Antiprotoscolices effect of methanolic extract of Zingiber officinale, Artemisia aucheri and Eucalyptus globulus against Echinococcus granulosus in vitro

FARIBA FAIZEI, AMIR HOSSEIN MAGHSOOD, FATEMEH PARANDIN, MOHAMMAD MATINI, SHIRIN MORADKHANI, MOHAMMAD FALLAH

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ABSTRACT
First choice in the treatment of hydatid disease is still surgery. Leakage the live protoscolices during surgery and the risk of secondary cysts have been a permanent fair for surgeons. Various methods and materials have been used to kill protoscolices as well as drug therapy that most of them had serious side effects. The main objective of this study was to evaluate the in vitro scolicidal effect of methanolic extract of eucalyptus, artemisia and ginger. Protoscolices were collected aseptically from the hydatid cysts of the sheep; washed three times in sterile PBS and stored at 4 °C until use. Live protoscolices were exposed for 5, 10, 25, 40 and 60 min for three concentrations of the each extract (25, 50, 100 mg/ml). The viability of the protoscolices were confirmed by 0.1% eosin staining method. The methanolic extract of ginger at concentration of 100 mg/ml, and eucalyptus at concentrations of 50, 100 mg/ml killed 100% of protoscolices after 40 min respectively. Scolicidal effect of all concentrations of the methanolic extract of ginger and eucalyptus was significant comparing to the control groups at all exposure times (P<0.001). No significant relationship was observed between the artemisia extract at different concentrations and exposure times comparing to the control groups (p=0.99). The results of this study showed that the eucalyptus and ginger extract have high scolicidal activity and can be used as natural scolicidal agents.

Keywords: Protoscolex, Hydatid cyst, Ginger, Eucalyptus, Artemisia, In vitro
this study conducted to evaluate the efficacy of in vitro protoscolicidal effect of artemisia, ginger and eucalyptus extracts on the protoscolices of hydatid cyst at different concentrations and exposure times.

**MATERIALS AND METHODS**

In this experimental study, hydatid cyst infected livers of sheep obtained from the Hamadan slaughterhouse carried to the research laboratory of Parasitology &Mycology, Faculty of Medicine, Hamadan University of Medical Sciences. In aseptic conditions, all the contents of the cyst were aspirated by a disposable syringe and transferred into the falcon tubes and left in stationary state for 30 min, then, supernatants were discarded. The cysts fluid which have the highest rate of viable protoscolices was used for experiment. After washing with sterile normal saline during three times, the percentage of early live protoscolices was determined by observing their flame cells movement (vibrations of a flame cell and contraction the body) and also using vital staining of eosin 0.1% and observing with a light microscope. The live protoscolices did not take the dye and stained protoscolices were considered dead. Finally, the live protoscolices were transferred into dark container at 4 °c temperature until use.

Plant materials, ginger rhizomes, eucalyptus leaves, and artemisia fruits, were purchased from the herbal market in Hamadan province, Iran, and was identified and authenticated at College of Pharmacy, Hamadan University of Medical Sciences. After washing with tap water and drying, each plant material was grinded to yield a fine powder. The powdered material of each plant (200 g) was extracted by maceration in methanol (3* 1 L) at room temperature for three times, each time for three days. Then, each methanolic extraction was evaporated under reduced pressure until completely dried. The dried extractions were kept in refrigerator until use. In this study, three concentrations (25, 50 and 100 mg/ml) and five different exposure times (5, 10, 25, 40 and 60 min) were examined for each plant extract. In order to prepare the methanolic extract, amount 200 gr of the eucalyptus leaves, artemisia fruits and ginger root each from with pure methanol were mixed at a ratio 3:1 slowly for one hour with shaker utterly blend. Obtained mixture was placed at room temperature for 24 hours. The obtained solution was smooth and it moved to Rotary devices for removing the solvent, then were placed in containers cristalizar at room temperature until completely dried and the materials were kept at refrigerator temperature. The scolicidal tests were carried out based on Moazeni and Nazer [8]. For preparation of extract, the concentrations 0.25, 0.5 and 1 g of dried extract were dissolved in 10 mL of normal saline, respectively. Then, 2 ml of each the concentration of extract was placed in a test tube and added a drop of solution suspension of parasite contains protoscolices (at least 1000 larvae) and mixed gently. The tube was incubated at 37°C and was examined at 5, 10, 25, 40 and 60 min. After finishing the exposure time for each tube, upper part of the solution was removed carefully by pipette and settled protoscolices two milliliters of 0.1% eosin stain was added and mixed gently. Upper part of the solution was discarded after 15 minutes. Then, to determine the protoscolices viability, they were examined under a light microscope with *10 objective and percent of dead larvae was determined. Also, in order to evaluate the possible factors affecting on the live protoscolices including the passage time, at the same times protoscolices were placed with the proximity of normal saline in the another test tube and the results were recorded. The experiments was repeated three times (triplicate). Analysis of the data was performed by SPSS software, using the chi-square for comparing between groups (P<0.05).

**RESULTS**

The mortality rate of protoscolices at concentrations and various exposure times of methanolic extract of these plants have been shown in Tables1-3.

According to the results of this study, the mortality rates of exposed protoscolices by ginger extract at concentrations of 25, 50 and 100 mg/ml was 80.61%, 85.21%, 100% after 60 min exposure respectively, while the death protoscoleces in the control group was 13%. Scolicidal effect of methanol extract of eucalyptus in concentrations of 25, 50 and 100 mg/ml after 40 minutes of exposure, was 84.78%, 100% and 100% respectively, while mortality rate was 15% in the control group. Artemisia extract had very low killing activity on the live protoscolices. So that after 60 min exposure at concentrations of 25, 50 and 100 mg/ml killed 5.19%, 11.29% and 17.33% of protoscolices, while mortality rate in the control group was 4%.

**DISCUSSION**

The present study showed that methanolic extract of ginger and eucalyptus had high scolicidal activity, while the methanolic extract of artemisia had low effect on the protoscolices of hydatid cysts. Until recent years, surgery was the treatment of choice for cystic echinococcosis. Different scolicidal materials such as formalin, hydrogen peroxide, cetrimide, ethanol, hypertonic saline and silver nitrate have used as scolicidal in hydatid cyst surgery, that most of these agents have serious side effects [9]. Z. officinale contains 1-2% of volatile oil and 5-8% of resinous matter, starch and mucilage. The volatile oil containing monoterpens, sesquiterpenes and sesquiterpen alcoholzingiberol, gingerol and shagools. These fractions are the most pharmacological active constituents in the volatile oils [10]. In this study, the obtained results of protoscolicidal effect of ginger extract showed that the methanolic extract of ginger with concentration of 100 mg/ml, killed all protoscolices in 40 min of exposure that was in accordance with Moazeni et al (2011) [8], which reported that at 50 mg/ml concentration after 40 min 100% of protoscolices were destroyed. Another study showed also, the killing effect of this herbal extract on the microorganisms.
### Table 1: Scolicidal effect of ginger extract at concentration and various exposure times

<table>
<thead>
<tr>
<th>Exposure time (min)</th>
<th>Con (mg/ml)</th>
<th>5</th>
<th>10</th>
<th>25</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PSC (Mean±SD)</td>
<td>Dead PSC (Mean±SD)</td>
<td>Mortality (%)</td>
<td>PSC (Mean±SD)</td>
<td>Dead PSC (Mean±SD)</td>
<td>Mortality (%)</td>
</tr>
<tr>
<td>25</td>
<td>426.51±74.77</td>
<td>540.69±14.33</td>
<td>12.37</td>
<td>543.17±36.36</td>
<td>128.37±9.39</td>
<td>23.60</td>
</tr>
<tr>
<td>50</td>
<td>479.98±40.36</td>
<td>119.42±6.97</td>
<td>25.25</td>
<td>557.12±46.95</td>
<td>205.22±17.4</td>
<td>36.83</td>
</tr>
<tr>
<td>100</td>
<td>422.88±84.80</td>
<td>189.62±31.20</td>
<td>45.58</td>
<td>534.79±179.79</td>
<td>374.13±14.56</td>
<td>66.83</td>
</tr>
</tbody>
</table>

Control (n) 500 20 4 500 36 7.2 500 50 10 500 59 11.8 500 65 13

Con: concentration, PSC: protoscolices, SD: Standard deviation

### Table 2: Scolicidal effect of eucalyptus extract at concentration and various exposure times

<table>
<thead>
<tr>
<th>Exposure time (min)</th>
<th>Con (mg/ml)</th>
<th>5</th>
<th>10</th>
<th>25</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PSC (Mean±SD)</td>
<td>Dead PSC (Mean±SD)</td>
<td>Mortality (%)</td>
<td>PSC (Mean±SD)</td>
<td>Dead PSC (Mean±SD)</td>
<td>Mortality (%)</td>
</tr>
<tr>
<td>25</td>
<td>502.10±71.44</td>
<td>75.22±15.41</td>
<td>14.32</td>
<td>574.14±97.37</td>
<td>173.13±31.52</td>
<td>29.97</td>
</tr>
<tr>
<td>50</td>
<td>545.92±70.74</td>
<td>183.59±18.09</td>
<td>33.98</td>
<td>596.99±14.02</td>
<td>304.17±12.52</td>
<td>50.96</td>
</tr>
<tr>
<td>100</td>
<td>472.42±77.57</td>
<td>185.11±36.68</td>
<td>37.92</td>
<td>529.10±31.07</td>
<td>307.19±7.80</td>
<td>58.33</td>
</tr>
</tbody>
</table>

Control (n) 300 20 4 6.66 500 50 10 500 65 13 400 60 15 500 15

Con: concentration, PSC: protoscolices, SD: Standard deviation

### Table 3: Scolicidal effect of artemisia extract at concentration and various exposure times

<table>
<thead>
<tr>
<th>Exposure time (min)</th>
<th>Con (mg/ml)</th>
<th>5</th>
<th>10</th>
<th>25</th>
<th>40</th>
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<td>Dead PSC (Mean±SD)</td>
<td>Mortality (%)</td>
<td>PSC (Mean±SD)</td>
<td>Dead PSC (Mean±SD)</td>
<td>Mortality (%)</td>
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<tr>
<td>25</td>
<td>365.36±48.86</td>
<td>12.56±1.64</td>
<td>3.43</td>
<td>367.77±42.22</td>
<td>13.55±1.64</td>
<td>3.67</td>
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<tr>
<td>50</td>
<td>341.31±47.77</td>
<td>21.38±2.95</td>
<td>6.26</td>
<td>380.71±49.56</td>
<td>27.27±3.79</td>
<td>7.13</td>
</tr>
<tr>
<td>100</td>
<td>370.78±43.50</td>
<td>41.98±4.92</td>
<td>11.32</td>
<td>410.62±36.89</td>
<td>52.13±5.46</td>
<td>12.67</td>
</tr>
</tbody>
</table>

Control (n) 300 9 3 300 9 3 306 11 3.59 300 12 4 300 12 4

Con: concentration, PSC: protoscolices, SD: Standard deviation
In vitro scolicidal effect of methanolic extract of eucalyptus...  

In Noor Nihad study, ethanolic extract of ginger at 50,100 and 150 mg/ml after 120, 90 and 60 min respectively; 100% of protoscolices killed [11]. Merawin et al. in 2010 showed strong microfilaricidal activity of aqueous extract of ginger against Dirofilaria immitis in vitro [12]. Other studies showed antidiabetic [13] and anti-oxidant effect of aqueous extract of ginger [14]. Eucalyptus belongs to the family Myrtaceae and the leaf extract has anti-cancer effects, anti-inflammatory, analgesic, anti-oxidant, anti-malarial, anti-fungal and antiviral effects. Eucalyptus globulus, the most represented species of eucalyptus genus in the international pharmacopeia, has many effects such as antiseptic, astringent, disinfectant, deodorant, expectorant, febrifuge, inhalant, and insect repellant, vermifuge. The eucalyptus leaf extract has eucalyptol (1,8-cineol), citronellol, citronell, citronellyl acetate, p-cymene, eucamalol, limonene, linalool, β-pinene, α-terpinene, α-terpinol, alloocimene, aromadendrene. Eucalyptol as the main component of eucalyptus leaf has antiparasitic and anti-fungal effects. In this research, eucalyptus showed profound protoscolicidal effects, which can be due to the presence of eucalyptol. Eucalyptus in concentrations of 50 and 100 mg/ml after 40 min of exposure killed all protoscolices. The results of Safarnejad Tameshkel showed that, the fatality effect of methanolic extract of eucalyptus on Giardia lamblia cyst after 60 min exposure with concentration of 200 mg/ml was 63.3% [15]. In Abdollahzade study, the result of in vivo and in vitro evaluation showed that aceton and Ethanolic extract of eucalyptus comparing to aqueous extract has the most effective antimicrobial activity on Brucella and they can be useful in treatment of human and animal Brucellosis [16]. The genus artemisia that is belonging to Aaraceae family [17], anti-tumoral activity in some species of this family has been attributed to the presence of flavonoids, sesquiterpene lactones, lignans, acetylenes, triterpenes or glycolipids [18]. Artemisia spp was used to treatment helminth infections and Enterobius, Ascaris in the first century [19].Various species of the genus Artemisia have been used for treatment of malaria, hepatitis, hypertension, inflammation and infections caused by fungi, bacteria and viruses[20]. Artemisia extract at none of surveyed concentrations (25,50 and 100 mg/ml) hadn’t effect on protoscolices that might be due to the resistance of protoscolices against extract and also used concentrations and times, because two variables of concentration and time have the major role in protoscolices mortality. Based on Zhuliang study and et al. the Artemisia lancea extract at concentration of 10 mg, causing restrain of larvae growth about 99% against Haemoncus contorus[21]. The results of Barati et al. showed that Artemisia extract in concentrations of 31.25, 62.5, 125, 250 and 500 mg/ml against promastigotes of Leishmania major is ineffective and only at concentration of 5000 mg/ml is in accordance with drug control [22]. No surveys about the effect of eucalyptus and artemisia extract on protoscolices of hydatid cysts are reported previously.

CONCLUSION

This study showed that the methanolic extract of ginger and eucalyptus may be considered as a natural and effective scolicidal agent. Therefore, we can recommend it as the complementary treatment and in surgery of cyst for patients with hydatid cyst after investigation on the animal models and clinical trials in human populations.

REFERENCES


**CURRENT AUTHOR ADDRESSES**

FARIBA FAIZEI, M.Sc. Department of Parasitology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.
AMIR HOSSEIN MAGHSOOD, Associate Professor, Department of Parasitology, School of Medicine Hamadan University of Medical Sciences & Health Services, Hamadan, Iran.
FATEMEH PARANDIN, M.Sc. Department of Parasitology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.
MOHAMMAD MATINI: Assistant Professor, Department of Parasitology, School of Medicine Hamadan University of Medical Sciences & Health Services, Hamadan, Iran.
SHIRIN MORADKHANI: Assistant Professor, Department of Pharmacognosy, Hamedan University of Medical Sciences, Hamedan, Iran.
MOHAMMAD FALLAH: Mohammad Fallah, Professor, Department of Medical Parasitology and Mycology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran, E-mail: fallah@umsha.ac.ir (Corresponding author)