

# Scolicidal effect of *Allium sativum* flowers on hydatid cyst protoscolices

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**Abstract. – OBJECTIVE:** Because there is no effective and safe drug therapy for hydatid cyst, finding of some new agents especially from herbal origin with a desired scolicidal effect attracts great attention for treatment and pre-surgical use to prevent the hydatid cyst recurrence. In this study, the scolicidal effect of ultrasonic methanol extract of Garlic (*Allium sativum*) flower is investigated.

**MATERIALS AND METHODS:** Protoscolices were collected aseptically from sheep livers containing hydatid cyst and were exposed to different concentrations of extract for various exposure times. The viability of protoscolices was confirmed by 0.1% Eosin staining.

**RESULTS:** The scolicidal activity of extract at a concentration of 50 mg ml<sup>-1</sup> was 59, 76, 81 and 86% after 10, 30, 60, and 180 min of exposure respectively. The scolicidal effect at 100 mg ml<sup>-1</sup> was 67, 78, 85 and 98% after various exposure times, respectively. The results of this study showed that the ultrasonic extract has high scolicidal activity and might be used as a natural scolicidal agent.

**CONCLUSIONS:** Garlic flower extracts is a potent protoscolicidal and might be used in hydatid cyst treatment and pre-surgery to prevent secondary cyst recurrence.

*Key Words:*

*Allium sativum*, Garlic, Protoscolices, Hydatid cyst.

## Introduction

*Echinococcus granulosus* is found in most sheep and cattle raising areas of the world and there are wild carnivore/wild herbivore cycles. Hydatid disease remains an important, challenging medical problem. *Echinococcosis* in humans and animals is an economic and public health concern in many parts of the world. The disease is endemic in the Mediterranean area, in the Middle East, the Baltic areas, South America, India, Northern China and other sheep-raising areas; however, considering the increased travels and tourism all over the world, it can be found any-

where, even in developed countries<sup>1</sup>. Its serious nature and the difficulty of cure make it an important public health problem in many countries. Symptoms and signs are those of slowly increasing pressure in the area of the cyst, resembling a slowly growing tumor. Surgery is still the most common form of treatment, particularly when the cysts are large and in a site such as the brain or heart<sup>2</sup>. Avoiding spillage of the cyst contents and the use of effective scolicidal agents are essential to lowering the recurrence rate. Currently, many medications are available to treat Hydatid cyst such as Albandazole, Mebendazole, Furazolidone and Nitazoxanide. These drugs can cause undesirable side effects also failures in treatment are frequently reported<sup>3,4</sup>. Therapeutic plants are the traditional treatment of disease and it was suggested by WHO<sup>5</sup>. The use of therapeutic plants by people in developing countries is common because these products are safe, available at low cost.

The genus *Allium* (Alliaceae) contains 600 to 700 species, but only a few species have been domesticated so far as vegetables, spices, or ornamental plants. We have recently reported biological activities of *A. paradoxum* and *A. scabriscapum*<sup>6-8</sup>. Garlic (*A. sativum*) has been shown to be antibacterial, antiviral and antifungal, as well as possessing both antitumor and antithrombotic properties<sup>9</sup>. There are some reports for scolicidal effects of *A. sativum* cloves<sup>2,9</sup>. In this present study, ultrasonic extracts from garlic flower were evaluated for their in vitro scolicidal effects. This is the first report on the scolicidal activity of garlic flower.

## Materials and Methods

### *Collection of Protoscolices*

Protoscolices of *E. granulosus* were aspirated from the infected livers of sheep slaughtered at Mazandaran slaughterhouse in Iran. The hydatid

fluid was transferred into glass cylinders under sterile condition and left to set for 30 min. The protoscolices settled down at the bottom of the cylinders. The supernatant was removed and the sedimented protoscolices were washed three times with normal saline. The viability of the protoscolices was confirmed by their motility under a light microscope and by Eosin 0.1%. The live protoscolices were finally transferred into a dark container containing normal saline solution and stored at 4°C for further use.

### **Plant material and Preparation of Ultrasonic Extract**

Garlic flower was obtained in spring of 2012 from Sari, Iran. Plant material was dried under dark condition at room temperature for 2 weeks. The dry material was milled to obtain 2-3 mm particles. Samples were extracted with methanol in an ultrasonic cleaning bath by indirect sonication at a frequency of 100 kHz and a temperature of  $25 \pm 3^\circ\text{C}$  for 1 h to yield ultrasonic extracts. The extracts were then separated from the samples residue and concentrated in a rotary evaporator until a crude solid extracts were obtained which were freeze-dried for complete solvent removal.

### **Effect of Extract on Protoscolices**

Four concentrations (10, 50, 100 and 200 mg ml<sup>-1</sup>) of extract were used for 10, 30, 60 and 180 min. 2 ml of each concentration was placed in test tubes, to which 10,000 washed protoscolices was added. The contents of the tubes were gently mixed. The tubes were then incubated at 37 °C for 10, 30, 60 and 180 min. At the end of each incubation time, the upper phase was carefully removed so as not to interrupt the protoscolices. Two milliliters of 0.1% eosin stain was then added to the remaining settled protoscolices. The remaining pellet of protoscolices was, then,

smear on a glass slide, covered with a cover glass and examined under a light microscope. The percentages of dead protoscolices were determined by counting a minimum of 250 (usually more than 500) protoscolices. Non treated protoscolices were considered a control group in each experiment. The experiments were performed in triplicate.

### **Viability Test**

In the present study eosin stain with the concentration of 0.1% (in distilled water) was used to check the viability of the protoscolices. The protoscolices with no absorbed dye were considered potentially viable; otherwise, they were recorded as dead.

### **Statistical Analysis**

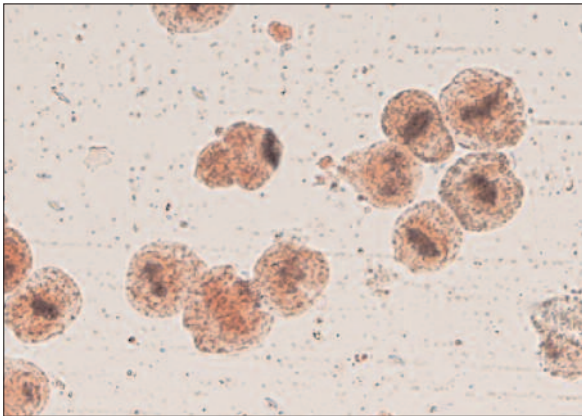
Statistical analysis was performed using GraphPadInStat software (Graph Pad Software, La Jolla, CA, USA). Differences between test and control groups were analyzed by  $\chi^2$  test. A  $p$  value less than 0.01 were considered significant.

## **Results**

The inhibitory capacity of ultrasonic extract of garlic flower was assayed using eosin 0.1%. The scolicidal effect of extract are summarized in Table I. Extract killed 53, 59, 67 and 69 % of cysts after 10<sup>th</sup> min at the concentration of 10-200 mg ml<sup>-1</sup>. Only 7% of untreated (control group) were died. Extract killed 69, 86, 98 and 100 % of the cysts after 180<sup>th</sup> min at the concentration of 10-200 mg ml<sup>-1</sup> (Figure 1). At the same time, only 9% of untreated (control group) were died. The scolicidal effect of any concentrations of ultrasonic extract of garlic flower was extremely significant compared to the control groups at all exposure times ( $p < 0.0001$ ). The

**Table I.** Effect of ultrasonic extract of garlic flower extract at different concentrations (10-200 mg ml<sup>-1</sup>) on *G. lamblia* cyst following various exposure times.

Time (min)	Mortality rate (%) at 10 mg/ml	Mortality rate (%) at 50 mg/ml	Mortality rate (%) at 100 mg/ml	Mortality rate (%) 200 mg/ml	Mortality rate (%) Control
10	53	59	67	69	7
30	63	76	78	81	7
60	68	81	85	87	8
180	69	86	98	100	9



**Figure 1.** Cysts were stained by eosin 0.1%. No-stained cysts were considered potentially viable and stained cysts recorded as dead. Extract killed all cysts at 180th min at the concentration of 200 mg ml<sup>-1</sup>.

results of our study indicated that this methanolic extract of garlic flower showed high scolicidal activity *in vitro*. Our results showed that as the concentration increased, the number of viable Protoscolices decreased. Moreover, if concentration being kept constant, longer exposure period decreases the number of viable cysts.

## Discussion

Various experimental studies have investigated the scolicidal effects of hypertonic saline, cetrimide, ethyl alcohol (95%), H<sub>2</sub>O<sub>2</sub> and 10% providone iodine, albendazole, chlorhexidine gluconate, honey, *Sambucus ebulus*<sup>10</sup> and *Allium sativum*<sup>2,9</sup>. Many of these scolicidal causes undesirable complications that limit their use. Hypertonic saline is one of the most common scolicidal agents<sup>11</sup>. A 3-6.5% saline is ineffective, even after 60 min. Complete (100%) *in vitro* scolicidal activity has been reported for 20% hypertonic saline at the end of 6 min, 15 min, and 45<sup>th</sup> min<sup>11</sup>. 100% scolicidal activity of albendazole sulfoxide has been reported<sup>12</sup> at concentrations of 100 mg ml<sup>-1</sup>. A rapid and complete scolicidal effect with no local or systemic side effects and also low cost are some properties of an ideal scolicidal solution. From this point of view, no ideal scolicidal agents have been described yet.

*Allium* spp. have been used as herbal medicine for thousands of years for different medical purposes<sup>7,9</sup>. Methanolic extract of *A. sativum* cloves at 25, 50 mg ml<sup>-1</sup> has been killed 100% of protoscolices following 60 and 10 min of application<sup>2</sup>.

Protoscolicidal activity of aqueous, chloroform, and hydroalcoholic extracts of garlic have been reported<sup>9</sup>. At the concentration of 50 mg ml<sup>-1</sup>, hydroalcoholic extract showed 92% scolicidal activity at 60<sup>th</sup> min of exposure. Our flower extract at the same time and concentration killed 81%. The results of our study revealed that the flower also have high scolicidal activity. Searching for an alternative new safe and effective treatment is extremely important because species are becoming resistant and synthetic pharmaceuticals often have severe side effects.

## Conclusions

Ultrasonic extract of garlic flower seems to be a good anti-giardial candidate for Echinococcosis treatment. However, some *in vivo* studies regarding to its effects on animals and human should be investigated.

## Conflict of Interest

The Authors declare that there are no conflicts of interest.

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