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# *Schistosoma mansoni*: Phytochemical Effect on Aquatic Life Cycle

**Ronaldo de Carvalho Augusto[1*], Gabriela Friani[1], Maurício Carvalho de Vasconcellos[1], Maria de Lurdes de Azevedo Rodrigues[2], Clélia Christina Mello-Silva[1]**

[1]Laboratório de Avaliação e Promoção da Saúde Ambiental, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brasil
[2]Departamento de Parasitologia Animal, Curso de Pós-Graduação em Ciências Veterinárias, Universidade Federal Rural do Rio de Janeiro, Seropédica, Brasil
Email: *augustorc@ioc.fiocruz.br

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## Abstract

**Background: Two aspects need to be considered for schistosomiasis control: morbidity and transmission. In this context, many soluble substances have been tested and *Euphorbia milii* latex is one of the most promising Brazilian molluscicides. Phytochemical studies involving simulation of the applicability of this latex on all aquatic forms of the *S. mansoni* life cycle are rare in the literature. The aim of this work was to evaluate the effect of *E. milii* latex on *S. mansoni* in the egg, miracidium and different developmental stages in *Biomphalaria glabrata*. Methods: The laboratory study was designed to simulate the different forms of exposure of the life cycle stages of *S. mansoni* to the $LC_{50}$ of *E. milii* latex; we tested the exposition from four situations of *S. mansoni* contact with the latex and observed the exposure on different snails' infection stage too. All snails were analyzed weekly for cercarial shedding and reproductive biology. Results: The results showed that contact of *S. mansoni* eggs and miracidia with the $LC_{50}$ of *E. milii* negatively influenced the development of the parasite life cycle in the intermediate host, with consequent reduction of cercarial shedding. The exposure of infected snails affected the reproductive biology and cercarial shedding in all intra-mollusk development stages of *S. mansoni*, but the reduction was greater in the first, fourth, fifth, sixth, seventh and eighth weeks of infection. The $LC_{50}$ of *E. milii* latex had toxic action on eggs and miracidia, and the number of cercariae shed by snails during the study period declined by about 80%. Conclusions: We can conclude that the use of natural biodegradable compounds containing low concentrations of substances already characterized as having eco-toxicological potential can be an important tool to reduce the transmission of Schistosomiasis.**

## Keywords

**Schistosomiasis, Cercariae, Miracidia, Eggs, *Euphorbia milii***

*Corresponding author.

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## 1. Introduction

For schistosomiasis control, two aspects need to be considered: morbidity and transmission. For morbidity control, chemotherapy using praziquantel is generally successful in reducing the number of severe cases, but chemotherapy is not enough for transmission control and it is also necessary to use water-soluble substances [1] [2]. In this context, many soluble substances have been tested. *Euphorbia milii* latex is one of the most promising Brazilian molluscicides [3] [4]. This compound has been tested under laboratory and field conditions and meets the recommendations of the World Health Organization for use in control programs on a large scale [5].

Several studies have shown different activities of *E. milii* latex on helminths. The molluscicidal effects of the crude latex of *E. milii* were a good tool against schistosomiasis dynamics [6], and among others factors, the latex's uptake by cercariae possibly occurred via absorption through the tegument or by ingestion. The input of different macromolecules was reported to induce changes in the genome of *S. mansoni*, affecting its growth and development in the intermediate host [7]. The exposure of intermediate host snails to the same latex caused several changes in the reproductive biology, carbohydrate content, nitrogen excretion products and survival [2] [3] [8] [9]. Although the action of *E. milii* on infected snails is well known, phytochemical studies to simulate applicability of this latex on all aquatic forms of the *S. mansoni* life cycle are rare in the literature. The aim of this work was to evaluate the effect of *E. milii* latex on the eggs, miracidia and different developmental stages of *S. mansoni* in *Biomphalaria glabrata*, the main intermediate host in Brazil.

## 2. Material and Methods

### 2.1. Determination of the Lethal ($LC_{90}$) and Sublethal ($LC_{50}$) Concentrations

The *E. milii* latex samples were collected in the Ilha do Governador district (22˚48'09"S/43˚12'35"W) of the city of Rio de Janeiro, Brazil, as described by Vasconcellos & Amorin (2003). The calculation of the lethal concentration (LC) of the aqueous extract of the latex was carried out by probit analysis [10] and the $LC_{50}$ and $LC_{90}$ values were 1.4 mg/L and 2.7 mg/L, respectively.

### 2.2. Experimental Design

The laboratory study was designed to simulate the different forms of exposure of the life cycle stages of *S. mansoni* to the $LC_{50}$ of *E. milii* latex. The situations analyzed were:

Infection of snails with eggs and/ormiracidia exposed to the $LC_{50}$ of the aqueous extract of *E. milli*: four situations of *S. mansoni* contact with the latex $LC_{50}$ were analyzed: Group 1—control (eggs and miracidia were maintained and the snails were infected in distilled water), Group 2—hatching of miracidia in distilled water, but the snails were infected in the presence of the latex $LC_{50}$; Group 3—hatching of miracidia in the presence of the $LC_{50}$ and infection of snails in distilled water; and Group 4—hatching of miracidia and infection of snails in the presence of the latex $LC_{50}$. All the groups were composed of 30 snails. After infection, the snails were kept individually in 50 mL beakers with distilled water and the reproductive parameters were recorded weekly. The snails were feds *ad libitum* with lettuce leaves three times a week.

Infected snails: The exposure to the latex $LC_{50}$ was varied according to the snails' infection stage ([1]-[8] weeks post infection). In each period analyzed, 10 exposed and 10 unexposed snails were used to compare the effects of the *E. milii* latex. Ten uninfected and exposed snails were maintained as positive control and 10 uninfected and unexposed snails were used as negative control. The snails were exposed for 24 hours to *E. milii* latex and then were washed in dechlorinated water.

All snails were analyzed weekly for cercarial shedding and reproductive biology. The results were expressed as mean ± standard deviation and analyzed by analysis of variance (ANOVA), the Tukey-Kramer test and Student's t-test for unpaired data to compare means ($\alpha$ = 5) (Instat, GraphPad, v.4.00, Prism, GraphPad, v.3.02, Prism Inc.).

## 3. Results

### 3.1. Infection of Snails with Eggs and Miracidia of *S. mansoni* Exposed to the Aqueous Extract $LC_{50}$

The survival of the snails in the control group (Group 1) was 45.3% greater at the end of the experiment than

observed in Group 4, in which the process of hatching and infection in the laboratory occurred in contact with the $LC_{50}$ of the *E. milii* latex. The latex contact at the time of hatching of the miracidia or infection caused reductions of 27.3% and 18.2% in the survival of the snails, respectively. The infection rate of the snails was 30% higher in Group 1 than in Group 4, where the hatching and infection occurred in contact with the latex. The reproductive parameters of *B. glabrata* infected with eggs and miracidia of *S. mansoni* exposed to $LC_{50}$ were observed during the entire experiment, but there were no significant differences ($\alpha$ = 5%).

The number of cercariae eliminated/snail in all treatment groups (Groups 2, 3 and 4) was significantly less than in the control group (Group 1) (**Table 1**). These results demonstrate that the presence of the latex in the water system is more damaging to hatching of the miracidia (Group 3) than to their infection of the intermediate hosts (Group 2). The influence of the latex on Group 3 and Group 4 was more critical to development of the life cycle of *S. mansoni*, with the elimination of cercariaewas reduced by 23.06% and 22.60%, respectively (**Table 1**).

### 3.2. Infected Snails in Different Phases of Infection by *S. mansoni* and Exposed to $LC_{50}$ of *E. milli* Latex

All infected snails used in the experiment shed *S. mansoni* cercariae. There was a significant decrease in the survival rate in all weeks of infection compared to the control group (unexposed and uninfected snails), but the survival was lower in snails infected and exposed to the latex, where survival ranged from 40% to 0% in the first and eighth week of infection, respectively.

**Table 2** shows there was a rapid decline in reproduction rate of snails exposed to $LC_{50}$ of *E. milii* latex. The infection reduced the number of eggs/snail and egg masses/snail in all groups. The most intense variation was observed in the infected and exposed snails and in the fourth, fifth and eighth week of infection there was no egg production. In the third week of infection, there production reduced the number of eggs/snail of the infected and exposed group by 87% (0.96 ± 0.89) in comparison with the infected and unexposed group (7.5 ± 5.1).

The above results show a clear reduction in the cercarial shedding per snail with longer time of infection. The mean cercarial shedding rates observed during 35 days demonstrated that exposure decreased the elimination of cercariae in all infected groups (**Table 3**). The overall effect of treatment on a population of 80 snails infected with *S. mansoni*, in laboratory conditions, showed 133,357 cercariae shed in different weeks of infection by the exposed snails, and 613.487 cercariae shed by the control group in the same period, an increase of 78%. The exposure to first, fourth, fifth, sixth, seventh and eighth week of infection significantly reduced the number of cercariae shed and the survival rate in comparison to snails of the control group.

## 4. Discussion

The present work demonstrated that contact with the $LC_{50}$ of *E. milli* latex by eggs, miracidia and infected snails can change the parasite dynamics of *S. mansoni.* The results show that contact of *S. mansoni* eggs and miracidia with the sublethal concentration of *E. milii* negatively influenced the development of the parasite'slife cycle in the intermediate host, with consequent reduction of cercarial shedding.

De Jong-Brink *et al.* (1988) elucidated the neuroendocrine mechanism of sporocysts in the snail. The development of sporocystsis responsible for increasing the concentration of the neuropeptide schistosomin in the snail's hemolymph, and the increase of this peptide is antagonistic to calfluxin, a peptide responsible for the

**Table 1.** Effect of exposure to the $LC_{50}$ of *Euphorbia milii* (*syn*. splendens) var. *hislopii* latex on the elimination of cercariae by *Biomphalaria glabrata* experimentally infected with *Schistosoma mansoni.* Data are mean ± standard deviation. Results in $Log_{10}(x)$.

| Groups | 3rd week of infection | 4th week of infection | 5th week of infection | 6th week of infection |
|---|---|---|---|---|
| **1) Control** | $1.59 \pm 1.17^{a}$ | $3.33 \pm 0.21^{a}$ | $3.37 \pm 0.2^{a}$ | $3.2 \pm 0.09^{a}$ |
| **2) Infection + latex** | $1.74 \pm 0.96^{a}$ | $2.82 \pm 0.38^{a,b}$ | $3.27 \pm 0.15^{a}$ | $3.11 \pm 0.5^{a}$ |
| **3) Hatching + latex** | $1.34 \pm 0.97^{a}$ | $1.87 \pm 1.08^{c}$ | $2.77 \pm 0.3^{b}$ | $2.86 \pm 0.42^{a}$ |
| **4) Hatching + infection + latex** | $1.11 \pm 0.95^{a}$ | $1.98 \pm 0.77^{b,c}$ | $2.74 \pm 0.51^{b}$ | $3.06 \pm 0.29^{a}$ |

a, b, c = Different letters indicate significant differences between the means ($\alpha$ = 5%).

**Table 2.** Effect of exposure to $LC_{50}$ of *Euphorbia milii* latex in different weeks of infection by *Schistosoma mansoni* on the reproductive biology of *Biomphalaria glabrata* infected in the laboratory.

| | | Eggs/snail | Egg masses/snail | Eggs/egg mass |
|---|---|---|---|---|
| | *Uninfected and unexposed* | 27.6 ± 13.1[a] | 5 ± 2.3[a] | 7.8 ± 6.5[a] |
| | *Uninfected and exposed* | 23.6 ± 14.5[a] | 4 ± 2.3[a,c] | 6.2 ± 1.8[a] |
| **1st week of infection** | *Infected and unexposed* | 6.3 ± 6.1[a] | 0.5 ± 0.4[b] | 19.9 ± 6.5[a] |
| | *Infected and exposed* | 2.8 ± 6.3[b] | 0.1 ± 0.3[b] | 10 ± 14[a] |
| **2nd week of infection** | *Infected and unexposed* | 6.6 ± 6.1[a] | 0.5 ± 0.4[b] | 11.1 ± 5.4[a] |
| | *Infected and exposed* | 1 ± 2.4[b] | 0.06 ± 0.1[b] | 3.6 ± 8[a] |
| **3rd week of infection** | *Infected and unexposed* | 7.5 ± 5.1[a] | 0.5 ± 0.4[b] | 14 ± 2.2[a] |
| | *Infected and exposed* | 0.92 ± 0.89[b] | 0.1 ± 0.1[b] | 6.5 ± 6.6[a] |
| **4th week of infection** | *Infected and unexposed* | 6.4 ± 3[a] | 0.5 ± 0.3[b] | 18.2 ± 13.2[a] |
| | *Infected and exposed* | 0.0 ± 0.0* | 0.0 ± 0.0* | 0.0 ± 0.0* |
| **5th week of infection** | *Infected and unexposed* | 12 ± 4[a] | 0.9 ± 0.2[b] | 13.2 ± 6.2[a] |
| | *Infected and exposed* | 0.0 ± 0.0* | 0.0 ± 0.0* | 0.0 ± 0.0* |
| **6th week of infection** | *Infected and unexposed* | 17.16[a] | 0.9 ± 0.2[b] | 21.8 ± 10.2[a] |
| | *Infected and exposed* | 13 ± 3[a] | 1 ± 0.1[b] | 13.6 ± 6.2[a] |
| **7th week of infection** | *Infected and unexposed* | 22.5 ± 23.7[a] | 1.7 ± 1.4[b,c] | 10.6 ± 3.6[a] |
| | *Infected and exposed* | 7.2 ± 4.3[a] | 1.2 ± 1.4[b] | 10.2 ± 6.8[a] |
| **8th week of infection** | *Infected and unexposed* | 28.8 ± 2[a] | 1.5 ± 0.7[b] | 20.8 ± 17.9[a] |
| | *Infected and exposed* | 0.0 ± 0.0* | 0.0 ± 0.0* | 0.0 ± 0.0* |

a, b = data followed by different letters showed statistical difference ($\alpha$ = 5%) among the groups. *= snails that did not lay eggs in the period analyzed.

**Table 3.** Effect of exposure to $LC_{50}$ of *Euphorbia milii* latex on the number of *Schistosoma mansoni* cercariae shed by *Biomphalaria glabrata* and number of snails at the end the experiment (35 days). The observation started with 10 snails in each group.

| | Control | | Exposed | | Reduction of cercariae shed |
|---|---|---|---|---|---|
| | Number of cercariae | Number of snails at the end | Number of cercariae | Number of snails at the end | (%) |
| **1st week of infection** | 239,157 | 4 | 35,705 | 0 | 85.07 |
| **2nd week of infection** | 12,410 | 5 | 11,430 | 4 | 7.89 |
| **3rd week of infection** | 45,130 | 7 | 30,680 | 3 | 32.01 |
| **4th week of infection** | 87,090 | 4 | 27,900 | 2 | 67.96 |
| **5th week of infection** | 57,130 | 4 | 7,300 | 1 | 87.22 |
| **6th week of infection** | 63,920 | 6 | 6,372 | 1 | 90.03 |
| **7th week of infection** | 79,290 | 5 | 11,040 | 4 | 86.07 |
| **8th week of infection** | 29,360 | 7 | 2,930 | 0 | 90.02 |
| **Total** | **613.487** | **42** | **133.357** | **15** | **78.26** |

reproductive activity of gastropods [11]. In the control group we observed a high number of cercariaeshed and a small reduction of the eggs produced per snail, similarly to that described above. On the other hand, the exposure of eggs and/or miracidia of *S. mansoni* can alter the sporocyst load, causing a small increase in the concentration of schistosomin in the intermediate host's hemolymph. This effect is indicted by the low number of cercariae shed and high reproductive biology of snails in Groups 2, 3 and 4.

The exposure of infected snails to the *E. milii* latex affected the reproductive biology in all intra-mollusk development stages of *S. mansoni*. The reduction in reproductive activity of snails infected with larval trematodes is due to metabolic changes in glucose and glycogen concentrations in the hemolymph, as described by Mello-Silva *et al.* (2010). During development of the parasite in the digestive gland (primary sporocyst → secondary sporocyst), glucose levels in the hemolymph decline, causing increased activity of glycogen at catalytic sites. Mello-Silva *et al.* (2010) compared the carbohydrate metabolism between snails infected or not by *S. mansoni* and exposed to this latex and observed that in infected snails exposed to the molluscicide, the reduction in glucose levels in the hemolymph occurs on the first day after exposure while the glycogen level in the digestive gland only declines after 1 week of exposure. We observed significant reductions in reproductive activity of snails exposed to $LC_{50}$in the first week of infection (55.6%), second week (84.8%) and third week (87.7%), after which the reduction was 100%. The increase and development of sporocysts pose energy needs and the increased activity of carbohydrate catalytic sites affects other events that require the same energy reserves, such as the snail's reproductive biology.

From an epidemiological standpoint, we observed snails infected and not infected with *S. mansoni*, but snails can be infected at different weeks of the parasite's development inside the intermediate host. In this sense, analyzing the effects of exposure to phytochemical products on snail population is very important to better understand the transmission dynamics during control actions

Over the 35 days, a population of 80 infected snails produced more than 613 thousand cercariae and the mortality rate was approximately 58% (**Table 3**). Furthermore, the stress caused by infection by *S. mansoni* in *B. glabrata* significantly reduces the snail's reproductive activity [12]. However, the exposure to $LC_{50}$ of *E. milii* latex of a similar population of 80 infected snails was strongly impacted by exposure. In the same period, the exposed snails produced around 133 thousand cercariae and the mortality was around 85% (**Table 3**), along with a drastic reduction or even interruption of reproductive activity in exposed snails. The numbers of cercariae shed varied according the weeks of infection, but we observed more pronounced reductions in the first,' fourth, fifth, sixth, seventh and eighth weeks of infection. Early in infection by primary sporocysts, severe structural changes occur, causing significant stress to the host snail. This caused mortality of 60% in the control group and 100% in the exposed group, besides reduction of 85% in cercarial shedding. After 18 days, the sporocysts start the process of migration to the digestive gland and after the fourth week of infection become cercariae [13]. Lima *et al.* (2012) observed *B. glabrata* in the patent period of cercariae shedding in *S. mansoni* exposed to the same latex and noted an increase in catalytic activity in the glycidic and protein pathway, with consequent changes in the levels of glucose, glycogen and nitrogen degradation products, changing the pattern of excretion of ureotelic to uricotelic products. In the patent period (fourth, fifth, sixth, seventh and eighth weeks of infection) we observed severe reduction in cercarial shedding with high rates of mortality.

## 5. Conclusion

We can conclude that the use of natural biodegradable compounds containing low concentrations of substances already characterized as having eco-toxicological potential can be an important tool to reduce the transmission of schistosomiasis. The $LC_{50}$ of the *E. milii* latex had toxic action on eggs and miracidia, and the number of cercariae shed by infected snails was approximately 80% less during the study period. Considering the parameters analyzed (survival, reproductive activity and number of cercariae shed), the latex was more toxic to infected snails, regardless of the week of infection, corroborating the hypothesis of selective action of this product.

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## Competing Interests

The author(s) declare that they have no competing interests.

## Authors' Contributions

RCA: Responsible to conception, design, acquisition and analysis of data and the construction of the manuscript.

GF: Have made substantial contributions to acquisition of data.
MCV: Have made substantial contributions to interpretation of data.
MLAR: Have made substantial contributions to interpretation of data.
CCMello-Silva: Have made substantial contributions of design, interpretation and construction of the manuscript.

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