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Attenuation of *Schistosoma mansoni* cercarial infectivity to albino mice by methanol extract of some plant species

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ABSTRACT

This study elucidates the activity of certain plants' methanol extract: *Anagallis arvensis*, *Solanum nigrum* (green fruits), *Chenopodium ambrosioides*, *Calendula officinalis* and *Sesbania sesban*, on the infectivity of *S. mansoni* cercariae to albino mice. Then, some parasitological parameters, e.g. the worm load/mouse, number of ova/g tissue in liver and intestine and the developmental stages of ova in the small intestinal wall (Oogram) of infected mice were determined. In addition, certain biochemical parameters of serum from infected mice (total protein, albumin, the activities of AlT, AsT, AcP and AkP enzymes) were, also, recorded.

The results showed that exposure of *S. mansoni* cercariae for 30 min to the tested plants' methanol extract before mice infection has a higher suppressive effect on their infectivity to albino mice then those exposed to this extract during mice infection. The number of worms recovered/infected mouse and the number of ova/g tissue from liver and intestine of mice groups infected with cercariae exposed to the tested plants' methanol extract either pre- or during mice infection were less than those of infected control groups (e.g. the reduction rates of worm load/mouse and number of ova/g tissue in the intestine were 46.1% and 76.8%, respectively, for mice infected with cercariae exposed to 5 ppm of *A. arvensis* during mice infection).

The results, also, indicated that exposing *S. mansoni* cercariae to methanol extract of the experimental plants either pre- or during mice infection reduced the activities of the enzymes AlT, AsT, AcP and AkP that were elevated in mice infected with untreated cercariae, meanwhile, the concentrations of total protein and albumin were increased in the serum of mice infected with these treated cercariae in comparison with those of mice group infected with untreated cercariae.

1. Introduction

Schistosomiasis is a public health problem in many developing countries. An estimated 80% of all infected people are now concentrated in Africa [1]. Water resource schemes for power generation and irrigation have resulted in a tremendous increase in the transmission and out breaks of schistosomiasis in several African countries [2,3].

Study of cercarial population in a natural water body may provide a useful mean for locating schistosome transmission foci and help in evaluating the success of bilharziasis control programs. Plant extracts with molluscicidal and cercicidal properties may provide cheap, locally produced, biodegradable and effective control agents in rural areas of developing countries where schistosomiasis is endemic [4]. Attenuation of *S. mansoni* cercariae with a molluscicide was previously achieved in vitro [5].

Most of the plants screened against schistosomiasis cercariae and miracidia were generally effective at levels less than that of their molluscicidal ones. It was recorded that no *S. mansoni* worms were detected from mice exposed to cercariae previously treated for one hour with 100 ppm of the plant *Anagalli. arvensis* dry powder [6]. *Solanum nigrum*, also, has a suppressive effect on the infectivity of *S. mansoni* cercariae to albino mice [7]. *S. mansoni* miracidia and cercariae were killed by 100 ppm dry powder of *Calendula micrantha* within 2 and 24 h of exposure, respectively [8].

Conyza dioscorides petroleum ether extract was toxic to *B. alexandrina* snails [9]. Later, the plant *Chenopodium ambrosioides* was recorded for ascariasis treatment and reduces more than 95% of the infective larvae of gastrointestinal nematodes of goats at 110.6 mg/ml [10]. The plant *Sesbania sesban* ethanol extract displayed a weak larvicidal activity against both *S. mansoni* miracidia and cercariae [11] .

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During molluscicidal operations in water bodies, some schistosomiasis transmission sites receive sublethal concentrations from the molluscicides under application. Therefore, the present study evaluates the attenuation effect of sublethal concentrations of methanol extract from some plant species on the infectivity of *S. mansoni* cercariae to albino mice.

2. Materials and methods

2.1. Cercariae

Schistosoma mansoni cercariae were from Schistosome Biological Supply Center (SBSC) at Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Egypt.

2.2. Experimental animals

Laboratory Swiss albino mice (*Mus musculus*), 20–25 g were from SBSC, TBRI. They were maintained on standard diet 24% protein content.

2.3. Plants

The five Egyptian plant species used were collected during spring 2007 and 2008. They are *A. arvensis* (Myrsinaceae) from Kafr Saqr, Sharkia Governorate, *S. nigrum* (Solanaceae) from Ashmoun, Menoufia Governorate, *C. ambrosioides* (Chenopodiaceae) and *S. sesban* (Fabaceae) from Nahia, Giza Governorate and *Calendula officinalis* (Asteraceae) from El-Qanater Gardens, Qaluobia Governorate. The plant specimens were shade dried, powdered by an electric grinder, stored in clean dry dark glass bottles.

2.4. Plant's extract

The dry powder of each plant species was extracted by soaking with 95% methanol (0.5 kg/L) for seven days [12]. Then the solvent was filtered and distilled under vacuum and the crude extract residues were stored in clean dry dark vessel till use.

2.5. Cercaricidal activity

Methanol extract of the five experimental plant species was used in the toxicity tests as aqueous solutions against *S. mansoni* cercariae.

A series of concentrations was prepared on basis of weight/volume using dechlorinated tap water. A 25 ml of dechlorinated water containing 100 fresh shed cercariae was mixed with another 25 ml of double concentration from the plants' methanol extract (using different gradual concentrations). During exposure period, stereomicroscopic observations on the cercarial movement and mortality were recorded at successive intervals 15, 30, 45 and 60 min. 50 ml of dechlorinated water containing 100 fresh cercariae were used as control [13]. The effectiveness of the tested plants' methanol extract on *S. mansoni* cercariae was determined [14].

2.6. Infectivity of S. mansoni cercariae to mice post exposure to the plants' extract either pre- or during mice infection

In this experiment, mice were infected with cercariae by tail immersion (80 cercariae/mouse) using two modes of mice exposure for 1 h to the treated cercariae. In the 1st mode, cercariae were exposed for 30 min to sublethal concentrations from the plants' extract pre-mice infection, and then mice were exposed to these cercariae. The 2nd mode includes cercarial treatment with the tested sublethal concentrations during-mice infection with these cercariae. After mice exposure to the treated cercariae, they were maintained in aquaria provided with food and water. Six mice were used for each experimental concentration. Another six mice were exposed to untreated cercariae in each case (infected control).

2.7. Sacrifice and perfusion of mice to detect adult worms

Eight weeks post infection, mice were sacrificed and perfused. The mean number of worms/mouse was considered as an indicator on the infectivity of cercariae to mice in each experiment [15].

2.8. Eggs' developmental stages (Oogram)

The percentages of immature, mature and dead eggs from the small intestinal wall of infected mice were computed from a total of hundred eggs per intestinal segment. Three segments/mouse were examined [16].

2.9. Tissue egg load

The number of eggs/gram tissue (liver and intestine) of infected mice was determined [17].

2.10. Biochemical parameters in serum of mice infected with S. mansoni cercariae

After 8 weeks of mice infection, some serum biochemical parameters (total protein, albumin, aspartate, and alanine transaminases-AsT and AlT- and Acid and Alkaline phosphatases-AcP and AkP) were evaluated spectrophotometrically. Determination of total protein [18], albumin [19], AsT and AlT [20], AcP [21] and AkP [22] were carried out.

2.11. Statistical analysis

The data are presented as mean ± standard deviation (Mean ± SD). The mean groups were compared by analysis of variance. Comparison of means was done by 2-tailed unpaired *t*-test [23]. SPSS computer program (version 13.0 windows) was used in data analyses.

3. Results

It is seen from Table 1 that *A. arversis* methanol extract was the most toxic one to *S. mansoni* cercariae, LC_{100} 20 ppm after one hour of exposure at 25 °C, followed by the extract of green fruits from *S. nigrum* (LC_{100} = 75 ppm) and *C. officinalis* (LC_{100} = 100 ppm). Thereafter, methanol extract of the other tested plant species showed a very low cercaricidal activity as their LC_{100} values ranged from 500 to 1250 ppm after 1 h of exposure.

For infection of albino mice with *S. mansoni* cercariae exposed to methanol extract of the tested plants, it was generally observed that the serum total protein and albumin levels were reduced in the infected mice, while the activities of AlT, AsT, AkP and AcP en-

Table 1
Cercaricidal activity of methanol extract from some Egyptian plant species against Shistosoma mansoni cercariae after 1 h of exposure.

Treatment	Lethal Concentrations (LC_{100} ppm)
Anagallis arvensis	20
Solanum nigrum (green fruits)	75
Calendula officinalis	100
Sesbania sesban	1000
Chinopodium ambrosides	1250