



Research Paper

Afr. J. Traditional,
Complementary and
Alternative Medicines
www.africanethnomedicines.net

ISSN 0189-6016©2009

EVALUATION OF THE *IN VIVO* ACTIVITY OF DIFFERENT CONCENTRATIONS OF *CLERODENDRUM UMBELLATUM* POIR AGAINST *SCHISTOSOMA MANSONI* INFECTION IN MICE

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Abstract

Clerodendrum umbellatum Poir (Verbenaceae) is traditionally used in Cameroon for the treatment of many diseases including intestinal helminthiasis. This study was undertaken to assess the *in vivo* antischistosomal activity of its leaves aqueous extract on a *Schistosoma mansoni* mice model and to determine the most effective dose of this extract. Mice showing a patent infection of *S. mansoni* were daily treated with *C. umbellatum* leaves aqueous extract at the doses of 40, 80 or 160 mg/kg body weight for 14 days. Seven days after administration of the extract, schistosomicidal activity was evaluated on the liver and spleen weights, faecal eggs releasing, liver egg count and worm burden. Treatment using *C. umbellatum* leaves aqueous extract resulted in an important reduction in faecal egg output by 75.49 % and 85.14 % for 80 mg/kg and 160 mg/kg of the extract respectively. These reduction rates did not differ significantly from the 100 % obtained in the group of infected mice treated with 100 mg/kg of praziquantel. *C. umbellatum* leaves aqueous extract was lethal to *S. mansoni* worm. A 100 % reduction rate was recorded in the group of infected mice treated with 160 mg/kg of the extract, as well as in praziquantel-treated mice. An amelioration of the hepatosplenomegaly was noticed in both the extract-treated mice and the praziquantel-treated mice. From these results, we can conclude that *C. umbellatum* leaves aqueous extract demonstrated schistosomicidal properties in *S. mansoni* model at doses of at least 80 mg/kg body weight.

Key words: *Clerodendrum umbellatum*, *Schistosoma mansoni*, faecal egg output, worm burden, mice.

Introduction

It is estimated that about 200 million people worldwide are currently affected by schistosomiasis, a disease caused by flatworms belonging to the genus *Schistosoma*. The disease is usually chronic and debilitating, with severe consequences on the urinary tract where *S. haematobium* is the organism involved and major damage to the intestinal tract where *S. mansoni*, *S. intercalatum* or *S. japonicum* is involved. For schistosomiasis, vaccine is nonexistent and drugs remain the mainstay of disease control. However, the current drug index is limited and/or inadequate, and the problem is being further exacerbated by the emergence of drug resistance (Ismail et al., 2002; Silva et al., 2003). This raises a need for complementary and alternative drugs that are both effective and safe. *Clerodendrum umbellatum* Poir (syn.: *Clerodendrum scandens* P. Beauv), family Verbenaceae, is a woody climbing shrub widely used in traditional medicine in Cameroon (Cheek et al., 2004),

for the treatment of several ailments including epilepsy, headache, intestinal helminthiasis, irregular menstruation, infective dermatitis, asthma, metaphysical powers, whitlow and vulvovaginitis (Adjanohoun et al., 1996). Some others species have been shown to possess anti-inflammatory and/or antioxidant properties (Panthong et al., 2003; Rajlakshmi et al., 2003; Choi et al., 2004; Chae et al., 2004, 2005; Devi et al., 2005). For the treatment of intestinal helminthiasis, it is recommended by traditional healers to crush some of the fresh leaves in a little water and to drink one part of the extract. The rest of the extract in which has been added five *Capsicum frutescens* fruits is used to purge the patient (Adjanohoun et al., 1996).

The aim of this study was to assess the *in vivo* antischistosomal activity of *Clerodendrum umbellatum* leaves aqueous extract on a *Schistosoma mansoni* mice model and to determine the most effective dose.

Material and methods

Plant material and extraction

Leaves of *Clerodendrum umbellatum* (voucher No.7405) were collected in april 2005 from Nkolenyen, near Djoum, South Province, Cameroon and identified by the Laboratory of Botanic and Ecology, Faculty of Science, University of Yaounde before a voucher specimen was deposited at the National Herbarium, Yaounde, Cameroon. The plant leaves were dried in the shade, powdered and mixed with water (300g of powder per 3 litres of water) for 24 hours of maceration. After filtration, the solution was evaporated at 45°C, aqueous extract weighed and extraction yield determined to obtain 63.62g of the aqueous extract, with an extraction yield of 21.21 %.

Phytochemical screening

Phytochemical properties of the leaves aqueous extract of *C. umbellatum* were analysed based on Asongalem et al. (2004). Chemical groups tested were alkaloids, triterpenes, saponins, saponosides, tannins, flavonoids, phenols, cardiac glycosides and lipids.

Animals

Ninety days old male BALB C mice, weighing 25 - 32 g, were used. They were housed in a standard environmental condition and fed with rodents' diet and water *ad libitum*. The study was approved by the institution's animal Ethical Committee.

Parasite

Snails of *Biomphalaria pfeifferi* used in this study were collected from Yaounde municipal lake (Cameroon), a *Schistosoma mansoni* focus. They were maintained in the laboratory in standardized conditions and screened for cercarial production. Infected snails were maintained in labelled bowls and used for mice infection.

Infection and treatment

Mice were individually infected with 50 cercariae of *S. mansoni* (Cameroonian strain) with tail and legs immersion technique. They were maintained in labelled cages and on day 60 post infection, screened by Kato Katz technique to identify infected mice for the experiment. Animals were divided into six groups and treated as follows:

Group NC: normal control receiving distilled water (5 mice)

Group IC: infected control receiving distilled water (5 mice)

Group PC: infected-treated orally with 100mg/kg of praziquantel (positive control) (5 mice)

Group IT₄₀: infected - treated orally with 40 mg/kg/day of *C. umbellatum* (5 mice)

Group IT₈₀: infected - treated orally with 80 mg/kg/day of *C. umbellatum* (6 mice)

Group IT₁₆₀: infected - treated orally with 160 mg/kg/day of *C. umbellatum* (6 mice)

The administration of the extract started on day 60 post infection and lasted fourteen (14) days. Animals were left for seven (07) days after treatment and sacrificed.

Body, liver and spleen weights

Animals were weighed once a week from the beginning of the treatment until the day of sacrifice. After sacrifice, the liver and spleen were removed from each mouse and weighed.

Eggs count

Faecal samples were collected from each mouse before sacrifice and processed for presence and number of eggs using the Kato-Katz technique (Katz et al., 1970) while the total number of eggs present in the liver was evaluated as described by Cheever and Anderson (1971).

Worm counting

After anaesthesia of mice, worms were recovered by liver perfusion as described by Duwall and Dewitt (1967). The percent of reduction in worm number was calculated by the method of Tendler et al. (1986) as follows: $P = [(C - V) / C] \times 100$

Where P = % of reduction; C = mean number of parasite recovered from infected animals; V = mean number of parasite recovered from treated animals.

Statistical analysis

Data are presented as mean \pm SEM. Statistical significance values were determined by one-way ANOVA with Dunnett's post test performed using GraphPad InStat version 3.05 for Windows 95/NT, GraphPad Software, San Diego California USA, www.graphpad.com.

Results

Phytochemical analysis

Phytochemical analysis of the leaves aqueous extract of *C. umbellatum* revealed the presence of alkaloids, flavonoids, saponins, saponosides, tannins and triterpenes.

Body, liver and spleen weights

The body weight between the various groups did not vary significantly (31.88 ± 0.67 g for group NC vs 26.82 ± 1.84 g for group IT₁₆₀). *S. mansoni* infection resulted in hepatomegaly (4.87 ± 0.02 g/100g for normal control vs 9.22 ± 0.36 g/100g for infected control) and splenomegaly (0.50 ± 0.07 g/100g for normal control vs 1.03 ± 0.05 g/100g for infected control) as shown in Table 1. Treatment with *C. umbellatum* or praziquantel induced a significant decrease in the liver weight of all the infected-treated animals when compared with that of infected control mice. In addition, the liver weight of infected mice treated with 160 mg/kg of *C. umbellatum* leaves aqueous extract did not showed significant difference with that of normal control mice (5.12 ± 0.33 g/100g vs 4.87 ± 0.02 g/100g). A significant reduction in splenomegaly was also noticed in all the infected mice treated with *C. umbellatum* leaves aqueous extract or praziquantel. When compared to the spleen weight of normal control mice, the one of all the infected mice treated with the plant extract or praziquantel did not differ significantly (Table 1).

Eggs load

As presented in Table 2, the mean number of *S. mansoni* eggs in the faeces of infected mice treated with *C. umbellatum* leaves aqueous extract at a dose of 80 mg/kg or 160 mg/kg decreased significantly by 75.49 % and 85.14 % respectively. The reduction rate obtained in the group of mice treated with 40 mg/kg of the extract was not significant. At the end of the treatment, no egg was detected in the faeces of mice treated with praziquantel, thus involving a 100 % percentage of reduction. At the end of the experiment, the eggs embedded in liver of each mice group were counted and a significant reduction was observed only in the group of mice treated with 160 mg/kg of the extract.

Worm burden

Table 3 presents the mean number of *Schistosoma mansoni* recovered after treatment with *C. umbellatum* leaves aqueous extract. Reduction rate was 73.62% and 88.74% in infected animals treated with 40mg/kg or 80 mg/kg of the extract respectively and 100% in those treated with either 160 mg/kg of the extract or praziquantel.

Table 1: Body and organs weights of *Schistosoma mansoni*-infected mice treated with *Clerodendrum umbellatum* leaves aqueous extract.

Animals	Body weight (g)	Organs weights (g/100g of body weight)	
		Liver	Spleen
Group NC	31.88 ± 0.67	4.87 ± 0.02	0.50 ± 0.07
Group IC	31.16 ± 0.54	9.22 ± 0.36 ^a	1.03 ± 0.05 ^a
Group PC	29.53 ± 1.75	7.10 ± 0.18 ^{**} , ^a	0.58 ± 0.04 ^{**} , ^b
Group IT ₄₀	29.12 ± 1.23	6.84 ± 0.64 ^{**} , ^a	0.63 ± 0.08 ^{**} , ^b
Group IT ₈₀	28.10 ± 1.89	6.95 ± 0.77 ^{**} , ^a	0.36 ± 0.03 ^{**} , ^b
Group IT ₁₆₀	26.82 ± 1.84	5.12 ± 0.33 ^{**} , ^b	0.43 ± 0.12 ^{**} , ^b

* Significantly different at P<0.05 from infected control group (group IC)

** Significantly different at P<0.01 from infected control group (group IC)

^a significantly different at P<0.01 from normal control group (group NC)

^b Statistically not different from normal control group (group NC)

Table 2: Mean number of *Schistosoma mansoni* eggs in the hepatic tissue and the faeces of mice treated with *Clerodendrum umbellatum* leaves aqueous extract.

Animals	Eggs count		Reduction rate in the faeces (%)
	Liver (eggs/g of liver)	Faeces (eggs/g of faeces)	
Group IC	5902.23 ± 1251.80	326.40 ± 100.00 ^a	
Group PC	6102.00 ± 969.61	00.00 ± 00.00 ^{**}	100 %
Group IT ₄₀	9741.83 ± 2312.80	283.20 ± 90.25 ^c	13.23 %
Group IT ₈₀	3433.83 ± 1421.30	80.00 ± 40.00 [*] , ^b	75.49 %
Group IT ₁₆₀	328.83 ± 328.83 [*]	48.50 ± 48.50 [*] , ^b	85.14 %

* Significantly different at P<0.05 from infected control group (group IC)

** Significantly different at P<0.01 from infected control group (group IC)

^a Significantly different at P<0.01 from positive control group (group PC)

^b Statistically not different from positive control group (group PC)

^c Significantly different at P<0.05 from positive control group (group PC)

Table 3: Mean number of *Schistosoma mansoni* recovered after treatment with *Clerodendrum umbellatum* leaves aqueous extract.

Animals	Total worm count	Reduction in worms (%)
Group IC	32.60 ± 22.78	
Group PC	0.00 ± 0.00 [*]	100.00 %
Group IT ₄₀	8.60 ± 4.90	73.62 %
Group IT ₈₀	3.67 ± 2.54	88.74 %
Group IT ₁₆₀	0.00 ± 0.00 [*]	100.00 %

* Significantly different at P<0.05 from infected control group (group IC)

Discussion

Schistosoma mansoni infection in mice caused excretion of eggs in the faeces and the liver by worms living in the portal and mesenteric veins. This also leads to hepatosplenomegaly. At the end of the treatment, the values of the liver weight of infected mice treated with 160 mg/kg of *C. umbellatum* leaves aqueous extract as well as the spleen weight of infected mice treated with *C. umbellatum* aqueous extract or praziquantel were not

statistically different from those of normal control mice. These results suggest a normalization of the liver and spleen weights after treatment with *C. umbellatum* leaves aqueous extract.

This study showed a high and significant reduction of faecal egg count by 75.49 % and 85.14 % after treatment of *S. mansoni*-infected mice with 80 mg/kg or 160 mg/kg of *C. umbellatum* leaves aqueous extract compared to infected mice. In the group of mice treated with praziquantel, the reduction rate was 100 %. The non statistically different results obtained with the doses of 80 mg/kg and 160 mg/kg of *C. umbellatum* leaves aqueous extract compared with praziquantel is a clear indication of the efficacy of these extract doses for the treatment of *Schistosoma mansoni* infection. This remarkable reduction of faecal egg count could be related to a possible lethal effect of the extract on eggs. Koko et al. (2005), El-Shenawy et al. (2006) and El-Ansary et al. (2007) also obtained considerable reductions of egg count in the faeces while treating *S.mansoni*-infected animals with *Balanites aegyptiaca* fruit mesocarp, *Cleome droserifolia* ethanolic extract and *Curcuma longa* oil extract. The reduction of ova count is probably the consequence of the noticeable decrease of worm burden.

C. umbellatum leaves aqueous extract was lethal to *S. mansoni* adult worms at the rate of 73.62 %, 88.74 % and 100 % for the doses of 40 mg/kg, 80 mg/kg and 160 mg/kg respectively. In addition to *C. umbellatum*, many other plants extracts have been recently investigated as potential sources of antischistosomal agents. Among these are artemether, a methyl ether derivative of artemisinin obtained from *Artemisia annua*, which exhibited antischistosomal properties alone and in combination with praziquantel (Shuhua and Catto, 1989; Utzinger et al., 2001; Lescano et al., 2004). *Balanites aegyptiaca* fruit mesocarp, phenolic extract of *Citrus reticulata* root and ethanolic extract of *Cleome droserifolia* are also lethal to adult worms of *S.mansoni* (Koko et al., 2005; Hamed and Hetta, 2005; El-Shenawy et al., 2006). It has been proved that antimicrobial and antiparasitic properties of plants extracts are assigned to some chemicals compounds as tannins, terpenes, flavonoids, phenols, and alkaloids present in plants extract (Perrett et al., 1995; Cowan, 1999; Lyddiard et al., 2002). The phytochemical screening of *C. umbellatum* leaves aqueous extract has revealed the presence of alkaloids, flavonoids, saponins, saponosides, tannins and triterpenes. Due to their antiparasitic property, some of these chemicals constituents could be responsible for the schistosomicidal effect of *C. umbellatum* leaves aqueous extract. Moreover, it has been found that some species of the genus *Clerodendrum* as *C. petasites* and *C. trichotomum* exhibited anti-inflammatory properties (Panthong et al., 2003; Choi et al., 2004). Given the fact that *S. mansoni* develops unique severe granulomatous inflammatory reactions in the liver, a plant extract with anti-inflammatory properties might be of great interest in the enhancement of the anti-inflammatory barrier. The immediate consequence could be a reduction of the number and the size of granulomas in the liver and then a reduction of hepatosplenomegaly. In fact, our study revealed an amelioration of the liver weight and a normalization of the spleen weight after the treatment of *S. mansoni*-infected mice with *C. umbellatum* leaves aqueous extract. Phenols, flavonoids and tannins are known to yield antioxidant effects (Weiner, 1994). Flavonoids and tannins present in *C. umbellatum* leaves aqueous extract could exert their antioxidant effect against the damage of the liver membrane as is usually the case in schistosomiasis.

All these findings suggest a possible antischistosomiasis activity of the *C. umbellatum* leaves aqueous extract. The effect of this extract is dose-dependent, the dose 160 mg/kg being the one showing the highest effect.

Acknowledgements

This study was carried out with the grant N° F/3622-1 from the International Foundation for Science (IFS).

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