IN VIVO ANTISCHISTOSOMAL AND TOXICOLOGICAL EVALUATION OF *SIDA PILOSA* RETZ ON MICE BALB/c

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Summary

The objective of this study was to evaluate the antischistosomal activity of the aqueous extract of *Sida pilosa* Retz (Malvaceae) on *Schistosoma mansoni*-infected mice and its toxicity profile. Sixty days post infection, animals were treated orally with the extract at 40, 80 and 160 mg/kg/day, for fourteen consecutive days. Unique administration of doses ranging from 4 to 20g/kg were used to assess acute toxicity while doses of 400, 800 and 1600 mg/kg/day for 8 weeks were used for sub-acute toxicity. One week after treatment, comparisons of body, liver and spleen weights, egg load and worm recovery were carried out between untreated and treated groups. Treatment with *S. pilosa* aqueous extract resulted in a significant reduction in egg-laying of at least 82% and in worms recovering of at least 88%. A significant reduction of hepatomegaly and splenomegaly ($p < 0.001$) were also seen in all treated-animals. The LD_{50} of *S. pilosa* aqueous extract was greater than 20g/kg and neither haematological nor biochemical parameters were modified after administration of the extract for eight weeks. These results indicate that *S. pilosa* aqueous extract exhibits antischitosomal activity in *S.mansoni*-infected mice and is toxicological safe.

Keywords: *Sida pilosa*, *Schistosoma mansoni*, egg load, worm load, toxicity.

Introduction

Despite considerable progress in pharmacology, epidemiology and clinical research, schistosomiasis remains a major public health concern in the tropics and subtropics. This disease remains endemic in 74 countries and infects more than 200 million people; of these 20 million suffer severe consequences from the disease.

In Cameroon, the number of infested people was estimated at 1.7 million in 2000 (1). Efficient chemotherapy is found to cure schistosomiasis and Praziquantel is now the drug of choice for the treatment of the disease caused by the three main species of the parasite. However, some cases of re-infection due to the relative resistance of larval stages of *Schistosoma mansoni* to schistosomicide drugs have been reported to occur after treatment (2, 3). The new trends nowadays are the use of natural plant extracts as new safe and effective drugs. In this regard, the role of plants extract has been recently investigated. Among others, Artemether, a methoxy derivate of the antimalarial agent quighaosu from *Artemisia annua* L. (4), *Citrus reticulata* roots (5), Myrrh from *Commiphora molmol* stem (6) and *Clerodendrum umbellatum* leaves (7) exhibited antibilharzial properties.

Sida pilosa Retz. (Malvaceae), locally known as "Obolsi", "Otounde" or "Ivangnoe" is a creeping plant founded mainly on the outskirts of dwelling areas and on wastelands. It is used in Cameroon for the treatment of intestinal helminthiasis and lower abdominal pains. For the treatment of intestinal helminthiasis, it is recommended by traditional healers to squeeze the whole plant in water and to take as often as possible until healed (8). The purpose of this study was to examine whether the aqueous extract of *Sida pilosa* whole plant have any activity against *Schistosoma mansoni* infection in mice BALB/c and to evaluate it's acute and sub acute toxicity profile.

Material and methods

Plant material and preparation of the extract

The whole plant of *Sida pilosa* was collected in March 2005 from Leboudi 2, near Yaoundé (Centre Province, Cameroon). The authenticity of the plant was confirmed against the specimen Lejoly n° 86/399 in the National Herbarium of Yaoundé, Cameroon, were a voucher specimen is conserved under the number 53202/HNC. Specimens were dried in the shade, powdered and 200g of the powder mixed with 2 litres of water for 24 hours of maceration. The solution was filtered and evaporated at 45°C to obtain 37.11g of the aqueous extract of *Sida pilosa*, with a recovery rate of 18.55%.

Phytochemical screening

The presence of alkaloids, terpenoids, tannins, flavonoids, phenols, saponins, cardiac glycosides and steroids in *S. pilosa* aqueous extract was detected by the qualitative method described by Trease and Evans (9).

Animals

Adult BALB/c mice, weighing 23 - 38 g were used. Male were used for the antischistosomal study and male and female for the toxicological evaluation. They were housed in a standard environmental conditions and feed with rodents' diet and water *ad libitum*. The experimental protocol was conducted in accordance with International Guidelines for Laboratory Animal Use and Care and was approved by the Institutional Animal Ethics Committee.

Antischistosomal activity

Male mice were individually infected by the tail and legs immersion technique by exposing them to 50 *S. mansoni* cercariae (Cameroonian strain) from naturally infected *Biomphalaria pfeifferi*. Sixty days post infection; infected mice were randomly divided into five groups of 5 to 6 animals each. Animals of experimental groups were treated orally with *S. pilosa* aqueous extract at 40, 80 or 160 mg/kg/day, for fourteen consecutive days. The positive control received 100mg/kg/day of praziquantel and the negative control, as well as the control group of non-infected animals received distilled water during the same period of treatment.

Animals were weighed once a week from the beginning of the treatment until the day of sacrifice. The day before sacrifice, faecal sample of each mouse was collected and eggs enumerated by the Kato Katz technique. Seven days after the end of the treatment, mice were killed by a lethal dose of sodium pentobarbital and worms recovered by perfusion as described by Duwall and Dewitt (10). Liver and spleen were removed from each mouse and weighed. After perfusion, a liver specimen was digested in 4% KOH for 4h at 37°C and the number of eggs/g of liver evaluated by the method of Cheever and Anderson (11).

Toxicological study

The evaluation of the toxicity profile of *S. pilosa* aqueous extract was performed according to the protocol described by W.H.O (12). For oral acute toxicity, mice were randomly divided into six groups of ten animals each (5 males and 5 females), consisting of one control group receiving distilled water and five groups receiving a unique dose of 4, 8, 12, 16 or 20 g/kg of *S. pilosa* aqueous extract. Mortality was evaluated in each group for 48h and the LD_{50} was then determined. Others symptoms of toxicity such as asthenia, hypoactivity (motor activity), anorexia, diarrhoea and syncope were also monitored. Survival animals were kept under observation for seven days.

For sub acute oral toxicity, mice were divided into four groups of ten animals each (5 males and 5 females). The control group received distilled water while doses of 400, 800 or 1600 mg/kg of *S. pilosa* aqueous extract were administered once a day, for 8 weeks to animals of the three experimental groups. Animals were weighed once a week while liver and kidney were weighed the day of sacrifice. The blood collected by decapitation at the end of the treatment was used for haematological and biochemical analyses. Haematological parameters including white blood cell (WBC) count , lymphocytes (LYM), red blood cell (RBC) count, hematocrit (HTC), haemoglobin (HGB) and platelet (PTL) count were determined using an Automated Haematology System (Hospitex Diagnostics, Hema - Screen 18, Firenze, Italy). Serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, total protein, and total cholesterol were determined by colorimetric methods. Liver and kidneys sections were obtained and stained with haematoxylin-eosin.

Statistical analysis

Results were analysed by one-way ANOVA with Tukey's post test performed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA. Values are expressed as the mean ± SEM and difference between groups were considered significant when $p < 0.05$.

Results

Phytochemical screening

Phytochemical screening of *S. pilosa* aqueous extract revealed the presence of alkaloids, phenols, tannins and terpenoids.

Body, liver and spleen weights

Schistosoma mansoni infection of BALB/c mice did not modified the body weight, but induced a significant increase of liver and spleen weights ($p < 0.001$) as shown in table 1. Treatment of *S. mansoni*-infected mice with 40, 80 or 160mg/kg/day for 14 days of *S. pilosa* aqueous extract resulted in a remarkable reduction of hepatomegaly and splenomegaly in all the treated groups ($p < 0.001$). The same result was obtained in the group treated with 100mg/kg/day for 14 days of praziquantel. At the end of the treatment, the liver and spleen weights of infected - treated animals with *S. pilosa* aqueous extract were comparable to the ones of non infected animals as proved by the non significant statistical difference between those groups.

Table 1. Effect of the administration of *Sida pilosa* aqueous extract on body and organs weights of *Schistosoma mansoni* - infected mice

Treatment	Dose (mg/kg)	Body weight (g)	Organs weights (g/100g of body weight)	
			Liver	Spleen
Control	0	30.38 ± 0.73	4.76 ± 0.01	0.52 ± 0.06
NC	0	28.84 ± 1.80	9.13 ± 0.29 [a]	1.05 ± 0.04 [a]
PZQ	100	29.53 ± 1.75	7.08 ± 0.14 [***, a]	0.56 ± 0.03 [**,b]
Sida pilosa	40	25.68 ± 1.02	5.07 ± 0.41 [***,b]	0.71 ± 0.05 [*,b]
	80	28.77 ± 0.94	6.02 ± 0.19 [***,b]	0.53 ± 0.10 [***,b]
	160	28.84 ± 1.78	5.96 ± 0.36 [***,b]	0.55 ± 0.06 [***,b]

Results are expressed as mean ± SEM, NC: Negative Control, PZQ: praziquantel
[*], [**], [***] Significantly different at $p < 0.05$, $p < 0.01$, $p < 0.001$ from infected animals (Negative Control)
[a] Significantly different at $p < 0.001$ from non infected animals (Control)
[b] Non significantly different from non infected animals (Control)

Egg and worm load

The egg and worm burden obtained with experimentally infected mice with *S. mansoni*, treated with 40, 80 or 160 mg/kg/day of *S. pilosa* aqueous extract for 14 days can be seen in table 2.

Table 2. Effect of the administration of *Sida pilosa* aqueous extract on egg and worm load in *Schistosoma mansoni*-infected mice

Treatment	Dose (mg/kg)	Egg count in the liver (eggs/g)	Egg count in the faeces (eggs/g)	Reduction rate of oviposition (%)	Total worm count	Reduction rate of worms (%)
NC	0	5902 ± 1252	331.20 ± 97.90		31.60± 13.90	
PZQ	100	4603 ± 942	4.80 ± 4.80 [***]	98.55 %	0.60 ± 0.40 [*]	98.10 %
Sida	40	2534 ± 1120	36.00 ± 12.00 [***]	89.13 %	1.17 ± 0.54 [*]	96.30 %
pilosa	80	3350 ± 685	28.00 ± 14.42 [***]	91.54 %	3.17± 1.68 [*]	89.97 %
	160	4500 ± 1394	57.60 ± 12.24 [**]	82.61 %	3.60 ± 1.94 [*]	88.61 %

Results are expressed as mean ± SEM, NC: Negative Control, PZQ: praziquantel,
[*], [**], [***] Significantly different at $p < 0.05$, $p < 0.01$ and $p < 0.001$ from infected animals (Negative Control)

Important reduction of the number of eggs in the faeces significantly occurred in the group of mice treated with 40 mg/kg by 89.13 %, in the group treated with 80mg/kg by 91.54 % and in the group treated with 160 mg/kg by 82.61 % ($p < 0.001$). In the praziquantel-treated animals, a 98.55 % of reduction of egg count could be observed. At the end of the treatment, eggs in the liver of mice in all experimental groups were counted, but did not present significant difference between treated groups and negative control group. The reduction of egg load follows the one of worm burden. In fact, treatment of *S. mansoni*-infected mice with 40, 80 or 160mg/kg/day of *S. pilosa* aqueous extract for 14 days induced 96.30, 89.97 and 88.61 % of worms' mortality respectively ($p < 0.05$). When praziquantel was administered, the mortality of the parasites was 98.10 %.

Acute and sub acute toxicity

Acute oral administration of *S. pilosa* aqueous extract to male and female mice at 4, 8, 12, 16 and 20 g/kg did not altered the growth, the food and the water intakes of animals. They did not exhibit any sign of toxicity such as asthenia, hypoactivity, anorexia and diarrhoea. Up to 20 g/kg, no mortality has been recorded. The LD_{50} of *S. pilosa* aqueous extract is therefore greater than 20 g/kg.

After a repetitive administration of 400, 800 or 1600 mg/kg of *S. pilosa* aqueous extract to male and female mice for 8 weeks, neither change in body, liver and kidney weights nor mortality could be observed. Haematological results after daily administration of *S. pilosa* aqueous extract to mice for 8 weeks are recorded in table 3. The levels of white blood cell, lymphocytes, red blood cell, hematocrit, haemoglobin and platelet count in all treated mice did not change significantly when compared to the control group.

Table 3. Haematological parameters of mice after daily administration of *Sida pilosa* aqueous extract for 8 weeks.

Parameters	Control	*Sida pilosa* (mg/kg)		
	0	400	800	1600
WBC (x 10^3/µL)	9.76 ± 1.40	12.29 ± 1.30	10.03 ± 0.91	14.33 ± 2.11
LYM (%)	56.23 ± 4.83	67.01 ± 3.59	57.20 ± 3.76	63.24 ± 7.24
RBC (x 10^6/µL)	9.65 ± 1.37	8.29 ± 0.30	7.81 ± 0.55	7.67 ± 1.08
HTC (%)	42.14 ± 5.87	35.61 ± 1.30	36.15 ± 1.48	33.84 ± 3.76
HGB (g/dL)	16.71 ± 2.51	12.97 ± 0.54	13.73 ± 0.66	12.42 ± 1.91
PLT (x 10^3/µL)	458.9 ± 67.84	431.00 ± 51.15	454.70 ± 31.22	307.00 ± 42.92

Results are expressed as mean ± SEM

Serum activities of total protein, cholesterol, creatinine, alanine aminotransferase and aspartate aminotransferase were not altered by repetitive administration of *S. pilosa* aqueous extract as shown on table 4.

Table 4. Biochemical parameters of mice after daily administration of *Sida pilosa* aqueous extract for 8 weeks.

	Control group	*Sida pilosa* (mg/kg)		
	0	400	800	1600
Total protein (g/dL)	4.16 ± 0.27	4.13 ± 0.31	4.37 ± 0.46	4.50 ± 0.15
Cholesterol (mg/dL)	72.86 ± 8.79	62.04 ± 3.34	82.39 ± 7.51	70.95 ± 9.26
Creatinine (mg/L)	0.51 ± 0.10	0.30 ± 0.02	0.32 ± 0.05	0.37 ± 0.03
ALT (UI/L)	22.90 ± 0.93	21.38 ± 1.27	22.38 ± 0.38	21.00 ± 0.67
AST (UI/L)	26.61 ± 0.51	26.91 ± 0.30	27.10 ± 0.28	27.13 ± 0.23

Results are expressed as mean ± SEM

Histopathological sections revealed slight inflammation in the liver and tubular clarification in the kidney when mice were treated with 1600 mg/kg of *S. pilosa* aqueous extract.

Discussion

Treatment of *S. mansoni*-infected mice with different doses of *S. pilosa* aqueous extract resulted in a significant decrease of liver and spleen weights. This result is in accordance with the one reported by Lescano *et al*. (4) after treatment of *S. mansoni*-infected mice with artemether. The major pathology of schistosomiasis is granulomatous inflammation, a cellular immune response to antigens secreted by schistosomes' ova trapped in organs as lung, liver and spleen. The reduction of hepatomegaly and splenomegaly after treatment might suggest a possible anti-inflammatory role of *S. pilosa* aqueous extract. In fact, *Sida cordifolia* and *Sida rhomboidea* have been reported to exhibit anti-inflammatory properties (13, 14). In this study, the number of eggs released in the faeces as well as the number of worms recovered; considerably decrease after treatment with *S. pilosa* aqueous extract.

The antischistosomal property of medicinal plants extracts has been reported in the literature. Some of these extracts such as *Ambrosia maritima* leaves (15), *Zingiber officinale* ethyl acetate extract (16) and *Jatropha curcas* leaves methanolic extract (17) exhibited negligible effect *in vivo* against *S. mansoni*. Egg load reduction and mortality of worms as recorded in this study, were also reported after administration of *Balanites aegyptiaca* fruit mesocarp (18), *Citrus reticulata* root phenolic extract (5), *Cleome droserifolia* ethanolic extract (19) and *Curcuma longa* oil extract (20) to *S. mansoni*-infected mice. Our findings constitute evidence that *S. pilosa* aqueous extract possess antischistosomal activity. Chemicals compounds present in this extract could be responsible for its bioactivity. In fact, phytochemical screening revealed the presence of classes of chemical compounds such as alkaloids, phenols, tannins and terpenoids. Karou *et al*. (21, 22) have demonstrated that the indoloquinoline alkaloids extracted from *Sida acuta* are responsible for its activity against *Plasmodium falciparum* and Gram-positive bacteria. Moreover, analgesic and anti-inflammatory activities of alkaloids and flavones from *Sida cordifolia* have been reported (23, 24). It has been shown that the sesquiterpenes lactones isolated from *Vernonia amygdalina* are the chemical constituents responsible for its activity against *S. japonicum* (25). The antischistosomal activity exhibited by *S. pilosa* aqueous extract could therefore be related to its alkaloids, phenolic compounds and terpenoids contents.

To assess the safety of *S. pilosa* aqueous extract, a toxicological study was conducted. Its LD_{50} was greater than 20 g/kg. According to Delongeas *et al*. (26), substances with LD_{50} greater than 5000mg/kg are low toxic. This is an indication of *S. pilosa* aqueous extract low acute toxicity when administered orally. Others species of the genus *Sida* have been shown to possess low acute toxicity (14, 27). Haematological and biochemical evaluations carried out after repetitive administration of *S. pilosa* aqueous extract, showed normal levels of the parameters evaluated. This indicates that the extract did not interfere with blood constituents, since the normal range of haematological parameters can be altered by the ingestion of some toxic plants (28). The lack of modification of biochemical parameters indicates that *S. pilosa* aqueous extract might interfere neither with the general metabolism nor with the renal and hepatic functions. Normal values of haematological and biochemical parameters were also reported after chronic administration of *Sida rhombifolia* roots aqueous extract to rats (27). Slight modifications observed in the liver and kidney parenchyma were not important enough to altered liver and renal functions.

These results could sustain the ethnomedical use of *S. pilosa* aqueous extract to treat intestinal helminthiasis. Phytochemical study of this extract in the aim to isolate bioactive compounds is currently in progress.

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References

1- Brooker S, Donnely CA, Guyatt HL. Estimating the number of helminthic infections in the Republic of Cameroon from data on infection prevalence in schoolchildren. Bull WHO 2000; 78: 1456-1465.

2- Ismail MM, Farghaly AM, Dyab AK, Afify HA, El-Shafei MA. Resistance to praziquantel, effect of drug pressure and stability test. J Egypt Soc Parasitol 2002; 32: 589-600.

3- Silva LM, Menezes RMC, Andrade de Oliviera S, Andrade ZA. Chemotherapeutic effects on larval stages of *Schistosoma mansoni* during infection and re-infection of mice. Rev Soc Bras Med Trop 2003; 36: 335-341.

4- Lescano SZ, Chieffi PP, Canhassi RR, Boulos M, Neto VA. Antischistosomal activity of artemether in experimental schistosomiasis mansoni. Rev Saude Publica 2004 ; 38: 71-75.
5- Hamed MA, Hetta MH. Efficacy of *Citrus reticulata* and Mirazid in treatment of *Schistosoma mansoni*. Mem Inst Oswaldo Cruz 2005; 100: 771-778.
6- Barakat R, Elmorshedy H, Fenwick A. Efficacy of Myrrh in the treatment of human schistosomiasis mansoni. Am J Trop Med Hyg 2005; 73: 365-367.
7- Jatsa HB, Ngo Sock ET, Tchuem Tchuente LA, Kamtchouing P. Evaluation of the *in vivo* activity of different concentrations of *Clerodendrum umbellatum* Poir. against *Schistosoma mansoni* infection in mice. Afr J Trad CAM 2009; 6: 216-221.
8- Adjanohoun JE, Aboubakar N, Dramane K et al. Traditional medicine and pharmacopoeia: contribution to ethnobotanical and floristic studies in Cameroon. CSTR/OUA, CNPMS, Porto-Novo, 1996.
9- Trease GE, Evans WC. A Textbook of Pharmacognosy (13th edn). Bailliere Tindall Ltd: London, 1989.
10- Duwall RH, Dewitt WB. An improved perfusion technique for recovering adult schistosomes from laboratory animals. Am J Parasitol 1967; 7: 293-297.
11- Cheever AW, Anderson LA. Rate of destruction of *Schistosoma mansoni* eggs in tissues of mice. Am J Trop Med Hyg 1971; 20: 62-68.
12- W.H.O. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. Geneva, 2000.
13- Venkatesh S, Reddy YS, Suresh B, Reddy B.M, Ramesh M. Antinociceptive and anti-inflammatory activity of *Sida rhomboidea* leaves. J Ethnopharmacol 1999; 67: 229-232.
14- Franzotti EM, Santos CV, Rodrigues HM, Mourao RH, Andrade MR, Antoniolli AR. Anti-inflammatory, analgesic activity and acute toxicity of *Sida cordifolia* L. (Malvabranca). J Ethnopharmacol 2000; 72: 273-277.
15- Abadome F, Geerts S, Kumar V. Evaluation of the activity of *Ambrosia maritima* L. against *Schistosoma mansoni* infection in mice. J Ethnopharmacol 1994; 44: 195-198.
16- Sanderson L, Bartlett A, Whitfield PJ. *In vitro* and *in vivo* studies on the bioactivity of a ginger (*Zingiber officinale*) extract towards adult schistosomes and their egg production. J Helminthol 2002; 76: 241-247.
17- Adamu SU, Kela SL, Suleiman MM. Antischistosomal properties of extracts of *Jatropha curcas* L. on *Schistosoma mansoni* infection in mice. Afr J Trad CAM 2006 ; 3: 37-41.
18- Koko WS, Abdalla HS, Galal M, Khalid HS. Evaluation of oral therapy on mansonial schistosomiasis using single dose of *Balanites aegyptiaca* fruits and praziquantel. Fitoterapia 2005; 76: 30-34.
19- El-Shenawy NS, Soliman M.F, Abdel-Nabi IM. Does *Cleome droserifolia* have anti-schistosomiasis mansoni activity? Rev Inst Med Trop S Paulo 2006; 48: 223-228.
20- El-Ansary AK, Ahmed SA, Aly SA. Antischistosomal and liver protective effects of *Curcuma longa* extract in *Schistosoma mansoni* infected mice. Indian J Exp Biol 2007; 45: 791-801.
21- Karou D, Dicko MH, Sanon S, Simpore J, Traore AS. Antimalarial activity of *Sida acuta* Burm. f. (malvaceae) and *Pterocarpus erinaceus* Poir. (fabaceae). J Ethnopharmacol 2003; 89: 291-294.
22- Karou D, Savadogo A, Canini A, et al. Antibacterial activity of alkaloids from *Sida acuta*. Afr J Biotech 2005; 4: 1452-1457
23- Sutradhar RK, Rahman AM, Ahmad M, Bachar SC, Saha A, Guha SK. Bioactive alkaloid from *Sida cordifolia* Linn with analgesic and anti-inflammatory activities. Iranian J Pharmacol Therap 2006; 5: 175-178.
24- Sutradhar RK, Rahman AKM, Ahmad MU, Bachar SC. Bioactive flavones of *Sida cordifolia*. Phytochemistry Letters 2008; 1: 179-182.

25- Jisaka M, Kawanaka M, Sugiyama H, et al. Antischistosomal activities of sesquiterpenes lactones and steroid glucosides from *Vernonia amygdalina*, possibly used by wild chimpanzees against parasite-related diseases. Biosc Biotech Bioch 1992; 56: 845-846.
26- Delongeas JL, Bunnel D, Netter P, et al. Toxicité et pharmacocinétique de l'oxychlorure de zirconium chez la souris et chez le rat. J Pharmacol 1983 ; 14 : 49-55.
27- Sireeratawong S, Lertprasertsuke N, Srisawat U, et al. Acute and subchronic toxicity study of the water extract from root of *Sida rhombifolia* Linn. in rats. Songklanakarin J Sci Technol 2008; 30: 729-737.
28- Ajagbonna OP, Onifade KI, Suleiman U. Hematological and biochemical changes in rats given extract of *Calotropis procera*. Sokoto J Vet Sci 1999; 1: 3-12.