

In vitro Lethal Effects of *Amygdalus eburnea* Spach Extract Against Hydatid Cyst Protoscoleces

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ABSTRACT

The goal of the present investigation is the *in vitro* scolical effects of *Amygdalus eburnea* Spach. Extract against protoscoleces of *Echinococcus granulosus*. Obtained protoscoleces from the animal (sheep) hydatid cysts were treated with some concentrations of the extract (87.5-700 mg/mL) for 10-60 min. the mortality rate of protoscoleces was assessed using the eosin exclusion test (0.1% eosin staining). The *A. eburnea* extract at the concentrations 700 mg/mL entirely killed protoscoleces after 10 min. Furthermore, the *in vitro* findings demonstrated that *A. eburnea* extract at the concentration of 350 mg/mL destroyed all protoscoleces after 20 min of incubation. Other concentrations of *A. eburnea* exhibited weaker activity against protoscoleces of *E. granulosus*. The results of this study indicate that the *A. eburnea* extract has protoscolicidal properties. Therefore it can be used as a natural scolical agent in the treatment of hydatid cyst during surgery. However, supplementary studies for further investigation of its efficacy and side effects in laboratory animals are necessary.

Key words: Hydatid cyst, *Echinococcus granulosus*, Protoscoleces, Plant

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INTRODUCTION

Hydatid disease as a widespread parasitic infection caused by the larval form of the *Echinococcus granulosus*. Due to its high epidemic, especially in developing countries, this disease annual brings a lot of health and economic damage to the human community [1,2]. Hydatidosis is transmitted from the wild and domestic dog and other carnivores to humans, by eating food, water, or fruit/vegetables contaminated with the eggs of this parasite [3]. After entering the body and penetrating the intestine, the parasite passes through the vascular or lymphatic system to the liver and lungs, and less commonly to other tissues. Although the liver is the main location of this parasite; however, there are also reports of contamination of other body organs such as the lungs, brain, muscle, spleen, etc. [4,5]. The onset of this disease is not usually associated with certain symptoms, and the occurrence of clinical signs may take years, depending on the location and size of the cysts [6,7].

The noteworthy point in the field of clinical management is that the treatment of different cysts requires diverse

methods. For example, in cases where cysts are enlarged or formed in vital organs and there is a risk of tearing, surgical procedure is selective treatment. Tiny and inactive cysts, if detected, are often treated with the drug of choice for the family of benzimidazoles; whereas these medicines are not free of side effects [8,9].

On the other hand, the fear of leakage of the contents of the cysts (protoscoleces) during surgery, the relapse of the disease in the organ involved, anaphylaxis shock and even death in severe cases, poses a serious risks to the surgical procedure [10]. Therefore, surgeons use chemical protoscolicidal materials such as 20% hypertonic saline, Ag-nitrate, and cetrimide to reduce these risks throughout surgery. However, according to researches, necrosis, fibrosis and impairment of performance of liver and gallbladder are examples of the dangerous side-effects of these chemicals that cannot be ignored [11-14]. So, based on what was said, achieving new scolical agents is essential to maintain and increase human health.

Plant resources have always been considered by traditional medicine because of their abundant diversity and their marvelous properties in the treatment of diseases [15]. *A. eburnea* Spach which is called "Ghosk" among Persian-speakers, is a plant of the *Rosaceae* family.

The species that are in this family are often known as almond and distributed in various parts of Europe to Asia and southern of Iran. *A. eburnea* is phenotypically a tree or small shrub, often without thorn, or with little thorns [16]. In addition to its role in controlling soil erosion and stabilized watersheds in semi-desert districts, this plant has long been used in the traditional medicine of Iran as an anti-worm, a treatment for burns and analgesics to reduce digestive and respiratory illnesses. New studies have also achieved to anti-oxidant, anti-bacterial, anti-fungal and anti-dermatophyte properties of this plant in modern medicine [17-21].

As stated above, considering the many attributes of this plant, the purpose of this study was to investigate the effect of protoscolicidal of *A. eburnea* extract against hydatid cyst protoscoleces *in vitro*.

MATERIALS AND METHODS

Plant collection

In April 2013, *A. eburnea* plant was collected from the rural regions of Shahr-e-Babak located in southeastern of Iran and were approved the plants by a botanist from of the Botany Department of Shahid Bahonar University, Kerman, Iran. A coupon specimen of plant materials for storage was given to the Herbarium of Department of Pharmacognosy, School of Pharmacy, Kerman University of Medical Science.

Preparation of the methanolic extract

Extraction was done on 100 g of powdered plant materials using penetration technique, methanol 80% and water at room temperature for 72 hours. The extract was passed through the filter paper for separating the desired materials from waste. Finally, the concentration of the extract was performed using a rotary evaporator in a vacuum at 50°C and kept at -20°C, until use [22-24].

Collection of protoscoleces

Protoscoleces were collected from the liver of sheep that were naturally infected with hydatid cysts by referring to slaughterhouses in Kerman. Methods for obtaining protoscoleces are described according to the methods described elsewhere [25]. The number of protoscoleces per mL was attuned to 3×10^3 protoscoleces in a 0.9% NaCl solution with an at least 90% viability rate.

In vitro protoscolicidal activity

To do this, in each test tube, we added 0.5 mL of the protoscoleces solution plus 0.5 mL of various concentrations of the extract (87.5, 175, 350, and 700 mg/mL) and then incubated at 37°C for 10, 20, 30, and 60 min. After this time, and removing the upper phase, 50 µl of 0.1% eosin stain was added to the remaining pellet of protoscoleces; in last step, smeared on a glass slide, covered with a cover glass and examined under a light microscope. The mean percentages of dead protoscoleces were recorded *via* counting 100 protoscoleces (Figure 1) [22].

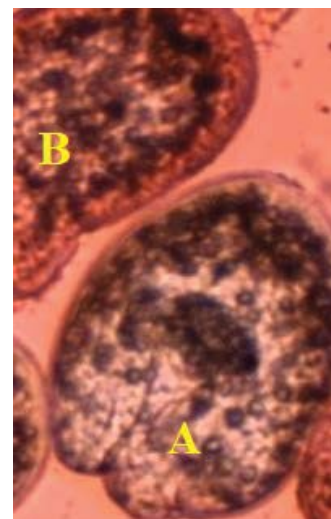


Figure 1: A) Live protoscoleces and B) Dead protoscoleces after exposure with various concentrations of *A. eburnea*

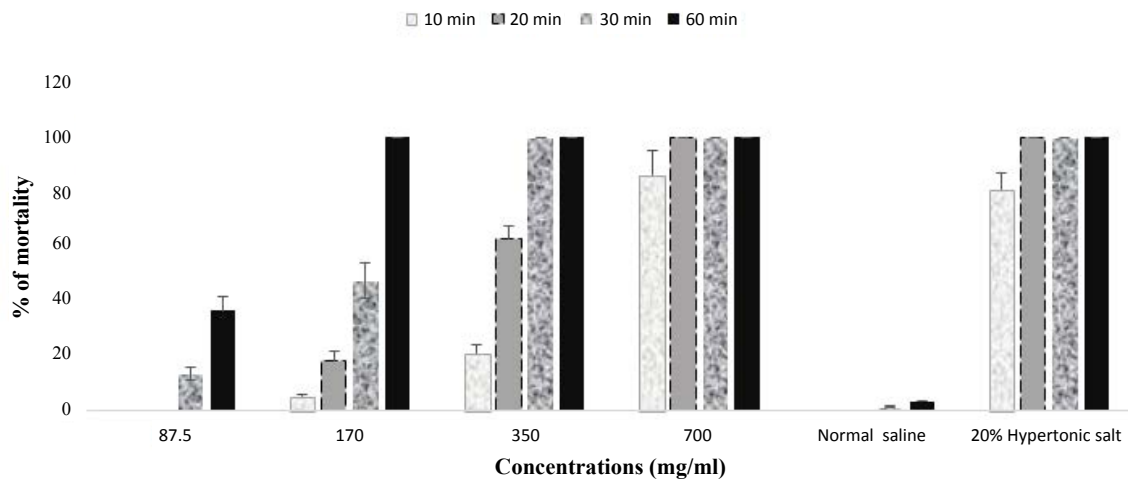


Figure 2: *In vitro* scolicidal effects of various concentrations of *A. eburnea* extract against protoscoleces of *E. granulosus* at different exposure time.

Statistical analysis

All the tests were performed in triplicate in the present study. Acquired data were analyzed by SPSS 17.0 statistical package. The difference between the test and control groups was determined by t-test and $P < 0.05$ was statistically significant [22].

RESULTS

Here we evaluated the *in vitro* lethal activity of *A. eburnea* extract at the different concentrations (87.5, 175, 350, and 700 mg/mL) against protoscoleces of *E. granulosus* for 10, 20, 30, and 60 min, respectively. Figure 2 shows the *A. eburnea* extract at the concentrations 700 mg/mL entirely killed protoscoleces after 10 min. Furthermore, the *in vitro* findings demonstrated that *A. eburnea* extract at the concentration of 350 mg/m destroyed all protoscoleces after 20 min of incubation. Other concentrations of *A. eburnea* exhibited weaker activity against protoscoleces of *E. granulosus*. These results indicating that the *A. eburnea* extract at all of above concentrations showed significantly higher ($p=0.001$) scolical effects than the control group.

DISCUSSION

Although there are drug treatments and techniques such as PAIR (Puncture Aspiration Injection Re-aspiration), many studies suggest surgery as the most appropriate treatment for hydatid cysts [26,27]. However, the recurrence and sometimes diffusion of the disease in the tissues adjacent to the organs involved, due to the leakage of protoscoleces, is one of the main concerns of surgeons during surgery [10]. To this end, different protoscolicidal use to minimize these risks. However, it should not ignore the warnings of new research on dangerous and sometimes irreparable complications of conventional chemical scolicals. For example, hypertonic saline can be associated with complications such as bile duct stenosis and Caustic sclerosing cholangitis [28]. According to the WHO reports, a good protoscolical, is a fast-acting, low-toxic, with highly efficacy and the least side effects substance. It should also be able to preserve its properties when diluted in hydatid fluid [29,30].

Given the well-known capabilities of herbal medicines, it may be possible to access a scolical agent from the plant sources described above. In this study, for the first time, we examined the scolical effects of *A. eburnea* extract against *E. granulosus* protoscoleces. Different concentrations of the extract were exposed to the protoscoleces from the infected sheep liver. Based on the obtained results the *A. eburnea* extract at the concentrations 700 mg/mL entirely killed protoscoleces after 10 min. Furthermore, the *in vitro* findings demonstrated that *A. eburnea* extract at the concentration of 350 mg/m destroyed all protoscoleces after 20 min of incubation. Other concentrations of *A.*

eburnea exhibited weaker activity against protoscoleces of *E. granulosus*.

Regarding the antimicrobial activity of *A. eburnea*, in studies conducted by Rezaeifar et al. The antibacterial anti-fungal effects of this plant against some bacterial pathogenic strains such as *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *Candida*, *Aspergillus* and *Cryptococcus* species have been proven [17,21].

Other studies have also been conducted to investigate the effects of protoscolical in other plants. Feizi et al. studied the scolical effects of Ginger (*Zingiber officinale*) and Artemisia (*Artemisia aucheri*) extracts on hydatid cyst protoscoleces *in vitro* [31]. In a similar study, Jafari et al. identified the *Punica granatum* aqueous extract as an effective protoscolical in the animal model and *in vitro* [32].

The results of the study by Salehi et al. showed that the scolical effect of aqueous and hydro-alcoholic extract of *Berberis vulgaris* fruit is significant at low dilution and low time [33]. Moreover, the effects of protoscolical a number of common herbal medicines such as *Nigella sativa*, *Pistacia vera*, *Pistacia khinjuk*, *Pistacia atlantica*, *Bunium persicum*, *Myrtus cumminus* have been proven [34-39].

CONCLUSION

The results of this study indicate that the *A. eburnea* Spach extract has protoscolical properties. Therefore it can be used as a natural scolical agent in the treatment of hydatid cyst during surgery. However, supplementary studies for further investigation of its efficacy and side effects in laboratory animals are necessary.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

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