

# Inhibitory activity of Fennel methanolic extract against hydatid cyst protoscolexes

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## ABSTRACT

Here we determine the effect of Fennel (*Foeniculum vulgare* Mill.) methanolic extract on *Echinococcus granulosus* protoscolexes in vitro condition. *E. granulosus* protoscolexes were obtained from the hydatid cysts of domestic livestock. Various concentrations of Fennel extract (25 - 200 mg/ml) and were applied for 10, 20, 30, and 60 minutes. Finally, the viability of hydatid cyst protoscolexes was assessed by Eosin test. The obtained results revealed that Fennel extract had remarkable protoscolicidal effects on hydatid cysts protoscolexes; so that at the concentration of 200 mg/ml after 10 min of killed completely all protoscolexes. Whereas, after 20 minutes incubation to concentration of 100 mg/ml the mortality mean of protoscolexes was 100%. The findings showed that Fennel extract had potent protoscolicidal effects against hydatid cyst protoscolexes indicating the potential of Fennel to produce a new scolocidal drug to apply in hydatid cyst surgery.

Keywords: Fennel; protoscolexes, Cystic echinococcosis, in vitro

## 1. INTRODUCTION

Hydatidosis (hydatid disease) which caused by the larval form of dog tape worm of *Echinococcus granulosus* sensu lato (s.l.) is as one of the most important neglected parasitic diseases globally. The disease affects humans and domestic livestock including cattle, sheep, camels, pigs, horses and others. At present, surgery along with chemotherapy (albendazole and/or mebendazole) is the most common choice of treatment in CE. During open surgical removal of the cysts to prevent the intraoperative linkage of the scoleces and following recurrence of hydatid cyst the use of proper protoscolicidal drugs are necessary. Nowadays, several scolocidal drugs have been applied during CE surgery; however, the most of them are along with some side effects such as sclerosane colangitis, and liver necrosis.

Historically, natural products, plant materials and their derivates have been one of the main resources of new drugs to treat a wide range of diseases. Fennel (*Foeniculum vulgare* Mill.) from Apiaceae family is a known medicinal plant with different therapeutic effects such as such as antioxidant, anti-inflammatory, antimutagenic, antimicrobial, gastroprotective, hepatoprotective and memory enhancing in folk and modern medicine.

This investigation designed to assess the in vitro inhibitory effects Fennel on *E. granulosus* protoscolexes.

## 2. MATERIALS AND METHODS

**Plant materials:** Fennel seeds were obtained from Khorramabad district in June 2015, Lorestan province, Iran. A botanist identified and confirmed plant materials and a voucher specimen was recorded at the Herbarium of Kerman University of Medical Science, Kerman, Iran.

**Preparation of methanolic extract:** The plant materials (seeds) were extracted by methanol (80%) for 72 h at room temperature using the percolation method. Then, the extract was passed via filter paper (Whatman No.3, Sigma, Germany) to delete plant debris. Finally the isolated extract concentrated in vacuum at 50°C using a rotary evaporator (Heidolph, Germany) and stored at -20°C, until use.

**Collection of protoscolexes:** *E. granulosus* protoscolexes were obtained from the livers of naturally infected livestock slaughtered at Kerman abattoir, Iran. The cyst fluid was aseptically aspirated and was left to set for 30 min. After washing of protoscolexes for two times with PBS (pH 7.2) solution the number of protoscolexes/ml was adjusted as  $2 \times 10^3$  protoscolexes in 0.9% NaCl solution with at least 90% viability rate.

**Scolocidal effects against protoscolexes:** To determine the inhibitory effects Fennel on hydatid cyst protoscolexes, various doses of Fennel extract were applied for 10, 20, 30 and 60 min. The protocol was performed according to the method described by Mahmoudvand (2014). Initially, 0.5 ml of the protoscolexes ( $2 \times 10^3$ /ml) and 0.5 ml of different doses of tested extract was added to each test tube. Tubes were mixed and then incubated at 37°C for 10-60 min. finally 50 µl of 0.1% eosin stain was added to protoscolexes and mixed. The percentages of dead protoscolexes were counted by counting 100 protoscolexes.

**Statistical analysis:** All the experiments were carried out in triplicate. Data analysis was carried out by SPSS software. Differences between test and control groups were analyzed by *t*-test. Furthermore,  $p < 0.05$  was considered statistically significant.

### 3. RESULTS

Table 1 shows the inhibitory effects of Fennel methanolic extract on hydatid cyst protoscoleces. The results indicated that Fennel extract had remarkable scolicidal activity on *E. granulosus* protoscoleces; so that at the concentration of 200 mg/ml after 10 min of killed completely all protoscoleces. After 20 min exposure, Fennel extract at the dose of 100 mg/mL killed also all of protoscoleces. The protoscolicidal effect of Fennel extract at the dose of 50 mg/ml was 18.3, 68.6, 100 and 100% after 10-60 min, respectively. In the negative control 7.1% and in the positive control 100% of protoscoleces killed after 60 and 10 min incubation, respectively; indicating that among tested drugs, the scolicidal effect Fennel extract was significant higher ( $p < 0.05$ ) than control groups at all incubation times.

**Table.1. Scolicidal effects of Fennel 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	18.3
	20	68.6
	30	100
	60	100
25	10	5.3
	20	21.3
	30	52.3
	60	96.6
Normal Saline	10	1.3
	20	2.6
	30	4.3
	60	7.1
20% Hypertonic Saline	10	100
	20	100
	30	100
	60	100

### DISCUSSION

This study aims to evaluate the scolicidal effects of Fennel extract against protoscoleces of hydatid cyst on an *in vitro* model. The obtained results showed that Fennel extract had remarkable protoscolicidal effects on protoscoleces of *E. granulosus*; so that the dose of 200 mg/ml at the time of 10 min killed all of protoscoleces. The dose of 100 mg/ml killed all of protoscoleces after 20 min of incubation. In recent years, scolicidal activity of some chemical and natural drugs such as hypertonic saline, silver nitrate and mannitol, cetrime, alcohol, Selenium NPs and some herbal derivatives have been indicated; but they are along with serious effects and their efficacy is controversial.

Plant extracts contain a large number of components, and it is probably that their mode of action involves several targets in the cell of microorganisms. In this investigation, we reported that Fennel extract had remarkable scolicidal effects in compared with positive control; so that, at concentrations of 200 and 100 mg/ml killed completely all protoscoleces after 10 and 20 min incubation, respectively. These findings are comparable with scolicidal effects aforementioned agents including of hypertonic saline, silver nitrate, cetrime, and ethyl alcohol which killed protoscoleces after 15, 20, 15, and 15 min incubation, respectively.

Several investigations have reported antibacterial, antiviral, antifungal and antiparasitic effects of Fennel. In addition, previous studies have shown the presence of alkaloids, flavonoids, tannins, saponins and cardiac glycosides in this plant. This shows that, phytoconstituents in the plant materials might be responsible for their antimicrobial activity; however, their exact mechanism of action is not fully understood.

Regarding cytotoxic effects of Fennel, previous investigation have revealed that essential oil of Fennel had no significant cytotoxicity against vero cells on an *in vitro* model.

#### 4. CONCLUSION

We found that Fennel as a natural source for the production of new scolicidal agent for use in hydatid cyst surgery. However, further investigations will be required to confirm our findings on *in vivo* model.

**Declaration of Interest:** The author declares that there is no conflict of interest in this study.

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