

# ***In vitro* lethal effect of *Zingiber officinale* R. on protoscolices of hydatid cyst from sheep liver**

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## **Abstract**

Cystic echinococcosis (Hydatid disease) is a zoonotic disease caused by *Echinococcus granulosus*. The aim of this study, was to evaluate the scolicidal activity of methanolic extract of *Zingiber officinale* (Roscoe.) family Zingiberaceae, against protoscolices of hydatid cyst. Protoscolices were collected aseptically from sheep livers containing hydatid cyst and were exposed to different concentrations of ginger extract for various exposure times. Scolicidal activity of *Z. officinale* extract at concentration of 25 mg/mL was 25.6%, 39.1%, 56.7%, 83.7%, 98.1% and 100% after 10, 20, 30, 40, 50, and 60 min of exposure respectively. Scolicidal effect of this extract at concentration of 50 mg/mL was 52%, 85.8 %, 99.6% and 100% after 10, 20, 30 and 40 min of exposure respectively. *Z. officinale* extract at concentration of 100 mg/mL killed 76.5 %, 87% and 100 % of protoscolices after 10, 20 and 30 min respectively. The results of this study showed that the methanolic extract of *Z. officinale* has high scolicidal activity and might be used as a natural scolicidal agent.

## **Introduction**

*Echinococcosis* (hydatid disease), a zoonosis, is characterised by worldwide distribution and frequent hepatic involvement. It is caused by the larval stage of *Echinococcus granulosus*, a parasite of the order Cestoda and family Taeniidae.<sup>1</sup> The disease is endemic in the Mediterranean area, in the Middle East, the Baltic areas, South America, India, Northern China and other sheep-raising areas; however, considering the increased travels and tourism all over the world, it can be found anywhere, even in developed countries.<sup>2</sup>

Surgery is still the mainstay of treatment for hydatid disease. Spillage of the cyst contents is a major cause of recurrence, which is seen in approximately 10% (8.5-22.0%) of post-operative cases. Instillation of a scolicidal agent into hepatic hydatid cysts to reduce the risk of spillage of viable protoscolices is an integral part of the surgical technique for many surgeons.<sup>3</sup> Scolicidal solutions remain indispensable in the treatment of hydatid cyst

disease and the surgeons need less harmful but more effective drugs in hydatid disease.<sup>4</sup>

*Zingiber officinale* (Roscoe.) family Zingiberaceae, commonly called ginger. The family Zingiberaceae contains a variety of compounds, which showed insecticidal, oviposition, antifeedant, growth regulating, reducing fecundity, development modifying properties and repellent activity against many tested insects.<sup>5</sup> Scientific reports show that *Z. officinale* has carminative, antipyretic, anticancer, cardio tonic, antispasmodic, antidiabetic, antioxidant and antihepatotoxic activities.<sup>6</sup> Among the herbal plants, only the scolicidal activity of garlic (*Allium sativum*) and *Zataria multiflora* have been previously addressed.<sup>7,8</sup> The present study was undertaken to evaluate the scolicidal activity of *Z. officinale*.

## **Materials and Methods**

### **Protoscolices**

Hydatid cyst protoscolices were obtained from the infected livers of sheep, naturally infected to hydatid disease, slaughtered at Shiraz slaughterhouse, South of Iran. The hydatid fluid of cysts were aseptically transferred into the glass cylinders and left to set for half an hour. The protoscolices were settled down at the bottom of cylinders. The supernatant was then removed and the yielded protoscolices were washed three times in normal saline. The viability of protoscolices was confirmed from their motility characteristics under light microscopy. The live protoscolices (Figure 1) were finally transferred into a dark container containing normal saline and stored at 4°C until use.

### **Extraction**

Fresh rhizomes of *Zingiber officinale* (Roscoe.) family Zingiberaceae, were purchased from a local herbal market in Shiraz (Iran) and was identified and authenticated at College of Pharmacy, Shiraz University of Medical Sciences. Then, the rhizomes were peeled, cut into pieces, dried under shade and powdered mechanically using a commercial electric blender. A total of 900 gr dried powder was extracted. The following method was used for preparation of methanolic extract of *Z. officinale*: 100 gr of dry powder was added to 400 mL of pure methanol and mixed gently for one hour using a magnetic stirrer. The obtained solution was left at room temperature for 24 h. The solution was stirred again, filtered and the solvent was removed by evaporation in a rotary evaporator. The remaining semisolid material was then freeze dried. The obtained residue was placed in a sterile glass container and stored in the refrigerator at 4°C for later use.<sup>7</sup>

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We obtained 19.2 gr dried extract from 900 gr of dried powder of *Z. officinale*.

### **Scolicidal tests**

The scolicidal tests were carried out based on Moazeni and Nazer (2010). In this study we examined three concentrations (25, 50 and 100 mg/mL) of the ginger extract for 10, 20, 30, 40, 50 and 60 min. For preparation of ginger extract solution with 25, 50 and 100 mg/mL concentration, 0.25, 0.5 and 1 gr of dried extract was dissolved in 10 mL of normal saline respectively. The dried extract was dissolved in 1% dimethyl sulphoxide (DMSO) before use. Two milliliters of each concentration was placed in a test tube, a drop of protoscolex rich sediment was added to the tube and mixed gently. The tube was then incubated at 37°C for 10, 20, 30, 40, 50 and 60 min. At the end of incubation time, the upper portion of the solution was removed with a pipette avoiding settled protoscolices. Then two milliliters of 0.1% eosin stain was added to the remaining settled protoscolices and mixed gently. After 15 min, the upper portion of the solution was discarded. The remaining settled protoscolices were then smeared on a manual scaled glass slide, covered with a cover glass (24×50 mm) and examined microscopically for viability. The percentages of dead protoscolices (Figure 2) were determined by counting a minimum of 350 (mostly more than 500) protoscolices. At least 1500 protoscolices with no exposure to ginger extract was considered as control group in each experiment. The experiments were performed in triplicate.

### **Viability test**

In the present study eosin stain with 0.1% concentration (1 gr of eosin powder in 1000 mL of distilled water) was used for the viability test of protoscolices. After 15 min of exposure, the protoscolices that did not take the dye

in, accepted as potentially viable and those done, were considered dead.<sup>7</sup>

### Statistical analysis

Differences between test and control groups were analyzed by the  $\chi^2$  test. A P value less than 0.01 was considered significant. Statistical analysis was performed with GraphPad InStat software.

## Results

Results of the effectiveness of different concentrations of *Z. officinale* extract as a scolicidal agent are shown in Tables 1, 2 and 3. While the death rate in the control group was 17.8%, scolicidal activity of *Z. officinale* extract at concentration of 25 mg/mL was 25.6 %, 39.1%, 56.7%, 83.7%, 98.1% and 100 % after 10, 20, 30, 40, 50, and 60 min of application respectively.

With death rate of 18.9 % for the control group, scolicidal effect of *Z. officinale* extract at concentration of 50 mg/mL was 52 %, 85.8 %, 99.6 % and 100 % after 10, 20, 30 and 40 min of exposure respectively.

*Zingiber officinale* extract at concentration of 100 mg/mL killed 76.52%, 86.98% and 100% of protoscolices after 10, 20 and 30 min respectively (the death rate in the control group was 19.6 %).

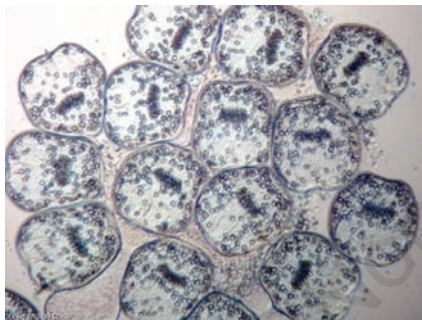


Figure 1. Live protoscolices after staining with 0.1% eosin.

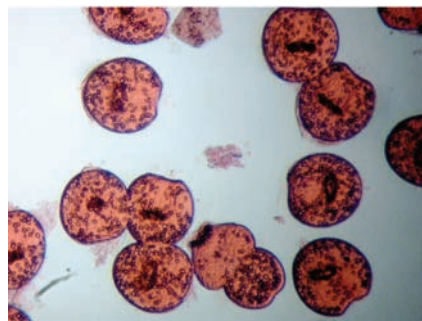


Figure 2. Dead protoscolices after exposure to *Zingiber officinale* extract and staining with 0.1% eosin.

Scolicidal effect of all three concentrations of the methanolic extract of *Z. officinale* was extremely significant comparing to the control groups at all exposure times ( $P < 0.0001$ ). The results of the present study indicated that methanolic extract of *Z. officinale* has high scolicidal activity and might be used as scolicidal agent in hydatid cyst surgery.

## Discussion

Up to date, many scolicidal agents have been used for inactivation of the hydatid cyst content, but there is no ideal agent that is both effective and safe.<sup>8</sup> An ideal scolicidal agent is define as being potent in low concentrations, acting in a short period time, being stable in

cyst fluid, not affected by dilution with the cyst fluid, being able to kill the scolex in the cyst, being non-toxic, having low viscosity, and being readily available and easily prepared, as well as being inexpensive.<sup>9</sup> New effective alternative treatment is extremely important in today's climate, where species are becoming resistant and there is a resurgence in the use of natural alternative therapies, instead of synthetic pharmaceuticals that often have severe side effects.<sup>10</sup>

Moazeni and Nazer (2010) investigated the *in vitro* scolicidal effect of methanolic extract of garlic (*Allium sativum*). At the concentration of 25 mg/mL, they reported 87.9, 95.6, 96.8, 98.7, 99.6, and 100% scolicidal activity following 10, 20, 30, 40, 50, and 60 min of application, respectively. Moreover, they reported 100% scolicidal activity for *Allium sativum*

Table 1. Scolicidal effect of ginger (*Zingiber officinale*) extract at concentration of 25 mg/mL after various exposure times.

Exposure time (min)	Protoscolices (Mean±SD)	Dead protoscolices (Mean±SD)	Mortality rate(%)
10	613.33±140.61	158.33±32.53	25.6%
20	572.66±108.52	223.66±48.58	39.1%
30	537±76.54	304.66±52.843	56.7%
40	455±85.28	384.33±88.05	83.7%
50	589±113.85	578±102.59	98.1%
60	602.66±177.43	602.66±177.43	100%
Control	1536	273	17.8%

SD, Standard deviation.

Table 2. Scolicidal effect of ginger (*Zingiber officinale*) extract at concentration of 50 mg/mL after various exposure times.

Exposure time (min)	Protoscolices (Mean±SD)	Dead protoscolices (Mean±SD)	Mortality rate(%)
10	528±219.04	274.33±112.10	52%
20	624±145.12	554.33±124.62	85.8%
30	712±25.63	709±28.05	99.6%
40	623.66±45.08	623.66±45.08	100%
Control	1838	349	18.9%

SD, Standard deviation.

Table 3. Scolicidal effect of ginger (*Zingiber officinale*) extract at concentration of 100 mg/mL after various exposure times.

Exposure time (min)	Protoscolices (Mean±SD)	Dead protoscolices (Mean±SD)	Mortality rate(%)
10	493.66±26.57	346.66±36.29	76.5%
20	461±72.38	401±67.35	87%
30	625.66±91.22	625.66±91.22	100%
Control	1838	349	18.9%

SD, Standard deviation.

extract at the concentration of 50 mg/mL after 10 min of application.<sup>7</sup>

Moazeni and Roozitalab (2011) investigated the *in vitro* scolicidal effect of methanolic extract of *Zataria multiflora*. *Z. multiflora* extract at a concentration of 10 mg/mL killed 68.9%, 93.7%, and 100% of protozoa after 1, 2, and 3 min respectively. They reported 100% scolicidal effect of this extract at a concentration of 25 mg/mL after 1 min of application.<sup>11</sup>

Ginger is a food plant known worldwide and is equally reputed for its medicinal properties.<sup>5</sup> Positive effect of aqueous extract of *Z. officinale* on the performance of broiler chicks in term of weight gain, feed conversion ratio, dressing percentage and weight of some other organs has been reported.<sup>12</sup> Consumption of one gram a day of ginger powder may alleviate clinical nausea of diverse causes including postoperative nausea.<sup>13</sup> The benzene fraction of the petroleum ether extract of ginger possesses antiemetic, anxiolytic and anticonvulsant activity with a wide margin of safety and does not produce neurological deficit.<sup>14</sup> Ethanol extract of *Z. officinale* has significant adaptogenic activity against a variety of biochemical and physiological perturbations in different stress models.<sup>6</sup> Ginger has been variously used as anti-diabetic agent.<sup>15</sup> The water extract of ginger has detoxifying and antioxidant effects.<sup>16</sup> *Z. officinale* has analgesic and anti-inflammatory effects.<sup>17</sup> and can be used as both prophylactic and therapeutic detoxificant on cadmium induced poison.<sup>18</sup> Ginger produced marked inhibitory effect on *Salmonella typhi* and *Escherichia coli* as representative of enteric microorganisms.<sup>19</sup> Significant anti-*Toxoplasma gondii* RH strain activity has been observed with methanolic extract of *Z. officinale*.<sup>20</sup> The larvicidal activity of a petroleum ether extract of *Z. officinale* against fourth instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* has been reported by Abdul Rahuman *et al.* (2008).<sup>5</sup> Aqueous extract of *Z. officinale* exhibited strong microfilaricidal activity against *Dirofilaria immitis in vitro*.<sup>21</sup> Ginger is among the 20 top-selling herbal supplements in the United States and today, pharmacopeias of a number of different countries list ginger extract for various digestive diseases.<sup>22</sup>

*Z. officinale* contains about 1-2% of volatile oil and 5-8% of resinous matter, starch and mucilage. The volatile oil contains monoterpenes, sesquiterpenes and sesquiterpene alcohol zingiberol, gingerol and shogaols. Most of the pharmacologically active constituents reside in the volatile oils.<sup>6</sup>

In the present study we investigated the effectiveness of methanolic extract of *Z. officinale* on the protozoa of hydatid cyst. The results of our study showed that *Z. officinale*

extract at concentration of 25, 50 and 100 mg/mL can kill all protozoa after 60, 40 and 30 min of application respectively.

Formalin, hydrogen peroxide, cetrimide, pure alcohol, hypertonic saline and silver nitrate have been used as effective scolecidal agents, but may cause unacceptable side-effects, limiting their use.<sup>23</sup> According to the results of our study, although *Z. officinale* showed lower scolicidal power in comparison with *Allium sativum* and *Zataria multiflora*, the scolicidal activity of *Z. officinale* extract at concentration of 25 mg/mL (60 min), 50 mg/mL (40 min) and 100 mg/mL (30 min) is comparable with scolicidal power of formalin,<sup>24</sup> H<sub>2</sub>O<sub>2</sub>,<sup>25</sup> cetrimide,<sup>26</sup> 95% ethyl alcohol,<sup>24</sup> hypertonic saline and silver nitrate.<sup>27</sup>

As far as we know, this is the first report on the scolicidal activity of methanolic extract of *Z. officinale*. The results of this study allowed us to suggest that *Z. officinale* is likely source of new compounds that could be used as an effective scolicidal agent. Further studies will be necessary to identify and isolate these active compounds. Ginger rhizome is edible,<sup>5</sup> therefore it is safe for human. Chronic administration of ginger (daily treatment with ginger powder at the dosages of 500, 1000 and 2000 mg/kg body weight for 35 days) in rats was not associated with any mortalities and abnormalities in general conditions, behavior, growth, and food and water consumption.<sup>28</sup> The results of present study open the possibility of more investigations of *in vivo* scolicidal effect of this traditional medicine.

## References

- Mehra BR, Thawait AP, Gupta DO, et al. Giant abdominal hydatid cyst masquerading as ovarian malignancy. *Sing Med J* 2007;48:284-6.
- Giorgio A, Sarno AD, Stefano GD, et al. Percutaneous Treatment of Hydatid Liver Cyst. *Rec Patents Anti-Infect Drug Discov* 2009;4:29-36.
- Puryan K, Karadayi K, Topcu O. Chlorhexidine gluconate: an ideal scolicidal agent in the treatment of intraperitoneal hydatidosis. *W J Surg* 2005;29:227-30.
- Adas G, Arikan S, Kemik O, et al. Use of albendazole sulfoxide, albendazole sulfone, and combined solutions as scolicidal agents on hydatid cysts (in vitro study). *W J Gastroenterol* 2009;15:112-6.
- Abdul Rahuman A, Gopalakrishnan G, Venkatesan P, et al. Mosquito Larvicidal Activity of Isolated Compounds from the Rhizome of Zingiber officinale. *Phytother Res* 2008;22:1035-9.
- Lakshmi BVS, Sudhakar M. Attenuation of acute and chronic restraint stress-induced perturbations in experimental animals by *Z. officinale* Roscoe. *Food Chem Toxicol* 2010;48:530-5.
- Moazeni M, Nazer A. In vitro Effectiveness of Garlic (*Allium sativum*) Extract on Scolices of Hydatid Cyst. *World J Surg* 2010;34:2677-81.
- McManus DP, Zhang W, Li J, et al. Echinococcosis. *Lancet* 2003;362:1295-304.
- Guide lines for treatment of cystic and alveolar echinococcosis in humans. WHO Informal Working Group on Echinococcosis WHO 1996;74:231-42.
- Harris JC, Plummer S, Turner MP, et al. The microaerophilic flagellate *Giardia intestinalis*: *Allium sativum* (garlic) is an effective anti-giardial. *Microbiology* 2000; 146:3119-27.
- Moazeni M, Roozitalab A. High scolicidal effect of *Zataria multiflora* on protozoa of hydatid cyst: an in vitro study. *Comp Clin Pathol* 2011; DOI 10.1007/s00580-010-1069-3
- Javed M, Durrani FR, Hafeez A, et al. Effect of aqueous extract of plant mixture on carcass quality of broiler chicks. *ARPN J Agric Biol Sci* 2009;4:37-40.
- Arfeen Z, Owen H, Plummer JL, et al. A double-blind randomized controlled trial of ginger for the prevention of post-operative nausea and vomiting. *Anaesth Inten Care* 1995;23:449-52.
- Vishwakarma SL, Pal SC, Kasture VS, et al. Anxiolytic and Antiemetic Activity of *Z. officinale*. *Phytother Res* 2002;16:621-6.
- Al-amin ZM, Thomson M, Al-qattan KK, et al. Anti-diabetics and hypolipidemic properties of ginger (*Zingiber officinale*) in streptozotocin-induced diabetic rats. *Br J Nutr* 2006; 96:660-6.
- Shati AA, Elsaid FG. Effects of water extracts of thyme (*Thymus vulgaris*) and ginger (*Z. officinale* Roscoe) on alcohol abuse. *Food Chem Toxicol* 2009;47:1945-9.
- Young HY, Luo YL, Cheng HY, et al. Analgesic and anti-inflammatory activities of [6]-gingerol. *J Ethnopharmacol* 2005;96:207-10.
- Egwurugwu JN, Ufearo, CS, Abanobi OC, et al. Effects of ginger (*Zingiber officinale*) on cadmium toxicity. *Afr J Biotechnol* 2007;6:2078-82.
- Ekwenye UN, Elegalam NN. Antibacterial activity of ginger (*Z. officinale* Roscoe) and Garlic (*Allium sativum* Linn) extract on *Escherichia coli* and *Salmonella typhi*. *Int J Mol Med Adv Sci* 2005;4:411-6.
- Choi KM, Gang J, Yun J. Anti-*Toxoplasma gondii* RH strain activity of herbal extracts used in traditional medicine. *Int J Antimicrob Agents* 2008;32:360-2.
- Merawin LT, Arifah AK, Sani RA, et al.

- Screening of microfilaricidal effects of plant extracts against *Dirofilaria immitis*. *Res Vet Sci* 2010;88:142-7.
22. Borrelli F, Capasso R, Pinto A, et al. Inhibitory effect of ginger *Z. officinale* on rat ileal motility in vitro. *Life Sciences* 2000;74:2889-96.
23. Rajabi MA. Fatal reactions and methaemoglobinemia after silver nitrate irrigation of hydatid cyst. *Surgical Practice* 2009;13:2-7.
24. Erzurumlu K, Hokelek M, Baris S, et al. Effect of albendazole sulfoxide solution on the scolices and the hepatobiliary system. *Eur Surg Res* 1998;30:433-8.
25. Besim H, Karayalçin K, Hamamci O, et al. Scolicidal agents in hydatid cyst surgery. *HPB Surgery* 1998;10:347-51.
26. Frayha GJ, Bikhazi KJ, Kachachi TA. Treatment of hydatid cysts (*Echinococcus granulosus*) by Cetrimide (R). *Trans R Soc Trop Med Hyg* 1981;75:447-50.
27. Caglar R, Yuzbasioglu MF, Bulbuloglu E, et al. In vitro effectiveness of different chemical agents on scolices of hydatid cyst. *J Invest Surg* 2008;21:71-5.
28. Rong X, Peng G, Suzuki T, et al. A 35-day gavage safety assessment of ginger in rats. *Regul Toxicol. Pharmacol* 2009;54:118-23.

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