Original Article

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Comparison of the Effect of Artemisia Sieberi Essential Oil and Albendazole Drug on Protoscolices of Hydatid Cyst under in Vitro Conditions

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ABSTRACT

BACKGROUND AND OBJECTIVE: Hydatidosis is a major zoonotic disease, and the best treatment for this disease is surgery. So far, many chemical protoscolex killers have been used to prevent protoscolices leakage during surgery, but it is necessary to consider the adverse effects of chemicals and the use of medicinal herbs. Considering the antiparasitic properties of Artemisia sieberi, this study was conducted to evaluate and compare the effect of Artemisia sieberi essential oil and albendazole drug on protoscolices of hydatid cyst under in vitro conditions.

METHODS: In this laboratory study, essential oils were prepared from aerial parts of the plant. Gas chromatography–mass spectrometry was performed to determine its components. Protoscolices were extracted from the livers infected with hydatid cyst and were exposed to Artemisia sieberi (2.5, 5 and 10 µg/ml) for 10, 30, 60 and 120 minutes. The viability of protoscolices was measured by eosin staining. Albendazole was used as a standard drug.

RESULTS: 31.5% alpha-Thujone was identified as the main composition pf essential oil. The amount of essential oil protoscolices at the concentration of 2.5 µg/ml in 10, 30, 60 and 120 minutes was 51.8, 71.5, 82.8 and 99.3%, respectively. Albendazole showed lower toxic effect with similar dose at similar intervals, but both treatments showed a significant effect ($p<0.05$). The highest toxic effect of essential oil was observed at 10 µg/ml concentration 30 minutes after treatment. For albendazole, this effect was observed at a dose of 10 µg/ml after 120 minutes.

CONCLUSION: The results of the study showed that Artemisia sieberi essential oil has an acceptable scolicidal effect compared to albendazole and can be used as a scolicidal agent.

KEY WORDS: *Hydatid cyst, essential oil, Artemisia, Gas chromatography–mass spectrometry.*

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Introduction

Hydatidosis is one of the most important zoonotic diseases with global spread and is caused by the larval form of the tapeworm *Echinococcus granulosus* (1). The disease is more common in rural areas where there is a closer relationship between dogs, livestock and humans. Hydatidosis reduces livestock products, causes serious damage to human health, has high cost treatment and increases the chance of death, and therefore is considered as a major challenge to public health and economics (2). Surgery is currently the best method for treating hydatid cysts. In the surgical treatment of cysts, if there is no provision for the necessary measures, there is a risk of leakage of protoscolices and its implantation in the intestines and tissues during the operation, which is one of the main causes of the recurrence of the disease and the formation of secondary cysts. To avoid leakage, it is essential to use effective scolicidal agent to reduce the disease recurrence rate (3).

So far, many chemical protoscolicidal agents such as formalin 2%, hypertonic saline 20 to 30%, cetrimide / scolicide solution, silver nitrate and betadine have been used. The use of these compounds in some cases can lead to severe side effects such as bile duct fibrosis, liver necrosis and healthy tissues adjacent to the cyst (4). Benzimidazole is used to treat human hydatid cysts with different reports of its success results. Mebendazole was the first of these compounds, but it has low absorption, its serum and intracystic levels are low and insufficient amounts of the drug penetrate into the cysts. Albendazole is a newer drug that is more effective in treating hydatid cyst compared to mebendazole (5).

An alternative method can be the formulation of new protoscolicidal agents using plant-derived compounds, including essential oils extracted from plants. Few studies have been conducted regarding the potential for the use of essential oils against hydatid cysts (6). The genus Artemisia (Astraceae) is a source of herbal molecules and products that are highly considered in parasitology, as the discoverer of artemisinin won the Nobel Prize. In addition to the anti-malaria drug, artemisinin, which is derived from the vulgaris species, other species of Artemisia have also shown good anti-parasitic effects (7).

Artemisia sieberi is a plant species specific to arid areas, which grows predominantly in the Southwest and Center of Asia. This plant is mostly found in desert and semi-desert areas of Iran and is one of the species recommended in Iranian folklore medicine for the treatment of parasitic diseases in humans and livestock (8). The antimicrobial and antifungal activity of this herbal species has been reported (8, 9). Furthermore, studies have demonstrated the anti-parasitic activity of this herb against different species of parasites, including protozoa and arthropods (10–12). Considering the above, the present study was conducted to investigate the protoscolicidal activity of *Artemisia sieberi* essential oil on protoscolices of hydatid cyst under in vitro conditions.

Methods

Preparation of Essential Oil: After the flowering stage, the aerial parts of *Artemisia sieberi* were collected from Arak, Markazi province, Iran. Plant specimens and species were determined and confirmed by Arak University of Agricultural Sciences, Iran. The plant was dried in shadow at room temperature and extraction of essential oil was done by Clevenger apparatus using hydro-distillation method. Gas chromatography-mass spectrometry (GC-MS) was performed by Shimadzu GC-9A with helium gas as carrier and on the DB-5 column. Mass spectrometry was performed on the GC-MS Varian 3400 system with a DB5 column and a temperature of 40 – 250 °C under the following conditions: transfer line temperature of 260 ° C, helium carrier gas with linear velocity of 31.5 cm/s, 1:60 split rate, ionization energy of 10 ev, scanning time of 1 s, and mass spectrometry of 40 – 300 amu. Identification of *Artemisia sieberi* essential oil was carried out based on commercial libraries NIST 98.1 and MassFinder 3.1. The concentration of each compound was calculated by chromatography (13).

Preparation of protoscolices of hydatid cyst: The livers infected with sheep hydatid cyst were obtained from a slaughterhouse in Sari, Mazandaran province, Iran, and were transferred to the parasitology lab of the Faculty of Veterinary Medicine, Azad University of Babol, Iran. The surface of these livers was washed and disinfected by 70% alcohol-impregnated cotton. After disinfection, the contents of cysts, including *Echinococcus granulosus* protoscolices and fluids, were drained into sterile Erlenmeyer flask. After complete emptying of the cysts and filling the Erlenmeyer flask, we waited a few minutes for the protoscolices to settle. After complete sedimentation of protoscolices, the fluid inside Erlenmeyer flask was

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washed three times with normal saline serum. On average, 10 minutes was spent for sedimentation, carefully draining the fluids and maintaining the protoscolices during each step of washing.

Protoscolices viability test: To determine the percentage of viable protoscolices, some of the prepared fluid containing protoscolices was poured onto the slide using Pasteur pipettes and eosin solution 0.1% was placed near the sample at the same volume. Then, protoscolices and the stain were slowly mixed by the side of the slide, and then, the slide was slowly placed on the prepared solution containing stain. The protoscolices were analyzed after 10 minutes by counting live protoscolices, i.e. the non-stained ones. At least about 500 to 700 protoscolices were counted for viability testing. The viability of protoscolices was determined to be 90% (14).

Investigating the protoscolicidal activity under in vitro conditions: In this study, the protoscolicidal effect of 3 concentrations of 25, 50 and 100 µg/ml of *Artemisia sieberi* essential oil on protoscolices of hydatid cyst was analyzed at 10, 30, 60 and 120 minutes. To prepare the concentrations, 25, 50 and 100 µg/ml *Artemisia sieberi* essential oil were dissolved in 9.7 ml normal saline plus 0.3 ml Tween 80 (Sigma-Germany) in each tube. The solution was mixed well for a few minutes with the help of a magnet.

Similar volume of solution containing the protoscolex of the hydatid cyst and containing about 1000 protoscolices was added to each of the tubes with the help of sterilized Pasteur pipettes and was gently mixed. All tubes were then incubated at 37 °C. After homogenizing each tube, 100 µl of the solution was separated with the aid of sterile pasteurized pipettes at 10, 30, 60 and 120 minutes and was placed on the slide and mixed with eosin 0.1% using a 24×24 slide. After 10 minutes, the protoscolices live-dead ratio was counted on the entire slide surface by optical microscope. The protoscolicidal effect of this essential oil was analyzed and compared with albendazole at similar concentrations (14). The control group did not receive any treatment except Tween in normal saline. Data were then collected and analyzed using one-way ANOVA and post-hoc Tukey tests in SPSS 16 software, while $p<0.05$ was considered significant.

Results

The results of gas chromatography -mass spectrometry analysis showed that the most significant essential oil components of *Artemisia sieberi* are α-Thujone, β-Thujone, and 1,8-Cineole, which constitute 31.5, 11.92, and 10.09% of the essential oil, respectively (Table 1).

Table 1. Chemical compounds of *Artemisia sieberi* Essential Oil

Components	Percentage of components of *Artemisia sieberi* Essential Oil	Inhibition index
α – thujene	0.6	922
α – Pinene	1.22	932
Camphene	8.72	945
Sabinene	0.3	969
β – Pinene	0.98	975
Myrcene	0.3	986
α – Terpinene	0.26	1012
P – Cymene	1.03	1019
1,8-Cineole	10.09	1028
Gamma-terpinene	0.62	1052
Linalool	0.64	1070
Artemisia alcohol	0.23	1080
α – Thujone	31.5	1102
β – Thujone	11.92	1112
Myrcenol	0.37	1123
Camphor	12.3	1140
cis-Verbenol	0.35	1143
Pinocarvone	1.22	1149
trans-Verbenol	0.89	1160
Borneol	1.2	1166
p-Cymene-8-ol	1.14	1176
Myrtenol	0.3	1192
Cis-Piperitol	0.26	1196
Trans-Piperitol	0.54	1206
Piperitone	1.8	1224
Thymol	0.3	1248

At the first 10 – minute time interval, the lowest concentration of *Artemisia sieberi* essential oil (2.5 µg/ml) resulted in the loss of more than half (51.8%) of protoscolices (Table 2). Albendazole showed less protoscolicidal effect at similar concentration (25.6%) and over the same period of time, but both treatments had a significant effect ($p=0.023$) compared to control

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(0%). As the concentration increases, the protoscolicidal activity increases; 5 µg/ml *Artemisia sieberi* essential oil killed 99% and albendazole killed 54.3% of the protoscolices ($p<0.05$) compared to controls. 10 µg/ml *Artemisia sieberi* essential oil killed 100% of protoscolices ($p<0.001$, compared to controls) after 30 minutes, while the similar concentration of albendazole showed a 73.3% toxicity after 30 minutes. 10 µg/ml albendazole was able to kill 100% of protoscolices 120 minutes after treatment (Fig 1).

Table 2. Comparison of Artemisia Sieberi Essential Oil and Albendazol against Echinococcus granulosus protoscolex

After treatment / Group		10 min Mean±SD	30 min Mean±SD	60 min Mean±SD	120 min Mean±SD
Artemisia sieberi (µg/ml)	2.5	51.8±2.8 [a]	71.5±6.3 [a]	82.8±5.1 [a]	99.3±0.6 [a]
	5	66.9±2.2 [c]	91.1±1.8[c]	100±0 [a]	100±0 [a]
	10	99.3±0.6[d]	100±0 [c]	100±0 [a]	100±0 [a]
Albendazole (µg/ml)	2.5	25.6±1.2[b]	43±2.6 [b]	58±5.5 [b]	74.3±4.8[b]
	5	35.6±3.4 [a]	62.2±2.8 [a]	86±3.4 [a]	97±2.5 [a]
	10	54.3±6.9[ac]	73.3±3.8 [a]	99.3±0.6 [a]	100±0 [a]
Control		0±0	8±0.2	10±0.46	10±1.03

Significant differences a, b, c are the meanings in a row with non-like letters = ($p<0.05$)

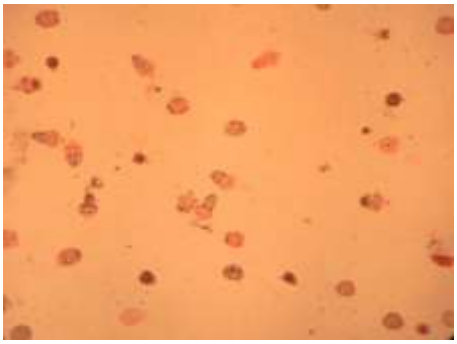
A   B

Figure 1. Staining with Eosin 0.01%. A: Protoscolices viability test B: Death of protoscolices after exposure to *Artemisia sieberi* essential oil.

Discussion

The results of this study showed that *Artemisia sieberi* essential oil has acceptable protoscolicidal effect compared to standard albendazole. Tabari et al. showed the toxicity of *Artemisia sieberi* essential oil against trichomonas galina protozoan under in vivo and in vitro conditions. These researchers reported 10 µg / ml *Artemisia sieberi* essential oil as the minimum inhibitory concentration during 24 hours under in vitro conditions (12). Azadbakht et al. showed that 0.001 concentration of Artemisia plant completely affected the trichomonas vaginalis at the beginning of culture (15). The present study demonstrated the toxic effect of *Artemisia sieberi* essential oil on the protoscolices of hydatid cyst.

The results of a study by Elissondo et al. to evaluate the protoscolicidal effect of thymol on the hydatid cyst were positive and thymol had toxic effect on protoscolexis of *Echinococcus granulosus*, but this toxicity took much longer than usual (16). The results of a study by Mahmoudvand et al. on analyzing the effect of *Carum carvil* extract on protoscolices of hydatid cyst during surgery showed that 100% of protoscolices were killed at 25 µl / ml concentration within 5 min. (6). Investigating the effect of oral pistachio extract on inhibition of the protoscolex of hydatid cyst, Mahmoudvand et al. showed that at 100 mcl / ml, 100% of the protoscolices disappeared in 10 minutes, showing pretty decent effectiveness (17).

The study by Rostami et al. showed that methanolic extract of ginger had a significant effect on protoscolices of hydatid cyst, but the methanolic extract of *Artemisia aucheri* did not have a significant protoscolicidal effect on hydatid cyst protoscolices (18). However, the present study showed acceptable protoscolicidal effect of *Artemisia sieberi* essential oil in killing protoscolices of hydatid cyst. The difference in the findings of these two studies is probably due to

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the different species of the plant and the chemical compounds being studied. María et al. demonstrated a positive effect of albendazole and thymol on the protoscolices of hydatid cyst simultaneously and described the synergistic effect of this medical compound (19).

The present study showed that *Artemisia sieberi* essential oil can be considered as an appropriate protoscolicidal agent and it should be used to eliminate and inhibit the protoscolices after being tested in terms of liver and biliary system toxicity. More studies are needed to analyze the components of *Artemisia sieberi* essential oil, their mechanism of action and the probable synergistic activity of *Artemisia sieberi* essential oil or its components, compared with the standard albendazole. It is to be hoped that due to the natural origin of this treatment and the widespread habitat of this species in Iran, further studies be carried out on the use of this substance alone or in combination with a standard drug during hydatid cyst surgeries.

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