



Evaluation of Protoscolicidal Effects of Nectaroscordum Koelzi Methanolic Extract Against Hydatid Cyst Protoscoleces

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ABSTRACT

Here, we studied *in vitro* scolicidal effects of *Nectaroscordum koelzi* (*N. koelzi*) extract against hydatid cysts protoscoleces. Collected protoscoleces from sheep livers with fertile hydatid cysts were exposed with different concentrations of the extract (62.5-500 mg/mL) for 10-60 min *in vitro*. Then viability of protoscoleces was evaluated using the eosin exclusion test (0.1% eosin staining). Findings revealed that *N. koelzi* extract, at the concentrations of 250 and 500 mg/mL completely killed protoscoleces after 10, 20 min of exposure, respectively. The results also indicated that the survival rate of protoscoleces in the negative and positive controls was 95.4% and 0% after 60 and 10 min of exposure, respectively; indicating that *N. koelzi* extract at all of these concentrations had significantly higher ($P < 0.05$) protoscolicidal activity than the control group. The findings indicated potential of *N. koelzi* methanolic extract as a natural source for the producing of new scolicidal agent for use in hydatid cyst surgery.

Keywords: Cystic Echinococcosis, Echinococcus Granulosus, Protoscoleces, Nectaroscordum Koelzi

HOW TO CITE THIS ARTICLE: Nasrin Galehdar, Massumeh Niazi, Sareh Jahanbakhsh, Hormoz Mahmoudvand, Abdolreza Rouientan, Evaluation of Protoscolicidal Effects of Nectaroscordum Koelzi Meth-anolic Extract Against Hydatid Cyst Protoscoleces, Entomol Appl Sci Lett, 2018, 5 (1): 72-76.

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Received: 08/12/2016

Accepted: 21/03/2018

INTRODUCTION

Cystic echinococcosis (CE, hydatid cyst) is a common neglected disease between humans and animals caused by the metacestode stage (larval stage) of *Echinococcus granulosus* [1]. CE in various countries, especially in developing countries of the world is well-known as a problem in the field of health and economy [2, 3]. Regarding to the epidemiology of CE, it can be said that the prevalence is the most common in areas with animal husbandry such as Iran [4]. Humans as the intermediate host can accidentally infected with eggs exerted from final hosts (dogs and other carnivores); where the hydatid cysts were developing in various organs [5].

Although the liver is the most common involved organ in hydatidosis (60-70%), but hydatid cysts may also be located in other organs such as, lung, heart, thyroid, breast, kidneys and soft tissues of the neck [6]. The clinical signs of hydatid cyst are dependent on the size and location of the replacement. However, most patients are asymptomatic due to the slow growth of cysts [7, 8].

Today, the clinical management of treatment is different for active and inactive cysts. Although surgery is a preferred method for treating live cysts, but methods such as subcutaneous techniques (PAIR) should not be ignored. Moreover, drug therapy with benzimidazoles is a recommended approach to treat high risk hydatid cysts or patients with special conditions [9]; however, the problem is that they bring a wide

collection of unwanted side effects, such as alopecia and hepatotoxicity [9, 10].

As stated above, surgery is the best treatment option that can lead to cysts' removal and patient healing. But there are serious risks in this method, such as leakage of the cyst contents (protoscoleces) during surgery and subsequently re-infection as well as anaphylactic reactions and even death [11, 12]. To solve this problem, surgeons today use some chemical protoscolicidal drugs for instance 20% hypertonic saline, Ag-nitrate, and cetrimide during CE surgery. However, previous studies show that these chemical agents have dangerous side effects such as fibrosis and necrosis of liver and gallbladder [13, 14]. Therefore, the discovery of new scoliosidal agents with fewer side effects seems to be necessary.

Due to the huge variety of plants and the long history of using them in traditional medicine, herbal medicines can be considered as an excellent resource with high availability and low toxicity in the treatment of diseases [15]. *Allium* is an important genus of one of the most chief families of medicinal plants called Alliaceae. *Nectaroscordum koelzi* is one of the subgenus of *Allium*, which grows broadly in Iran and its neighboring countries like Iraq [16]. In traditional Iranian medicine, due to the warm nature of this plant, it is often an appropriate treatment for rheumatic pains, joints, kidney and bladder stones as well as improvement of the common colds [16, 17]. With the knowledge of the above items, the aim of this study was to investigate the effect of protoscolicidal of *N. koelzi* extract on in vitro.

MATERIALS AND METHODS

Collection of plant materials

The plants materials (aerial parts of wild rising *N. koelzi*) in April 2017 from mountainous areas of Lorestan Province, West of Iran were collected. Following the collection, the plant was identified by a botanist in Herbarium of Agriculture and Natural Resource Research Center, Khorramabad, Iran.

Preparation of extract

Extraction of plant was performed by percolation procedure on 200 g of air dried plant materials using methanol (80%). To remove the artifacts, the extract was passed

through filter paper (Whatman No.3, Sigma, Germany). As the next step, the extract was concentrated in vacuum at 50°C by means of a rotary evaporator (Heidolph, Germany) and kept at -20°C, until use [18, 19].

Collection of protoscoleces

With referring to slaughterhouses in Khorramabad, Iran, protoscoleces were obtained from the liver of naturally infected sheep and goats. The collection of protoscoleces was done according to the methods described elsewhere [20]. The number of protoscoleces per mL was attuned to 2×10^3 protoscoleces in a 0.9% NaCl solution with an at least 90% viability rate.

In vitro protoscolicidal activity

To study lethal effects of *N. koelzi* extract against protoscoleces of hydatid cysts, we used several concentrations of the extract (62.5, 125, 250, and 500 mg/mL) for 10, 20, 30, and 60 min. In each test tube, we added 0.5 mL of the protoscoleces solution plus 0.5 mL of various concentrations of the extract and then incubated at 37 °C for 10, 20, 30, and 60 min. After this time, and removing the upper phase, 50 µl of 0.1 % eosin stain was added to the remaining pellet of protoscoleces; finally 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. The normal saline and hypertonic saline 20 % as negative and positive control group, respectively.

Statistical analysis

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

RESULTS

In this investigation we studies the lethal effects of *N. koelzi* extract at various concentrations (62.5, 125, 250, and 500 mg/mL) against protoscoleces of hydatid cysts for 10, 20, 30, and 60 min, respectively. As shown in Table 1, *N. koelzi* extract at the concentrations of 250 and 500 mg/mL completely killed protoscoleces after 10, 20 min of exposure, respectively.

The results also showed that the mean survival rate of protoscoleces was zero after treatment with *N. koelzi* extract at the concentration of 125 mg/mL after 30 min of incubation. While, at the concentration of 62.5 mg/ml showed weak protoscolicidal effects so that, at this concentration of 7.6, 16.6, 46.3, and 68.3% of protoscoleces were killed after 10, 20, 30, and 60 min of incubation, respectively. The obtained results also demonstrated that the survival rate of protoscoleces in the negative and positive controls was 95.4% and 0% after 60 and 10 min of exposure, respectively; indicating that the essential oil of *N. koelzi* extract at all of these concentrations had significantly higher ($P < 0.05$) protoscolicidal activity than the control group.

Table 1. Protoscolicidal effects of *N. koelzi* extract against protoscoleces of hydatid cyst at various concentrations following various exposure times. Data are expressed as the mean \pm SD (n = 3).

Concentration (mg/mL)	Exposure time (min)	Mean of mortality rate (%)
500	10	100 \pm 0.0
	20	100 \pm 0.0
	30	100 \pm 0.0
	60	100 \pm 0.0
250	10	66.6 \pm 6.1
	20	100 \pm 0.0
	30	100 \pm 0.0
	60	100 \pm 0.0
125	10	22.6 \pm 3.15
	20	61.6 \pm 3.15
	30	100 \pm 0.0
	60	100 \pm 0.0
62.5	10	7.6 \pm 1.51
	20	16.6 \pm 2.51
	30	46.3 \pm 2.88
	60	68.3 \pm 6.1
Normal saline	10	0.0 \pm 0.0
	20	2.6 \pm 1.15
	30	3.0 \pm 0.5
	60	5.6 \pm 0.5
20% hypertonic salt	10	100 \pm 0.0
	20	100 \pm 0.0
	30	100 \pm 0.0
	60	100 \pm 0.0

DISCUSSION

CE is a threat to human health and the economy of societies and Iran is a hyper-endemic country for this disease [21]. Given the surgical

procedure is the proposed option for treatment of this disease and the drugs used during surgery to prevent the cysts being reversed are associated with serious complications, Therefore, the achievement of new protoscolicidal agents with less side effects and high ability is necessary [22].

Herbal medicines are considered due to high availability, appropriate cost, low toxicity and a large variety in their chemical nature as reliable resources for the replacement of drugs with side effects. Therefore, in this study, the evaluation of the scolical effect of *N. koelzi* extract on the hydatid cyst protoscoleces was selected as the target.

Different concentrations of the extract under study were exposed to protoscoleces extracted from the livers of contaminated sheep. The results indicated a significant scolical activity of *N. koelzi* extract compared with scolical agents such as 20% hypertonic saline and Ag-nitrate.

Regarding protoscolicidal effects of medicinal herbs, previous studies have shown high potency of *Nigella sativa*, *Pistacia vera*, *Pistacia khinjuk*, *Pistacia atlantica*, *Bunium persicum*, *Myrtus cumminus* against the hydatid protoscoleces [23-28]. There are few studies about the antimicrobial activities of Alliacea family. According to study conducted by Ezatpur et al., *N. tripedale* extract as an Alliacea family has significant antibacterial effects on *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa* [29]. In the other study conducted by Mahmoudvand et al., it has been shown the anti-leishmanial effect of *N. tripedale* extract on promastigotes and amastigotes of *Leishmania tropica*. The results showed substantial anti-leishmanial effects of this extract [30]. In another study by Mahmoudvand et al., the effects of protoscolicidal *N. tripedale* extract against hydatid cyst have been proven [31].

CONCLUSION

Thus, the findings confirm that the *N. koelzi* extract as a herbal source can be used as a new scolical agent during hydatid cyst surgery due to the presence of certain effective substances; although, the assessment of the chemical

structure of this plant and its mechanism of action require further studies.

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