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 (Rosc.) 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.1 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.2 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 postoperative 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.3 Scolicidal solutions remain indispensable in the treatment of hydatid cyst disease and the surgeons need less harmful but more effective drugs in hydatid disease.4 Zingiber officinale (Rosc.) family Zingiberaceae, commonly called ginger. The family Zingiberaceae contains a variety of compounds, which showed insecticidal, oviposition, antifeedant, growing regulation, reducing fecundity, development modifying properties and repellent activity against many tested insects.5 Scientific reports show that Z. officinale has carminative, antipyretic, anticaner, cardio tonic, antispasmodic, anti diabetic, antioxidant and antipathotopic activities.6 Among the herbal plants, only the scolicidal activity of garlic (Allium sativum) and Zataria multiflora have been previously addressed.7,8 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 (Rosc.) 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.9

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 sulfoxide (DMSO) before use. Two milliliters of each concentration was placed in a test tube, a drop of protoscolices 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

We obtained 19.2 gr dried extract from 900 gr of dried powder of Z. officinale.
in, accepted as potentially viable and those done, were considered dead.7

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.

\(Z.\) 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%).

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.8 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.9 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.10

Moazeni and Nazer (2010) investigated the \textit{in vitro} scolicidal effect of methanolic extract of garlic (\textit{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, and 60 min of application, respectively. Moreover, they reported 100% scolicidal activity for \textit{Allium sativum} extract.

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

<table>
<thead>
<tr>
<th>Exposure time (min)</th>
<th>Protoscoleces (Mean±SD)</th>
<th>Dead protoscoleces (Mean±SD)</th>
<th>Mortality rate(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>613.33±140.61</td>
<td>158.33±32.53</td>
<td>25.6%</td>
</tr>
<tr>
<td>20</td>
<td>572.66±108.52</td>
<td>223.66±48.58</td>
<td>39.1%</td>
</tr>
<tr>
<td>30</td>
<td>537±76.54</td>
<td>304.66±52.84</td>
<td>56.7%</td>
</tr>
<tr>
<td>40</td>
<td>455±85.28</td>
<td>384.33±88.05</td>
<td>83.7%</td>
</tr>
<tr>
<td>50</td>
<td>589±113.85</td>
<td>578±102.59</td>
<td>98.1%</td>
</tr>
<tr>
<td>60</td>
<td>602.66±177.43</td>
<td>602.66±177.43</td>
<td>100%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Exposure time (min)</th>
<th>Protoscoleces (Mean±SD)</th>
<th>Dead protoscoleces (Mean±SD)</th>
<th>Mortality rate(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>528±219.04</td>
<td>274.33±112.10</td>
<td>52%</td>
</tr>
<tr>
<td>20</td>
<td>624±145.12</td>
<td>554.33±124.62</td>
<td>85.8%</td>
</tr>
<tr>
<td>30</td>
<td>712±25.63</td>
<td>709±28.05</td>
<td>99.6%</td>
</tr>
<tr>
<td>40</td>
<td>623.66±45.08</td>
<td>623.66±45.08</td>
<td>100%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Exposure time (min)</th>
<th>Protoscoleces (Mean±SD)</th>
<th>Dead protoscoleces (Mean±SD)</th>
<th>Mortality rate(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>493.66±26.57</td>
<td>346.66±36.29</td>
<td>76.5%</td>
</tr>
<tr>
<td>20</td>
<td>461±72.38</td>
<td>401±67.35</td>
<td>87%</td>
</tr>
<tr>
<td>30</td>
<td>625.66±91.22</td>
<td>625.66±91.22</td>
<td>100%</td>
</tr>
</tbody>
</table>

SD, Standard deviation.
extract at the concentration of 50 mg/mL after 10 min of application.7

Moazeni and Roozitalab (2011) investigated the in vitro scoliciidal 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 protoscolecites 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.11

Ginger is a food plant known worldwide and is equally reputed for its medicinal properties.5 Positive effect of aqueous extract of Z. officinale on the performance of broiler chicks in terms of weight gain, feed conversion ratio, dressing percentage and weight of some other organs has been reported.12 Consumption of one gram a day of ginger powder may alleviate clinical nausea of diverse causes including postoperative nausea.13 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.14 Ethanolic extract of Z. officinale has significant adaptogenic activity against a variety of biochemical and physiological perturbations in different stress models.5 Ginger has been variously used as anti-diabetic agent.15 The water extract of ginger has detoxifying and antioxidant effects.16 Z. officinale has analgesic and anti-inflammatory effects.17 and can be used as both prophylactic and therapeutic detoxifier on cadmium induced poison.18 Ginger produced marked inhibitory effect on Salmonella typhi and Escherichia coli as representative of enteric microorganisms.19 Significant anti-Toxoplasma gondii RH strain activity has been observed with methanolic extract of Z. officinale.20 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 Rahman et al. (2008).5 Aqueous extract of Z. officinale exhibited strong microfilaricidal activity against Dirofilaria immitis in vitro.21 Ginger is among the 20 top-selling herbal supplements in the United States and today, pharmacopoeias of a number of different countries list ginger extract for various digestive diseases.22

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 alcohols zingeriberol, gingerol and shagool. Most of the pharmacologically active constituents reside in the volatile oils.5

In the present study we investigated the effectiveness of methanolic extract of Z. officinale on the protoscoleces 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 protoscolecites 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 scolicidal agents, but may cause unacceptable side-effects, limiting their use.23 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), 30 mg/mL (40 min) and 100 mg/mL (30 min) is comparable with scolicidal power of formalin,24 H2O2,25 cetrimide,26 95% ethyl alcohol,24 hypertonic saline and silver nitrate.27

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,5 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.28 The results of present study open the possibility of more investigations of in vivo scolicidal effect of this traditional medicine.

References