RESEARCH NOTE

# Effects of *Euphorbia milii* Latex on *Schistosoma mansoni* Eggs, Miracidia and Cercariae

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The latex of "Crown-of-Thorns" (*Euphorbia milii* var *hislopii*) is a potent plant molluscicide and a promising alternative to niclosamide (NCL), today`s mostly used molluscicidal compound (MC Vasconcellos & VT Schall *1986 Mem Inst Oswaldo Cruz 81*: 475-476). In addition to being an effective molluscicide, NCL also kills miracidia and cercariae, the two free living stages of *Schistosoma* trematodes. It is active against other helminths as well. Owing to its prominent cestocide activity NCL has been used in human and veterinary medicine as a drug of choice to treat several tapeworm infections (P Andrews et al. 1983 *Pharmac Ther 19*: 245-295). However, the effects of *E. milii* on helminths have not been studied so far. In this study we investigated the effects of *E. milii* latex on *S. mansoni* eggs, miracidia and cercariae. Data on the toxicity of NCL to these developmental stages of *S. mansoni* were also obtained for comparative purposes.

*E. milii* latex was obtained from plants cultivated in the district of "Ilha do Governador", Rio de Janeiro, Brazil, in July-August, 1995. Lyophilized latex and NCL ethanolamine salt (BayluscideWP70â) were dissolved in dechlorinated water. *S. mansoni* eggs, miracidia and cercariae were from the "Belo Horizonte" (BH) strain maintained in *Biomphalaria glabrata* snails and Swiss-Webster mice (WL Paraense & L Corrêa 1989 *Mem Inst Oswaldo Cruz 84*: 281-288).

*Eggs hatching inhibition test* - *Schistosoma* eggs were recovered from infected Swiss mice. Livers were homogenized in NaCl 1.7% w/v. The homogenate was filtered and left to stand for 1 hr. The sediment containing eggs was resuspended in NaCl 1.7% w/v and again left to stand for 1 hr. The procedure was carried out in the dark at 4ºC. The eggs were then incubated in the dark at 26ºC for 2 hr in the presence of latex (10, 25, 50 and 100 mg/l), NCL (0.05, 0.1 and 10mg/l) or dechlorinated water. Solutions were placed under a tungsten lamp (60 W) during 1 hr for egg hatching. Hatching was stopped by adding an alcohol-formalin-acetic acid solution. The proportions of hatched and unhatched eggs were scored in samples of 30 eggs.

*Lethality to miracidia* - The sediment containing eggs was resuspended in dechlorinated water and exposed to artificial light (60W). Owing to their phototaxic behaviour, swimming miracidia flocked to the illuminated side-arm of a 150 ml glass flask where they were collected. thirty miracidia per concentration of latex or NCL were then exposed to testing solutions in multi-well plates. The number of living miracidia was recorded after 2, 3 and 4 hr of exposure to testing solutions. All miracidia were tested within 2 hr after hatching.

*Lethality to cercariae* - Freshly shed cercariae were exposed to testing solutions (200 ± 2 cercariae per concentration) in tissue culture plates (60 x 15 mm) for 4 hr. Evans Blue dye was used to stain dead cercariae. The numbers of living and dead cercariae were recorded. The experiment was repeated three times.

*Effect on cercariae infectivity* - Freshly shed cercariae were exposed to testing solutions for 2 hr in 5 ml assay tubes. Female Swiss Webster mice (4 to 6 weeks-old) had their tails immersed in assay tubes containing 100 pretreated cercariae for 30 min. Ten mice per concentration of latex or NCL were used. The number of cercariae that failed to penetrate the mouse skin was counted, and all infected mice were killed eight weeks later. Adult worms were recovered from mesenteric veins after a portal-hepatic perfusion.

The present study findings suggested that NCL (0.05 mg/l) does not interfere with *S. mansoni* eggs hatching. Approximately 50% of eggs hatched during the 3 hr test period in the control group as well as in NCL - and latex-treated groups. No dif-

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ference between control and treated groups was found. Contrasting with the lack of action on eggs, NCL (0.05 mg/l) proved to be lethal to swimming miracidia (Table I) and cercariae (Table II). No previous investigation of NCL effects on eggs was found in the literature, but its miracidicidal and cercaricidal properties have been observed by several authors. For instance, it was reported that *S. mansoni* miracidia were killed by NCL at concentrations as low as 0.3 mg/l (within minutes) and 0.1 mg/l (after longer exposures) (Andrews et al. *loc. cit*.). More recently, a 4 hr-$LC_{50}$ for NCL miracidicidal effect as low as 0.03 mg/l was obtained by PB Tchounwou et al. (1991 *J Environ Sci Health B26*: 69-82). On the other hand, it was reported that *S. mansoni* cercariae were rapidly killed by NCL at concentrations as low as 0.1-0.2 mg/l (Andrews et al. *loc. cit*). PB Tchounwou et al. (1992 *Environ Toxicol Water Qual 7*: 107-117) also investigated the cercaricidal effect of NCL and found a 4 hr-$LC_{50}$ equal to 0.04 mg/l. In the present study, miracidicidal and cercaricidal effects of NCL were noted at a concentration as low as 0.05 mg/l. It is of note that this concentration of NCL is well below its 24hr-$LC_{50}$ (0.16 mg/l) and 24hr-$LC_{90}$ (0.31 mg/l) for adult *B. glabrata* snails (EC Oliveira-Filho 1995 *Estudo Ecotoxicológico do Látex Moluscicida da Coroa de Cristo, Euphorbia milii var hislopii*, MSc Thesis, Fundação Oswaldo Cruz, Rio de Janeiro, 157 pp.). The data presented in this paper thus suggest that *S. mansoni* free living stages are killed by NCL at concentrations which are not lethal to their intermediate host snails. However, results also showed that, at such a low concentration of NCL (0.05 mg/l), a relatively long exposure period is necessary to induce cercarial deaths. While exposure of cercariae to NCL (0.05 mg/l) for 4 hr resulted in 68.8% mortality rate in one experiment (Table II), exposure for just 2 hr was ineffective in increasing cercarial mortality in the infectivity test (Figure). Neither the cercarial survival, nor the proportion of cercariae that penetrated the mouse skin was affected after a 2 hr exposure to NCL (0.05 mg/l). On the other hand, none of these penetrating NCL-treated cercariae developed into adult worm in infected mice. These findings suggest that NCL does not just kill cercariae, at higher concentrations and longer exposures, but also render most of the surviving ones incapable of developing into adult worms in the final host. Similar results were also obtained by Tchounwou et al. (1992 *loc. cit*.) with a different sample of *S. mansoni*. These authors noted that the percentages of worms recovered from NHI mice infected with cercariae pre-exposed to dechlorinated water alone, and NCL 0.02 mg/l and 0.04 mg/l, were 61.6%, 16.3% and 1.5%, respectively. It should be pointed out that infectivity of their *S. mansoni* sample (untreated controls: 61.6%,) was higher than that observed with the BH strain used in our experiment (untreated controls: 23%). On the other hand, RMF De-Oliveira (1996 *Características Parasitológicas e Perfil Isoenzimático de Amostras de Schistosoma mansoni Sambon 1907*, MSc Thesis, Fundação Oswaldo Cruz, Rio de Janeiro, 112 pp.) obtained a similar infectivity rate (25.5%) with the same BH strain. As demonstrated by RMF De-Oliveira, infectivity in mice of *S. mansoni* cercariae varies with the origin of the worm sample used.

TABLE I

Mortality (cummulative) of *Schistosoma mansoni* miracidia exposed to *Euphorbia milii* lyophilized latex and niclosamide for 2, 3 and 4 hr. Data are shown as percentage (%) of dead miracidia. (N=30 miracidia per concentration)

| Concentration (mg/l) | Duration of exposure (hr) | | |
|---|---|---|---|
| | 2 | 3 | 4 |
| *E. milii* latex | | | |
| 0 | 10 | 20 | 23 |
| 10 | 33* | 63* | 77* |
| 25 | 53* | 63* | 67* |
| 50 | 47* | 57* | 73* |
| 100 | 43* | 57* | 80* |
| Niclosamide | | | |
| 0.05 | 87* | 90* | 100* |

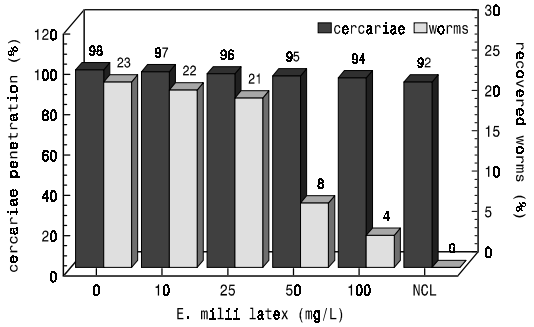
Statistical comparisons were made by the chi-square test. Mortality indices (%) significantly different ($P<0.05$) from those found for dechlorinated-water controls (0 mg/l) are indicated by an asterisk (*).

TABLE II

Mortality of *Schistosoma mansoni* cercariae exposed to aqueous solutions of *Euphorbia milii* lyophilized latex and niclosamide for 4 hr

| Concentration (mg/l) | *S. mansoni* cercariae | | |
|---|---|---|---|
| | Tested (Nr) | Dead (Nr) | Dead/tested (%) |
| *E. milii* latex | | | |
| 0 | 602 | 28 | 4.6 |
| 10 | 606 | 66 | 10.9* |
| 25 | 602 | 53 | 8.8* |
| 50 | 602 | 33 | 5.5 |
| 100 | 605 | 87 | 14.4* |
| Niclosamide | | | |
| 0.05 | 606 | 417 | 68.8* |

Statistical comparisons were made by the chi-square test. Mortality indices (%) significantly different ($P<0.05$) from those found for dechlorinated-water controls (0 mg/l) are indicated by an asterisk (*).




Effects of *Euphorbia milii* latex and niclosamide (NCL) on the infectivity of *Schistosoma mansoni* cercariae. Freshly shed cercariae (BH strain) were exposed to dechlorinated water (0 mg/l), lyophilized latex (10, 25, 50 and 100 mg/l) or NCL (0.05 mg/l) for 2 hr. Mice were next exposed to cercariae for 30 min by the tail immersion technique. On the left (dark columns): % of cercariae that penetrated the mouse skin. On the right (gray columns): % of worms recovered eight weeks later. Data were analyzed by the chi-square test. % of recovered worms was significantly different ($P<0.05$) from controls (0 mg/l) at the two highest concentrations of *E. milii* latex (50 and 100 mg/l) and NCL. No other statistically significant difference was found.

*E. milii* latex (10-100 mg/l) did not show any inhibitory effect on *S. mansoni* eggs hatching. Furthermore, latex proved to be only slightly toxic to miracidia (Table I) and cercariae (Table II). Miracidicidal and cercaricidal effects of latex were tested at concentrations ranging from 10 to 100 mg/l, i.e. concentrations which are 15 to 150 times higher than the 24 hr-$LC_{90}$ (0.67 mg/l) for adult *B. glabrata* snails (Oliveira-Filho *loc. cit.*). Miracidium mortality was found to be somewhat higher in latex solutions than in untreated controls, but no concentration-effect relationship was observed at any exposure time. Moreover, even at the highest concentration tested (100 mg/l), latex was ineffective in achieving a 100% mortality rate during a 4 hr exposure period (Table I). Similar results were obtained with regard to cercarial lethality. In most instances cercarial mortality was slightly higher in latex solutions than in controls, but again no concentration-effect relationship was found (Table II). Even at the highest concentration of latex tested (100 mg/l) mortality rate was as low as 14.4%, a lethal effect well below that obtained with 0.05 mg/l of NCL (68.8%). Contrasting with the absence of consistent lethal effects of latex on swimming cercariae, a concentration-dependent effect was observed in the cercariae infectivity test (Figure). Neither cercarial survival, nor the percentage of cercariae that succeeded in penetrating the mice skin was reduced by latex (10-100 mg/l, for 2 hr). Nonetheless, the percentage of recovered worms was substantially reduced in mice infected with cercariae exposed to 50 and 100 mg/l of latex. This result suggested that *E. milii* latex - at least at the two highest concentrations tested - was toxic to *S. mansoni* cercariae rendering them incapable of developing into adult worms in the final host. From these observations one has to conclude that latex is slightly toxic to *S.mansoni* free living stages at concentrations much higher than those which are sufficient to kill their intermediate host snails. Thus, in contrast to NCL, *E. milii* molluscicidal latex does not present the additional advantage of also affecting the viability of schistosoma miracidia and cercariae.

In conclusion, *E. milii* latex had no effect on *S. mansoni* eggs and was slightly toxic to miracidia and cercariae. NCL, on the other hand, was effective in killing miracidia and cercariae at a concentration which is not lethal to their intermediate host snails. These findings seems to support the view that, in comparison with the reference molluscicide NCL, *E. milii* latex has a narrower spectrum of biocidal actions.

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