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## ***Nectaroscordum tripedale* essential oil: Protoscolicidal effects against hydatid cyst protoscoleces**

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### **ABSTRACT**

The present investigation was designed to evaluate the protoscolicidal effects of *Nectaroscordum tripedale* L. essential oil against hydatid cyst protoscoleces. Different doses of *N. tripedale* essential oil (3.75 - 15 %) and were used for 10, 20, 30, and 60 minutes. The viability of hydatid cyst protoscoleces was assessed by Eosin test. The findings exhibited that *N. tripedale* essential oil showed considerable protoscolicidal effects on hydatid cysts protoscoleces; at the dose of 15% after 10 min of killed 100% of protoscoleces; while at the dose of 7.5% after 10 min of killed 100% of protoscoleces. This study revealed the potential of *N. tripedale* essential oil to produce a new scolicidal agent.

**Keywords:** Essential oil; protoscoleces, Cystic echinococcosis, *in vitro*

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### **INTRODUCTION**

To date, herbal medicines have been indicated to present interesting biological and pharmacological activities and are used as chemotherapeutic agents [1-4]. Historically, plants have been applied in treating infectious diseases and are recognized for their ability to produce secondary metabolites [5-7]. Hydatid disease is a parasitic infestation which caused by a tapeworm of the genus *Echinococcus*. The disease is one of the most important neglected parasitic diseases around the world [8-10] which 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 [11-13]. 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 scolicidal 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 [14-17].

In folk medicine, *Nectaroscordum tripedale* L. (family Alliaceae) have been broadly used for treatment and prevention of various diseases such as rheumatic and joint pains[15], bladder and kidney stones, laxative, expectorant diuretic, parasite repellent, appetizer, stimulant, and muscle ache [16]. This investigation was designed to assess protoscolicidal effects of *N. tripedale* essential oil against protoscoleces of hydatid cysts.

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**MATERIALS AND METHODS*****Plant materials***

Fruits of wild *N. tripedale* were collected from the Khorramabad Mountains (Lorestan, Iran) in May 2012. The plant materials were identified by a botanist at the Razi Herbal Medicine Research Center Khorramabad, Iran.

***Preparing of essential oil***

Two hundred grams of dried materials of *N. tripedale* were subjected to hydro-distillation for 3 h using an all-glass Clevenger-type apparatus. The essential oil obtained was dried over anhydrous sodium sulfate, and stored in darkness at 4 °C in airtight glass vials closed under nitrogen gas until testing [17-19].

***Collection of protoscoleces***

Protoscoleces were collected 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 protoscoleces for two times with PBS (pH 7.2) solution the number of protoscoleces/ml was adjusted as  $2 \times 10^3$  protoscoleces in 0.9% NaCl solution with at least 90% viability rate [20].

***Scolicidal effects against protoscoleces***

Protoscolicidal effects of *N. tripedale* against hydatid cyst protoscoleces, various doses of *N. tripedale* essential oil were applied for 10, 20, 30 and 60 min and performed based on the method described by Mahmoudvand et al (2014). At first, 0.5 ml of the protoscoleces ( $2 \times 10^3$ /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 protoscoleces and mixed [17]. The percentages of dead protoscoleces were counted by counting 100 protoscoleces [21-23].

***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 [20].

**RESULTS**

The results of protoscolicidal effects of *N. tripedale* essential oil against hydatid cyst protoscoleces were shown in Table 1. The obtained findings revealed that *N. tripedale* essential oil shows significant protoscolicidal effects on hydatid cyst protoscoleces. The concentration of 15% of essential oil after 10 min of killed completely 100% of protoscoleces; whereas the concentration of 7.5% of essential oil after 20 min of killed completely 100% of protoscoleces. The protoscolicidal effect of *N. tripedale* essential oil at the dose of 3.75% was 29.3, 76.6, 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; which demonstrated that the protoscolicidal effect of *N. tripedale* essential oil was significant higher ( $p < 0.05$ ) than control groups at all incubation times.

**Table 1: Protoscolicidal effects of *N. tripedale* essential oil against protoscoleces of hydatid cyst at various concentrations following different exposure times**

Concentration (%)	Exposure time (min)	Mean of mortality rate (%)
15	10	100
	20	100
	30	100
	60	100
	10	78.6
7.5	20	100
	30	100
	60	100
	10	29.3
	20	76.6
3.75	30	100
	60	100
	10	1.6
	20	2.6
	30	3.3
Normal saline + Tween 20	60	7.1
	10	100
	20	100
20% Hypertonic saline	30	100
	60	100
	10	100
	20	100

## DISCUSSION

Recent studies have also revealed promising results from using of plants in the treatment or prevention of a wide variety of diseases such as infectious ones [24-18]. This work was aimed to determine the *in vitro* protoscolicidal effects of *N. tripedale* essential oil against hydatid cysts protoscoleces. The obtained results demonstrated that the *N. tripedale* essential oil at concentrations of 15% and 7.5% after 10 and 20 min of exposure killed 100% protoscoleces. However, lower concentrations of *N. tripedale* essential oil indicated a lower protoscolicidal effects. Various studies have reported the protoscolicidal activity of various chemical products and also some herbal medicines such as *Nigella sativa*, *Berberis vulgaris*, *Zataria multiflora*, Myrtle, cardamom, *Pistacia vera*, *Pistacia atlantica*, and garlic [29-40]. However, the efficacy of these natural products is controversial. We found that protoscolicidal effects of *N. tripedale* essential oil is similar with the current scolicidal agents including hypertonic saline and silver nitrate; indicating that *N. tripedale* extract might be a natural source for the production of a new scolicidal agent to apply in hydatid cyst surgery.

Previous studies showed the presence of terpenoids, flavonoids, tannins and fatty acids in *N. tripedale* [41]. Moreover, antimicrobial effects of these components and their derivatives such as  $\alpha$ -pinene, limonen, piperitone oxide, terpinene, Hexadecanoic acid, thymol and carvacrole have been previously reported [42, 43]. Thus, these compounds might be responsible for protoscolicidal activity of *N. tripedale essential* though their exact mechanism of action is not clearly understood. Of course, some researchers have shown that these components especially terpenoids diffuse into pathogen and damage cell membrane structures, [44, 45].

To conclude, this study showed that *N. tripedale* essential oil might be a natural source for the production of new scolicidal agents to reduce the risk of protoscoleces spillage during hydatid cyst surgery. However, more investigations are needed to evaluate exact biological activity of *N. tripedale* essential oil in clinical setting as a new protoscolicidal drug.

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