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In Vitro Assessment of the Protoscolicidal Activities of the *Ephedra major* Methanol Extracts

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Abstract

Background: The basic approaches for the treatment of human hydatidosis are surgery and chemotherapy. The risk of spillage of protoscoleces cannot be underestimated during surgery.

Objectives: This study aimed to evaluate the scolicidal activity of *Ephedra major* methanol extracts against protoscoleces of hydatid cysts.

Materials and Methods: Various concentrations of the methanolic extracts (0.01%-0.001%, mg/mL) of different parts of *E. major* were used for exposure times of 10, 20, 30, and 60 minutes.

Results: Different extracts of *E. major* were tested, 0.1% concentration had strong scolicidal activity in 60 minutes. The stem extracts of 0.1% had very strong scolicidal effects in 60 minutes of exposure time and the mortality rate decreased with the lower concentration.

Conclusion: Findings showed that scolicidal effects of *E. major* root extracts against cystic echinococcosis protoscoleces were less effective, while the stem and leaf extracts demonstrated more activities, respectively.

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Background

Cystic echinococcosis is a widely zoonotic infection caused by larval stages (metacestodes) of the tapeworm *Echinococcus granulosus*. Hydatidosis affects mainly the intermediate hosts viscera, including the liver, lungs, and less frequently the muscle, kidney, bone, spleen, and other organs.[1] Hydatidosis is more prevalent in the countries in the Middle-East, Arabic, North Africa, and it is endemic in Iran.[2] Currently, the main methods for the treatment of hydatidosis are surgery (percutaneous aspiration, injection and reaspiration—PAIR) and chemotherapy (using benzimidazoles such as mebendazole and albendazole).[3,4] The surgical treatment of hydatid diseases is still the most effective approach. It can be done successfully in a large number of patients if a cyst does not have a risky location. Injection of protoscolicidal agents into the cysts preoperatively is a traditional method.[5] Spillage of protoscoleces can cause relapse or secondary infection that occurs in approximately 10% of postoperative cases.[6] However, the absence of enough evidence about the efficacy and the presence of toxicity associated with the protoscolicidal agents led many surgeons to leave this routine stage in the operative management of hydatidosis.[7,8] Therefore, the cystic fluid including many of protoscoleces has the potential to grow into new cystic echinococcosis.[9]

Recently, there has been a considerable attention in finding natural protoscolicidal agents from indigenous herbs to replace synthetic ones. Results of investigations have shown that the plant contains a large variety of substances that possess scolicidal effects.[10-14] *Ephedra*, a medicinal plant belonging to the Ephedraceae Dum. family, is genus of non-flowering seed plants belonging to the Gentlales, the closest living relative of the angiosperms.[15,16] In our area, this indigenous plant that is called "Hamisheh Zaroor" means "Always necessary" has been used for many years in the treatment of allergies, bronchial asthma, chills, coughs, and has been a source of natural product for alkaloids and other related compounds.[17-19] There are several reports concerning in vitro antibacterial effect against different bacterial species, including *Staphylococcus aureus*, *Bacillus antheracis*, *B. diphtheria*, *B. dysenteriae*, *B. typhosus* and *Pseudomonas aeruginosa*.[20,21]

Objective

The purpose of this study was to determine the in vitro activity of *Ephedra major* methanolic extracts against pro-

toscoleces of hydatid cysts based on different concentrations and exposure times.

Materials and Methods

Plant Materials

The root, stem and leaf of *Ephedra major* were collected between 2014 and 2015 in their places of origin in Lorestan province in southwest of Iran. They were authenticated by Razi Herbal Medicine Research Center, Lorestan University of Medical Sciences, Iran.

Extracts Preparation

Different parts of the plants were dried at room temperature and powdered. About 10 g of powdered subjects were extracted with 100 mL of absolute methanol (10% w/v) in a conical flask for maceration. The mixture was shaken for 3 days at room temperature. The suspension was filtered with a Whatman Filter Paper No.1.[22] The procedure of extraction was operated via rotary evaporation at 37°C and the extracts were stored at 4°C until use.

Protoscoleces

Hepatic hydatid cysts from naturally infected sheep were obtained from an abattoir located in Haftjuy in the central district of Qods county, Tehran province, Iran. Protoscoleces were collected in aseptically conditions and washed at least 3 times with phosphate-buffered saline (PBS). The concentration of protoscoleces per milliliter of the hydatid fluid in normal saline solution (0.9% NaCl) containing 5000 protoscoleces in milliliter with more than 90% viability was used.[23]

Viability

After adding 10 µL eosin solution (0.1% w/v) to 10 µL, the viability of protoscoleces was reviewed microscopically for 15 minutes. Stained protoscoleces were considered as dead and unstained protoscoleces were recorded as alive. The control group included non-treated protoscoleces with the plant extracts.

Statistical Analysis

All experiments were done three times in each group. Findings are shown as means ± standard error of mean (SEM). Statistical differences were analyzed using Mann-Whitney non-parametric test. Probability (*P*) values of less than 0.05 were considered to be statistically significant.

Results

The mortality rates of protoscolices of hydatid cysts after exposure to various concentrations of *E. major* extracts in different times are demonstrated in Table 1. Obtained findings showed that *E. major* stem extracts at the concentration of 0.1% after 60 minutes of exposure time killed 99.09% of protoscoleces. Similarly, the mean of mortality rate of protoscoleces after 60 minutes exposure with *E. major* leaves extract in the concentration of 0.1% was 90.25%. The protoscolicidal rate in the control group was 4.99% after in the some exposure times. Findings also revealed that *E. major* extracts at all concentrations had significant protoscolicidal activity ($P=0.001$) compared with the control group (Table 2).

Discussion

There is a particular interest in studies about protoscolicidal activity in order to prevent the formation of secondary echinococcosis. An appropriate protoscolicidal effects

Table 1. Scolicidal Activities of Different Concentrations of *Ephedra major* Root, Stem and Leaf Extracts in Different Exposure Time[a]

Concentrations (%)	Exposure Time (min)			
	10	20	30	60
Group 1[b]				
0.1	63.19	64.90	50.20	69.22
0.01	20.94	11.27	7.93	12.27
0.001	10.40	9.91	7.13	9.54
Group 2[c]				
0.1	99.71	98.92	99.39	99.09
0.01	8.57	7.19	41.37	35.63
0.001	44.99	4.21	26.36	28.57
Group 3[d]				
0.1	80.56	84.80	97.10	93.50
0.01	51.14	73.21	70.28	83.09
0.001	17.72	21.09	43.33	48.11
Control				
1	0.23	2.45	5.78	8.89
2	1.53	1.34	7.90	8.73
3	1.06	2.67	6.23	8.97

[a] Concentrations of all of the plants extract perpetrated with one batch.
[b] Root.
[c] Stem.
[d] Leaf.

Table 2. The Mean of Scolicidal Effects of Various Extracts of *Ephedra major* Root, Stem and Leaf Extracts in Exposure Times[a]

Concentrations (%)	Mean ± SD (min)
Roots	
0.1	66.39 ± 34.61
0.01	13.21 ± 11.50
0.001	9.51 ± 8.40
Control	3.43 ± 3.76
Stems	
0.1	99.09 ± 2.27
0.01	20.26 ± 28.77
0.001	13.30 ± 28.67
Control	5.19 ± 3.51
Leaves	
0.1	90.25 ± 19.42
0.01	68.53 ± 28.79
0.001	30.78 ± 27.61
Control	6.35 ± 4.20

[a] *P* value = 0.001

demonstrated by its ability for rapid preparation, higher availability, stronger effects at low concentrations, high efficacy in shorter time after exposure, and less toxic effects.[24] Recently, the scolicidal effects of several herbal extracts such as *Olea europaea*, *Pistacia atlantica*, *Allium sativum*, *Zataria multiflora*, and some chemicals including silver nitrate, cetrimide, ethyl alcohol, peroxide hydrogen, manithol, and hypertonic saline have been.[25] We found that *E. major* stem extracts in various concentrations had potent protoscolicidal effects which are comparable with the protoscolicidal activity leaf and root extracts in different exposure times. It is noteworthy that increasing concentrations and exposure times showed more protoscolicidal efficacy. Findings indicated that stem extracts killed almost all of protoscoleces and its 0.1% concentration revealed strong scolicidal effects at 60 minutes, 0.001% concentration did not have enough lethality activity at the same time. In this in vitro experiment, we showed that the concentrations (0.1%-0.001%) of root extracts had less activity. Thus, the protoscoleces that were killed by root extracts (0.001% concentration) reduced the mortality rate to 9.54% at 60 minutes exposure time.

Although, to our knowledge, there is no report on antiparasitic activities of *E. major* methanolic extracts. Previous studies have proven antimicrobial properties of this plant. The *Ephedra strobilacea* showed activity against most of microorganisms. Based on the results, the methanolic extracts of *Ephedra pachyclada* significantly revealed antimicrobial effects against *Klebsiella pneumonia* and *Bacillus subtilis* as gram positive bacteria. Furthermore, the result revealed the methanolic extract of *Ephedra procera* wild plant significantly had highest antifungal activity against *Candida albicans* microorganisms. Amazingly, all methanolic extracts of *Ephedra callus* culture showed anti-*Pseudomonas aeruginosa* effects.[25-29] A survey which focused solely on the protoscolicidal activity of *E. major* leaves aquatic extracts demonstrated that the scolicidal effect against protoscoleces was not suggested and more research is also necessary to investigate by other extracts.[30]

Finally, the present study demonstrated that the methanolic extracts of *E. major* might be a natural source and be used as new scolicidal agents during hydatid cysts surgery (PAIR) to reduce the risk of protoscoleces spillage. However, more researches will be needed to evaluate the in vivo effects of these extracts in a clinical setting on animals and humans.

Authors' Contributions

MZ: the study design, management and supervision; SS and SB: sampling, processing and performing the conventional and procedures; ZJ: provided advice; FF: provided advice, read and arranged the final manuscript; MS: plant collection.

Ethical Approval

Approval of the study protocol was received from the Ethical Committee of Alborz University of Medical Sciences (No. 5348).

Conflict of Interest Disclosures

The authors declare that they have no competing interests.

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References

1. Ammann RW, Eckert J. Cestodes. *Echinococcus*. Gastroenterol Clin North Am. 1996;25:655-659.
2. Rokni MB. Echinococcosis/hydatidosis in Iran. Iran J Parasitol. 2009;4:1-16.
3. Moazeni M, Nazer A. In vitro effectiveness of garlic (*Allium sativum*) extract on scolices of hydatid cyst. World J Surg. 2010;34:2677-2681. doi:10.1007/s00268-010-0718-7.
4. Markin D, Duek L, Berdicevsky I. In vitro antimicrobial activity of olive leaves. Mycoses. 2003;46:132-136.
5. Belghiti J, Benhamou JP, Houry S, Grenier P, Huguier M, Fékété F. Caustic sclerosing cholangitis: a complication of the surgical treatment of hydatid disease of the liver. Arch Surg. 1986;121:1162-1165.
6. Rajabi MA. Fatal reactions and methaemoglobinaemia after silver nitrate irrigation of hydatid cyst. Surg Pract. 2009;13:2-7.
7. Prasad J, Bellamy PR, Stubbs RS. Instillation of scolicidal agents into hepatic hydatid cysts: can it any longer be justified? N Z Med J. 1991;104:336-337.
8. Jakubowski M, Barnard D. Anaphylactic shock during operation for hydatid disease. Anesthesiology. 1971;34:197-199.
9. Besim H, Karayalçin K, Hamamci O, Güngör C, Korkmaz A. Scolicidal agents in hydatid cyst surgery. HPB Surg. 1998;10:347-451.
10. Sadjjadi SM, Zoharizadeh MR, Panjeshahin MR. In vitro screening of different *Allium sativum* extracts on hydatid cysts protoscoleces. J Invest Surg. 2008;21(6):318-322. doi:10.1080/08941930802348261.
11. Ahmadian-Attari MM, Amini M, Farsam H, et al. Isolation of major active antibacterial compounds of Sumac fruit (*Rhus coriaria* L.). Int J Enteric Pathog. 2016;4(4):e37101. doi:10.15171/ijep.2016.11.
12. Hosseini SV, Ghanbarzadeh K, Barzin Z, Sadjjadi SM, Tanideh N, Mehrabani D. In vitro protoscolicidal effects of hypertonic glucose on protoscolices of hydatid cyst. Korean J Parasitol. 2006;44:239-242.
13. Ciftci IH, Esme H, Sahin DA, Solak O, Sezer M, Dilek ON. Effect of octenidine dihydrochloride on viability of protoscoleces in hepatic and pulmonary hydatid diseases. J Natl Med Assoc. 2007;99(6):674-677.
14. Moazeni M, Larki S. In vitro effectiveness of acidic and alkaline solutions on scolices of hydatid cyst. Parasitol Res. 2010;106(4):853-856. doi:10.1007/s00436-010-1723-3.
15. Friedman WE. Introduction to biology and evolution of the Gnetales. Int J Plant Sci. 1996;157:S1–S2.
16. Friedman WE. The evolution of double fertilization and endosperm a historical perspective. Sex Plant Reprod. 1998;11:6-16.
17. Konar RN, Singh MN. Production of plantlets from female gametophytes of *Ephedra foliates* Boiss. Z Pflanzenphysiol. 1979;95:87-90.
18. Nawwar MAM, Barakat HH, Buddrust J, Linscheidt M. Alkaloidal, lignan and phenolic constituents of *Ephedra alata*. Phytochemistry. 1985;24:878-879.
19. O'Dowd NA, McCauley G, Wilson JAN, Parnell TAK, Kavanaugh D. In vitro culture, micropropagation and the production of ephedrine and other alkaloids. In: Bajaj YPS, ed. Biotechnology in Agriculture and Forestry. Berlin, Germany: Springer;1998:41.

20. Soltan MM, Zaki AK. Antiviral screening of forty-two Egyptian medicinal plants. J Ethnopharmacol. 2009;126:102-107.
21. Ramawat KG, Arya HC. Growth and morphogenesis in callus cultures of *Ephedra gerardiana*. Phytomorphology. 1976;26:395-403.
22. Zibaei M, Sarlak A, Delfan B, Ezatpour B, Azargoon A. Scolicidal effects of *Olea europaea* and *Satureja khuzestanica* extracts on protoscolices of hydatid cysts. Korean J Parasitol. 2012;50:53-56.
23. Zibaei M, Rostamipour R, Nayebzadeh H. Effect of *Pistacia atlantica* fruit and leaf extracts on hydatid cyst protoscolices. Recent Pat Antiinfect Drug Discov. 2016;11:53-58.
24. Shahnazi M, Azadmehr A, Latifi R, Hajiaghaee R, Saraei M, Alipour M. In vitro protoscolicidal effects of various concentrations of *Ziziphora tenuior* L. extract at different exposure times. Avicenna J Phytomed. 2016;6:376-382.
25. Mahmoudvand H, Kheirandish F, Ghasemi Kia M, Tavakoli Kareshk A, Yarahmadi M. Chemical composition, protoscolicidal effects and acute toxicity of *Pistacia atlantica* Desf. fruit extract. Nat Prod Res. 2016;30(10):1208-1211. doi: 10.1080/14786419.2015.1046868.
26. Rice-Evans CA, Miller NJ, Paganaga G. Antioxidant properties of phenolic compounds. Trends Plant Sci. 1997;2:152-159.
27. Wojdylo A, Oszmianski J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 2007;105:940-949.
28. Mi-Jung Y. Anti-arthritic effects of *Ephedra sinica* STAPF herb acupuncture: inhibition of lipopolysaccharide-induced inflammation and adjuvant-induced polyarthritis. Pharmacol Sci. 2006;100:41-50.
29. Parsaeimehr A, Sargsyan E, Javidnia K. A comparative study of the antibacterial, antifungal and antioxidant activity and total content of phenolic compounds of cell cultures and wild plants of three endemic species of *Ephedra*. Molecules. 2010;15(3):1668-1678. doi:10.3390/molecules15031668.
30. Roshan A, Nayebzadeh H, Zibaei M, Shokrani H, Tarahi M. In vitro evaluation of aqueous solution of *Ephedra major* on protoscloces of hydatid cysts (Persian). Sci J Alborz Univ Med Sci. 2016;5:236-241.