Effect of leaf extracts of *Dendrosicyos socotrana* and *Jatropha unicostata* on the viability of *Echinococcus granulosus* protoscoleces

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Abstract
The present work evaluates the effect of aqueous and methanolic extracts of *Dendrosicyos socotrana* and *Jatropha unicostata* on the viability of *Echinococcus granulosus* protoscoleces *in vitro*, and on the development of secondary hydatid cysts, *in vivo*. Three different concentrations of each leaf extract were used. Concentrations of 5000 and 1000 μg/mL, for *D. socotrana* and *J. unicostata*, respectively, exhibited the highest protoscolicidal activity, significantly reducing and/or stopping protoscolex viability. Oral and intraperitoneal administration of the extracts in white mice invoked noticeable inhibitory effects on the *in vivo* development of secondary hydatid cysts. These effects were compared with those of albendazole sulfoxide, a commonly used treatment for hydatidosis.

Keywords: *Dendrosicyos socotrana*, *Echinococcus*, hydatidosis, *Jatropha unicostata*, Socotra Island.

INTRODUCTION

Hydatidosis, or echinococcosis is a disease caused by the metacestodes of different species of *Echinococcus* Rudolphi, 1801. Tapeworm eggs are passed with the feces of infected carnivores and may subsequently infect humans who inadvertently ingest them (Andersen 1997). Clinical manifestation of hydatidosis is characterized by tumor-like growths that occur mostly in the liver and lungs, with varying degrees of infestation of other organs (Abdel-Hafez and Al-Yaman 1989). These growths are usually filled with a watery fluid known as ‘hydatid cyst fluid.’ Hydatidosis is a major world zoonosis affecting humans as well as domestic animals (Thompson 1995).

Because of the slow progression of the disease, it may initially be asymptomatic or show very slight manifestations (Lyagoubi et al. 1997). However, serious clinical symptoms may eventually develop, which vary, depending on the extent of infestation, the site of infection, and the size of the cyst(s) (Shambesh 1997).

As with other parasitic diseases, hydatidosis must be treated. Chemotherapy, using benzimidazole carbamate derivatives is the standard protocol (Liu and Weller 1996). However, chemotherapy is not always effective and may cause serious side effects in some patients; therefore, in certain cases surgical intervention becomes mandatory (Zhou et al. 1988).

Treatment of a variety of diseases with plant extracts has been practiced for many centuries, with numerous plant species having been used (Ye et al. 1990, Al-Saimary and Zeki 1999, Aghwan 1999, Al-Aboody 2001, Al-Eryani 2002). Extracts of *Dendrosicyos socotrana* Balf f. and *Jatropha unicostata* Balf f., both of which are endemic to Socotra Island (Yemen), are used locally for a variety of medical treatments. Extracts of the former

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are used by the islanders for the treatment of urinary retention, cystitis symptoms of diabetes, liver problems, and burns (Miller and Morris 2004). Ali et al. (2003) failed to show any antimicrobial effect of D. socotrana and only a weak effect for J. unicostata. However, a methanolic extract of J. unicostata demonstrated a marked antiviral effect against Influenza virus type A and Herpes simplex type 1 (Mothana et al. 2006). Remarkably, the methanolic extract of D. socotrana exhibited noteworthy cytotoxic activity against human cancer cell lines (Mothana et al. 2007).

According to Miller and Morris (2004), J. unicostata has various uses. The red sap is used as a haemostatic in humans and livestock, while the colorless sap is used to treat livestock infections. The latter is also used to treat the skin, 'tender' glands, eye infections, chest pain, stomach pain, retching, vomiting, and is used as a laxative and vermifuge. The present work aimed therefore, to evaluate the effect of both plants on the viability of Echinococcus granulosus (Batsch, 1786) protoscoleces, and on the development of secondary hydatid cysts.

**MATERIAL AND METHODS**

**The parasite**

Fresh hydatid cysts were obtained from livers of naturally-infected sheep, which had been slaughtered at local abattoirs in Sana’a. They were wrapped carefully in clean plastic bags, placed in an ice box, and transported to the Department of Biology, Faculty of Science, Sana’a University, where protoscoleces were extracted. The outer surfaces of the cysts were sterilized with 70% ethanol before being dissected. Protoscoleces were extracted according to Smyth (1967). Extracted protoscoleces were maintained in a sterile medium made of a mixture of Kreb’s Ringer Solution (KRS) and hydatid cyst fluid (4:1), which was prepared according to Rotunno et al. (1974) and Al-Eryani (2002). Crystalline penicillin ‘G’ and streptomycin sulphate were added to the mixture to keep it free from contamination (Schwabe et al. 1963, Lorenzinii and Ruggieri 1990, Casado et al. 1992, 1996, Hemphill and Gottstein 1995, Urrea-Paris et al. 2000).

**Animals**

Sixty five, 6-8 week old white mice (20 males and 45 females), were used in this study. These were descended from eight pairs that were originally obtained from Al Azhar University Animal House, Cairo, Egypt.

**Plant collection and extraction**

Fresh leaves of Dendrosicyos socotrana (Cucurbitaceae) and Jatropha unicostata (Euphorbiaceae) were collected from Socotra Island at the end of April 2004. Voucher specimens of both plants were deposited in the herbarium of the Faculty of Science, Sana’a University. The leaves were cleaned and air-dried at room temperature. They were then pulverized in a grinder to obtain a fine powder. Seventy grams of powdered leaves of each of the two plant species were extracted with methanol by using a soxhlet extraction apparatus. The residue was dried overnight and extracted with water at 60°C. The methanol extracts were evaporated using a rotary evaporator and the water extracted with a freeze-dryer. The dried crude extracts were kept in dark plastic bags, sealed under vacuum, and kept in a freezer at -20°C until use.

**Determination of in vitro and in vivo effect**

Stock solutions of methanolic and aqueous crude extracts of D. socotrana were prepared by dissolving 0.36 g of the powdered extract in 10 mL of 50% dimethyl sulfoxide (DMSO). Concentrations of 1000, 2000, and 5000 μg/mL of the stock solutions were used to treat the protoscoleces. The stock solutions of J. unicostata were prepared by dissolving 0.08 g of the methanolic and aqueous crude extracts in 10 mL of 60% DMSO. Concentrations of 500, 750, and 1000 μg/mL of the stock solutions were used for in vitro treatment of protoscoleces. A mixture of 50% DMSO and the maintenance medium (1:1000) was used for D. socotrana and 60% DMSO and the maintenance medium (1:1000) for J. unicostata were used as positive controls. Kreb’s Ringer solution was used as a negative control.

The efficacy of methanolic and aqueous extracts of both plants on the viability of protoscoleces was compared with different doses of albendazole sulfoxide (ABZSO), the standard medicine used in treating hydatidosis (Ingold et al. 1999, Urrea-Paris et al. 2000, Stettler et al. 2001).
Both in vitro and in vivo experiments on *E. granulosus* protoscoleces were performed during this study. The former were carried out in sets of 21 Eppendorf tubes at each of the three concentrations for each of the two plant species. Three positive control and three negative control tubes were used for each concentration set. 1.0 mL of the maintenance medium, supplemented with penicillin and streptomycin was added to each tube. Approximately 3000 viable protoscoleces were placed in each tube, and incubated at 28°C. The effect of ABZSO was tested in vitro using three tubes for the drug, together with three tubes for each of the positive and negative controls.

The effects of plant extracts on the in vivo development of protoscoleces and secondary hydatid cysts was studied in the sixty five 6-8 week old experimentally infected mice. The animals were divided into 13 groups with five mice in each group. Mice in each of four groups received methanolic and aqueous extracts of each plant, and one group received ABZSO by oral administration. Another batch of five groups received the same treatment but intraperitoneally (IP). Mice in two groups received oral and IP doses of the positive control. The last group of mice received the negative control. All animals used in the experiment were sacrificed 60 days post-treatment and examined for the presence of secondary hydatid cysts.

**Statistical analysis**

The data were expressed as mean values ± S.E.M. and tested with one-way ANOVA followed by the Tukey’s test for multiple comparisons using Statistical Package for Social Sciences (SPSS) computer package. Results with P<0.05 were considered significant.

**RESULTS**

**Viability of protoscoleces**

Untreated protoscoleces remained viable for 24 h prior to treatment, with no significant differences in viability between 0 and 24 h (t = 0.69, P<0.005).

**In vitro treatment of protoscoleces**

The viability of protoscoleces was significantly affected when treated with the methanolic extract of *D. socotrana* leaves. Protoscoleces treated with concentrations of 1000, 2000, and 5000 μg/mL started showing decrease in viability at 96, 12, and 3 h post treatment, respectively. All treated protoscoleces perished at h 480, 408, and 360 post treatment, respectively (Fig. 1). This result was highly significant (P<0.05). The inhibitory effect of an aqueous extract of the same plant was also significant (P<0.05). All protoscoleces died at h 504 and 408 when treated with 2000 and 5000 μg/mL, respectively, while 85.2% and 75.0% of the untreated controls remained viable until the end of the experiment at h 504 (Fig. 2).

Both methanolic and aqueous extracts of *J. unicostata* inhibited the viability of protoscoleces. All protoscoleces treated with the methanolic extract at concentrations of 500, 750, and 1000 μg/mL, died at hours 408, 336 and 288, respectively. The majority of protoscoleces in the negative control and positive control groups remained viable until the end of the experiment (Fig. 3). The same trend was observed in protoscoleces treated with similar concentrations of the aqueous extracts (Fig. 4).

ABZSO exhibited a significant effect on the viability of protoscoleces (P<0.05). 96 h from the onset of treatment, 90.5% of the 3000 protoscoleces were dead. All of the remaining protoscoleces had died by h 360. Negative and positive control groups showed a slight decline in viability whereas 91.2% and 90.2% of the protoscoleces were still viable by h 360 post-treatment. A comparison between the effects of high concentrations of both *D. socotrana* and *J. unicostata* and that of ABZSO is summarized in Fig. 5.

Oral treatment of aqueous and methanolic extracts of the two plants showed varying effects on the growth of secondary hydatid cysts, growing on different organs of the mice. Generally, the methanolic extract of *J. unicostata* exhibited a high noticeable effect similar to that of ABZSO. However, effects demonstrated by the other extracts were generally weak or not noticeable (Table 1). Moreover, there was little discernable difference between the effects of extracts administered intraperitoneally and orally (Table 2).
Cystic echinococcosis (CE) is a subject of major health concern in many countries. Each confirmed case could potentially cost the health authorities thousands of dollars (Nasrieh et al. 2003, Torgerson 2003). Although surgery is the most effective choice of treatment, a number of drugs are being used and various degrees of success have been claimed. However, the metabolites of certain drugs including benzimidazole, mebendazole, albendazole, and albendazole sulfoxide are potentially toxic in some subjects (Davis et al. 1989, Whittaker and Faustman 1991, Pawlowski 1997).

The use of plant extracts against CE has received critical attention in recent years. Some studies have shown that extracts of certain plant species, belonging to different families, may affect the viability of protoscoleces and/or the survival of secondary hydatid cysts. For instance, seeds of *Peganum harmala* L. (Zygophyllaceae) affected *E. granulosus* in mice (Al-Eryani 2002). Al-Saimary and Zeki (1999)
demonstrated that extracts of *Eucalyptus* and *Myrtus communis* L., (Myrtaceae), *Apium graveolens* L. (Umbelliferae) and *Trigonella foenum-graecum* L. (Leguminosae) can kill the larval stages of *Echinococcus* sp. Al-Rubaei (1999) reported that water extracts of garlic *Allium sativum* L. (Liliaceae) and Artemisia herba-alba Asso (Compositae) affected viability and killed protoscoleces. Also Al-Aboody (2001) showed that *Ruta chalepensis* L. (Rutaceae) and *Plantago lanceolata* L. (Plantaginaceae) stopped the viability of protoscoleces.

*D. socotrana* and *J. unicostata* were selected for this study because they are two of the endemic plants on Socotra island that are used widely for medicinal purposes (Miller and Morris 2004). The former plant contains a group of compounds known as curcurbitacins, a special group of highly oxygenated tetracyclic triterpenes, with a wide range of bioactivity (Dinan et al. 1997, Ito et al. 2002, Hussein et al. 2004). Curcurbitacins are extremely bitter and are toxic to most organisms (Smyth et. al. 2002). *J. unicostata* on the other hand was shown to contain a mixture of sterols and ketosteroids and terpenoids (Franke et al. 2004), which might have an effect on the viability of certain organisms.

In conclusion, our data suggest that medicinal plants can be a promising source of potential anti-protoscoleces. The data suggest that *J. unicostata* should be selected for further investigation into the potential discovery of new natural bioactive compounds. Studies aimed at the isolation of active anti-protoscoleces constituents are in progress and may lead to the discovery of compounds with improved therapeutic value.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Malcolm Potts for the valuable comments and corrections to the manuscript.

<p>| Table 1. Effect of various treatments on the development of secondary hydatid cysts in the different albino mice organs (oral administration). |</p>
<table>
<thead>
<tr>
<th>Organ/Region</th>
<th>Treatment with <em>D. socotrana</em> 50 mg/kg/day</th>
<th>Treatment with <em>J. unicostata</em> 50 mg/kg/day</th>
<th>ABZSO 200 µg/kg/day</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanolic</td>
<td>Aqueous</td>
<td>Methanolic</td>
<td>Aqueous</td>
</tr>
<tr>
<td>Liver</td>
<td>31.17 ± 0.58</td>
<td>31.17 ± 1.42</td>
<td>5.19 ± 0.58*</td>
<td>9.09 ± 1.16*</td>
</tr>
<tr>
<td>Lungs</td>
<td>2.59 ± 0.71</td>
<td>3.89 ± 0.71</td>
<td>0.00 ± 0.00*</td>
<td>1.29 ± 0.58</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.59 ± 0.71</td>
<td>3.89 ± 0.71</td>
<td>1.29 ± 0.58</td>
<td>1.29 ± 0.58</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.89 ± 0.71</td>
<td>5.19 ± 1.08</td>
<td>0.00 ± 0.00*</td>
<td>0.00 ± 0.00*</td>
</tr>
<tr>
<td>Mesentery</td>
<td>36.36 ± 0.71</td>
<td>40.26 ± 1.08</td>
<td>5.19 ± 0.58*</td>
<td>14.3 ± 0.58*</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>3.89 ± 0.71</td>
<td>3.89 ± 0.71</td>
<td>0.00 ± 0.00*</td>
<td>0.00 ± 0.00*</td>
</tr>
</tbody>
</table>

* = (P<0.05)

<p>| Table 2. Effect of various treatments on the development of secondary hydatid cysts in the different albino mice organs (I.P. administration). |</p>
<table>
<thead>
<tr>
<th>Organ/Region</th>
<th>Treatment with <em>D. socotrana</em> 50 mg/kg/day</th>
<th>Treatment with <em>J. unicostata</em> 50 mg/kg/day</th>
<th>ABZSO 200 µg/kg/day</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanolic</td>
<td>Aqueous</td>
<td>Methanolic</td>
<td>Aqueous</td>
</tr>
<tr>
<td>Liver</td>
<td>35.62 ± 1.15</td>
<td>34.25 ± 0.97</td>
<td>8.22 ± 0.61*</td>
<td>17.8 ± 0.75*</td>
</tr>
<tr>
<td>Lungs</td>
<td>4.11 ± 0.75</td>
<td>4.1 ± 1.23</td>
<td>0.00 ± 0.00*</td>
<td>2.74 ± 0.75</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.11 ± 0.75</td>
<td>4.1 ± 0.75</td>
<td>2.74 ± 0.75</td>
<td>2.74 ± 0.75</td>
</tr>
<tr>
<td>Spleen</td>
<td>6.85 ± 0.97</td>
<td>6.8 ± 0.97</td>
<td>0.00 ± 0.00*</td>
<td>4.1 ± 0.75</td>
</tr>
<tr>
<td>Mesentery</td>
<td>43.83 ± 0.75</td>
<td>42.5 ± 1.15</td>
<td>5.5 ± 0.61*</td>
<td>13.7 ± 0.97*</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>5.48 ± 0.61</td>
<td>5.48 ± 0.61</td>
<td>1.4 ± 0.61</td>
<td>1.4 ± 0.75</td>
</tr>
</tbody>
</table>

* = (P<0.05)
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Dendrosicyos socotrana ve Jatropha unicostata Yaprak Özütlerinin Echinococcus granulosus Protoskolekslerinin Canlıligina Etkisi

Özet
Bu çalışma; Dendrosicyos socotrana ve Jatropha unicostata’nın su ve metanolde elde edilen özütlerinin, Echinococcus granulosus protoskoleksleri üzerine in vitro ve sekonder hidatit kistlerin gelişimi üzerine in vivo etkisini incelemektedir. Her yaprak özütünün üç farklı konsantrasyonu kullanildi. Sirasiyla D. socotrana ve J. unicostata’nın 5000 and 1000 μg/mL yoğunlukları en yüksek protoskolisidal aktivite sergiledi ve protoskoleks canlıligini önemli ölçüde azalttı ve/veya durdurdu. Beyaz farelerde, yaprak özütlerinin oral veya intraperitoneal uygulanması, sekonder hidatit kistlerin in vivo gelişimi üzerinde farkedilir oranda inhibitör etki oluşturdu. Bu etkiler, hidatidosiz tedavisinde yaygın olarak kullanılan albendazol sülfoksid’inkilerle kıyaslandı.

Keywords: Dendrosicyos socotrana, Echinococcus, hidatidosiz, Jatropha unicostata, Socotra Adası.