ORIGINAL ARTICLE

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

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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 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

Introduction
Hydatidosis is a common zoonotic disease caused by the larval stages of Echinococcus granulosus [1]. In the life cycle of this parasite, domestic and wild carnivores, mainly dogs are the definitive hosts, and herbivores and occasionally humans are the intermediate hosts of this parasite [2]. While infection of carnivores with adult stage of E. granulosus cannot cause important disease unless in heavy infection, the establishment of larvae (cyst) in various organs of intermediate host (especially liver, lungs, and sometimes the brain, heart and spinal cord), can cause severe disease and it can be even fatal [3]. This disease is more common in rural areas where there is a close relationship between dog and livestock and human [4]. This disease, according to the significant mortality rate caused great economic losses, can be considered as a public and the socio-economic health concern in both the public health and livestock industry [2]. Surgery is still the main method of the treatment of cystic echinococcosis [5]. Although at surgical treatment, if surgeons ignore the necessary measures, will be a risk of leakage of protoscolices and it will seeded in the internal organs and tissues during surgery that is one of the main causes of recurrence and the formation of secondary hydatid cysts [6]. To avoid the leakage of cyst contents, it is necessary to use the effective scolicidal agents in order to reduce the secondary infection rate [7]. Since anti-parasitic properties of some of herbal extracts have been well proven as well as the chemical scolicidales that have toxic and side effects; therefore,
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 &Mycolagy, 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 artesia 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 at 4 °C temperature until use.

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In vitro scolicidal effect of methanolic extract of eucalyptus.  

### Table 1: Scolicidal effect of ginger extract at concentration and various exposure times

<table>
<thead>
<tr>
<th>Exposure time (min)</th>
<th>5</th>
<th>10</th>
<th>25</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con (mg/ml)</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>
</tr>
<tr>
<td>25</td>
<td>426.51±74.77</td>
<td>54.99±14.33</td>
<td>12.37</td>
<td>543.17±36.18</td>
<td>128.37±9.39</td>
</tr>
<tr>
<td>50</td>
<td>479.98±40.36</td>
<td>119.42±6.97</td>
<td>25.25</td>
<td>537.12±46.95</td>
<td>205.22±17.4</td>
</tr>
<tr>
<td>100</td>
<td>422.88±84.00</td>
<td>189.62±31.20</td>
<td>45.58</td>
<td>537.91±197.79</td>
<td>374.13±136.8</td>
</tr>
</tbody>
</table>

Control (n): 500 20 4 500 36 7 250 40 15 500 59 11 500 65 15

P<0.001

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>5</th>
<th>10</th>
<th>25</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con (mg/ml)</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>
</tr>
<tr>
<td>25</td>
<td>502.10±71.44</td>
<td>75.22±15.41</td>
<td>14.32</td>
<td>547.14±97.37</td>
<td>173.13±31.52</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>
</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>
</tr>
</tbody>
</table>

Control (n): 300 20 4 500 36 7 300 39 11 300 59 11 300 65 15

P<0.001

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>5</th>
<th>10</th>
<th>25</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con (mg/ml)</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>
</tr>
<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>13.53±1.64</td>
</tr>
<tr>
<td>50</td>
<td>341.31±47.77</td>
<td>21.38±2.95</td>
<td>6.26</td>
<td>380.71±49.58</td>
<td>27.23±3.79</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>52.15±4.96</td>
</tr>
</tbody>
</table>

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

P=0.99

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

In Noor Nihad study, ethnolic 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 microfilaricial activity of aqueous extract of ginger against Dirofilaria imitis in vitro [12]. Other studies showed anti-diabetic [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 repellent, vermifuge. The eucalyptus leaf extract has eucalyptol (1,8-cineol), citronellol, citronell, citronellyl acetate, p-cymene, eucamal, limone, linalool, β-pinene, α-terpinene, α-terpinol, alloocimene, aromadendrene. Eucalyptol as the main component of eucalyptus leaf has anti-parasitic and anti-fungal effects. This research, eucalyptol showed profound protoscolocidal 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 mortality effect of methanolic extract of eucalyptus on Gaerdia lambia 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 acetone 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 Astarceae 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 of 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


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