

Scolicidal effects of squash (*Corylus* spp) seeds, hazel (*Curcubia* spp) nut and garlic (*Allium sativum*) extracts on hydatid cyst protoscolices

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Background: Because there is no effective drug therapy for hydatid cyst yet, assessment and 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. Hazelnut, squash seeds and garlic chloroformic and hydro-alcoholic extracts' scolicidal effects were examined. **Materials and Methods:** Suspension of protoscolices was obtained from infected liver and or lung of sheep and goats from Ziyaran abattoir. The chloroformic and hydro-alcoholic extracts from hazelnut, squash seeds and garlic were extracted using the succinate method. Scolicidal effect of each extract assessed in different concentrations and effected time using microscopy and 0.1% eosin solution stained only killed protoscolices. **Results:** Present study showed that garlic had more potent scolicidal effects among all the 3 plants and the chloroformic extract of garlic was the most potent protoscolicid among all of the extracts and killed 98% of protoscolices in 50 mg/ml on a minimum of 20 minutes exposure. **Conclusion:** Garlic chloroformic extract is a safe and potent protoscolicid and might be used in hydatid cyst treatment and pre-surgery to prevent secondary cyst recurrence.

Key words: Chloroformic, cystic echinococcosis, extract, garlic, hazelnuts, hydro-alcoholic, protoscolicid effect, squash seed

INTRODUCTION

Protoscolex, the larval stage of a Cestoda worm, *Echinococcus granulosus* in herbivores and human (as intermediate host) produces cystic echinococcosis (CE). The preferred method for treatment of CE is surgery, although it increases the risk of intra-operative spillage of protoscolices.^[1] In approximately 10% of the postoperative cases, new cysts will reproduce almost due to hydatid fluid spillage. This may also lead to the secondary hydatidosis in the site of operation.^[2] There is a severe need to some safe and more effective scolicids for killing or inactivation of protoscolices in CE surgeries.^[3-8]

Garlic or *Allium sativum* - a member of *Liliaceae* family has been reported as the source of some antihelminthic substances and hazelnut and squash seeds as well.^[9-11] Garlic is a well-known and important dietary and medicinal plant with several useful properties.^[9-20] For

instance, its antioxidant and antitumor,^[14] antiviral,^[15-17] antifungal,^[18,19] antibacterial,^[19,20] antiprotozoal,^[21,22] antihelminthic^[9-11] and antiprotoscolex^[23,24] effects have been reported.

There are some folkloric believes about the correlation between eating of squash seeds and exertion of some cestoda especially in members of *Taenidae* family, like *Taenia saginata* in old Iranian medicinal culture, so pumpkin and *Taenia saginata* are called "Kâdu" and "Kerm-e Kâdu" in Persian (Kadu = pumpkin and Kerm = worm), respectively due to the observation of exerted *Taenia saginata* and probably *Hymenolepis nana* subsequent of eating pumpkin seeds in some infected individuals.

The present study was designed to evaluate the *in situ* scolicidal effects of (i) hydro-alcoholic extract, (ii) chloroformic extract, (iii) 1:1 mixture of both extracts of squash (*Corylus spp*) seeds, hazel (*Curcubia spp*) nut and garlic (*Allium sativum*).

MATERIALS AND METHODS

Collection of protoscolices

Protoscolices of *Echinococcus granulosus* were obtained from the infected livers of sheep and goats slaughtered at Ziyaran abattoir in Qazvin, central Iran. The hydatid fluid aspirated by a 20 ml syringe and aseptically

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transferred into an Erlen Meyer flask was left to set for 30 min for protoscolices to settle down. The supernatant was discarded and the protoscolices were washed 2 times with PBS (pH 7.2) solution. The viability of the protoscolices was confirmed by their flame cell motility and impermeability to 0.1% eosin solution under a light microscope.

Preparation of extracts

The extracts were prepared as follows: Small slices of garlic were taken and dried under shade, and powdered mechanically using an electrical blender. The squash seeds completely and the edible part of hazelnut were powdered mechanically. Hydro-alcoholic and chloroformic extracts of *Corylus*, *Curcubia* spp. and *Allium sativum* were extracted using the succinate method. After evaporating the solvent completely, a 50 mg/ml suspension in de-ionized water of each extract was prepared as the working mixture.^[24]

Treatment method

The amount of 100 µl of sheep/goat hydatid fluid after preparation, containing 1.5×10^3 protoscolices per/ml, was treated with the same volume of (i) hydro-alcoholic extract, (ii) chloroformic extract, (iii) 1:1 mix of both extracts, (iv) saturated salt (NaCl) solution as a positive control and (v) hydatid fluid as a negative control, respectively in separate 1 ml micro tubes. The micro tubes were incubated at 37°C.

With 20-minute intervals, a 20 µl sample was taken from each tube and transferred to the wells of a glass plate containing 20 µl of 0.1% (w/v) eosin in water.^[24]

Viability test

In the present study, eosin solution with a concentration of 0.1% (1 g of eosin powder in 1000 ml distilled water) was used to check the viability of protoscolices. Fifteen minutes after exposure to the stain, protoscolices that excluded the eosin dye were considered potentially viable otherwise, they were recorded as dead [Figure 1].

The scolicidal effects of different extracts were assessed by

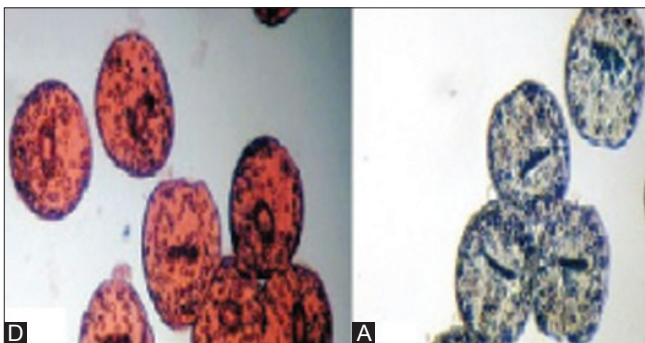


Figure 1: Alive (A) and dead (D) protoscolices were evaluated after 0.1% eosin treatment

calculating before and after 60 minute treatment death ratios (R1 and R2). Each ratio was equal to the number of dead protoscolices divided by all of the dead and alive number multiplied by 100.^[23,24]

Data analysis

The experiments were done in triplicate and their average percent of protoscolex death were calculated and considered for each extract. The significant differences for all extracts were tested against the negative control ($\alpha < 0.05$).

RESULTS

The results of all 6 different extracts of 3 plants in 60 minutes - the time during which albendazole has maximum scolicidal effect (99%) - are summarized comparably in Table 1. It shows that all the extracts have some scolicidal effects but garlic extracts (chloroformic) have a potent effect compared with albendazole - a drug of choice for chemotherapy against hydatid cyst (98% against 99%, respectively).

The rate of scolicidal effect against the time of exposure at 37°C is shown in Table 2. All protoscolices were killed upon 60 minutes of exposure to 50 mg/ml (final concentration) of *Allium sativum* extract. *Allium sativum* extracts have the maximum killing effect on protoscolices among all the extracts. It killed all the scolices at the concentration of 50 mg/ml or less in about 30 minutes. Table 2 shows the scolicidal activity of *Allium sativum* extract at the concentration of 50 mg/ml, which was close to 100% after 60 minutes of treatment.

The results of this study revealed that the chloroformic extract of garlic (*Allium sativum*) has the maximum protoscolicidal effect among all the extracts in the same

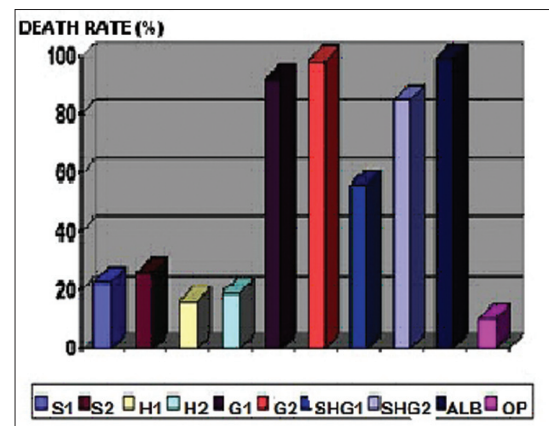


Figure 2: The scolicidal effects of different extracts and controls under the same condition; S1: Squash seeds hydro-alcoholic extract; S2: Squash seeds chloroformic extract; H1: Hazelnut hydro-alcoholic extract; H2: Hazelnut hydro-alcoholic extract; G1: Garlic hydro-alcoholic extract; G2: Garlic chloroformic extract; SHG1: Mixture of equal parts of 3; hydro-alcoholic extracts; SHG2: Mixture of equal parts of 3; chloroformic extract; ALB: Albendazole; P: Only protoscolex

concentration and treatment time, although other extracts also have some effects. The effect of chloroformic extract of garlic is similar to that of albendazole and may be used as an alternative for it [Figure 2].

DISCUSSION

There are a lot of documents in recent and ancient Iranian medical texts about several de-worming materials for humans and domestic animals.^[23,24] This study takes some ideas from those documents. However, almost all of these evidences are historical and/or traditional.

Based on our searches, we couldn't find any special study on the protoscolicidal effect of squash seeds and hazelnuts, although they have some other medicinal effects. As finding a favorite scolicide has been an essential query in treatment of CE, there are several experimental studies for finding such scolicides. For instance, hypertonic saline,^[25,26] silver nitrate,^[27] albendazole,^[28,29] chlorhexidine gluconate^[30,31] and honey^[32] were examined. From this point of view, no ideal scolicidal agents have been described yet. Many of these scolicidal

agents may cause undesirable complications that limit their use, because they need a higher concentration or longer time to have a notable effect. For instance, a 3% solution of NaCl has no scolicidal effect even after 60 minutes but its saturated solution kills 100% scolices in less than 5 minutes.^[25-27]

We investigated the potency of hydro-alcoholic and chloroformic squash seeds, hazelnuts and garlic extracts on the scolices of hydatid cyst. The results of our study showed that garlic has a high scolicidal activity at a concentration of 50 mg/ml after 60 minutes with a significant priority to 2 other plants. One of the most important medicinal plants in a long time in human history is garlic.^[18-24] Furthermore, previous studies have revealed the antiprotozoal^[21-24] and scolicidal activities of the garlic extract.^[23,24] New effective alternative treatment is extremely important nowadays due to emergence of resistance to a variety of antiparasitic drugs, as well as the remarkable side effects and re-emergence of using medical plants.^[25-27,29] Sadjjadi *et al.*^[23] investigated the protoscolicidal activity of aqueous, chloroformic and hydro-alcoholic extracts of garlic. They concluded that the chloroformic extract of garlic had a 97.9% scolicidal effect at a concentration of 200 mg/ml after 30 minutes of exposure. Moazeni observed a higher scolicidal effect (100%) with methanolic extract of garlic at a lower concentration (50 mg/ml) and in a shorter exposure time (10 minutes).^[24] The results of these *in vitro* studies are in agreement with our findings and showed that extracts of garlic are effective scolicidal agents and therefore may be used in hydatid cyst surgeries. However, the *in vivo* efficacy of this extract remains to be explored. Even though garlic is edible, its possible side effects when used as a scolicidal agent need more investigation.

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Table 1: Comparative scolicidal effects of different extracts with a constant concentration (50 mg/ml) after 60 minutes

Extracts with concentration of 50 mg/ml in 60 minutes	R1	R2
Saturated NaCl solution (positive control)	4	100
Squash seeds hydro-alcoholic extract	4	23
Squash seeds chloroformic extract	4	26
Hazelnut hydro-alcoholic extract	5	16
Hazelnut chloroformic extract	4	19
Garlic hydro-alcoholic extract	6	92
Garlic chloroformic extract	5	98
Mixture of equal parts of 3 hydro-alcoholic extracts	5	56
Mixture of equal parts of 3 chloroformic extracts	7	85
Albendazole (a recommended drug for cyst chemotherapy)	6	99
Protoscolex only (negative control)	6	10

R1 = Dead protoscolices at beginning of treatment/total number of protoscolices × 100,
R2 = Dead protoscolices after treatment/total number of protoscolices × 100^[23,24]

Table 2: The effects of constant concentration (50 mg/ml) of different extracts on protoscolices in 20, 40 and 60 minutes of treatment in 37°C

Extract solution (50 mg/ml) final concentration	Dead scolices at the beginning of treatment (%)	Dead scolices after 20 min (%)	Dead scolices after 40 min (%)	Dead scolices after 60 min (%)
Squash seeds hydro-alcoholic	10	16	21	33
Squash seeds chloroformic	10	18	23	36
Hazelnut hydro-alcoholic	10	20	29	44
Hazelnut chloroformic	10	23	29	47
Garlic hydro-alcoholic	10	40	75	92
Garlic chloroformic	10	45	84	98
Albendazole - a drug of choice for cyst chemotherapy	10	65	92	99
Only protoscolices (negative control)	10	11	13	14
Saturated NaCl solution (positive control)	10	100	100	100

The most scolicidal effects are highlighted in bold

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