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Susceptibility of Protoscoleces of Hydatid Cyst to Various Concentrations of Oak Gall (*Quercus infectoria* Olivier) Extract at Different Exposure Times *In Vitro*

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Abstract

Background: Chemotherapy is currently used in treatment of different diseases, yet its various adverse effects has led to difficulties in its use for treating hydatid cysts. This leads to use of different non-chemical materials, such as plant extracts as alternatives to chemotherapy in order to cure hydatid cysts.

Objectives: The aim of the present study was to investigate *Quercus infectoria* Olivier extract effects on hydatid cysts.

Methods: In this experimental study, various concentrations of *Quercus infectoria* Olivier extract at different exposure times were evaluated under laboratory conditions for their scolicidal effects on hydatid cysts. To this end, protoscoleces were collected from the livers of sheep affected by hydatid cysts and they were placed under exposure of various concentrations of *Q. infectoria* extract (10, 25, and 50 mg/mL) for various durations of 10, 20, 30, and 60 minutes. Staining with 0.1% eosin was used to specify the viability of protoscoleces. The SPSS software (version 19, Chicago) was the software used to perform the statistical analysis.

Results: The obtained results indicate that *Q. infectoria* extract with the concentration of 50 mg/mL is able to kill all protoscoleces during 20 minutes. It is understood that the scolicidal effects of *Q. infectoria* on hydatid cysts was significant compared to the control groups ($P < 0.05$).

Conclusions: The obtained results delineate higher scolicidal efficacy of *Q. infectoria*'s methanolic extracts; nevertheless, more research should be conducted to confirm the *in vivo* effects of *Q. infectoria* on curing hydatid cysts in human beings and different herbivorous animals.

Keywords: *Quercus infectoria* Olivier Extract, Hydatid Cyst, *In Vitro*, Protoscolex

1. Background

Cystic echinococcosis (CE), also known as hydatid cyst, is one of the main zoonosis infections, which is caused by *Echinococcus granulosus* at the larval stage (1). This disease is widespread in most countries of the world, including Australia, South America, Middle East, Eastern Europe, South Africa, and the Mediterranean region (2).

Three different treatment options to eliminate hydatid cysts, include surgery, chemotherapy, and percutaneous aspiration (3). Inoperable cases are treated using benzimidazole chemotherapy, which is recommended as the best alternative to surgical methods (4). In spite of its efficacy, chemotherapy poses different side effects, such as hepatotoxicity, severe leucopenia, thrombocytopenia, and alopecia (5). Furthermore, resistance to synthetic anthelmintics in treating cystic echinococcosis has made researchers look for other beneficial scolicidal agents, such as medicinal herbs, which pose less adverse effects (3, 6).

Oak is a plant that is related to the genus Quercus in the Fagaceae family. It grows in Asia Minor, Iran, and Greece and it is well-known for its medical properties all over the world (7, 8). Furthermore, *Q. infectoria* was once used as a natural remedy for curing various diseases and it is abundantly found in Zagros mountains in the west of Iran (7).

Several studies have investigated *Q. infectoria* and its derivative antibacterial (9), antifungal (10), antiviral (11), and anti-parasitic (12) effects. Analgesic quality of *Q. infectoria's* methanol extract has also been confirmed previously (13).

Presence of polyphenolic compounds in crude extract of *Q. infectoria* engendered antioxidant, anti-inflammatory, antimicrobial, and anticancer qualities. These compounds include gallic acid and tannins, and some other flavonoid compounds, such as quercetin (12, 14).

2. Objectives

To the best of the author's knowledge, there are no researches that have investigated the scolicidal effects of *Q. infectoria*. Hence, the present study aimed at investigating *Q. infectoria* Olivier extract's in vitro effects on eliminating hydatid cysts.

3. Methods

3.1. Collecting Protoscoleces

In this experimental study, the protoscoleces were taken from livers of infected sheep, which were slaughtered at Urmia abattoir, Northwest of Iran. The hydatid fluid was transferred under aseptic conditions to a flask and left for 30 minutes in order to settle down the protoscoleces (15). Phosphate buffered saline (PBS) solution (pH = 7.2) was used to wash the protoscoleces for three times after removing the supernatant. The viability of the protoscoleces was confirmed from the motility features of protoscoleces, using an ordinary light microscope following staining with 0.1% eosin.

3.2. Collecting Plant Materials and Extraction

The oak plants were obtained from mountains of Sardasht (West Azerbaijan, Iran) on September, 2015. The obtained samples were identified and confirmed by the agriculture faculty of Urmia University (Urmia, Iran).

Some modification was done on the method described by Moazeni and Roozitalab (15) to prepare the methanolic extract of *Q. infectoria*. Briefly, a commercial electric blender was utilized to powder the dried plant. A small amount of the obtained powder (100 g) was added to 400 mL of pure methanol and gently mixed for one hour by a magnetic stirrer. The resulting solution was left intact for 24 hours at room temperature. After 24 hours, the solution was stirred again and filtered. Using a rotating evaporator, the solvent was removed. The material was left in semisolid form and it was freeze dried. It was stored at 4°C for further usage.

3.3. Scolicidal Effects of Quercus inferctoria

Quercus infectoria extract with three different concentrations of 10, 25, and 50 mg/mL were used with different exposure times (10, 20, 30, and 60 minutes) in order to study their efficacy in eliminating protoscoleces of hydatid cysts. Furthermore, 0.5 mL of protoscoleces (2×10^3/mL) solution was placed in test tubes. Then 0.5 mL of each concentration was added to each test tube (16). They were gently mixed and then left intact for 10, 20, 30, and 60 minutes at room temperature. At each interval, the upper phase of the mixture was taken by a pipette. This was done very carefully to avoid disruption of the protoscoleces. Staining was done by adding 2 mL of 0.1% eosin to the settled protoscoleces in the tube and it was gently mixed (15). The upper position of the remaining solution was discarded. The remaining protocoleces were smeared on a glass slide to be examined with a light microscope in order to investigate their viability. Dead protoscoleces' percentage was obtained by counting a minimum of 450 (mostly more than 500) protoscoleces. Normal saline was selected for utilization as the control group and the experiments were performed in triplicates.

This study used 0.1% eosin (1 g eosin in 1000 mL of distilled water) in order to analyze protoscoleces viability. Fifteen minutes of exposure to eosin had no significant effect on the color of viable cells, yet it made the dead cells turn red due to absorbing eosin. By dividing the percentage of dead protoscoleces to their sum total, the mortality rate was measured (16).

3.4. Statistical Analysis

The SPSS software (version 19, Chicago) was the software used to perform the statistical analysis. Chi-square test was utilized to analyze the difference between test and control groups. Values less than 0.05 ($P < 0.05$) were considered significant.

4. Results

Results of the effectiveness of different concentrations of *Q. infectoria* extract as a scolicidal agent are summarized in Tables 1-3. It was observed that the *Q. infectoria* extract at all concentrations exhibited significant scolicidal effects in comparison with the control group ($P < 0.05$).

Furthermore, *Q. infectoria* extract at a concentration of 25 mg/mL killed 47.16%, 55.1%, 73.08%, and 92.57% of the protoscoleces after 10, 20, 30 and 60 minutes of application, respectively, while the mortality rate of protoscolices in the control group was 11.7%. In addition, all protoscoleces were killed after 20 minutes of exposure to 50 mg/mL of *Q. infectoria* extract (Figure 1). By increasing the exposure time with *Q. infectoria* extract at all concentrations, the mortality rate was significantly increased ($P < 0.05$) (Figure 2). Therefore, the results of the current study indicated that the methanolic extract of *Q. infectoria* has high scolicidal activity *in vitro*.

5. Discussion

In this study, the researchers investigated the potency of *Q. infectoria* extract on prtoscolices of hydatid cyst.

Table 1. Scolicidal Effect of *Quercus infectoria* Extract Against Protoscoleces of Hydatid Cyst at the Concentration of 10 mg/mL Following Various Exposure Times

Exposure Time (Min)	Experiments			Total
	1	2	3	
10				
Protoscoleces	430	400	421	1251
Dead protoscoleces	105	90	94	289
Mortality rate (%)	25.41	22.5	22.32	23.1
20				
Protoscoleces	421	429	457	1307
Dead protoscoleces	178	167	162	507
Mortality rate (%)	42.28	38.92	35.44	38.7
30				
Protoscoleces	467	469	478	1414
Dead protoscoleces	282	283	279	844
Mortality rate (%)	60.38	60.34	58.36	59.6
60				
Protoscoleces	450	445	457	1352
Dead protoscoleces	328	321	333	982
Mortality rate (%)	72.8	72.1	72.8	72.6
Control				
Protoscoleces	624	502	521	1647
Dead protoscoleces	76	60	58	194
Mortality rate (%)	12.17	11.9	11.13	11.7

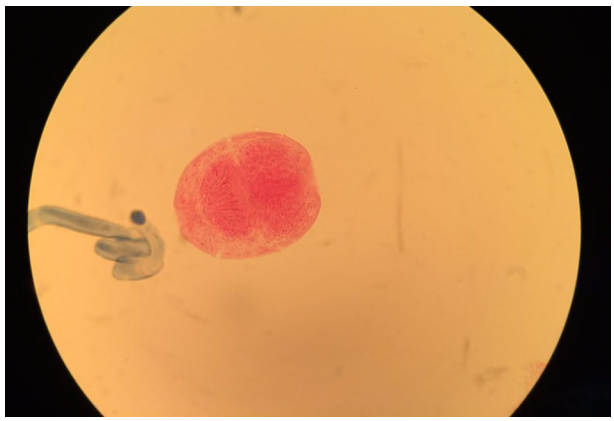

Figure 1. Dead protoscolex after staining with 0.1% eosin

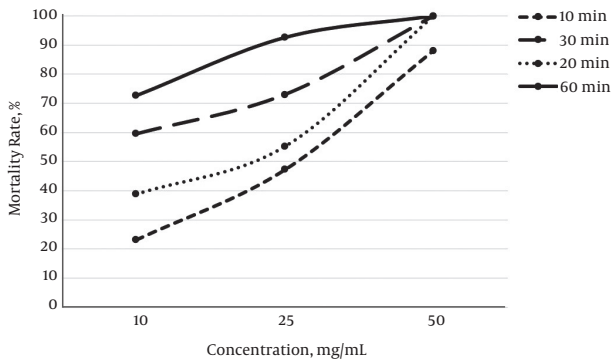

Figure 2. Scolicidal effects of *Quercus infectoria* extract against protoscolices of *Echinococcus granulosus* at various concentrations following various exposure times.

Surgery is the most preferred method for removing *E. granulous* cysts in order to cure hydatid cysts (17). So far, different sclocidal agents were utilized to counteract the contents of hydatid cysts, yet no one proper strategy has been figured out to eradicate this disease (18, 19). Qualities, such as low cost, potency at low concentrations, lack of adverse effects, quick action, and non-toxicity are among the desired qualities for a good scolicidal agent (20).

Less adverse effects, low price, and high availability are qualities that made herbal extracts a good alternative for treating various diseases. There have been numerous stud-

Table 2. Scolicidal Effect of *Quercus infectoria* Extract Against Protoscoleces of Hydatid Cyst at the Concentration of 25 mg/mL Following Various Exposure Times

Exposure Time (Min)	Experiments			Total
	1	2	3	
10				
Protoscoleces	400	478	426	1304
Dead protoscoleces	202	210	203	615
Mortality rate (%)	50.5	43.93	47.65	47.16
20				
Protoscoleces	414	409	425	1248
Dead protoscoleces	232	231	225	688
Mortality rate (%)	56.03	56.4	52.9	55.1
30				
Protoscoleces	462	455	465	1382
Dead protoscoleces	35	37	29	101
Mortality rate (%)	75.7	81.3	62.3	73.08
60				
Protoscoleces	413	425	415	1253
Dead protoscoleces	392	377	391	1160
Mortality rate (%)	94.91	88.7	94.2	92.57
Control				
Protoscoleces	624	502	521	1647
Dead protoscoleces	76	60	58	194
Mortality rate (%)	12.17	11.9	11.13	11.7

Table 3. Scolicidal Effect of *Quercus infectoria* Extract Against Protoscoleces of Hydatid Cyst at the Concentration of 50 mg/mL Following Various Exposure Times

Exposure Time (Min)	Experiments			Total
	1	2	3	
10				
Protoscoleces	475	471	482	1427
Dead protoscoleces	405	425	427	1257
Mortality rate (%)	85.26	90.23	88.58	88.08
20				
Protoscoleces	459	467	475	1383
Dead protoscoleces	459	467	475	1383
Mortality rate (%)	100	100	100	100
Control				
Protoscoleces	624	502	521	1647
Dead protoscoleces	76	60	58	194
Mortality rate (%)	12.17	11.9	11.13	11.7

ies on scolicidal effects of different plants, such as *Nigella sativa* (16), *Allium sativum* (21), *Curcuma long*, and *Zingiber officinale* (22), *Salvadora persica* (23), and *Zataria multiflora* (15) to use them in curing hydatid cysts.

Quercus infectoria has been widely used in traditional medicine for analgesic, CNS depressant, antidiabetic, anti-

inflammatory, and antiparkinsonian purposes (24). In addition, *Q. infectoria* is considered a safe and nonpathogenic plant for cells of mammalian species (12).

Reports on the effects of this herbal extract delineate that *Q. infectoria* is composed of various components, which target several points in microorganism cells (25). As mentioned earlier, polyphenolic compounds like tannins and gallic acid, along with flavonoid compounds, such as quercetin, are among the most significant components of *Q. infectoria* (12, 14), and it is assumed that they are responsible for its biological efficacy. Studies has also revealed antiplasmodial, antileishmanial, and trypanocidal effects of flavonoid compounds (26, 27).

There haven't been any studies investigating scolocidal qualities of *Q. infectoria*. This is the first study analyzing the effects of oak extract *in vitro* condition on hydatid cysts.

This study showed that *Q. infectoria's* scolicidal activity is significant at concentrations of 50 mg/mL after 20 minutes of exposure, which leads to a 100% mortality rate. In other studies, Moazeni and Roozitalab (15) investigated the protoscolicidal activity of *Zataria multiflora* extract. They concluded that *Zataria multiflora extract* had 100% scolicidal effect at a concentration of 25 mg/mL after one minute of exposure, whereas the application of Garlic (*Allium sativum*) extract produced excellent results (100%) over killing of protoscolices at a concentration of 50 mg/mL after 10 minutes (21).

Quercus infectoria's scolicidal effects at the concentration of 50 mg/mL after 20 minutes of exposure was similar to some other agents, such 95% ethyl alcohol (15 minutes) (28), 0.5% to 1% cetrimide (10 minutes) (29), 3% H_2O_2 (15 minutes) (30), and 20% hypertonic saline (15 minutes) (31). In the present study, the researchers observed a higher scolicidal effect (100%) with *Q. infectoria* extract at a higher concentration (50 mg/mL).

Furthermore, this research showed higher *in vitro* concentrations of *Q. infectoria* extract were toxic for protoscoleces of *E. granulosus*, which might be due to the presence of flavonoid compounds. In line with the current results, Kheirandish et al. (12), showed that high amounts of phenolic compounds extract of *Q. infectoria* might be the active compound responsible for anti-leishmanial activity.

In conclusion, the current findings demonstrated that *Q. infectoria* could be used as an effective scolicidal agent. Further investigations are required to determine the potential adverse effects of *Q. infectoria* and to confirm the scolicidal efficacy of this herbal medicine *in vivo*.

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Footnotes

Authors' Contribution: This study was designed and managed by Farnaz Malekifard. The experiments were performed and analyzed by Fatemeh Keramati.

Conflict of Interests: The authors disclose no potential conflicts of interest.

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