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Scolicidal effects of Myrtle methanolic extract on hydatid cyst protoscoleces

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ABSTRACT

This investigation aims to evaluate the protoscolicidal effects of Myrtle (Myrtus communis L.) 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 500 and 25 mg/mL killed 100% protoscoleces after 10 and 20 minutes of exposure, respectively. Obtained results in this investigation for the first time demonstrated that Myrtle might be a natural source for the production of new scolicidal agents.*

Key words: Cystic echinococcosis; Prtotoscoleces; Extract

INTRODUCTION

Traditional medicine has a long history of serving peoples worldwide [1]. The use of medicinal plants to treat diseases and maintain public health is highly prevalent globally [2].

Since last decades, various parts of Myrtle *[Myrtus communis* L.] have been used widely as a traditional medicine for the treatment of several diseases such as infectious ones [3]. In addition, various investigations have reported anti-inflammatory, antinociceptive, antioxidant, anti-hepatic ischemia, neuro-protective and antimicrobial effects of this plant [4, 5].

Hydatid disease [cystic echinococcosis, CE] is a parasitic infestation by a tapeworm of the genus *Echinococcus [6]*. Surgery is considered the first choice of treatment for CE [7]. The use of effective scolicidal agents during CE surgery is necessary to reduce the risk of intraoperative spillage of the cyst contents [protoscoleces] and subsequently recurrence of CE and secondary infection [8, 9]. The present scolicidal agents have indicated various side effects such as liver necrosis and sclerosane colangitis [10]. This investigation aims to evaluate the protoscolicidal effects of *Myrtle* extract against protoscoleces of hydatid cysts on *in vitro* model.

MATERIALS AND METHODS

Collection of plant materials

The Myrtle leaves were collected from rural regions of Baft district of Kerman Province, southeastern Iran, in September 2012 (11). The identity was confirmed by a botanist at the Botany Department of Shahid Bahonar University, Kerman, Iran. A voucher specimen of the plant materials was deposited at the Herbarium of Department of Pharmacognosy, School of Pharmacy, Kerman University of Medical Science, Kerman, Iran (KF1356).

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).

Collection of protoscoleces

The hydatis cyst protoscoleces 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 (13).

Effect on protoscoleces

To determine the scolicidal effects of *Myrtle*, and its main components against protoscoleces of hydatid cyst**,** various concentrations of the essential oil, thymol and carvacrol were used for 5, 10, 20 and 30 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 were 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, whereas dead protoscoleces absorbed eosin and colored red. In addition, normal saline containing Tween 80 and 20% hypertonic saline were used as negative and positive control, respectively (14).

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 (15).

RESULTS

As shown in Tables 1, Myrtle extract demonstrated the scolicidal activity against the protoscoleces of hydatid cyst. The results revealed that extract at the concentrations of 500 and 250 mg/mL killed 100% protoscoleces after 10, 20 minutes of exposure. Moreover, all of the protoscoleces were killed after 30 and 60 minutes of exposure to 125 and 62.5 mg/mL concentration of extract, respectively. 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. The obtained results demonstrated all the concentrations of extract had significant ($p<0.05$) protoscolicidal effects compared with the control group.

DISCUSSION

According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary health care needs (16). We found that Myrtle extract at the concentrations of 500 and 250 mg/mL killed 100% protoscoleces after 10, 20 minutes of exposure. A proper protoscolicidal agent is explained 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 (16).

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

Concentration (mg/mL)	Exposure time (min)	Mean of mortality rate (%)
500	10	100
	20	100
	30	100
	60	100
250	10	61.3
	20	100
	30	100
	60	100
125	10	33.6
	20	71.3
	30	100
	60	100
62.5	10	7.3
	20	25.3
	30	61.3
	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

Nowadays, reviews have shown the scolicidal effects of hypertonic saline, silver nitrate and mannitol, cetrimide, ethyl alcohol, H_2O_2 and providone iodine, chlorhexidine gluconate, selenium nanoparticles, honey and some plant extracts (17-32). The current findings 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. Therefore, the obtained results suggested the idea that Myrtle extract 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 Myrtle extract are not clear and further studies are needed to elucidate these mechanisms, particularly on *in vitro* models. Previous studies have reported the presence of terpenoids, flavonoids, and tannins in this plant. in the case of antimicrobial mechanism of some terpenoids compounds such as monoterpens, as the main components of Myrtle (4, 5, 33). Some researchers indicated that they diffuse into pathogen and damage cell membrane structures (32, 34). On the other hand, other reports suggested that the antimicrobial activity is related to ability of terpenes to affect not only the permeability but also other functions of the cell membranes, these compounds might cross the cell membranes, thus penetrating into the interior of the cell and interacting with critical intracellular sites (35-38).

Declaration of Interest

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

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