PHARMACOLOGICAL PROPERTIES AND TOXICOLOGY OF *SECURIDACA LONGIPEDUNCULATA* FRESEN (POLYGALACEAE)

***HARUNA, Y [1]., C.M. ELINGE [2]**

[1,2] Department of Pure and Applied Chemistry, Kebbi State University of Science and Technology, P.M.B. 1144, Aliero, Birnin Kebbi, Nigeria.

Abstract

Some medicinal plants are potential sources of new drugs to improve in the treatment of trypanosomiasis, malaria, etc whose treatment is still a challenge. This study established the LD_{50} for both aqueous and methanol roots extracts using oral and intra-peritoneal routes respectively, investigated the anti-malarial and the anti-trypanosomal effects of the plant in mice. Swiss albino mice (19-23 g) of both sexes were used to perform a four day suppressive standard test and employing chloroquine sensitive P. berghei NK 65 strain. For anti-trypanosomal screening, thirty healthy Swiss albino mice of both sexes were randomly selected and divided weight dependently into groups of 5 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 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 and produced parasitaemia. 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 to the standard group at a therapeutic dose of 3.5 mg/kg once.

Keywords: *medicinal, Mice, Parasitaemia, S. longepedunculata, Treatment.*

Introduction

Securidaca longipedunculata Fresen (Polygalaceae) is one of the most popular of all traditional medicinal plants in South Africa and is used for almost every conceivable ailment (Dapar *et al*., 2007). It is a multi-purpose plant with a long history of use in African traditional medicine to treat various sexually transmitted infections, hernias, coughs, fever, ascariasis, constipation, headaches, rheumatism, stomach ache, malaria, tuberculosis, pain, epilepsy, pneumonia, skin infections, and it is also used as an aphrodisiac for men (Mongalo *et al*., 2015). It is reported that almost all parts of *S*. *longepedunculata* (leaves, twigs, stem, bark, root and seeds) are used by man for different purposes such as medicine (Hutchings, 1996). The plant is employed in traditional medicine principally for its psychotropic

properties various rheumatic and inflammatory diseases, antihelminthic and purgative agent (Belmain *et al*., 2001; Neuwinger, 1996). The use of the plant against snakebites, fish poisoning have been documented (Burkill, 1997; Neuwinger, 1996; Olajide *et al*., 1998). It is also used in bacterial and malarial chemotherapy (Belmain *et al*., 2001; Atawodi *et al*., 2003).

Trypanosomiasis being a critical disease of both man and domestic animals has been an obstacle to livestock industry and economic development of Africa. Intervention by chemotherapeutic agents is fast failing and vector control is impossible. However, potent efficacious ethno-medicinal plants have been identified and are freely available. There is therefore, a need for the development and use of new drugs to overcome this disease(s) for mankind in Africa.

MATERIALS AND METHOD

Plant Collection

The root bark of *Securidaca longepedunculata* was collected from Zuru, in Kebbi State, North-Western Nigeria. The identity of the whole plant was confirmed by Mr U.S. Gallah, a taxonomist of the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria; where a voucher specimen number 900149 was deposited for future reference purposes.

Preparation of Plant Extract

The root of *S. longepedunculata* plant was air-dried, pounded with a mortar and pestle and sieved. Two hundred grams (200g) of the root powder of *S. longepedunculata* was weighed and macerated into 500 ml of distilled water in an extraction bottle with the bottom packed with cotton wool with a clipped cap. The macerated material was left for 72 hours with intermittent shaking, after which the tap was opened, and the filtrate was collected into a beaker. The mixture was finally filtered into a 500 ml conical flask using a funnel and Whitman filter paper no.125 mm. The filtrate obtained was concentrated in an oven at a temperature between 40^{o}C and 45^{o}C. The concentrate was preserved in Bijou bottles in a refrigerator until needed for experimental use.

Trypanosome and Plasmodium Stocks

T. brucei was obtained from Nigerian Institute for Trypanosomiasis Research and Onchocerciasis (NITR), Kaduna, Nigeria. The organisms were maintained by serial passages in rats. All the experimental animals were inoculated through intraperitoneal (i.p.) route with 0.2 ml of blood solution made in normal saline that contains approximately 1.0 X 10^{6}-10^{7} infected red cells. The parasitaemia was checked daily by microscopic method at X 40 magnification using wet blot film, and with the blood collected from the tail veins as described by Peter and Anatoli (1998) and David *et al*., (2004).

Plasmodium berghei NK 65 chloroquine sensitive strain obtained from the Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria was used for this study. The *P. berghei* was subsequently maintained in the laboratory by serial blood passage from mouse to mouse. A donor mouse with a rising parasitemia of 20% was sacrificed and its blood was collected (in a slightly heparinized syringe) and diluted with trisodium citrate (TC) medium so that each 0.2 ml contained approximately 1 X 10^7 infected red blood cells (Peter and Antoli, 1998; and David *et al.,* 2004). Each animal received inoculums of about 1 X 10^7 parasites per gram body weight through needle passage and it produced infection in the mice.

Experimental Animals

Swiss albino mice (19-23 g) of both sexes were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria were used for this study. The animals were housed in metallic cages. The animals were examined and confirmed to be free of trypanosomes and plasmodia organisms. They were fed with standard rodent feed (Vital Feeds, Jos, Nigeria), and allowed access to tap water *ad libitum.*The animals were allowed to acclimatize to the laboratory conditions for at least 3 days before the experiments. All 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.

Acute toxicity study

Lorke's method (1983) was used for the LD_{50} determination. Three animals were given widely differing doses for each of the test groups in phase one as; 10 mg/kg, 100 mg/kg, 1,000 mg/kg respectively and a control group was given normal saline according to the weight of the animal. The animals were observed for toxic signs, and for further 24 hours, the number(s) of death(s) were recorded. The doses of second phase were selected based on the outcome of the first phase and observed further closely for yet another 24 hours too. The number(s) of death(s) or none in the groups were used to calculate the $LD_{50.}$

***In-vivo* Anti-malarial Studies**

Tests were performed using a four day suppressive standard test (WHO, 1980; Peter and Anatoli, 1998 and David *et al.*, 2004); and employing chloroquine sensitive *P. berghei* NK 65 strain.

Thin smears of blood films were obtained from the tail end of each mouse on day 4 after infection and treatments (WHO, 1980 and David *et al*., 2004). The smears were placed on microscopic slides, fixed with methanol and stained with 10% Gemsa at pH 7.2 for 15

minutes, and examined under the microscope at X 100 magnification to assess the level of parasitemia.

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

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:

Estimation of Parasitemia in Mice Infected with *T. brucei*

Complete elimination of motility or reduction in motility of parasites when compared to the control group was taken as an index of trypanocidal activity. This study revealed that *T. brucei brucei* organisms manifest in the blood of mice between 3 to 4 days post inoculation and reached peak parasitaemia between 5 to 6 days post inoculation. Parasitemia following trypanosome infection with *T. brucei*, was estimated according to the rapid 'matching' method of Herbert and Lumsden (1976) as described by Atawodi *et al.,* (2003) and Atawodi (2005).

Statistical Analysis

Results were presented as mean ± SEM. Tests of significance between means were done using Student t-test and ANOVA.

Results and Discussions

In vivo Trypanocidal Effects Methanol Root Extracts of *Securidaca longepedunculata* in Mice

Table 2 revealed a steady increase of parasitaemia in all the animals of the negative control group (infected with *T. brucei brucei*) the animals died between Day 13 and Day 15. Diminazene aceturate 3.5 mg/kg cleared the parasites completely on Day 1 post treatment and the animals survived throughout the study period. The extract demonstrated a significant reduction ($P<0.05$) parasites in Day 6 post administration. This was as low as 6.48 ± 0.51, 4.86 ± 0.15, 3.20 ± 0.13 at doses of 0.14, 0.28, and 0.56mg/kg respectively against 24.64 ± 0.66 of the infected but not treated group on Day 13 post treatment (Table 2). They also survived up to Day 20 and above.

In Vivo Anti-Malarial Investigation of Methanol Root Extract of *Securidaca longepedunculata* in Mice Infected with *Plasmodiun berghei*

The infected normal saline group showed high parasitaemia when compared to 10 mg/kg chloroquine; 1.6 mg/kg artemether and various doses of the extract (0.14, 0.28, 0.56 mg/kg) groups see Table 3. The results revealed that chloroquine, arthemeter as well as the extract gave very good anti-malarial effect (suppression) that was statistically significant ($p<0.05$) when compared to the normal saline group. Table 3.

This study investigated the anti-trypanosomal, anti-malarial and toxicological effects of the plant in mice. Generally, the data presented in this study, provided evidence that the root bark of *S. longepedunculata* may contain biologically active principles with potential values in the treatment of Trypanosomiasis and Malaria. The study also provided data confirming the safety of this medicinal plant. The acute toxicity study indicated that the crude methanol root extract of *S. longepedunculata* is toxic when administered to mice by intraperitoneal routes but relatively safe when administered orally. However, the intraperitoneal LD_{50} of the aqueous and methanol extracts is low suggesting that the extracts are toxic when administered intraperitoneally.

References

Atawodi, S.E; Bulus, T; Ibrahim, S; Ameh, D.A; Nok, A.J; Mamman, M. and Galadima, M. (2003). *In vitro* trypanocidal effect of methanol extract of Some Nigerian savannah plants. *African Journal of Biotechnology*, 2: 317-321.

Atawodi, S.E. (2005) Antioxidant potential of African medicinal plants. *African Journal of Biotechnology.* 4(2):128-133.

Herbert, W.J. and Lumsden, W.H.R. (1976). *Trypanosoma brucei*: A rapid ''matching'' method for estimating the host's parasitemia. *Experimental Parasitology*, 40:427-431.

Belmain, S.R. and Steveson, P.C. (2001). Ethnobotanicals in Ghana, reviving and modernizing age old farmer practice. *Pesticide out look,* 12: 233-235.

Burkil, H.M. (1997). The useful plants of west tropical Africa*, Royal Botanical Gardens Families*, U.K, 4: 969.

Dapar, L.P.M; Aguiyi, C.J; Wannang, N.N; Gyang, S.S. and Tanko, M.N. (2007). The histopathologic effects of *Securidaca longepedunculata* on heart, liver, kidney and lungs of rats. *African Journal of Biotechnology* 6 (5): 591-595.

David, A.F; Philip, J.R; Simon, L.C; Reto, B. and Solomon N. (2004). Anti-malarial Drug Discovery: Efficacy models for compound screening, *Nature Reviews.* 3: 509-520.

Lorke, D. (1983). A new approach to practical acute toxicity. *Achieves of Toxicology,* 54:275-287

Mongalo, N.I., McGaw, L.J., Finnie, J.F. and Staden, J.V. (2015). Journal of Ethnopharmacology Volume 164,

Neuwinger, H.D. (1996). African ethno-botany poisons and drugs, chemistry, pharmacology, toxicology. *Pharmaceutical Biology*, Chapman & hall, New York. Newsholme, c-w, 941.

Olajide, O.A; Awe, S.O. and Makinde, J.M. (1998). Pharmacological screening of the root extract of *Securidaca longepedunculata*. *Fitoterapia*, 69(3): 245-248.

Peter, L.T. and Anatoli, V.K. (1998). The current global malaria situation. *In*: *Malaria parasite Biology*, *pathogenesis and protection*. ASM Press. Washington D.C. pp. 11-22.

WHO (1980). The biology of malaria parasites: report of a WHO scientific group WHO *Technical Report series*: 2.

Table 1: Median Lethal Doses of Extracts of *S. longepedunculata* in Mice using Oral and Intraperitoneal Routes (mg/kg)

	Oral	Intraperitoneal
Aqueous	1,131.4	19.3
Methanol	565.7	2.8

Table 2: Effects of Methanol Root Extracts of S*ecuridaca longepedunculata* in Mice Infected with *Trypanosoma brucei brucei*

		Average Percentage Parasitemia								
Infection	Treatment Groups	D4	D5	D6	D7	D8	D9	D10	D11	D12
–	No treatment	0	0	0	0	0	0	0	0	0
+	Normal saline	2.00±0.32	4.66±0.43	7.34±0.50	9.86±0.11	11.52±0.32	13.45±0.41	15.08±0.36	15.72±0.47	19.72±0.49
+	D. A. (3.5mg/kg)	2.18±0.27	4.28±0.32	7.26±0.42	0	0	0	0	0	0
+	SLE (0.14mg/kg)	1.84±0.29	4.10±0.17	6.68±0.28	7.92±0.30	8.60±0.30	8.24± 0.22*	7.50±0.30*	7.06± 0.33*	6.84±0.51*
+	SLE (0.28mg/kg)	2.12±0.22	3.84±0.30	7.04±0.52	8.84±0.48	9.10±0.31	8.40± 0.26*	6.96±0.12*	6.04± 0.12*	5.02±0.07*
+	SLE (0.56mg/kg)	2.50±0.24	3.90±0.17	7.24±0.33	8.34±0.38	8.40±0.20	7.12± 0.36*	5.40±0.23*	4.60±0.26*	3.70±0.29*

....Continued

		Average Percentage Parasitemia								
Infection	Treatment Groups	D13	D14	D15	D16	D17	D18	D20	D25	D30
–	No treatment	0	0	0	0	0	0	0	0	0
+	Normal saline	24.64±0.66	35.06±0.50	D	D	D	D	D	D	D
+	D. A. (3.5mg/kg)	0	0	0	0	0	0	0	0	0
+	SLE (0.14mg/kg)	6.84 ± 0.43*	7.72 ± 0.10*	8.68±0.19	9.86±012	11.97±0.28	15.80±0.41	27.10± 0.60	D	D

+	SLE (0.28mg/kg)	4.86 ±0.15*	5.68 ± 0.19*	6.76±0.32	7.74 ±0.36	9.40 ± 0.24	12.32±0.57	30.46± 1.45	D	D	
+	SLE (0.56mg/kg)	3.20 ± 0.13*	3.58 ± 0.18*	4.18±0.22	4.64 ±0.28	6.08 ± 0.24	8.96±0.35	20.04± 1.20	26.9±1.77	31.34±1.78	

- = No infection; + = Infection; Dx = Days post infection; D. A. = Diminazine aceturate; SLE = *Securidaca longepedunculata* extract; Drug administration started on Day 6; 0 = No parasite; D = Death of infected mice; *= Significant parasitaemia reduction ($P<0.05$) compared to the control group; See Appendix 2 for corresponding graph; Statistical tool used ANOVA

Table 3: Parasitaemia Suppressive Test of Methanol Root Extract of *S. longepedunculata* against *P. berghei* in Mice after 4 Days

Infection	Treatment Groups	Percent Parasitaemia	Percent suppression
+	Normal saline	16.1 ± 3.00	-
+	SLE (0.14 mg/kg)	6.7 ± 0.34*	58.4
+	SLE (0.28 mg/kg)	6.0 ± 0.57*	62.7
+	SLE (0.56 mg/kg)	2.8 ± 0.80*	82.6
+	Chloroquine (10.00 mg/kg)	0.9 ± 0.40*	93.8
+	Artemether (1.60 mg/kg)	2.5 ± 0.60*	84.4

+ = Infection; SLE *=S. longepedunculata* extract*;* *Data are means ± SEM; N = 5 animals per group