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Short Communication

In vivo and *in vitro* activity of oil extract of garlic (*Allium sativum* Linnaeus) against *Schistosoma japonicum* cercariae

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Abstract

Introduction: The activity of garlic oil extract against *Schistosoma japonicum* cercariae was evaluated. **Methods:** The *in vitro* and *in vivo* cercaricidal activities against *S. japonicum* larvae were determined. **Results:** Exposure to ≥ 10^{-6} (*v*/*v*) garlic emulsions for 30 min led to 100% cercariae mortality; pre-exposure treatment with ≥ 10^{-4} (*v*/*v*) garlic emulsions showed 100% preventive efficacy against *S. japonicum* infection, while pre-treatment with 10^{-5} and 10^{-6} (*v*/*v*) emulsions achieved 20%-40% preventive efficacy and 35.2%-63.6% worm burden reduction. **Conclusions:** Garlic oil extract has activity against *S. japonicum* larvae and a promising preventive efficacy against *S. japonicum* infection.

Keywords: *Schistosoma japonicum*. *Allium sativum*. Cercaricidal activity.

Schistosomiasis japonica, a neglected tropical parasitic disease caused by *Schistosoma japonicum*, is mainly endemic in China, the Philippines, and parts of Indonesia[1]. Infections may occur following contact with the free-swimming *S. japonicum* cercariae that float on water surfaces; therefore, *S. japonicum* infections may be prevented by avoiding exposure to cercariae-contaminated water[1].

Garlic is the bulb of the plant *Allium sativum* Linnaeus, and its extracts have shown antimicrobial, anti-inflammatory, antihypertensive, anticancer, and blood lipid-lowering actions, as well as prevention of atherosclerosis, and improvement of immunologic function[2]. Recently, garlic was reported to be active against *Schistosoma* and its intermediate host snails[3-7]. However, little information is currently available on the cercaricidal action of garlic oil extract[5,7]. Therefore, the purpose of this study was to evaluate the *in vivo* and *in vitro* activity of garlic oil extract against *S. japonicum* larvae.

Female mice of the Institute of Cancer Research (ICR) strain aged 6 to 8 weeks and weighing 20 to 25 g were purchased from Shanghai Laboratory Animal Center (Shanghai, China). All animals were housed in a clean facility and were given free access to food and water. All animal procedures were performed according to the Guidelines for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of the People's Republic of China.

Oncomelania hupensis snails infected with Jiangsu isolate of *S. japonicum* were provided by Jiangsu Institute of Parasitic Diseases (Wuxi, China). The infected snails were transferred to a 100 mL glass beaker containing dechlorinated water under a lamp, and freshly released cercariae were used for the subsequent experiments. Garlic oil was extracted using distillation method[8]. Then, a 0.1mL stock solution of garlic oil extract was transferred to 1.0mL 0.5% Tween-80, mixed evenly, and distilled water was added to a final volume of 10mL to yield a 10^{-2} (*v*/*v*) emulsion formulation. Working solutions at concentrations of 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} (*v*/*v*) were obtained by dilution with dechlorinated water for the subsequent experiments.

For the *in vitro* assay, approximately 100μL garlic emulsions at 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} (*v*/*v*) concentrations were placed on glass coverslips and then freshly released *S. japonicum* cercariae were added, while 0.5% Tween-80 (1:100 dilution in dechlorinated water) and dechlorinated water served as controls. The cercariacidal activity was observed at 1 min, 30 min, 1h, and 4h post treatment using a dissecting microscope and all experiments were repeated in quintuplicate. For *in vivo* experiments, the mouse abdomen was shaved, cleaned, and pre-treated with garlic formulations at 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} (*v*/*v*) for 5 min, prior to exposure. Then, the mice were challenged by applying *S. japonicum* cercariae to their abdomens for 30 min at 40 ± 1 cercariae/mouse, and then they were caged and bred in the Key Laboratory on Technology for Parasitic Disease Prevention and Control, Ministry of Health, People's Republic of China (Wuxi, China). All the mice were

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euthanized by CO_2 inhalation 35 days post infection and subsequently dissected. Worms in the portal and mesenteric veins were recovered, and the worm burden reduction was estimated in the treated and control samples. The experiments were performed in duplicate, and all the data are presented as mean ± standard deviation (SD).

Following exposure to garlic emulsions at concentrations of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} (*v/v*) for 30 min, 1h, and 4h, all the *S. japonicum* cercariae were dead, while treatment with 10^{-2} and 10^{-3} garlic emulsions for 1 min also resulted in 100% mortality. In addition, 98.7%, 14.7%, and 7.8% mortality of *S. japonicum* cercariae was observed following exposure to garlic emulsions at concentrations of 10^{-4}, 10^{-5}, and 10^{-6}, respectively for 1 min, and the median lethal dose (LC_{50}) of garlic oil extract against *S. japonicum* cercariae was 1.9×10^{-5} for 1 min. However, 96.8% and 98.7% of the cercariae remained active in Tween-80 and dechlorinated water 4h post treatment, respectively (**Table 1**).

Pre-exposure treatment with garlic emulsions at concentrations of 10^{-2}, 10^{-3}, and 10^{-4} (*v/v*) resulted in 100% prevention of *S. japonicum* infection in mice in both experiments. However, 60% and 70% infection in the first experiment were observed after pre-exposure treatment with garlic emulsions at concentrations of 10^{-5} and 10^{-6}, respectively, and 80% infection was observed at both concentrations in the second experiment. In addition, pre-treatment with garlic emulsions at concentrations of 10^{-2}, 10^{-3}, and 10^{-4} reduced the worm burden by 100% in both experiments while concentrations of 10^{-5} and 10^{-6} achieved 62.1% and 35.2% worm burden reduction in the first experiment and 63.6% and 38.8% reduction in the second experiment, respectively (**Table 2**).

More than 98% of mature *S. japonicum* cercariae float on water surfaces and human and mammalian animals are infected following contact with cercariae-contaminated water[1]. Currently, niclosamide formulations are the major chemical cercaricides used; however, these formulations have the disadvantages of high cost, toxicity to aquatic animals, and pollution of aquatic environments[9]. In addition, laboratory experiments have shown the promising activity of some plant-derived agents against *S. japonicum* cercariae[10-11]; however, there are currently no commercial plant-derived cercaricides available in China.

Oil extracted from the garlic plant belongs to the class of volatile organic sulfur compounds, which are difficult to dissolve in water[12]. Garlic oil extract is composed of garlicin, sulfides, citric acid, linalool, phellandrene, propylaldehyde, and pentanal[12]. Laboratory and clinical studies have shown the biological activity of garlic oil extract[2]. Furthermore, garlic was found to show activity against various developmental stages of *S. mansoni*[5] and *S. japonicum* larvae[7] and was reported to suppress granuloma formation and ameliorate the histological and histochemical changes caused by *S. mansoni* infection[13]. In addition, garlic showed molluscicidal actions against *Biomphalaria alexandrina* and *O. hupensis*, the intermediate host snails of *S. mansoni* and *S. japonicum*[6-7]. Further studies to assess the toxicity of garlic oil extract against aquatic animals that would likely inhabit the same environment as *B. alexandrina* or *O. hupensis* such as crabs are warranted.

In the current study, exposure to garlic emulsions at concentrations of 10^{-6} (*v/v*) and higher for 30 min induced a 100% mortality of *S. japonicum* cercariae. To evaluate the toxicity of garlic oil extract against *S. japonicum*, the mice were pre-treated with garlic emulsion on the shaved abdomen, followed by an *S. japonicum* cercariae challenge[14]. The *in vivo* assay revealed that pre-exposure treatment with garlic emulsions at concentrations of 10^{-4} and greater showed a 100% inhibition of *S. japonicum* infection in mice while a 5-min pre-exposure treatment with 10^{-5} and 10^{-6} garlic emulsions resulted in 20% to 40% inhibition of *S. japonicum* infection and 35.2% to 63.6% worm burden reduction, respectively. Our findings demonstrate the high activity of garlic oil extract against *S. japonicum* cercariae. In this study, we did not investigate the effective concentration of garlic oil extract required to maintain the infection-free status of mice and the potential toxicity to humans at this concentration. However, previous studies have

TABLE 1

In vitro activity of garlic oil extract against *Schistosoma japonicum* cercariae.

Treatment*	Mortality of cercariae (mean ± SD, %)			
	1 min	30 min	1h	4h
10^{-2} garlic oil extract	100.0	100.0	100.0	100.0
10^{-3} garlic oil extract	100.0	100.0	100.0	100.0
10^{-4} garlic oil extract	98.7 ± 0.8	100.0	100.0	100.0
10^{-5} garlic oil extract	14.7 ± 3.8	100.0	100.0	100.0
10^{-6} garlic oil extract	7.8 ± 2.1	100.0	100.0	100.0
Tween-80	0.0	0.0	1.1 ± 0.1	3.2 ± 1.3
Dechlorinated water	0.0	0.0	0.0	1.3 ± 0.2

SD: standard deviation. * 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} (*v/v*) indicate concentrations of garlic oil extract (volume percent).

TABLE 2

Preventive efficacy of pre-exposure treatment with garlic oil extract against *Schistosoma japonicum* infection in mice.

Pre-exposure treatment*	Experiment 1			Experiment 2		
	infection rate (%)	mean worm burden per mouse (mean ± SD)	worm burden reduction (%)	infection rate (%)	mean worm burden per mouse (mean ± SD)	worm burden reduction (%)
10^{-2} garlic oil extract	0.0	0	100.0	0.0	0	100.0
10^{-3} garlic oil extract	0.0	0	100.0	0.0	0	100.0
10^{-4} garlic oil extract	0.0	0	100.0	0.0	0	100.0
10^{-5} garlic oil extract	60.0	14.1 ± 4.1	62.1	70.0	13.8 ± 2.7	63.6
10^{-6} garlic oil extract	80.0	24.1 ± 3	35.2	80.0	23.2 ± 2.8	38.8
Tween-80	100.0	35.1 ± 3.7	5.6	100.0	36.3 ± 4.5	4.2
Dechlorinated water	100.0	37.2 ± 2.9	-	100.0	37.9 ± 1.9	-

SD: standard deviation.*10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} (*v/v*) indicate concentrations of garlic oil extract (volume percent).

demonstrated that garlic oil extract is nontoxic to humans and no mutagenic effects or deformities have been observed[15].

In conclusion, the results of this study indicate that the oil extract of the edible garlic plant is highly active against the cercariae of *S. japonicum* and may be used as an agent to prevent *S. japonicum* infection. Since garlic oil is insoluble in water, emulsion or ointment formulations of garlic oil extract may be more convenient to use prior to contact with schistosome-infested water in schistosomiasis-endemic areas. Further studies to investigate the field preventive efficacy of garlic oil extract against *S. japonicum* infection are warranted.

Ethical considerations

This study was approved by the Institutional Review Board of Nanjing Medical University Affiliated Wuxi Second Hospital (Permission number: WXEY201300129) and was performed in the Key Laboratory on Technology for Parasitic Disease Prevention and Control, Ministry of Health, People's Republic of China (Wuxi, China).

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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