. Powdered roots are used to treat head ache by rubbing them on the fore head. Infusions of the roots are used for washing topical ulcers. In Limpopo the Venda people take the roots for mental disorders and against children's illness during breast feeding. They also mix the powdered roots with maize and sorghum beverages for men being sexually weak. In Zimbabwe, the roots are given to people who are believed to be possessed by evil spirits. The root extracts are also used for menstrual pains and gonorrhoea in Nigeria (Akiniyi and Sultanbawa, 1986). Powdered with salt and water they are used against snake bites and cough. In few cases, metabolites responsible for the associated activity have been isolated, and potent bioactive compounds have been reported (Mann *et al*, 2004). The claims of the medicinal application of the root of this plant are numerous. The aim of this study was to establish the median lethal dose and then investigate the *in-vivo* anti-trypanosomal activity of *S. longepedunculata* methanolic crude extracts on *T.b. brucei*, using laboratory animal species which data may be extrapolated to human species.

Materials and Methods:

Plant Material

The roots of *S. longepedunculata* were collected from Zuru, Kebbi State, Northern Nigeria. The plant was authenticated by a taxonomist at the herbarium section of Biological Sciences Department, Ahmadu Bello University Zaria, Nigeria. Where a voucher specimen was deposited and it was given a voucher number: 900149. The roots were thoroughly washed with water, air dried and pounded into powder using pestle and mortar.

Animals and Animal husbandry

Healthy adult Swiss albino mice of both sexes weighing approximately ±21.20g were used for this study; the animals were obtained from the animal house of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. They were housed in cages at the same Departmental animal house, fed on standard rats feed (vital feeds, Jos, Nigeria) and allowed access to water *ad libitum*. The animals were allowed to acclimatize to the laboratory conditions for at least 14 days before being subjected to the experiments. All the experiments were carried out in a Conducive laboratory setting that has ambient illumination and a temperature that is close to that of the animal house, according to OECD guidelines.

Test Organisms

Trypanosoma brucei brucei organisms were obtained from National Institute of Trypanosomiasis and Onchocerciasis Research (NITR) No. 1, Sulame Road, Unguwan rimi, Kaduna, Kaduna State, Nigeria. They were maintained in the animal house of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zairia, Kaduna State Nigeria by continuous passage into donor rats intraperitoneally.

Extract preparation

The methanol crude extracts of the roots of *S. longepedunculata* was obtained by Soxhlet extraction using methanol as a solvent for 48 hours. The extract was concentrated to dryness on a water bath between 40-45°C. The extracts were stored in sealed bottles at room temperature.

Determination of the Median lethal dose of Methanol crude extract of *S. longepedunculata* (LD_{50}) in mice.

The method of Locke (1983) was modified and used to determine the dose of the extract that would be lethal to 50% of the population of the animals. Three dose points were (10, 100 & 1000 mg/kg) chosen for the pilot experiment, from

which doses of 1, 2, 4 & 8 mg/kg respectively were given to one animal per group in the second phase. The square root of the geometric mean of the highest non-lethal dose and the lowest lethal dose was used to calculate the $LD_{50.}$

Estimation of trypanosome parasites in the blood

The parasites in the blood were estimated according to the ''rapid matching'' method of Herbert and Lumsden (1976); as described by Atawodi *et al* (2003 & 2005). The method involves a matching technique in which microscopic fields were compared with a range of standard logarithm values. Counting of parasites per field in pure blood or blood approximately diluted with buffered phosphate saline (PBS PH 7.2) a drop of blood was obtained on a slide by pinching the tip of the pre-sterilized tail with a sterile needle, immediately covered with a cover slip and the wet mount observed under the microscope at X40 magnification. The number of trypanosomes per microscopic field was compared with the table of logarithmic values. The logarithmic values which matched the microscopic observation were then converted to antilogarithm, from where the absolute number of trypanosomes per ml of blood was obtained.

In-vivo* Trypanocidal Activity of Methanol root Extracts of *Securidaca longepedunculata* on mice infected with *T. brucei brucei.

Experimental Procedure:

A standard protocol was drawn up in accordance with the Good Laboratory Practice (GLP) regulations of the World Health Organisation (WHO Document, 1998).

Thirty healthy Swiss albino mice of both sexes were randomly selected for this study, and divided weight dependently into groups of 5 mice each, consisting of three methanol extracts groups of 5%, 10%, and 20% of the extract's LD_{50} which is equivalent to (0.14, 0.28, & 0.56 mg/kg) respectively, and also a standard control drug (diminazene aceturate 3.5 mg/kg), infected and not treated group and no infection no treatment group. Except the no infection no treatment group, all other groups were infected with *T. brucei.* Invariably, each animal received inoculums of about 1.0×10^7 parasites per gramme body weight through needle passage and produced parasitaemia in the mice. On commencement of the medications (i.e. at peak parasitaemia) the methanol root extract of *S. longepedunculata* was given to the three groups in divided doses as shown below for seven days and the diminazene aceturate was given to the standard group at a therapeutic dose of 3.5 mg/kg just once, while the infected not treated and the no infection no treatment received no treatment, all

the drugs were given through intra-peritoneal routes after confirming parasitaemia:

GROUP 1: Is a negative control infected with *T. brucei* but no treatment.

GROUP 2: Is a positive control group, not infected and no treatment.

GROUP 3: Infected with *T. brucei* and treated with diminazene aceturate 3.5 mg/kg once (therapeutic dose) (i.p).

GROUP 4: Infected with *T. brucei* and treated with 5% of extracts LD_{50} 0.14 mg/kg of methanol root extract of (SLE) daily for 7 days.(i.p)

GROUP 5: Infected with *T. brucei* and treated with 10% of extracts LD_{50} 0.28 mg/kg of (SLE) methanol extract daily for 7 days.(i.p)

GROUP 6: Infected with *T. brucei* and treated with 20% of extracts LD_{50} 0.56 mg/kg of (SEL) methanol extract daily for 7days (i.p.)

Statistical analysis

Data were analysed using Analysis of variance (ANOVA) and student t-test using SPSS-computer package. In all cases the level of statistical significance was considered at ($P< 0.05$).

Results

Microscopic examination of this work showed that peak parasitaemia was reached in 5 to 6 days post inoculation in (table 1). Medications in all the infected groups commenced on the 6^{th} day post inoculation (peak parasitaemia), similarly microscopic examination revealed a steady increase of parasitaemia in all animals of the negative control group (infected with *T.bruce* but not treated) until all the animals died on day 14 post inoculation due to parasitaemia (Table 1). After administration of diminazene aceturate (intraperitoneally) microscopic examination showed that parasites in the group were completely cleared and the mice remained alive throughout the period of the preliminary studies.

Effect of extract administration on lifespan in trypanosome-infected mice

The work showed a steady decrease in parasitaemia in the plant treated groups dose dependently (0.14, 0.28, 0.56 mg/kg) respectively. It also showed that the extract treated animals survived for 25 days before they died. Though, relapse parasitaemia occurred, but results showed that on daily administration of the extract of *S. longepedunculata* by day 8 post administration there was a statistically significant reduction ($P<0.05$) in parasitaemia load due to the effect of the extract to as low as 3.58 ± 0.18, 5.68 ± 0.19, & 7.72 ± 0.10 of 1.78, 0.89 & 0.45 mg/kg dose respectively when compared to the negative control group with 35.06 ± 0.50 parasite value of the same day 8 post medication (Table 1).

Packed cell volume and parasitaemia in animals administered methanol root extracts of *S. longepedunculata*

Packed cell volume (PCV) and anaemia are critical issues in the pathogenesis of African trypanosomiasis contributing to morbidity and mortality thus curtailing the absence longevity (Jennings *et al* 1977). Infected mice given *S. longepedunculata* root extracts in this study showed significantly ($P<0.05$) higher level in PCV recovery compared to the infected not treated groups given normal saline only (tables 1 &2).

As the parasitaemia increased with time there was also a corresponding drop in % PCV levels. This suggests that haemoglobin concentration decreases with increase severity of infection. However, the extract treated group helped in recovering the PCV and Hb levels dose dependently (table 3).

Discussion

The present study demonstrates that *trypanosoma brucei brucei* when inoculated into mice develop parasitaemia within three days and reach peak parasitaemia within 5-6 days. This is in agreement with the reports of Ajagbonna *et al* (2005)., Anene *et al*, (2006) and Ameh *et al*, (2006).The observed *in-vivo* anti-trypanosomal activity of the methanol root extract of *Securidaca longepedunculata* is not suprising since previous reports (Nok *et al*, 1993.,Asuzu *et al*, 1990., and Owolabi *et al*, 1990) have clearly demonstrated that plants of different families could possesses anti-trypanosomal activity and vice versa due to the biotransformation of plant materials that may convert active therapeutic molecules to in active ones. The study indicates that there is significant trypanocidal activity of *S. longepedunculata* root extracts as shown by the steady clearance of parasitaemia by the plant extract (Tables/Figures 1), which also supports the studies of Atawodi (*et al*, 2003) and Ameh *et al* (2006). This work also agrees with Adebaubaurer *et al* (2008), who reported that the roots of *Securidaca logepedunculata* Fresen (Polygalaceae) were able to reduce parasitaemia in mice experimentally infected with *trypanosoma brucei brucei* by 42 and 48% at the dose of 150 mg/kg body weight intraperitoneally two times daily for three days etc. Table 1 and 2 gives the parasitaemia pattern among three groups treated with the extracts dose dependently, five days post treatment, a statistically significant ($P<0.05$) dose dependent parasite suppression was observed, while the diminazine-treated mice were cleared of their parasites 24 hours post treatment. The relapse seen immediately on withdrawal of treatment in the extract treated group after the parasites were statistically significantly reduced ($P<0.05$) could be as a result of resistance put forward by the parasites or some limitations on the part of the extract itself such as the inaccessibility of the extract to other tissues where the flagellates are known to hide as a way of evading trypanolytic action of drugs (Anosa,

1998). Therefore, this plant provides a readily available cost effective alternative trypanocide. However, further investigation may explore more of the plant's usefulness to humanity.

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Figure 1: *In Vivo* Anti-trypanosomal effect of Methanol Root Extract of *S.longepeunculata* in Mice Infected with *T.brucei*

Table 1: Median Packed Cell Volume (%)

Phase I

Group	Dose (mg/kg)
Normal Saline	
1	
2	
3	

Phase II

Group	
1	2
2	4
3	8
4	16

Extracts were administered through Intra-Peritoneal route.

LD_{50} = 2.8 mg/kg

PCV Values in Mice

infected with *T. brucei* and Treated with Methanol Root Extracts of *S. longepedunculata*

Infection	Treatment Groups	D0	D4
+	Normal saline	57.40 ± 1.66	48.60 ±0.75
+	SLE(0.14 mg/kg)	52. 80 ± 0.86	52.00 ±1.60
+	SLE (0.28 mg/kg)	55.00 ± 1.84	54.00 ±1.70
+	SLE (0.56 mg/kg)	57.00 ± 0.84	55.60 ±1.17
+	Arthemeter (1.6 mg/kg)	51.60 ± 1.21	48.4 ± 2.46
+	Chloroquine (10 mg/kg)	52.80 ± 1.88	48.4 ± 0.93

+ = Infection; SLE= *Seccuridaca longepedunculata* extract; N = 5 animals per group; D = Days post infection