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
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 at 4 °c temperature until use.

The obtained solution was smooth and it moved to Rotary devices for removing the solvent, then were placed in containers cristalizer 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], which reported that 50 mg/ml concentration of 100 mg/ml, killed all protoscolices in 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%.

**RESULTS**

The mortality rate of protoscolices at concentrations and various exposure times of methanolic extract of these plants have been shown in Tables 1 and 2.

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 protoscolices 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 monoterpene s, sesquiterpenes and sesquiterpenes alcoholizingiberol, gingerol and shagoals. 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.
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>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>452.51±74.77</td>
<td>54.69±14.33</td>
<td>12.37</td>
<td>543.17±36.18</td>
<td>125.37±9.39</td>
<td>25.60</td>
</tr>
<tr>
<td>50</td>
<td>479.98±40.36</td>
<td>119.42±6.97</td>
<td>25.25</td>
<td>577.12±46.95</td>
<td>205.22±17.4</td>
<td>36.83</td>
</tr>
<tr>
<td>100</td>
<td>422.88±84.00</td>
<td>189.62±31.20</td>
<td>45.58</td>
<td>534.79±116.46</td>
<td>464.31±95.07</td>
<td>89.79</td>
</tr>
</tbody>
</table>

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

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>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>29.77</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 4 500 36 7.2 500 50 10 500 59 11.8 500 65 13

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>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>365.36±48.86</td>
<td>12.56±1.64</td>
<td>3.43</td>
<td>367.77±22.22</td>
<td>13.55±1.64</td>
<td>3.67</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>27.27±3.79</td>
<td>7.13</td>
</tr>
<tr>
<td>100</td>
<td>370.78±31.50</td>
<td>41.98±4.92</td>
<td>11.32</td>
<td>410.62±36.89</td>
<td>52.13±4.46</td>
<td>12.67</td>
</tr>
</tbody>
</table>

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

P<0.001

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 Dicrofilaria 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 repellant. Verminfuge. The eucalyptus leaf extract has eucalyptol (1,8-cineol), citronellol, citronellal, citronellyl acetate, p-cymene, eucamalol, limonene, linalool, β-pinene, α-terpene, α-terpinol, alloocimene, aromadendrene. Eucalyptol as the main component of eucalyptus leaf has antiparasitic and antifungal effects. In this research, eucalyptus 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 Tameskkel 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 Atracaceae 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 helmith infections and Enterobius, Ascaris in the international pharmaceutical system. Viszeralmedizin. 2006;22(3):185-90.

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

In vitro scolicidal effect of methanolic extract of eucalyptus. 


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)

Published online: July 12, 2015