

THE MOLLUSCICIDAL ACTIVITY OF CROWN OF CHRIST (*EUPHORBIA SPLENDENS* VAR. *HISLOPII*) LATEX ON SNAILS ACTING AS INTERMEDIATE HOSTS OF *SCHISTOSOMA MANSONI* AND *SCHISTOSOMA HAEMATOBIIUM*

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Abstract. The present study describes the action of the latex of *Euphorbia splendens* var. *hislopii* (*E. milli*) on species of the genus *Bulinus* and on *Biomphalaria pfeifferi*, intermediate hosts of schistosomiasis in Africa, and the Brazilian snails *B. glabrata*, *B. tenagophila*, and *B. straminea*, intermediate hosts of schistosomiasis in Brazil. The impact of the latex on the egg masses and embryos of *B. glabrata* was also evaluated. Using the standardized methodology of the World Health Organization for testing plant-derived molluscicides, we obtained a 90% lethal dose (LD₉₀) ranging from 0.13 ppm for *B. glabrata* subjected to lyophilized latex to 4.0 ppm for *B. pfeifferi* tested with the natural latex. This material has proved to be one of the most potent and specific plant molluscicides discovered thus far, presenting advantages in terms of application so that it could be used in programs involving community participation in endemic areas in both Brazil and Africa.

The use of materials with molluscicidal activity has been studied since the 1930s with the perspective of becoming a more adequate and effective method in the control of snails acting as the intermediate hosts of *Schistosoma mansoni*. The investigation of the molluscicidal properties of plants has recently been greatly expanded, with more than 1,400 species studied thus far.^{1–3} The extracts of 20 species have been found to have a high molluscicidal potential when tested at low concentrations.

The Crown of Christ (*Euphorbia splendens* var. *hislopii*), a plant originating from Madagascar, is especially interesting since the aqueous extract of its latex was found to have a lethal action on *Biomphalaria glabrata* and *B. tenagophila* at a concentration of less than 0.5 ppm under laboratory conditions.⁴ This concentration is much lower than that recommended by the World Health Organization (WHO) for plants to be tested for molluscicidal activity.⁵

The chemical fractionation of the latex revealed eight substances derived from the active fraction, with one of them, milliamine L, showing a 90% lethal dose (LD₉₀) for the snails at a concentration of 0.01 ppm.⁶ This substance was evaluated with other milliamines and was found not to be carcinogenic in tests performed on the shaved back skin of NMRI mice using a single dose of dimethylbenz(a)anthracene as an initiator.^{7,8} Other toxicologic tests have been carried out, including studies of skin and eye irritability in rabbits,⁹ acute toxicity (Microtox System, Beckman Instruments, Carlsbad, CA), mutagenicity (TA98 and TA100 strains of *Salmonella typhimurium*; Ames test) and cytotoxicity (Chinese hamster ovary cell assay),^{10,11} embryofetotoxicity (using Wistar treated with latex solution during the second stage of the embryogenic period from the sixth to the 15th day of pregnancy),¹² carcinogenicity (using V79 cells),¹³ ecotoxicology (using plankton species, fish, mollusk species, and insect larvae),¹⁴ and subchronic toxicology (giving sublethal doses to mice for 90 days) studies (Lopes MC and others, unpublished data), revealing no toxic effects in the concentrations used as molluscicide.

Laboratory tests have demonstrated that the molluscicidal activity of the natural latex remains unchanged after storage for 124 days in a closed assay tube at room temperature and

after 736 days in a closed bottle in a refrigerator at 10–12°C (lyophilized latex).¹⁵ Field tests with the natural latex in lentic and lotic environments showed 100% mortality of *B. glabrata* and *B. tenagophila* at concentrations of 5 and 12 ppm, respectively.^{16,17}

In the present study, we carried out bioassays in the laboratory investigating the action of Crown of Christ latex on *B. glabrata*, *B. tenagophila*, and *B. straminea*, snails that act as host of *S. mansoni* in Brazil, and on *B. pfeifferi* and *Bulinus* sp., snails that act as hosts of *S. haematobium* in Africa. The objective was to determine the possible use of this product as one of the methods for the control of the disease, which currently affects approximately 12 million people in Brazil and 120 million on the African continent.¹⁸

MATERIALS AND METHODS

Collection of the latex. Two biologic assays were carried out using two samples of Crown of Christ latex collected at the same site (Ilha do Governador, Rio de Janeiro, Brazil, where this species is used as an ornamental plant in several large gardens). Currently, this plant is cultivated in gardens throughout the country, demonstrating its great adaptability to Brazilian soil. The latex samples used were collected during the same season of the year (spring) in different years (1993–1994).

The latex sample of the plant was obtained by collecting drops from cuts on the plant stem. One milliliter of latex was collected in 9 ml of distilled water, the solution from which the required concentrations were obtained.

In the first assay, natural plant latex that was stored in the refrigerator at 10°C for 63 days after collection was used. In the second assay, performed one year after the first, latex subjected to lyophilization on the day of collection and stored at 10°C for seven days in a desiccator was used. The lyophilized latex was used in the second experiment to avoid problems with the transportation of the product. The greater level of stability of material in this form was previously demonstrated.¹⁵

Snail species. The animals tested in the first experiment

TABLE 1

Mortality of *Biomphalaria glabrata*, *B. straminea*, and *B. pfeifferi* exposed for 24 hr to different aqueous concentrations of the natural latex of crown of Christ*

Concentration (ppm)	<i>B. glabrata</i>	<i>B. straminea</i>	<i>B. pfeifferi</i>
Control	0 (0)	0 (0)	0 (0)
0.2	0 (0)	0 (0)	0 (0)
0.4	5 (1)	5 (1)	0 (0)
0.6	30 (6)	10 (2)	15 (3)
0.8	75 (15)	15 (3)	25 (5)
1.0	90 (18)	15 (3)	25 (5)
1.5	100 (20)	60 (12)	75 (15)
2.0	100 (20)	65 (13)	75 (15)
2.5	100 (20)	65 (13)	75 (15)
3.0	100 (20)	80 (16)	75 (15)
3.5	100 (20)	85 (17)	85 (17)
4.0	100 (20)	90 (18)	90 (18)
LD ₉₀	0.99	4.0	4.0

* Values are percentages except for the 90% lethal dose (LD₉₀). The number of dead animals is given in parentheses. Analysis of variance demonstrated significant differences in mortality rate among species ($F = 24.21$, $P < 0.001$) and concentrations ($F = 32.92$, $P < 0.001$).

were two Brazilian species of snails: *B. glabrata* (10–12 mm in diameter), which originated in Paulista, Brazil and *B. straminea* (5–10 mm in diameter), which originated in Petrolandia, Brazil. It was also tested on another species, *B. pfeifferi* (5–7 mm in diameter), of unknown origin. These animals were reared in Field Station of the Schistosomiasis Program, Aggeu Magalhaes Research Center (Sao Lourenco da Mata, Pernambuco, Brazil). Twenty specimens per concentration were exposed (10 per flask), giving a total of 240 animals per species, distributed in 11 different concentrations (ranging from 0.2 ppm to 4.0 ppm) (Table 1). Two flasks of controls (20 animals) were also tested.

In the second experiment, the concentrations of lyophilized latex was tested on adult Brazilian species: *B. glabrata* (10–12 mm in diameter), *B. tenagophila* (10–12 mm in diameter), both from Belo Horizonte, Minas Gerais, *B. straminea* (5–10 mm in diameter) from Paracatu, Minas Gerais, and *Bulinus* sp. (4–5 mm in length) of unknown origin, all of them reared in the Malacology Laboratory of the Rene Rachou Research Center (Belo Horizonte, Brazil). Newly hatched specimens (1–3 days) and egg masses of *B. glabrata* from the same laboratory were also exposed. The egg masses were obtained using cellophane sheets as substrates and egg viability was observed up to five days after the beginning of the experiment. Twenty specimens of adult snails were used per concentration, giving a total of 200 animals per species, distributed in nine different concentrations (ranging from 0.05 ppm to 12.0 ppm) (Table 2) and two flasks of controls (20 animals). For the newly hatched snails, 40 animals per concentration were used. For the egg masses (1 day old), five spawns with a mean number of 30 eggs per spawn were tested. Eight spawns were used as controls.

Dilutions of the latex, exposure, and counting of snails.

The experiments were performed according to the methodology standardized by WHO,^{19,20} as described in a previous report.¹⁰ In both assays, adult snails were exposed to various concentrations of the molluscicide in duplicate in flasks for a period of 24 hr, with 20 animals per concentration divided into two groups of 10 animals per flasks. The animals in the

TABLE 2

Mortality of *Biomphalaria glabrata*, *B. tenagophila*, *B. straminea*, and *Bulinus* sp. adults and of newly hatched *B. glabrata* individuals (1–3 days) exposed to different aqueous concentrations of the lyophilized latex of crown of Christ*

Concentration (ppm)	<i>B. glabrata</i>	<i>B. tenagophila</i>	<i>B. straminea</i>	<i>Bulinus</i> sp.	<i>B. glabrata</i> (1–3 days)
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.05	15 (3)	0 (0)	0 (0)	0 (0)	27.5 (11)
0.1	75 (15)	45 (9)	30 (6)	75 (15)	62.5 (25)
0.2	100 (20)	90 (18)	95 (19)	95 (19)	100 (40)
0.4	100 (20)	100 (20)	100 (20)	100 (20)	100 (40)
0.5	100 (20)	100 (20)	100 (20)	100 (20)	100 (40)
1.0	100 (20)	100 (20)	100 (20)	100 (20)	100 (40)
3.0	100 (20)	100 (20)	100 (20)	100 (20)	100 (40)
6.0	100 (20)	100 (20)	100 (20)	100 (20)	100 (40)
12.0	100 (20)	100 (20)	100 (20)	100 (20)	100 (40)
LD ₉₀	0.13	0.20	0.18	0.15	0.20

* Values are percentages except for the 90% lethal dose (LD₉₀). The number of dead animals is given in parentheses. No significant difference was observed among the species tested $F = 2.60$, $P > 0.05$, by analysis of variance.

control group (20 animals, 10 per flask) were exposed only to the diluent, i.e., distilled water.

The concentrations were obtained by dilutions made from a stock solution of 1,000 ppm (1 ml of natural or 1 g of lyophilized material) diluted in 1,000 ml of distilled water and ranged from 0.2 ppm to 4.0 ppm in the first experiment (1993, Table 1) and from 0.05 ppm to 12 ppm in the second experiment (1994, Table 2) for the adults and the newly hatched snails. For the egg masses, the concentrations ranged from 50 ppm to 2,000 ppm. In the first experiment, 1,000 ml of the solution were prepared for each concentration and divided into two 1,000-ml glass beakers, each containing 500 ml of solution. The control flasks received the same volume of diluent, i. e., distilled water. In the second experiment, the same preparations were used, but 500-ml glass beakers used and were completely filled with the solutions. The egg masses were placed individually in 50-ml beakers.

During the 24-hr exposure, the flasks containing the latex were kept at room temperature and covered with netting, with the snails receiving no food. The snails were then removed from the flasks, washed with distilled water, and returned to the same flasks that had also been rinsed and refilled with distilled water only. Small pieces of lettuce were added as food and the animals were left to stand for a recovery period of 24 hr. Dead and surviving animals were counted at the end of day 2.

Differences in mortality rates among the species tested were analyzed by the two-way analysis of variance test, after arcsine-square root transformation.²¹

RESULTS

The molluscicidal activity of aqueous extracts obtained from the natural latex of the Crown of Christ on *B. glabrata*, *B. straminea*, and *B. pfeifferi* adults is presented in Table 1. The data demonstrate a significant difference among species ($F = 24.21$, $P < 0.001$) and among the concentrations tested ($F = 32.92$, $P < 0.001$). The mortality rate of *B. glabrata* was significantly higher than that of the other two species, demonstrating a differential susceptibility to the latex. The

TABLE 3

Mortality of *Biomphalaria glabrata* eggs (0–1 days) exposed to various aqueous concentrations of lyophilized crown of Christ latex*

Concentration (ppm)	<i>B. glabrata</i> eggs
Control	0 (0/193)
50	0 (0/155)
200	0.5 (1/201)
400	2.2 (4/179)
800	52.6 (59/112)
1,000	70.0 (117/167)
1,500	100 (157/157)
2,000	100 (138/138)
LD ₉₀	1,200

* Values are percentages except for the 90% lethal dose (LD₉₀). The number of dead embryos/total number of embryos is given in parentheses.

LD₉₀ values were 0.99 ppm for *B. glabrata* and 4.0 ppm for *B. straminea* and *B. pfeifferi*. No mortality occurred in the controls placed in distilled water only.

Table 2 presents the molluscicidal effect of lyophilized Crown of Christ latex on adult snails of the three Brazilian host species, on the African host species, and on newly hatched animals. The mortality rate was 100% for *B. glabrata* adults and newly hatched specimens starting at a concentration of 0.2 ppm. For *B. tenagophila*, *B. straminea*, and *Bulinus* sp., 100% mortality was obtained with a concentration of 0.4 ppm.

As shown in Table 2, the LD₉₀ values did not vary among the Brazilian host species and were 0.13 ppm for *B. glabrata*, 0.20 ppm for *B. tenagophila*, and 0.18 ppm for *B. straminea*. A similar value was obtained for the African *Bulinus* sp. (0.15 ppm) and for newly hatched *B. glabrata* specimens (0.20 ppm). No significant difference was found among adults and the newly hatched specimens ($F = 2.60$, $P > 0.05$). No mortality occurred in the controls placed in distilled water only.

Table 3 shows the mortality rates of embryos inside *B. glabrata* eggs (0–1 days), with 100% mortality being obtained with concentrations greater than 1,500 ppm. The LD₉₀ value was 1,200 ppm.

DISCUSSION

The results of the first experiment with the natural latex show a differential susceptibility between *B. glabrata* and the other two species (*B. straminea* and *B. pfeifferi*), with the latter requiring high concentrations as lethal doses when compared with the first species. Different susceptibilities among various species was previously observed for field snails, which require higher lethal doses than the animals colonized for long periods in the laboratory.⁴ Previous data showed that *B. straminea* is more resistant than *B. glabrata* to other molluscicides used.²² The high lethal dose for embryos of *B. glabrata* eggs (100% lethality at 1,500 ppm) has been reported in the literature for other products tested.²³ In contrast, the LD₉₀ value for newly hatched animals was similar to that obtained for adults (0.2 ppm).

Despite this variability, even for species for which the LD₉₀ value was 4.0 ppm, the validity of the product tested is maintained if we consider the recommendation of WHO that a good plant-derived molluscicide should be lethal at a

concentration of less than 20 ppm.⁵ Furthermore, only 20 plant species among the more than 1,000 tested showed lethal doses below this recommended value.⁵

When one considers plants that have an effective molluscicide action at a concentration less than 20 ppm, few of them have had this efficacy verified in the field.⁵ In the case of *E. splendens*, the lethal doses tested in the field (5 ppm and 12 ppm) confirmed the efficacy of the plant in the control of the snail vectors of schistosomiasis in Brazil.^{16, 17, 24}

The results of the present study on the natural latex of *E. splendens*, which demonstrate an effective lethal dose of 4.0 ppm for *B. pfeifferi* (a snail vector of schistosomiasis in Africa) and 0.15 ppm for *Bulinus* sp. (which belongs to a genus in which there are some species transmitting *S. haematobium* in Africa), can open even wider possibilities for its use in that continent when one considers 1) the high prevalence of schistosomiasis in Africa (approximately 120 million people),¹⁸ and 2) the fact that the plant originates from the African continent (Madagascar), with the possibility of large-scale cultivation in the region. In addition, data reported²⁵ concerning the cost-benefit ratio of the product confirm the potential use of the plant and its large-scale culture using simple and feasible operational processes.

Following the WHO guidelines⁵ devised for the study of plant molluscicides, it is recommended that a useful molluscicidal plant not be a source of prejudice (fear or superstition). There is no known superstition that would preclude the use of *E. splendens*. In addition, the Brazilian rural population in some regions has used this substance to treat caluses on the feet and also as a facial skin treatment. Extracts of *E. splendens* also contain compounds with anti-inflammatory activity²⁶ as well as anticancerous compounds.²⁷ It has also been reported that the plant is used in China as a treatment for hepatitis and abdominal edema.²⁷

Despite the good results obtained in the present study, as recommended by WHO,^{5, 20} it is necessary to carry out an extensive toxicologic evaluation of each new substance before its use in schistosomiasis control programs. As a result, several studies are being conducted and initial results indicate that aqueous solutions of the latex are not irritating to rabbit skin at concentrations less than 0.5% and to rabbit eyes at concentrations less than 0.35%.⁹ In addition, there was no observed embryofetotoxicity with this latex in pregnant Wistar rats.¹² Other studies showed that the latex had no cytotoxic effect on Chinese hamster ovary cells and no mutagenic effect in the Ames test with *Salmonella typhimurium* strains TA98 and TA100 with and without the S9-mixture (a mixture that contains the S-9 fraction, which is the floating portion obtained by centrifugation [at 9,000 × g] of homogenated hepatic tissue, and contains enzymes that metabolically degrade promutagens and procarcinogens).^{10, 11} The latex is also biodegradable and has proved to be less harmful to nontarget organisms than niclosamide, the most commonly used molluscicide compound.¹⁴ It was also verified that the minimum concentration of crude latex required for tumor-promoting activity in an in vitro assay is 10 µg/ml, which is 20-fold higher than the effective molluscicidal concentration obtained in the laboratory (0.5 µg/ml).¹³ Additional in vivo studies are underway, such as a tumor-promoter assay on mouse back skin to better eval-

uate the dose-response relationships of the molluscicidal latex.

In the control of schistosomiasis, it is important to point out that the control of snail vectors continues to be one of the viable strategies for use together with the other measures, such as treatment of clinical cases. This is particularly relevant in endemic localities in rural areas of Brazil, where the lack of sanitation and good housing shows little prospect for improvement in the near future. The use of the latex by communities associated with health education programs, as previously suggested,²⁵ can help to keep schistosomiasis at low levels since eradication is not considered realistic in current conditions in Brazil.¹⁸

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