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Evaluations of protoscolicidal activity of *Cardamom* extract against hydatid cyst protoscoleces

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ABSTRACT

The present study aims to evaluate the scolicidal effects of Elettaria cardamomum L. (cardamom) extract on the protoscoleces of *hydatid cysts on an in vitro model. Protoscoleces were aseptically aspirated from the livers of naturally infected sheep. Various concentrations of extract were used for 10-60 minutes. Eosin exclusion test was used to determine the viability of protoscoleces. Findings showed that extract at the concentrations of 200 and 100 mg/mL killed 100% protoscoleces after 10 and 20 minutes of exposure, respectively. Obtained results in this investigation for the first time demonstrated that cardamom might be a natural source for the production of new scolicidal agents.*

Key words: Cystic echinococcosis; Prtotoscoleces; Extract

INTRODUCTION

Cystic echinococcosis (CE) is the larval cystic stage (called echinococcal cysts) of a small taeniid-type tapeworm (*Echinococcus granulosus*) that may cause illness in intermediate hosts, generally herbivorous animals and people who are infected accidentally [1]. Nowadays, the main treatment modality for CE is surgery; however, other methods such as chemotherapy with benzimidazoles and PAIR (puncture, aspiration, injection and reaspiration) are present as alternative treatments [2]. During CE surgery, use of effective scolicidal agents is necessary to reduce the risk of intraoperative spillage of the cyst contents (protoscoleces) and subsequently recurrence of CE and secondary infection [3, 4]. Current chemical scolicidal agents have demonstrated serious side effects including liver necrosis and sclerosane colangitis [5]. This shows, the necessity of development of new scolicidal agents with low side effects and more efficacies.

From the past decades, natural products and their compounds have been the most productive source for new drug development [6, 7]. *Elettaria cardamomum L. (*cardamom*)* belonging to the Zingiberaceae family is a traditional native spice from the humid Asian areas [8]. Different pharmacological activity such as, anti-inflammatory, analgesic, and antispasmodic effects have been related to this plant [8]. Moreover, in the folk medicine the plant has been used as gut modulatory, blood pressure lowering, diuretic, sedative, gastroprotective, antimicrobial, antiplatelet, antioxidant, and anti-cancer effects [8-10].

The present work aims to evaluate the scolicidal effects of Cadamom extract against the protoscoleces of hydatid cysts on an *in vitro* model.

MATERIALS AND METHODS

Plant materials

Dry cardamom seeds were purchased from the market. The plant materials were identified by Dr. Mirtajaldin, a botanist at Department of Botany, Shahid Bahonar University of Kerman, Kerman, Iran [11]. A voucher specimen of the plant materials was deposited at the herbarium of Department of Pharmacognosy, School of Pharmacy, Kerman University of Medical Science, Iran (KF1375)***.***

Preparing the methanolic extract

The dried aerial parts of the plant (100 g) were grinded and extracted by methanol (80%) for 72 h at room temperature using the percolation method. The extracts were passed through filter paper (Whatman No.3, Sigma, Germany) to remove plant debris. The extracts were finally concentrated in vacuum at 50 °C using a rotary evaporator (Heidolph, Germany) and stored at -20 °C, until use [12, 13].

Collection of protoscoleces

The protoscoleces of *E. granulosus* were obtained from the livers of naturally infected sheep and goats slaughtered at Kerman abattoir, southeastern Iran and carried to the Parasitology Laboratory at the Department of Parasitology and Mycology, Kerman University of Medical Sciences (Kerman, Iran). The hydatid fluid aspirated by a 20 mL syringe and aseptically transferred into a flask was left to set for 30 minutes for protoscoleces to settle down. The supernatant was discarded and the protoscoleces were washed two times with PBS (pH 7.2) solution. The number of protoscoleces per ml was adjusted as 2×10^3 protoscoleces in 0.9% NaCl solution with at least 90% viability rate. The viability of the protoscoleces was confirmed by their flame cell motility and impermeability to 0.1% eosin solution under a light microscope [14].

Effect on protoscoleces

For evaluation of scolicidal effects of cardamom against protoscoleces of hydatid cyst**,** various concentrations were used for 10, 20, 30 and 60 minutes. At first, 0.5 mL of the protoscoleces (2×10^3/mL) solution was placed in test tubes. Then 0.5 mL of various concentrations of the extract was added to each test tube. The contents of the tubes were gently mixed and then incubated at 37°C for 5, 10, 20 and 30 minutes. At the end of each incubation time the upper phase was carefully removed so as not to interrupt the protoscoleces. Fifty µL of 0.1% eosin stain was then added to the remaining settled protoscoleces and mixed gently. The upper portion of the solution was discarded after 10 minutes of incubation. The remaining pellet of protoscoleces was then smeared on a glass slide, covered with a cover glass and examined under a light microscope. The percentages of dead protoscoleces were determined by counting 300 protoscoleces. Eosin exclusion test was used to investigate the viability of protoscoleces. Eosin solution with a concentration of 0.1% (1 g of eosin powder in 1000 mL distilled water) was used. After exposure to the stain, alive protoscoleces remained colorless and showed characteristic muscular movements and flame cell activity (Figure. 1), whereas dead protoscoleces absorbed eosin and colored red (Figure. 2). In addition, normal saline containing Tween 80 and 20% hypertonic saline were used as negative and positive control, respectively [15].

Statistical analysis

All the tests were performed in triplicate. Data analysis was carried out by using SPSS statistical package version 17.0 (SPSS Inc., Chicago, IL, USA). Differences between test and control groups were analyzed by *t*-test. In addition, $p<0.05$ was considered statistically significant (16).

RESULTS

According to the results given in Tables 1, cardamom extract had a potent scolicidal activity against the protoscoleces of hydatid cyst. Findings showed that extract at the concentrations of 200 and 100 mg/mL killed 100% protoscoleces after 10, 20 minutes of exposure. Similarly, all of the protoscoleces were killed after 30 and 60 minutes of exposure to 50 and 25 mg/mL concentration of essential oil, respectively. The results also revealed that the mortality rate of protoscoleces in the negative and positive controls was 9.1% after 60 min and 100% after 10 min of exposure, respectively. These results also demonstrated that all the concentrations of extract had significant ($p<0.05$) scolicidal effects compared with the control group.

Table 1 Scolicidal effects of *cardamom* extract against protoscoleces of hydatid cyst at various concentrations following various exposure times

Concentration (mg/mL)	Exposure time (min)	Mean of mortality rate (%)
200	10	100
	20	100
	30	100
	60	100
100	10	53.3
	20	100
	30	100
	60	100
50	10	28.6
	20	58.3
	30	100
	60	100
25	10	6.3
	20	28.3
	30	71.6
	60	100
Normal saline	10	1.3
	20	2.6
	30	4.3
	60	9.1
20% Hypertonic saline	10	100
	20	100
	30	100
	60	100

DISCUSSION

Nowadays, an ideal scolicidal agent is defined by its potency at lower concentrations, high efficacy in a shorter time of exposure, stability in the presence of cystic fluid, scolicidal ability inside a cyst, lower toxicity, higher availability, and ability for rapid preparation [1, 17]. Several investigations have reported the scolicidal effects of hypertonic saline, silver nitrate and mannitol, cetrimide, ethyl alcohol (95%), H_2O_2 and 10% providone iodine, chlorhexidine gluconate, selenium nanoparticles, honey and some plant extracts [18-32]. The present findings showed that cardamom extract with the concentrations of 200 and 100 mg/mL killed 100% protoscoleces of hydatid cyst after 10 minutes of exposure. These results were comparable with the scolicidal activity of 20% hypertonic saline, 20% silver nitrate, 0.5–1% cetrimide, H_2O_2 3%, and 95% ethyl alcohol. Thus, this study supported the idea that cardamom might be a natural source for the production of a new scolicidal agent for use in hydatid cyst surgery. However, main mechanisms of scolicidal effects of cardamom are not clear and further studies are needed to elucidate these mechanisms, particularly on *in vitro* models.

Studies have reported the presence of terpenoids, flavonoids, and tannins in this plant [11]. Currently, the biological activities of these compounds have been demonstrated [12, 13]. Thus, the phytoconstituents in this plant could be responsible for their scolicidal effects though their exact mode of action is poorly understood. However, previous studies have suggested that some terpenoids compounds such as monoterpens can diffuse into pathogen and damage cell membrane structures [33-35]. On the other hand, previous studies suggested that not only the antimicrobial activity is related to ability of terpenes to affect permeability but also other functions of cell membranes; these compounds might cross the cell membranes, thus penetrating into the interior of the cell and interacting with critical intracellular sites [35-37].

In conclusion, the findings showed that cardamom extract might be a natural source for the production of new scolicidal agents for use against hydatid cyst surgery.

Declaration of Interest

The author declares that there is no conflict of interest in this study.

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