**REPORT**

# Evaluation of the scolicidal effects of *Nectaroscordum tripedale* extract and its acute toxicity in mice model

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**Abstract**: Current scolicidal agents, which have been used for inactivation of protoscoleces during hydatid cyst surgery are associated with adverse side effects. This study aims to evaluate the *in vitro* scolicidal effects of *Nectaroscordum tripedale* L. leave extract against protoscoleces of hydatid cysts and its acute toxicity in mice model. Various concentrations of the extract (12.5-100 mg/mL) were used for 5 to 30 min. Viability of protoscoleces was confirmed using eosin exclusion test (0.1% eosin staining). In addition, the acute toxicity of *N. tripedale* extract was determined for 2 days in mice model. The results showed that the *N. tripedale* extract at the concentration of 100 mg/mL after 5 min of exposure killed 100% protoscoleces. Similarly, the mean of mortality rate of protoscoleces after 10 min of exposure to concentration of 50 mg/mL was 100%. The $LD_{50}$ values of intraperitoneal injection of the *N. tripedale* extract was 3.36 g/kg body wt. and the maximum nonfatal doses were 2.98 g/kg body wt. The results showed the potential of *N. tripedale* extract as a natural source for the production of new scolicidal agent for use in hydatid cyst surgery.

**Keywords**: *Nectaroscordum tripedale* L.; Toxicity*;* Cystic echinococcosis*;* Hydatid cyst; Protoscoleces.

## INTRODUCTION

Hydatid disease or cystic echinococcosis (CE) is an oral transmitted parasite infection, which caused by the larval form of the *Echinococcus granulosus* tapeworm. CE has a worldwide distribution, affecting people of working age and can cause high levels of morbidity and even death (WHO, 1996). Nowadays, the main clinical methods for CE treatment are based on surgery, percutaneous techniques and antiparasitic treatment for active cysts (Eckert and Deplazes, 2004). To reduce the risk of intraoperative spillage of the cyst contents (protoscoleces) during surgery and subsequently recurrence of CE and secondary infection, the use of effective scolicidal agents are obligatory (Brunetti *et al*., 2003; Junghanss *et al*., 2008). Now, several scolicidal agents such as hypertonic saline, silver-nitrate, cetrimide and ethanol have been used for inactivation of the cyst contents during surgery, but most of them have demonstrated serious side effects (Hosseini *et al*., 2006; Rajabi, 2009). *Nectaroscordum tripedale* L. (family Alliaceae) grows in Iran, Iraq, Turkey, North Caucasus and Transcaucasus called "Piaze tabestaneh" or "Aneshk" in Persian. In traditional medicine, different parts of this plant including root, leaf, bark and fruit have been widely used for treatment and prevention of various diseases including rheumatic, joint pains, bladder and kidney stones (Shafie-Zadeh 2002). Moreover, various pharmacological properties such as laxative, expectorant diuretic, parasite repellent, appetizer, stimulant, muscle ache and joint pain reliever and sedative have related to this plant (Zargari, 1996; Shafie-Zadeh, 2002). This study aims to evaluate scolicidal activity of *N. tripedale* leave extract against protoscoleces of hydatid cysts and also its acute toxicity in mice model.

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## MATERIALS AND METHODS

### *Plant collection and extraction*

Aerial parts (leaves) of wild *N. tripedale* were collected from the Khorramabad Mountains (Lorestan, Iran) in May 2013. The plant materials were identified by a botanist at the Razi Herbal Medicine Research Center, Khorramabad, Iran. A voucher specimen (LR12343) of the plant materials was deposited at the Herbarium of Agriculture and Natural Resource Research Center, Khorramabad, Iran. One hundred g of powdered plant materials were separately extracted by percolation method with 70% ethanol successively for 72h in room temperature (Mahmoudvand *et al*., 2014a).

### *Phytochemical analysis*

The preliminary phytochemical analysis of *N. tripedale* extract was carried out to determine the presence of tannins, saponins, alkaloids, terpenoids, phenols and glycosides as described elsewhere (Ezatpour *et al*., 2015).

***Collection of protoscoleces***

The protoscoleces of hydatid cysts 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, Kerman University of Medical Sciences, Iran. The hydatid fluid aspirated by a 50 mL syringe and aseptically transferred into a flask was left to set for 30 min for protoscoleces to settle down. The supernatant was discarded and the protoscoleces were washed two times with phosphate buffered saline (PBS, pH 7.2) solution. The number of protoscoleces per mL was adjusted as $2\times10^3$ protoscoleces in 0.9% NaCl solution with at least 90% viability rate (Mahmoudvand *et al*., 2016a).

***Scolicidal effect on protoscoleces***

To determine the scolicidal activity of *N. tripedale* extract against protoscoleces of hydatid cysts, various concentrations of the extract (12.5, 25, 50 and 100 mg/mL) were used for 5, 10, 20, and 30 min (Mahmoudvand *et al*., 2016b). Initially, 0.5mL of the protoscoleces ($2\times 10^3$/mL) solution was placed in test tubes. Then 0.5mL 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 30min. 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 (Sigma-Aldrich, St Louis, MO, USA) was then added to the remaining settled protoscoleces and mixed gently. 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. We also used normal saline and hypertonic saline 20% as negative and positive control group, respectively (Mahmoudvand *et al*., 2014b). In this study, eosin exclusion test was used to determine the viability of protoscoleces of hydatid cysts (Smyth, 1980). After exposure to the stain, live protoscoleces remained colorless and displayed characteristic muscular movements and flame cell activity, while dead protoscoleces absorbed eosin and colored red (fig. 1).

***Acute toxicity test***

Twenty male NMRI mice (6-8 weeks old) were obtained from the Animal Breeding Stock Facility of Razi Institute of Iran (Karaj, Iran). Animals were housed in a colony room with a 12:12h light/ dark cycle at 21±2˚C and were handled according to standard protocols for the use of laboratory animals. The protocol of this study was approved by the Committee on the Ethics of Animal Experiments of the Lorestan University of Medical Science (91/271, 2013). To determine the acute toxicity, various doses of *N. tripedale* extract (0.5, 1, 2 and 4g/kg) were injected as intraperitoneally into groups of five mice. The number of deaths was recorded at 48 h after treatment. $LD_{50}$ values were determined by the Probit test in SPSS software (Hosseinzadeh *et al*., 2011).

## 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). The difference between groups was evaluated by ANOVA, which followed by *t*- test and *p* values less than 0.05 were considered significant.

## RESULTS

***Phytochemical analysis***

The findings of primary phytochemical screening of the *N. tripedale* extract demonstrated the presence of high amount of terpenoids, flavonoids, tannins and fatty acids in this plant.

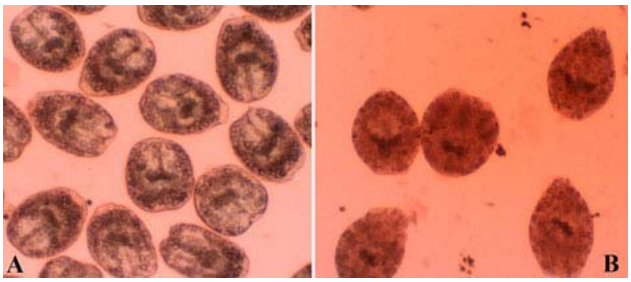


**Fig. 1**: Live (A) dead (B) protoscoleces of hydatid cysts after exposure with 0.1% eosin

***Effect on protoscoleces***

As shown in Table 1, *N. tripedale* extract at various concentrations following different exposure times had remarkable scolicidal effects against protoscoleces of hydatid cysts. *N. tripedale* extract at the concentration of 100 mg/mL after 5 min of exposure killed 100% protoscoleces. Similarly, the mean of mortality rate of protoscoleces after 10 min of exposure to concentration of 50mg/mL was 100%. The obtained results also demonstrated that lower concentrations of *N. tripedale* extract provoked a delayed scolicidal effects. The mortality rate of protoscoleces in the negative and positive control was 5.2% after 30 min and 100% after 5min exposure, respectively. Findings also indicated that *N. tripedale* extract at all of concentrations had significant ($p<0.05$) scolicidal effects compared with the control group.

***Acute toxicity***

The $LD_{50}$ values of intraperitoneal injection of the *N. tripedale* extract was 3.36g/kg body wt. and the maximum nonfatal doses were 2.98g/kg body wt.

## DISCUSSION

This work aims to determine the *in vitro* scolicidal effects of *N. tripedale* extract against hydatid cysts protoscoleces.

**Table 1**: Scolicidal effects of *N. tripedale* extract against protoscoleces of hydatid cyst at various concentrations (12.5, 25, 50 and 100 mg/mL) following different exposure times (5, 10, 20 and 30 min).

| Concentration (mg/mL) | Exposure time (min) | Mean of mortality rate (%) |
|---|---|---|
| | 5 | 100 |
| | 10 | 100 |
| 100 | 20 | 100 |
| | 30 | 100 |
| | 5 | 58.6 |
| 50 | 10 | 100 |
| | 20 | 100 |
| | 30 | 100 |
| | 5 | 21.6 |
| 25 | 10 | 56.3 |
| | 20 | 83.6 |
| | 30 | 100 |
| | 5 | 9.3 |
| 12.5 | 10 | 20.3 |
| | 20 | 44.6 |
| | 30 | 61.3 |
| | 5 | 1.3 |
| Normal saline + Tween 20 | 10 | 2.6 |
| | 20 | 3.3 |
| | 30 | 5.2 |
| | | |
| 20% Hypertonic saline | 5 | 63.3 |
| | 10 | 100 |
| | 20 | 100 |
| | 30 | 100 |

The findings of the present study exhibited that the *N. tripedale* extract at the concentrations of 100 and 50 mg/mL after 5 and 10 min of exposure killed 100% protoscoleces. However, lower concentrations (12.5 and 25mg/mL) of *N. tripedale* extract provoked a delayed protoscolicidal effects**.** According to WHO (1996), a proper scolicidal drug is described by its high efficacy at lower concentration and in a shorter time of exposurelower toxicity, higher availability and rapid preparation. Based on previous investigations, the scolicidal effects of various chemical and natural products including 20% hypertonic saline, silver nitrate, mannitol, cetrimide, ethyl alcohol (95%), $H_2O_2$, 10% providone iodine, chlorhexidine gluconate, selenium nanoparticles, honey and some plant extracts have been reported (Mahmoudvand *et al*., 2014c,d; Mahmoudvand *et al*., 2015a,b). However, the use of these scolicidal drugs because of possessing adverse side effects such as liver necrosis, sclerosing colangititis and methaemoglobinaemia is discussed. Our results revealed that scolicidal activity of *N. tripedale* extract is comparable with the current scolicidal agents such as 20% hypertonic saline (15 minutes), 20% silver nitrate (20minutes), 0.5-1% cetrimide (10 minutes), $H_2O_2$ 3% (15 minutes) and 95% ethyl alcohol (15 minutes). Therefore, results of the present study suggested the idea that *N. tripedale* extract could be a natural source for the production of a new scolicidal agent for use in hydatid cyst surgery.

The phytochemical screening of extract demonstrated the presence of terpenoids, flavonoids, tannins and fatty acids in this plant. Previously, Cown (1999) demonstrated the individual effects of these components. Therefore, phytoconstituents in this plant might be responsible for their scolicidal effects though their exact mechanism of action is not clear. Regarding antimicrobial mechanisms of terpenoids, some researchers have reported that terpenoids diffuse into pathogen and damage cell membrane structures, penetrating into the interior of the cell and interacting with critical intracellular sites, induces DNA damage and inhibition of fatty acid synthesis (Ismail *et al*., 2013; Saedi Dezaki *et al*., 2015).

With respect to the toxicity effects of *N. tripedale* extract, we found that The $LD_{50}$ values of intraperitoneal injection of the *N. tripedale* extract was 3.36g/kg body wt. and the maximum nonfatal doses were 2.98g/kg body wt. According to a toxicity classification, the *N. tripedale* extract had no significant toxicity against male NMRI mice (Loomis, 1968). Thus, these findings suggest that *N. tripedale* extract at the concentrations used in the present study had no significant toxicity and could be consider safe for host.

In conclusion, our findings demonstrated that *N. tripedale* extract might be a natural source for the production of new scolicidal agents to reduce the risk of protoscoleces spillage during hydatid cyst surgery. However, further studies are required to evaluate exact biological activity of *N. tripedale* extract in clinical setting as a new scolicidal agent.

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