

# Scolicidal agents for protoscolices of *Echinococcus granulosus* hydatid cyst: Review of literature

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Hydatid cyst is the larval stage of dog tape worm *Echinococcus granulosus*. Protoscolices are parasite larvae that develop into adult worms in the final host intestine. During surgical treatment of human hydatidosis spillage of live protoscolices is the major cause of hydatidosis recurrence. To prevent this problem scolicidal agent such as hypertonic salt are used to kill the protoscolices that may disseminate into the patient's tissues during surgery. However, they may have some unacceptable side effects. To find scolicidal agents with high efficacy, the effect of different compounds on protoscolices of hydatid cyst *in vitro* has been reviewed. Using PubMed, Scopus, Google Scholar, and SID databases articles about scolicidal effects of different agents on protoscolices of hydatid cyst *in vitro* were collected. *Foeniculum vulgare* after 5 min, metallic extracts of *Allium sativum* and hypertonic saline after 10 min, and warm water after 2 min kill all live protoscolices. The above agents that in minimum time and minimum concentration have 100% scolicidal activity, could be good candidates for further investigations.

**Key words:** Hydatid cyst, protoscolices, scolicidal agents, surgery, surgery

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## INTRODUCTION

Hydatid cyst is the larval stage of *Echinococcus granulosus* dog tapeworm. It is endemic and is still an important economic and public health concern in some parts of the world, especially in the Middle East.<sup>[1-3]</sup> It is estimated that about 20% of sheep in some parts of this area infected with hydatid cyst. In human, the seroepidemiology of infection in some countries such as Iran is about 4.8%, also up to 4.2 surgical operation per 100,000 population has been reported for this country.<sup>[1]</sup> Although different genotypes of the parasite have been reported, the main structure of the hydatid cyst is the same in all genotypes.<sup>[4-6]</sup> Hydatidosis is mainly diagnosed by imaging technique and confirmed by immunological methods.<sup>[7]</sup> Hydatid cyst which is located in different tissues outwardly included laminated layer, germinal layer, brood capsules containing protoscolices,

and the cyst fluid.<sup>[8]</sup> Protoscolices are microscopic larvae that capable to develop to adult worms in the final host intestine or to secondary hydatid cyst in the intermediate host viscera. One of the treatment options for CE is surgical removal of the cyst<sup>[9]</sup> combined with chemotherapy using albendazole (ABZ) or mebendazole before and after surgery.<sup>[10]</sup> Surgical treatment of human hydatidosis involves the use of various scolicidal agents to kill infective *E. granulosus* protoscolices that may disseminate into the peritoneal cavity during surgery and potentially re-infect the patient. Spillage of live protoscolices during the operation is the major cause of recurrence. Hence, instillation of scolicidal agent such as hypertonic salt into hydatid cyst is the most commonly employed measure to prevent this complication.<sup>[10,11]</sup>

To date, many scolicidal agents including some plant extracts, mannitol, ABZ, chlorhexidine gluconate (Chx-Glu), honey, hypertonic saline, silver nitrate, cetrime, ethyl alcohol, H<sub>2</sub>O<sub>2</sub>, and

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povidone-iodine have been used for inactivation of the hydatid cyst content.<sup>[12-18]</sup> However, most common scolicidal agents may cause unacceptable side effects<sup>[19-21]</sup> which may result in limiting their use. In different studies, the effect of various protoscolicides agents has been investigated. However, currently, no scolicidal agent is completely effective in killing intracystic protoscolices in humans. Therefore, finding new scolicidal agents with fewer side effects, low cost, and higher efficacy are an urgent need for surgeons.<sup>[22]</sup> In this paper to find convenient protoscolicides agents to be used during surgery, the effect of different agents on protoscolice *in vitro* has been reviewed.

## Methods

Using PubMed, Scopus, Google Scholar and SID databases articles about scolicidal effects of different agents on protoscolices of hydatid cyst *in vitro* were collected.

## DIFFERENT AGENTS WITH SCOLICIDAL ACTIVITIES

ABZ and ABZ sulfoxide (ABZ-SO), when used separately had protoscolicidal activity after a longer incubation period (30 days) than when used as combined compounds.<sup>[23]</sup> In an investigation protoscolices of *E. granulosus* were incubated *in vitro* with praziquantel (PZ), ABZ, or a combination of both (PZ + ABZ). PZ and ABZ displayed slower protoscolicidal activity when applied separately than when used in combination.<sup>[24]</sup> In another work, it has been shown that after 5 min, effectiveness of ABZ sulfone, ABZ sulfoxide and combined solution was 97.3%, 98.4%, and 98.6%, respectively. Hence, none of the solutions of ABZ killed 100% of the scolices following 5–10 min.<sup>[22]</sup> Erzurumlu *et al.* also showed that ABZ has 50%–100% scolicidal activities.<sup>[25]</sup>

In an investigation, Caglar *et al.* showed that silver nitrate, dextrose, NaCl and mannitol have 100% scolicidal activities following 20, 30, 45, and 45 min, respectively.<sup>[26]</sup> In another study, protoscolices of *E. granulosus* were incubated in a 10  $\mu$ m solution of monensin. All movement of protoscolices stopped after 1 h and all protoscolices were dead at 36 h.<sup>[27]</sup> Various concentrations of amphotericin B (AmB) (2.5–20 mg/ml), silver nanoparticles (Ag-NPs) (0.5–4 mg/ml), *Foeniculum vulgare* essential oil (0.125–1 mg/ml), and hypertonic saline (10%–20%) were incubated with live protoscolices. Maximum protoscolicidal effect of AmB and Ag-NPs was found at concentrations of 20 and 4 mg/ml. While AmB and Ag-NPs killed only 82.3% and 71.6% of the protoscolices after 60 min, *F. vulgare* essential oil at concentration of 1 mg/ml and hypertonic saline 20% killed 100% protoscolices after 5 and 10 min of exposure, respectively.<sup>[28]</sup>

Fungal chitosan could be a good scolicidal for hydatid cysts control.<sup>[29]</sup> Furthermore, it has been shown that commercial

chitosan with the highest degree of deacetylation has high scolicidal activity *in vitro*.<sup>[30]</sup> Scolicidal activity of *Endophytic pestalotiopsis* has also been shown. There may be some bioactive compounds on it that have a selective action on the tegument layer of protoscoleces.<sup>[31]</sup> In another study, it has been shown that Biogenic selenium nanoparticles have scolicidal effects, especially at concentrations of 500 and 250  $\mu$ g/ml after 10 and 20 min of application, respectively.<sup>[32]</sup> When protoscolices of hydatid cysts was treated with an extract of endophytic fungi *Eupenicillium* and *Chaetomium* sp. isolated from *Azadirachta indica* and *Piper longum* plants, a sharp decrease in viability of protoscolices was observed after 6 h of incubation.<sup>[33]</sup> Colebrook *et al.* showed that cyclosporin A (CsA) with dose of 100  $\mu$ g/ml killed all activated protoscolices for 3 days and 50 or 20  $\mu$ g/ml for 5 days. The lethal effect of CsA on unactivated protoscolices reached 100% over 15 days in culture with dose of 100 or 50  $\mu$ g/ml. They concluded that CsA is an effective scolicidal agent *in vitro* that may have potential application as a new therapeutic agent in the treatment of human hydatid disease.<sup>[12]</sup> Rahimi *et al.* investigated the effect of synthesized Ag-NPs derived from the aqueous aerial extract of *Penicillium aculeatum* on hydatid cyst protoscolices. They showed that the Ag-NPs has high scolicidal effects.<sup>[13]</sup> Gholami *et al.* investigated the scolicidal effect of methanolic extract of *Sambucus ebulus* fruit. They showed that methanolic extract of *S. ebulus* fruit showed a high scolicidal activity *in vitro*.<sup>[14]</sup> Moazeni and Alipour-Chaharmahali investigated the effect of warm water at different degree and also different incubation time on protoscolices. They showed that warm water at 50, 55, and 60°C after 5, 2, and 1 min, respectively, had 100% scolicidal effect.<sup>[15]</sup> Zibaei *et al.* investigated scolicidal effects of hydroalcoholic extracts of *Satureja khuzestanica* leaves and aqueous extracts of *Olea europaea* leaves on hydatid cyst protoscolices. They showed that *S. khuzestanica* 0.1% had very strong scolicidal effects in 30, 60, and 120 min of exposure times. Olive leaf extracts with 0.1% and 0.01% concentrations had strong scolicidal effects in 120 min.<sup>[18]</sup> Moazeni *et al.* investigated the scolicidal effect of methanolic extract of *Allium sativum*. They showed that *A. sativum* extract at the concentration of 25 mg/ml killed 100% of protoscolices following 60 min of application. Furthermore, the scolicidal activity of *A. sativum* extract at the concentration of 50 mg/ml was 100% after 10 min of incubation.<sup>[17]</sup> In another investigation, scolicidal effect of the different acidic solution with pH 1 after 5 min and with pH 2 and 3 after 10 min was 100% has been showed. Acidic solution with pH 2 and 3 after 5 min had scolicidal effect of 99.6% and 98.7%, respectively. The alkaline solution with pH 14 after 5 min and with pH 13 after 15 min killed all protoscolices. In addition, alkaline solution with pH 13 after 5 and 10 min had scolicidal effect of 97.5% and 99.7%, respectively.<sup>[16]</sup> Mahmoudvand *et al.* evaluated the

*in vitro* scolicidal effect of *Nigella sativa* (*Ranunculaceae*) essential oil and also its active principle, thymoquinone, against protoscolices of hydatid cysts. They showed that the essential oil of *N. sativa* at the concentration of 10 mg/ml and its main component, thymoquinone, at the concentration of 1 mg/ml had scolicidal activities after 10 min of exposure.<sup>[32]</sup> Moazeni *et al.* investigated scolicidal power of ajowan (*Trachyspermum ammi*). They showed that this compound killed 100% of protoscolices after 60 min. Treatments by 20% silver nitrate in 20 min, by 50% dextrose and by aminomix-1 solution in 30 min, and by 20% NaCl and by 20% mannitol in 45 min showed 100% scolicidal activity.<sup>[34]</sup>

In another investigation, it has been shown that all activated protoscolices were killed in 100 µg/ml of CsA after 3 days and 50 or 20 µg/ml following 5 days. The lethal effect of CsA on nonactivated protoscolices reached 100% over 15 days with 100 or 50 µg/ml of CsA.<sup>[12]</sup> Propolis which is a resinous material collected by bees from various plants, has been shown to killed all of the protoscolices in concentration of 1 µg/ml at the end of the 3<sup>rd</sup> min.<sup>[26,35]</sup>

Zeghir-Bouteldja *et al.* showed scolicidal activity of nitric oxide metabolites (NO<sup>2-</sup>) and (ONOO<sup>-</sup>) on human hydatid

cyst protoscolices.<sup>[36]</sup> Topcu *et al.* investigated the effect of Chx-Glu on 45 human hydatid cyst. They showed that all protoscolices in 45 cysts were killed by 5 min of exposure to 0.04% Chx-Glu.<sup>[37]</sup> Polyvinylpyrrolidone-iodine has also been shown to have protoscolocidal activities.<sup>[38]</sup> Aydin *et al.* showed that taurolidine in the concentration of (5 mg/ml) killed all protoscolices following 90 min of incubation.<sup>[39]</sup> Elissondo *et al.* showed that thymol caused severe damages to the protoscolices even after short incubation times and its protoscolocidal effect was dose- and time-dependent.<sup>[40]</sup> In another investigation, it has been shown that chloroformic extract of garlic was able to kill 98% of protoscolices in concentration of 50 mg/ml following 20 min incubation.<sup>[41]</sup>

Rouhani *et al.* investigated scolicidal activity of *Berberis vulgaris* and showed that it has an effective scolicidal activity in concentration of 4mg/ml and following 5 min incubation.<sup>[42]</sup> Finally, Verma *et al.* showed that *E. pestalotiopsis* sp fungi has 97% protoscolocidal after 30 min of incubation.<sup>[31]</sup> Moreover, scolicidal activities of Iodine and 3% H<sub>2</sub>O<sub>2</sub> has also been reported.<sup>[19]</sup> Protoscolocidal activities of different agents are summarized in Table 1.

**Table 1: Effect of different scolicidal agents on protoscolices of *Echinococcus granulosus* hydatid cyst *in vitro***

Scolicidal agent	Concentration	Time	Results	References
ABZ or ABZ-SO	50, 10, 1, and 0.1 µg/mL	-	Combined compounds had better protoscolocidal activity	[23]
PZ, ABZ	Low PZ + ABZ concentrations	Within 15 days	PZ and ABZ displayed better protoscolocidal activity when applied in combination	[24]
Monensin	10 µm	36 h	All protoscoleces were dead	[27]
AmB	AmB (20 mg/mL)	60 min	82.3%	[28]
AgNPs	AgNPs (0.5-4 mg/mL)	60 min	71.6%	[28]
<i>Foeniculum vulgare</i>	1 mg/mL	5 min	100%	[28]
Hypertonic saline	20%	10 min	100%	[28]
Fungal chitosan isolated from <i>Penicillium waksmanii</i> and <i>Penicillium citrinum</i>	50, 100, 200, 400 µg/mL	10, 30, 60 and 180 min	Showed strong scolicidal activity	[29]
Chitosan from <i>Penicillium viridicatum</i> , <i>Penicillium aurantiogriseum</i> and commercial chitosan	50, 100, 200, 400 µg/mL	10, 30, 60, and 180 min, respectively	Commercial chitosan showed highest degree of scolicidal activity	[30]
Endophytic <i>pestalotiopsis</i> spp. from neem plant	-	Within 30 min	Up to 97% scolicidal activity	[31]
Se NPs	Concentrations 500 and 250 µg/mL	10 and 20 min of application, respectively	Had potent scolicidal effects	[32]
Extract of endophytic fungi <i>Eupenicillium</i> and <i>Chaetomium</i> spp.	-	6 h	Had significant anti-cestodal activity	[33]
CsA	100 µg/mL and 50 or 20 µg/mL	For 3 days and 5 days, respectively	CsA is an effective scolicidal agent <i>in vitro</i>	[12]
AgNPs derived from the aqueous aerial extract of <i>Penicillium aculeatum</i>	0.1 and 0.15 mg/mL	120 min	Showed 83% and 90% scolicidal activity, respectively	[13]

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Table 1: Contd...

Scolicidal agent	Concentration	Time	Results	References
Methanolic extract of <i>Sambucus</i> (S.) ebulus fruit	1, 10, 50, and 100 mg/mL	5, 10, 30, and 60 min	Showed a high scolicidal activity <i>in vitro</i>	[14]
Warm water	50°C, 55°C, and 60°C	1, 2, 5 min	100% scolicidal activity	[15]
a) Aqueous extracts of <i>Olea europaea</i> leaves	a) 0.1%	a) 30, 60, and 120 min	Strong	[18]
b) Hydroalcoholic extracts of <i>Satureja khuzestanica</i> leaves	b) 0.1% and 0.01%	b) 120 min		
Methanolic extract of <i>Allium sativum</i>	50 mg/mL	10 min	100% scolicidal activity	[17]
a) Acidic solutions and b) alkaline solutions	a) With pH 1, 2, 3, and 4 b) With pH, 13, and 14	a) 5 and 10, min b) 5 and 15 min	100%	[16]
<i>Nigella sativa</i> ( <i>Ranunculaceae</i> ) essential oil	Various concentrations of the essential oil (1 mg/ml)	10 min	Had potent scolicidal activities	[43]
Ajowan ( <i>Trachyspermum ammi</i> ) the essential oil	5 mg/mL	60 min	100% scolicidal activity	[34]
Chx-Glu	0.04%	6 months	All protoscolices most potent, nontoxic agent were killed	[29]
Ethyl alcohol extract of propolis	1 µg/mL	3 min	Killed all of the protoscoleces	[35]
NO <sub>2</sub> (-), ONOO (-)	With 320 µm and 80 µm	24 h and 3 h respectively	Showed protoscolicidal activity	[36]
a) Polyvinylprolidone-iodine (Betadine, PVP-I)	a) 1%	a) 2 and 5 minutes	Taurolidine was ineffective as a scolicidal agent	[38,39]
b) Taurolidine	b) 2%	b) 2 minutes, and 5 minutes		
Thymol	250 µg/mL	2 min	Viability of protoscoleces was approximately 1.3%	[40]
<i>Berberis vulgaris</i> (Zereshk)	4 mg/mL	5 min	The scolicidal activity was very effective	[42]
Endophytic fungi <i>Pestalotiopsis</i> spp.	-	30 min	97% mortality	[31]

ABZ = Albendazole; ABZ-SO = Albendazole sulfoxide; PZ = Praziquantel; AmB = Amphotricin B; AgNPs = Silver nanoparticles; Se NPs = Selenium nanoparticles; CsA = Cyclosporin A; Chx-Glu = Chlorhexidine gluconate; PVP-I = Polyvinylpyrrolidone-iodine

The mechanism of scolicidal activities of agent that are able to kill the protoscolices is not clear. However, some of them like *N. sativa* by inhibiting histone deacetylase enzyme which interacts with the chromosomes may inhibit DNA synthesis.<sup>[43]</sup>

## CONCLUSION

In this work to find new scolicidal agents with more efficacies and low side effects, lethal effect off different agents on protoscolices of *E. granulosus* was reviewed. It seems that agents that in minimum concentration and minimum time have 100% scolicidal activity could be good candidates for further investigations. In this context, it has been shown that *F. vulgare* after 5 min,<sup>[28]</sup> metalonic extracts of *A. sativum* after 10 min,<sup>[17,41]</sup> hypertonic saline after 10 min<sup>[28]</sup> and warm water after 2 min<sup>[15]</sup> kill all alive protoscolices. These compounds could be good candidates to be used as protoscolisieds agents. Other compounds such as monensin although has 100% protoscolicidal activities, but it take a long time to show their effects. Hence, they could not be considered candidates to be used in practice. Finally, the side effects of candidate agents should be investigated on cells *in vitro* and also in animal models.

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## Conflicts of interest

The authors have no conflicts of interest.

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