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Scolicidal Effects of *Olea europaea* and *Satureja khuzestanica* Extracts on Protoscolices of Hydatid Cysts

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Abstract: Treatment of hydatid disease is mainly surgical, with medical treatment being reserved as a coadjuvant treatment. Use of effective scolicidal agents during surgery of cystic echinococcosis is essential to reduce the recurrence rate. The goal of this study was to evaluate the in vitro scolicidal effects of hydroalcoholic extracts of *Satureja khuzestanica* leaves and aqueous extracts of *Olea europaea* leaves on hydatid cyst protoscolices. *Echinococcus granulosus* protoscolices were collected from the liver of sheep infected with the hydatid cyst. Various concentrations of plant extracts were used in different exposure times for viability assay of protoscolices. Among the olive leaf extracts tested, 0.1% and 0.01% concentrations had strong scolicidal effects in 120 min. *S. khuzestanica* 0.1% had very strong scolicidal effects in 30, 60, and 120 min of exposure times and the mortality rate decreased with the lower concentration. The finding have shown that the scolicidal activity of *S. khuzestanica* against cystic echinococosis protoscolices were more effective, while the *O. europaea* extract showed less effects.

Key words: *Echinococcus granulosus*, *Olea europaea, Satureja khuzestanica*, protoscolicidal activity, hydatid cyst

INTRODUCTION

Hydatidosis caused by *Echinococcus* spp. is a major zoonotic infection that is detrimental to both humans and animal husbandries in many countries. Cystic echinicoccosis affects mainly the intermediate host's viscera, including the liver, lungs, and less frequently, the spleen, kidneys, bone, brain, and other organs [1]. Currently the basic approaches for treatment of hydatid disease are surgery and chemotherapy. However, operative leakage may lead to dissemination of viable protoscolices to adjacent tissues and thus to intrapritoneal hydatid disease [2,3].

The olive (*Olea europaea*) tree, a plant which can survive for hundreds of years, is known to naturally possess strong resistance to microbial attack [4]. Natural olive leaf and olive leaf extracts are now marketed as anti-aging agents, immunostimulators, and even antibiotics. Olive leaf extracts have been used throughout history for their medical properties, for instance, treatment of infections. Several studies have shown a decreased risk of bacterial and parasitic protozoan diseases with an increasing consumption of olive products [4,5]. Although antiprotozoal activities of the competent Oleuropein (*O. europaea*) have been examined, anthelmintic potential of olive leaf extracts have not been reported.

Satureja khuzestanica Jamzad is an endemic plant that is widely distributed in the southern part of Iran. It is famous for its medical uses as an analgesic and antiseptic in folk medicine [6]. Recently, antiviral, antibacterial, antifungal, and antiprotozoal effects were investigated from various species of *Satureja* [7-15].

Therefore, the aim of this investigation was to examine the activity of Iranian medical plants, including *O. europaea* and *S. khuzestanica*, against protoscolices of hydatid cysts and to determine the exposure time and concentrations of the extracts providing scolicidal activities.

MATERIALS AND METHODS

Plant material

Leaves of *O. europaea* and the aerial parts of *S. khuzestanica* were collected in June 2010 from Khorram Abad (center of Lorestan province) in southwestern Iran, and identified by Botanical Section, Lorestan University of Medical Sciences. The

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plants were dried in open air and shady conditions until completely dried and then ground to a powder. All experiments were performed with 1 batch of olive leaf and *S. khuzestanica* extracts, separately.

Preparation of aqueous plant extracts

A 200 *g* based on dry weight powdered leaves added to adequate amount water (1,000 ml) to concentration of 20% (w/v). The products were squeezed through gauze cloth to remove the practice and the extract was passed through a 0.2 µm filter (Millipore™ Membrane Filter, USA). The procedure of extraction and filtration were operated at room temperature. The extract was stored at 4˚C until use.

Preparation of hydroethanolic plant extract

S. khuzestanica extracts were prepared according to the method of Zarrin et al. [16]. Briefly, about 10 g powdered leaves of *S. khuzestanica* was extracted with adding 100 ml of 80% ethanol (1:10 w/v). After 72 hr at room temperature, the suspension was filtered though a Whatman paper No.1 and the crude ethanol extracts were evaporated at 37˚C. One gram of extract was dissolved in 1 ml of 100% dimethylsulfoxide (DMSO), and the final concentration of each extract was adjusted to 1,000 mg/ml.

Collection of protoscolices

Sixty hydatid cysts were collected from the liver of infected sheep slaughtered at Khorram Abad and carried to the Parasitology Laboratory at the Department of Parasitology and Mycology, Lorestan University of Medical Sciences. The protoscolices were obtained from the hydatid fluid and washed 3 times in PBS (pH 7.2). The concentration of protoscolices was confirmed as the number of protoscolices per ml of the hydatid fluid in a saline solution (0.9% NaCl solution) containing 5×10^3 protoscolices in 1 ml with more than 90% viability was used in further use [2].

Viability assay

A 0.1 mg of eosin stain was dissolved in 100 ml distilled water at a 0.1% (w/v) concentration. The viability was checked microscopically after adding 10 µl of eosin solution to 10 µl of protoscolices for 15 min. Stained protoscolices were considered as dead while unstained protoscolices were recorded as alive. Non-treated protoscolices (with plant extracts) were considered as the control group.

Statistical analysis

The statistical analysis was performed with the use of SPSS version 15.0.1. The protoscolicidal activity was calculated as means ± SD.

RESULTS

The mortality rates of protoscolices of hydatid cysts after exposure to different concentrations of *O. europaea* extracts in various time periods are demonstrated in Table 1. Olive leaf extracts 0.1% had strong scolicidal effects in 120 min, and 0.01% also revealed the same effects at the same time. A 96.7% of protoscolices lost viability at 120 min (0.01% diluted). The mortality rate at 0.001% decreased to 53.1% at 30 min, while many of the protoscolices died at 0.1% at 120 min (Table 2).

The experiment conducted with *S. khuzestanica* showed all of the protoscolices died in 0.1% concentrations. On the other hand, the mortality rate was low by increasing the time of exposure and decreasing concentration. The effects of different concentrations of *S. khuzestanica* extracts on the viability of *E. granulosus* protoscolices in different exposure times is in shown Table 3.

DISCUSSION

The surgical treatment of *E. granulosus* cyst is still the current-

Table 1. Protoscolicidal effects of various concentrations of *Olea europaea* leaf extract in different time periods

Concentration[a] (%)	% Mortality rates (dead/total) after exposure		
	30 (min)	60 (min)	120 (min)
Group 1 (%)			
0.1	61.7 (127/206)	77.5 (159/205)	91.5 (183/200)
0.01	60.3 (126/209)	71.9 (148/206)	78.7 (172/218)
0.001	51.2 (125/205)	66.6 (136/204)	73.9 (153/207)
Group 2 (%)			
0.1	74.0 (153/207)	97.6 (202/207)	100.0 (208/208)
0.01	70.1 (141/201)	95.8 (205/214)	99.5 (202/203)
0.001	55.5 (111/200)	65.1 (140/215)	98.1 (214/218)
Group 3 (%)			
0.1	49.5 (106/214)	77.1 (162/210)	98.5 (202/205)
0.01	52.5 (107/204)	76.4 (159/208)	80.3 (159/198)
0.001	48.3 (100/207)	63.8 (129/202)	79.4 (166/209)
Control			
1	0.0 (0/199)	3.5 (7/202)	6.9 (15/215)
2	0.0 (0/208)	2.4 (5/202)	8.1 (17/209)
3	0.0 (0/215)	3.5 (7/201)	7.3 (15/205)

[a]Concentration of all of the plant extract perpetrated with 1 batch.

Table 2. The protoscolicidal activity of different extracts of *Olea europaea* and *Satureja khuzestanica* at 30, 60, and 120 min of exposure times

Concentrations (%)	Rate of death[a] (No. dead/No. tested)		
	30 (min)	60 (min)	120 (min)
Olea europaea			
0.1	61.7±12.3 (386/627)	84.1±11.7 (523/622)	96.7±4.5 (593/613)
0.01	60.9±8.8 (374/614)	81.4±12.7 (512/628)	89.2±9.7 (533/619)
0.001	51.8±3.6 (336/612)	65.2±1.4 (405/621)	83.8±12.7 (533/634)
Satureja khuzestanica			
0.1	100.0±0.0 (611/611)	100.0±0.0 (611/611)	100.0±0.0 (631/631)
0.01	77.1±7.2 (514/614)	83.3±3.4 (525/630)	68.6±3.7 (426/621)
0.001	75.6±5.6 (474/627)	74.3±4.9 (459/617)	67.6±2.1 (419/620)

[a]Results are expressed as mean±SD.

Table 3. Scolicidal effects of different concentrations of *Satureja khuzestanica* after 30, 60, and 120 min of application

Exposure time (min)	Tests	Concentration[a] (%)	% M.R.[b] (No. dead/No. examined)
30	1	0.1	100.0 (205/205)
	2	0.1	100.0 (201/201)
	3	0.1	100.0 (205/205)
	1	0.01	88.9 (185/209)
	2	0.01	80.3 (163/203)
	3	0.01	82.1 (166/202)
	1	0.001	78.2 (158/202)
	2	0.001	79.4 (170/214)
	3	0.001	69.1 (146/211)
60	1	0.1	100.0 (203/203)
	2	0.1	100.0 (203/203)
	3	0.1	100.0 (205/205)
	1	0.01	85.5 (177/207)
	2	0.01	85.1 (178/209)
	3	0.01	79.4 (170/214)
	1	0.001	79.2 (172/217)
	2	0.001	69.5 (141/203)
	3	0.001	74.1 (146/197)
120	1	0.1	100.0 (216/216)
	2	0.1	100.0 (208/208)
	3	0.1	100.0 (207/207)
	1	0.01	71.6 (151/211)
	2	0.01	64.4 (134/208)
	3	0.01	69.8 (141/202)
	1	0.001	69.8 (143/205)
	2	0.001	65.6 (133/203)
	3	0.001	67.5 (143/212)
Control	1		0.5 (1/200)
	2		3.3 (7/208)
	3		5.3 (11/205)

[a]Concentration of all of the plant extract perpetrated with 1 batch.
[b]Mortality rate (%)

ly most effective method. It can be performed successfully in majority of the patients, if a cyst does not have a risky localization or if the disease is not too far advanced. It has been traditional to inject protoscolicidal agents into hydatid cysts perioperatively. However, lack of objective evidence about the efficacy and the presence of toxicity associated with the protoscolicidal agents led many surgeons to abandon this routine step in the operative management of cystic echinococosis [17,18]. However, the cystic fluid contains a large number of protoscolices and they have the potential to grow into new hydatid cysts [19].

In recent years, there has been a considerable interest in finding natural scolicidal agents from plant materials to replace synthetic ones [2,3,20-22]. Information from previous studies has shown that the plant contains a large variety of substances that possess antimicrobial activity. Hydroalcoholic extracts of *Satureja* had antiprotozoal properties on *Trypanosoma cruzi* and *Plasmodium falciparum* [14,15]. In addition, *O. europaea* aqueous extracts have been used as herbal medicine for several years for different medical purposes, and an in vitro study focused solely on the antimicrobial and antifungal properties of olive leaf extracts [4].

In the present investigation, protoscolicidal effects of the 2 herbal agents were observed, separately. We found that the protoscolicidal efficacies of *S. khuzestanica* extracts in various concentrations and different time periods were appropriate, whereas olive leaf extracts had less effects on protoscolices of hydatid cysts. These plants are known to have antimicrobial effects and naturally grow in the Lorestan province of Iran.

We realized that *S. khuzestanica* killed all of the protoscolices and its 0.1% dilution revealed strong scolicidal activity at the 30, 60, and 120 min, although 0.01% diluted *S. khuzestanica* did not reveal enough protoscolicidal effects at the same time. The results of our study proved that protoscolices killed with *S. khuzestanica* (0.001% diluted) showed a decrease in the mor-

tality rate to 67.6% at 120 min exposure time. Low concentrations (0.1%) of *O. europaea* leaf extracts had effective protoscolicidal efficacy. It is remarkable that increasing exposure time showed more scolicidal activities.

In conclusion, the present study is the first report demonstrating the effectiveness of *S. khuzestanica* and *O. europaea* on protoscolices. *S. khuzestanica* had the greatest scolicidal effect against cystic echinococosis. This plant may be useful as an agent in the PAIR (Puncture-Aspiration-Injection-Reaspiration) method for cystic echinococcosis because of its rapid and strong scolicidal effects. It seems that *O. europaea* leaf extracts have a less scolicidal activity, but it could be used as an agent with surgery techniques. However, more research is necessary to evaluate mode of actions and in vivo effects of these plant extracts, and also possible side effects on animals and humans.

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