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Original article

Assessment of prevalence of hydatidosis in slaughtered Sawakny sheep in Riyadh city, Saudi Arabia

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ABSTRACT

Hydatidosis, or echinococcosis, is a serious medical and veterinary problem in many countries, particularly those with rural communities where there is a greater contact between dogs and domestic animals. Domestic livestock act as intermediate hosts which are the main reservoir for the disease in humans. It is therefore very important to estimate the prevalence of hydatid cysts in slaughtered animals since it can be transmitted to humans through dogs, which act as the final host for the disease. From this point of view, the present study was suggested to determine the prevalence of hydatidosis in Sawakny sheep slaughtered in Riyadh city, Saudi Arabia. During the course of the study 12,569 Sawakny sheep were inspected for hydatidosis infection. An overall prevalence of 1.06% was detected among the examined sheep, with the highest prevalence occurring in winter (1.38%) and lowest prevalence in summer (0.67%). Sheep aged 6–12 months had a higher rate of infection than older animals, and males were the predominant carriers of infection (97.7%) compared to females (2.3%). The liver was the most infected organ (79.1%), followed by the lungs (14.6%), while concurrent infections of both the liver and the lungs occurred in 6% of cases. The fertility and viability rates of hydatid cysts in the liver (70.1% and 85.1% respectively) were higher than that in any other organs. In conclusion, it is evident that fertile cysts in slaughtered sheep could have an important role in the continuation of hydatid cyst transmission to humans through dogs. Considerable effort should be devoted to controlling the transmission of cysts from abattoirs by the secure disposal of infected offal. In addition, plans are required for further epidemiological studies and control programs.

1. Introduction

Echinococcosis (hydatidosis) is one of the common zoonotic and severe clinical forms of disease caused by the larval (metacestode) and adult stages of cestodes of the genus Echinococcus and the family Taeniidae (Eckert and Deplazes, 2004). The normal life cycle of Echinococcus species requires two mammalian hosts; adult worms inhabit the small intestine of canids as definitive hosts, while larval stages or the hydatid cyst occur in herbivorous intermediate hosts and, occasionally, in humans (Thompson and McManus, 2002). Since Echinococcosis is an important zoonosis, the identification of the incidence and prevalence of infection in various intermediate hosts is essential for determining the significance of each animal species in the maintenance of the parasite life cycle and, subsequently, in the spread of the disease (Cadavid Restrepo et al., 2016). Infections with hydatid cysts in intermediate hosts (goat, sheep, cattle, camels, etc.) are normally asymptomatic, and there are usually no dependable methods for the routine diagnosis of the infection in living animals except in a few cases where cysts have been distinguished by ultrasonography (Eckert and Deplazes, 2004; Hayajneh et al., 2014). Accordingly, the most reliable demonstrative technique is cyst detection during meat investigation or at post mortem inspection and, therefore, the slaughter house is the best place to survey hydatidosis in livestock. From this perspective, we provide here a survey undertaken in Riyadh city, Saudi Arabia, to determine the spread of hydatidosis in food animals, especially Sawakny sheep.
2. Materials and methods

This work was conducted on Sawakny sheep slaughtered in slaughter houses in the city of Riyadh in the central region of Saudi Arabia. A total of 12,569 Sawakny sheep were examined for cystic hydatidosis over the course of one year from November 2015 to October 2016. Data including age, sex and site of infection were recorded for each animal. Slaughtered sheep were examined at the slaughterhouse by visual inspection and palpation for hydatid cysts in visceral organs including the liver, lungs, spleen, heart and kidneys according to guidelines recommended by WHO/FAO/UNEP (1994). Infected organs were transferred to the Parasitology Laboratory, Zoology Department, College of Science, King Saud University, and all cysts in each organ were measured and examined for fertility and viability of protoscolices. Fertility was detected by the examination of cyst content for the presence of protoscolecites. The viability of the protoscolecites was assessed using 0.1% aqueous solution of eosin staining; unstained protoscolecites were considered as viable while stained protoscolecites were considered as non-viable (Fig. 1) (Moazeni and Nazer, 2010).

3. Statistical analysis

Statistical significance differences were assessed with a one-way ANOVA using a statistical package program (Sigma Plot version 11.0). Data are presented as mean ± standard deviation from the mean (SD) and P ≤ 0.005 was considered significant.

4. Results

The results showed that the infection prevailed throughout the year with an overall prevalence of 1.06% (134/12,569). The highest prevalence was recorded in winter (1.38%; 42/3009) followed by spring (1.15%; 36/3100) and autumn (1.08%; 33/3069), while the lowest prevalence was reported in summer (0.67%; 23/3391). Statistically, however, the prevalence of infection was not significantly different between the seasons (P = 0.139) (Table 1, Fig. 2).

The prevalence of hydatidosis did differ significantly by sheep sex though, with males dominating the incidences of infection (97.7%; 131/134) compared to females (2.3%; 3/134) (P < 0.001) (Fig. 3).

The results also showed that infection differed according to the sheep’s age, with the highest prevalence in sheep aged 6–12 months (58.2%; 78/134) followed by sheep aged 13–24 months (30.6%; 41/134) while the lowest prevalence was

<table>
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<tr>
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<tr>
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<tr>
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<td>3069</td>
<td>33</td>
<td>1.08</td>
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<tr>
<td>Total</td>
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<td>134</td>
<td>1.06</td>
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Fig. 1. Representative photomicrographs for viability test; (A) viable non-stained protoscolecites, (B) viable protoscolecites after staining with 0.1% eosin, (C) dead protoscolecites after staining with 0.1% eosin. Scale-bar = 100 μm.

Fig. 2. Mean seasonal prevalence (% ± SD) of hydatid cyst in slaughtered sheep.

Fig. 3. Mean prevalence (% ± SD) of hydatid cyst per sex of slaughtered sheep.
in sheep aged more than 2 years (11.2%: 5/134) (Fig. 4). A highly significant difference in terms of prevalence of infection was found between sheep aged 6–12 months and sheep aged 13–24 months ($P<0.001$), between sheep aged 6–12 months and sheep aged more than 2 years ($P=0.001$), but not between sheep aged 13–24 months and sheep aged more than 2 years ($P=0.051$).

The examination of cyst locations indicated that the liver (Fig. 5A) was the most frequently infected organ with a prevalence of 79.1% (106/134), followed by the lung (Fig. 5B) with a prevalence of 14.9% (20/134), while concurrent infection of both liver and lungs and other visceral organs showed the lowest prevalence of 6% (8/134) (Fig. 6). The location of the cysts revealed a significant difference between liver and lung ($P<0.001$), liver and concurrent infection of both liver and lungs and other visceral organs ($P<0.001$), but not between lung and synchronous infection of both liver and lungs and other visceral organs ($P=0.393$). In all organs the cysts ranged from 1 to 7 cm in diameter.

Fertile cysts were the most common (70.1%: 94/134), and 85.1% (80/94) of these were viable, mostly located in the liver. 29.9% (40/134) of the examined cysts were sterile and no calcified cysts were reported.

5. Discussion

Echinococcosis has a world-wide geographic distribution and is found in every continent except Antarctica in a wide variety of hosts at different levels of prevalence (WHO, 2002). The highest prevalence of the parasite in humans and livestock hosts is found in temperate and rural areas, including Eurasia (southern and central parts of Russia, Mediterranean regions, central Asia and China), Africa, Australia and South America (Grosso et al., 2012). Livestock hosts such as sheep, camels and goats, are the essential reservoirs of hydatidosis disease for humans (Daryani et al., 2007). In this context, and since hydatidosis is of public health significance, as well as of economic importance in the Kingdom of Saudi Arabia (Hussein et al., 2012; Abdel-Baki et al., 2016), the objective of the present work was to study the prevalence of hydatidosis infection in slaughtered Sawakny sheep in Riyadh city slaughter houses.

The results showed an overall prevalence of 1.06% (134/12,569) with the highest incidence in winter and the lowest in summer. Previous studies in Saudi Arabia, and in other parts of the world, have reported that the prevalence of echinococcosis is high in sheep compared to some other animals like cattle and goats (Christodouloupolous et al., 2008; Kebede et al., 2009; Ibrahim, 2010; Toulah et al., 2012; Hayajneh et al., 2014). The prevalence observed in this study (1.06%: 134/12,569) is much lower than those reported in other regions in Saudi Arabia e.g. Ibrahim (2010) [12.61% (823/6525)] in Al Baha, Toulah et al. (2012) [69.6% (29,108/41,822)] in Jeddah and Hayajneh et al. (2014) 13.5% (162/1198) in Al Taif. The lower prevalence in Riyadh city compared to other locations could be attributed to the fact that Riyadh is a large urban area while the other locations are rural to semi-urban characterized by the presence of bedouins and their herds, often with guard dogs living near sheep runs. The amelioration of the standards of living and the augmentation of the consciousness of zoonotic disease threat may also contribute to this diminished rate of prevalence in Riyadh.
The variability in prevalence could also be generally related to the differences in the strain of sheep, age factors, the number of examined sheep, the different sources of sheep in the kingdom, culture differences, social activities and availability of dogs (Macpherson et al., 1985; Budke et al., 2006; Ibrahim, 2010; Hayajneh et al., 2014). The present study proved that infection prevailed throughout the year in all seasons but that there was no statistical difference between any of the seasons. Similarly, Elmajdoub and Rahman (2015) found no significant differences in seasonal infection rates in slaughtered sheep in Libya. Interestingly, Daryani et al. (2007) reported significant differences in the prevalence of infection between winter and autumn in Iran while Ibrahim (2010) found significant differences in rates of infection between autumn and spring in Saudi Arabia. These variations could be related to variations in climatic factors, for example rainfall, temperature, humidity and the nature of the pasture (Ibrahim, 2010; Elmajdoub and Rahman, 2015).

In the present study, the prevalence of hydatid cysts in the liver was found to be higher than that in the lung and other visceral organs, which is in accordance with the previous findings of Azlaf and Dakkak (2006), Haridy et al. (2006), Ibrahim (2010) and Toulah et al. (2012). This may due to the fact that liver is the first organ to which the blood flows having left the digestive tract, which results in the greater part of the oncospheres being transported to the liver, with only the ones that are not separated in the liver moving on to lungs and other organs (Al-Khalidi, 1998). In contrast, Azami et al. (2013) found that the infection was spread predominantly in the lungs of sheep slaughtered in Iran. The present study also found that male sheep have a higher prevalence of cysts than females, which is in agreement with previous studies from Jordan, Saudi Arabia and Libya (Al-Yaman et al., 1985; Ibrahim, 2010; Elmajdoub and Rahman, 2015).

Information on the fertility and viability of hydatid cysts in different domesticated animals is important to give an indication of the significance of each livestock type as a conceivable wellspring for infection of final hosts, especially dogs. Generally, the hydatid cysts have different fertility rates depending on their size and location and the type of host (Elmajdoub and Rahman, 2015). In the current study, it was observed that the cysts in the liver had higher fertility rates than those in the lung. Similarly, Dalimi et al. (2002), Ibrahim (2010) and Elmajdoub and Rahman (2015) reported that cysts in the livers of sheep were more fertile than those in the lungs.

The study demonstrated that sheep aged 6–12 months had a higher rate of infection compared to older ones, which is in agreement with the results of Hayajneh et al. (2014). This finding, however, is opposite to that in most previous studies (e.g. Ibrahim, 2010; Al-Qurashi and Bahnass, 2012; Elmajdoub and Rahman, 2015) in which the rate of infection was higher among older animals than younger ones. The reason why young sheep were most commonly infected in the present study is probably that the urban residents of Riyadh prefer to slaughter young sheep rather than older ones and therefore most of the sheep slaughtered in Riyadh abattoirs were young (Al-Qureishy, 2008).

In conclusion, fertile cysts in slaughtered sheep could have an important role in the continuation of hydatid cyst transmission to humans through dogs. Considerable effort should therefore be devoted to controlling the transmission of cysts from abattoirs by the secure disposal of infected offal. In addition, plans are required for further epidemiological studies and control programs.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

We extend our appreciation to the Dean of Scientific Research, King Saud University, for funding the work through the research group project number RGP-002.

References

In vitro effectiveness of *Curcuma longa* and *Zingiber officinale* extracts on *Echinococcus* protoscoleces

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**Keywords**

Hydatid cyst; Scolicidal; Ethanolic extract; Turmeric; Ginger

**Abstract**

Hydatid disease is an important economic and human public health problem with a wide geographical distribution. Surgical excision remains the primary treatment and the only hope for complete cure of hydatosis. The most important complications arising from surgical excision, however, is recurrence, which is due to dissemination of protoscoleces during the surgery. Pre-surgical inactivation of the contents of the hydatid cyst by injection of scolicidal agent into the cyst has been used as adjunct to surgery in order to overcome the risk of recurrence. In the present study, ethanolic extracts of turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) were tested as scolicidal agent for *Echinococcus* protoscoleces. Protoscoleces were collected aseptically from sheep livers containing hydatid cysts. Three concentrations (10, 30 and 50 mg/ml) of each extract were investigated and viability of the protoscoleces was tested by 0.1% eosin staining. Ginger extract showed the strongest scolicidal effect (100%) after 20 min at a concentration of 30 mg/ml and 10 min at 50 mg/ml. The maximum scolicidal effect of turmeric was 93.2% after 30 min at a concentration of 50 mg/ml. It is concluded that turmeric and ginger extracts have high scolicidal activity and could be used as effective scolicidal agents against *Echinococcus* protoscoleces.

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1. Introduction

Echinococcosis, caused by the larval stage of the tapeworm *Echinococcus granulosus*, is considered to be one of the most important global zoonotic diseases and widespread worldwide (Rajabloo et al., 2012; Pensel et al., 2014). The adult worms occur in the small intestine of dogs, and occasionally other...
carnivores, while the larval stage can establish itself in a wide range of intermediate hosts, including cattle, sheep, pigs, horses and humans (Gholami et al., 2013). Infection of these hosts may occur after ingestion of infective eggs in contaminated food or water (Moazeni and Roozitalab, 2012). Infection is a result of the development of hydatid cysts, mainly in the liver and lungs, although cysts can arise anywhere in the body (Budke et al., 2009).

Surgical removal of the intact hydatid cyst remains the preferred method of therapy (Gholami et al., 2013) but surgery can increase the chance of intraoperative spillage of scolices, which is a major cause of recurrence and multiple secondary echinococcosis (Kilicoglu et al., 2008; Moro and Schantz, 2009). Many scolicidal agents have been used as an adjunct to surgery as a prophylactic means of preventing spillage of the contents of the cyst (Blanton et al., 1998; Spicher et al., 2008). The most frequently used agents are formalin, hypertonic saline, alcohol and povidone iodine (Karaoglanoglu et al., 2011). However, it has been reported that the use of these agents are accompanied in most cases with toxicity and severe hepatobiliary complications and also they may cause fatal hyperthermia (Yetim et al., 2005; Topcu et al., 2006; Adas et al., 2009; Karaoglanoglu et al., 2011).

Therefore, there is an urgent need for scolicidal agents that are less harmful to the patient, and also more effective for use in hydatid cyst surgery (Adas et al., 2009). Recently, efforts have been made to discover new anti-scolicidal compounds from sources such as plants and microorganisms (Moazeni et al., 2012). Zingiberaceae is one of the largest families in the plant Kingdom and is an important natural resource that offers several useful products including food, spices, medicines, dyes, perfume and aesthetics (Sirirugsa, 1999). Members of the Zingiberaceae family such as turmeric (Curcuma longa) and ginger (Zingiber officinale) have been used for many years as spices and in traditional forms of medicine to treat a variety of diseases (Flores-Sanchez and Gang, 2013). Both plants are rich in phyto-constituents such as alkaloids, saponins, flavonoids, terpenes and steroids, which are widely used as drug components in medicine (Singh et al., 2011). Since both plants have been shown to have a number of medicinal properties, the present study aimed to evaluate the in vitro scolicidal effects of the ethanolic extracts of both plants on the protoscoleces of hydatid cysts.

2. Materials and methods

2.1. Protoscoleces collection

Hydatid cysts were collected from the liver of naturally infected sheep that had been slaughtered in a Riyadh abattoir, Saudi Arabia. The hydatid fluid was aseptically transferred into glass cylinders and left to set for 30 min to allow the protoscoleces to settle to the bottom of the cylinders. The supernatant was then removed and the settled protoscoleces were washed three times in normal saline. The viability of the protoscoleces was confirmed by their motility characteristics and a 0.1% eosin staining test under light microscopy. Finally, the live protoscoleces were transferred into a dark container containing normal saline and stored at 4 °C for further use.
counting a minimum of 450 and mostly more protoscolices and then calculating the number of viable protoscolices divided by the total number of protoscolices (Haghani et al., 2014). A percentage of viable protoscolices within the sediment of 95% or more, was considered to be appropriate for further experiments.

2.3. Preparation of extracts

Turmeric (C. longa) and ginger (Z. officinale) were collected from a local market in Riyadh city, Saudi Arabia. Powder totalling 500 g from each plant was extracted separately with 70% ethanol as follows: 100 g of dry powder was added to 400 ml of 70% ethanol and mixed gently for one hour using a magnetic stirrer. The obtained solution was left at room temperature for 24 h before being stirred again and filtered. The solvent was then removed by evaporation in a rotary evaporator. The alcohol free residue of each extract was weighed to give 5.86 g in the case of turmeric and 7.30 g in the case of ginger.

2.4. Determination of in vitro effects

In the present study, we tested three concentrations (10, 30 and 50 mg/ml) of both extracts for 10, 15 and 30 min on protoscolices. In order to prepare these different concentrations, respectively, 0.1, 0.3 and 0.5 g of dried extract was dissolved in 10 ml of distilled water. In each experiment, 2.5 ml of each concentration was placed in a test tube. Approximately 5 \times 10^3 protoscolices was then added to the tube and mixed gently, and the tube was then incubated at 37 °C for 10, 15 and 30 min. At the end of each incubation period, the upper portion of the solution was discarded, taking care to avoid disturbing the settled protoscolices. One millilitre of 0.1% eosin stain was then added to the remaining settled protoscolices and mixed gently. After 5 min, the upper portion of the solution was again discarded. The remaining settled protoscolices were smeared on a glass slide, covered with a cover glass and examined microscopically for viability. At least 5 \times 10^3 protoscolices in 2.5 ml distilled water with no exposure to either of the extracts formed a control group and each experiment was performed in triplicate.

2.5. Statistical analysis

One-way ANOVA was performed using a statistical package programme (Sigma Plot version 11.0). All p values are two-tailed, and P ≤ 0.001 was considered as significant.

3. Results

The scolicidal effects of different concentrations of C. longa and Z. officinale extracts are summarized in Tables 1 and 2.

Regarding C. longa, given a concentration of 10 mg/ml, mortality rates of 39.2%, 48.1% and 57.2% were observed following treatment periods of 10, 20 and 30 min, respectively. When the concentration of C. longa was increased to 30 mg/ml, mortality rates of 46.0%, 53.0% and 61.9% were observed at the same time intervals. A C. longa concentration of 50 mg/ml, meanwhile, led to mortality rates of 71.0%, 81.3% and 93.2% after 10, 20 and 30 min, respectively. The mortality rates of protoscolices following exposure to Z. officinale extract at a concentration of 10 mg/ml were 46.7%, 68.4%, and 79.2% after 10, 20 and 30 min of application, while for a concentration of 30 mg/ml, the mortality rates were 74.7%, 94.3% and 100% at the same time intervals. The highest mortality rate, however, was observed at a concentration of 50 mg/ml, where treatment durations of 10, 20 and 30 min returned mortalities of 92.7%, 100% and 100%, respectively. Compared to the control group, the difference between the mortality rates due to effects of C. longa and Z. officinale extracts was statistically highly significant (P < 0.001) for all three concentrations of both and at each of the various application times.

### Table 1 Scolicidal effect of turmeric (Curcuma longa) extracts on the viability of protoscolices of E. granulosus.

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<thead>
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<th>Concentrations</th>
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<th>% of mortality rates after exposure</th>
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<td>10 mg/ml</td>
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### Table 2 Scolicidal effect of ginger (Zingiber officinale) extracts on the viability of protoscolices of E. granulosus.

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4. Discussion

Surgical removal is the preferred method for the treatment of hydatid cysts (Gholami et al., 2013), and inactivation of the parasite with protoscolicidal agents is an important component of surgical treatment in order to avoid recurrence and multiple secondary echinococcosis (Haghani et al., 2014). Many protoscolicidal agents have been used, including hypertonic saline, alcohol, and povidone-iodine (Karaoglanoglu et al., 2011). However McManus et al. (2003) have argued that there is no ideal agent which is both effective and safe. It is extremely important, therefore, to identify an effective alternative protoscolicidal agent, especially to overcome the severe side-effects of the synthetic pharmaceuticals (Moazen and Nazer (2011); Abdel-Baki et al., 2016). Since herbal extracts have been recognised as having the potential to be effective and safe alternative agents (Elissondo et al., 2008), the present study has sought to investigate the protoscolicidal effect of ethanolic extracts of turmeric and ginger for use against protoscolices in hydatid cysts.

The results proved that Curcuma longa extract induced a significant scolicidal effect at all concentrations and exposure times. C. longa has long been used as a herbal medicine for different medical purposes (Maheshwari et al., 2006), and previous studies have revealed C. longa possess a multitude of beneficial effects in the treatment of cancers, cardiovascular disease and inflammation (Akram et al., 2010). C. longa extract has also been proven to have anti-parasitic activities against Leishmania, Giardia lamblia, Trypanosoma and Schistosoma (Morais et al., 2013). This study, however, is the first report that testifies the scolicidal activity of Ethanolic extract of C. longa.

Regarding ginger (Z. officinale), it has been previously been established that ginger has anthelmintic activity against Dirofilaria immitis, Anisakis simplex, Schistosoma mansoni and Hymenolepis nana (Lina et al., 2014), while Moazen and Nazer (2011) investigated the protoscolicidal activity of methanolic extracts of Z. officinale. They found that methanolic extract of Z. officinale had a 100% mortality rate at concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml after 60, 40 min and 30 min of exposure respectively. In the present study, however, we observed 100% mortality rate at concentrations of 30 mg/ml and 50 mg/ml of Ethanolic extract of Z. officinale after 30 min and 20 min of exposure respectively. This means that Ethanolic extract of Z. officinale has a greater scolicidal effect in a shorter exposure time than the methanolic extract.

Our results strongly suggest, therefore, that Ethanolic extracts of C. longa and Z. officinale have an anti-helminthic effect against protoscolices of hydatid cysts, with Z. officinale having a considerably greater effect than that observed with C. longa. Further in vivo and in vitro studies are needed to more fully evaluate the potential of these extracts, or some of their pure components, as useful alternatives for the treatment of hydatidosis.

Acknowledgement

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In Vitro Scolicidal Effects of Salvadora persica Root Extract against Protoscolices of Echinococcus granulosus

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Abstract: It has been known that Arak, Salvadora persica, has a number of medicinal properties. We tried to investigate in vitro scolicidal effect of root extracts of this plant against protoscolices from hydatid cysts of Echinococcus granulosus. Protoscolices were aseptically collected from sheep livers containing hydatid cysts. S. persica root extract was used in 10, 30, and 50 mg/ml concentration for 10, 20, and 30 min. The viability of protoscolices was ascertained by 0.1% eosin staining. Scolicidal activity of S. persica extract at a concentration of 10 mg/ml was 36.3%, 50.3%, and 70.8% after 10, 20, and 30 min of exposure, respectively. The scolicidal effect of this extract at a concentration of 30 mg/ml was 52.9%, 86.7%, and 100% after 10, 20, and 30 min of exposure, respectively. S. persica extract at a concentration of 50 mg/ml, meanwhile, killed 81.4%, 100%, and 100% of protoscolices after 10, 20, and 30 min, respectively. Also, the cytotoxic potential of S. persica was assessed on human liver cells (HepG2) using trypan blue exclusion test. No cytotoxic effect was observed on HepG2 cell line. The present study confirmed for the first time that the ethanolic extract of S. persica has high scolicidal power in vitro. However, in vivo effect of this material remains to be studied for treatment of echinococcosis in humans and herbivorous animals.

Key words: Echinococcus granulosus, hydatidosis, sheep, liver, Arak, scolicidal agent, cytotoxicity

INTRODUCTION

Echinococcosis or hydatidosis is a serious zoonotic infection with a worldwide distribution, caused by larval stages of cestodes belonging to the genus Echinococcus [1]. Infection with Echinococcus granulosus, the causative agent of cystic echinococcosis, is found on all continents, with the highest prevalence in parts of Eurasia (especially Mediterranean countries, the Russian Federation and adjacent independent states, and China), north and east Africa, Australia, and South America [2]. Hydatid cysts of E. granulosus develop in internal organs (mainly the liver and lungs) of humans and other intermediate hosts as unilocular fluid-filled bladders [3]. Although the initial phase of the infection is always asymptomatic for many years or even permanently [2]. Without efficient treatment, the continuous development of the cyst will ultimately result in organ malfunction and even death in many cases [4].

Currently, there are 3 treatment choices for hydatidosis: surgery, which remains the foremost effective treatment, percutaneous aspiration, and medicinal treatment [5]. Surgery is still the preferred method of treatment but one of the major surgical complications of hydatidosis is the recurrence after operation for primary hydatid disease [6]. The spillage of protoscolices-rich fluid during surgery is a major cause of recurrence and multiple secondary hydatidosis [7]. The use of effective scolicidal agents, while avoiding spilling the cyst contents is an integral part of the surgical technique for this intervention, helping to reduce the risk of spilling viable protoscolices [8]. Many scolicidal agents have been used to inactivate the content of cysts, but most are not safe due to their side effects such as sclerosane cholangitis (biliary tract fibrosis), liver necrosis, and methemoglobinemia [9]. The development of safe and effective new scolicidal agents, especially from natural sources, is therefore of great interest [10].

Arak (Salvadora persica) is one of the most commonly used medicinal plants for treatment of rheumatism, leprosy, gonor-
rhea, ulcers, scurvy, tumors, and dental diseases [11]. Arak has been used for centuries as a natural toothbrush (as Mesiwak or Siwak), especially in Muslim countries as a sunnah of the prophet, while its fibrous branches have been promoted by the World Health Organization for use in oral hygiene [12].

No laboratory studies have documented the effect of *S. persica* on protoscolices of hydatid cysts. Therefore, the present study was conducted to investigate the in vitro scolicidal effect of root extracts of this plant against protoscolices of *E. granulosus* at different exposure times. Also, the cytotoxic effect of *S. persica* extracts was evaluated using trypan blue exclusion test on human liver cells (HepG2).

**MATERIALS AND METHODS**

Collection of protoscolices and viability test

Hydatid cysts of *E. granulosus* naturally infected in the liver of sheep were obtained from a slaughterhouse in Riyadh city, Saudi Arabia. The intact cysts were immediately placed in an ice-box and transported to the Parasitology Laboratory, Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia. Hydatid fluid along with protoscolices was collected according to Smyth and Barrett [13]. The collected fluid with protoscolices was transferred into glass cylinders and left to set for 30 min to allow the protoscolices to settle to the bottom of the cylinders. The supernatant was then discarded, and the yielded protoscolices were washed 3 times in normal saline.

The viability of protoscolices (Fig. 1A) was confirmed from their motility characteristics under light microscopy. The viability was also assessed using 0.1% aqueous solution of eosin stain (1 g of eosin powder in 1,000 ml distilled water). Five min after exposure to the stain, unstained protoscolices were considered as viable (Fig. 1B), while stained protoscolices were considered as non-viable (Fig. 1C) [14]. When 95% or more viable protoscolices were present in the sediments, the sample was considered to be suitable for further experiments, and these were transferred into a dark container containing normal saline and stored at 4˚C until use.

**Preparation of *S. persica* extract**

* S. persica L. (Salvadoraceae) roots (Arak) were collected from Jizan, Saudi Arabia. The identification was confirmed by a local expert at the herbarium of the Botany and Microbiology Department, College of Science, King Saud University. Roots were cut into small pieces, and ground into a fine powder using an electrical blender. About 100 g of root powder was extracted using 400 ml of 70% ethanol. The mixture was agitated for 30 min [15], and then maintained at rest for 24 hr. The resulting extract was filtered using a sterile filter paper, and then the solvent was completely removed using a rotary evaporator.

**In vitro scolicidal tests**

We tested 3 concentrations (10, 30, and 50 mg/ml) of *S. per-
sica extract for 10, 15, and 30 min. To prepare these concentrations, respectively, 0.1, 0.3, and 0.5 g of dried extract was dissolved in 10 ml of distilled water. Then, 2.5 ml of each concentration was placed in a test tube to which about 10,000 washed protoscolices was added. The contents of the tube were mixed gently, and the tube was then incubated at 37°C for 10, 15, or 30 min. At the end of each incubation period, the supernatant was discarded carefully to avoid disturbing the settled protoscolices. One ml of 0.1% eosin stain was then added to the remaining settled protoscolices and mixed gently. After 5 min, the upper portion of the solution was again discarded. The remaining settled protoscolices were smeared on a glass slide, covered with a cover glass, and examined microscopically for viability. The percentages of dead protoscolices were determined by counting at least 450 protoscolices. Non-treated protoscolices in normal saline were considered as a control group. Each experiment was performed in triplicate.

Cytotoxicity assessment

**Cell culture**

Human live cell line (HepG2) that used in the experiment was obtained from American Type Culture Collection (ATCC) (Manassas, Virginia, USA). HepG2 cells were cultured in DMEM, supplemented with 10% FBS, 0.2% sodium bicarbonate, and antibiotic/antimycotic solution (1 ml/100 ml of medium). Cells were grown in 5% CO₂ at 37°C in high humid atmosphere. The results of the present experiment revealed highly significant differences between the test and control groups were analyzed with chi-square test using a statistical package program (Sigma Plot version 11.0). P-values less than 0.01 were considered to be significant.

**RESULTS**

The test was based on the principles that live cells with an intact cellular membrane that exclude the trypan blue dye while dead cells uptake the dye and stained blue. The test was performed following the protocol of Siddiqui et al. [16]. In brief, HepG2 cells were exposed to various concentrations (10, 30, 50 mg/ml) of ethanol extracts of *S. persica* for different time intervals (10, 20, and 30 min). After the respective exposure, cell suspensions were aspirated, centrifuged at 2,000 rpm for 3 min and washed with sterile PBS twice immediately after the incubation. The cells were stained with trypan blue (0.4% solution at a ratio of 1:5, dye: cell suspension) and counted using a hemocytometer. The counting for live (unstained) and dead (blue stained) cells was made at 100× magnification under a phase-contrast inverted microscope. The untreated cells were also run simultaneously under the identical conditions and served as the control. Each experiment was performed in triplicate.

### Table 1. Scolicidal effect of *Arak (Salvadora persica)* extracts

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Experiment no.</th>
<th>Total proto-scolices</th>
<th>Dead proto-scolices</th>
<th>Mortality rate (%)</th>
<th>Total proto-scolices</th>
<th>Dead proto-scolices</th>
<th>Mortality rate (%)</th>
<th>Total proto-scolices</th>
<th>Dead proto-scolices</th>
<th>Mortality rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>10 mg/ml</strong></td>
<td>1</td>
<td>500</td>
<td>175</td>
<td>35</td>
<td>480</td>
<td>240</td>
<td>50</td>
<td>470</td>
<td>320</td>
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<td></td>
<td>2</td>
<td>480</td>
<td>192</td>
<td>37.9</td>
<td>480</td>
<td>233</td>
<td>48.5</td>
<td>490</td>
<td>343</td>
<td>70.9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>500</td>
<td>180</td>
<td>36</td>
<td>480</td>
<td>225</td>
<td>46.4</td>
<td>490</td>
<td>350</td>
<td>76.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1,480</td>
<td>537</td>
<td>36.3</td>
<td>1,460</td>
<td>735</td>
<td>50.3</td>
<td>1,470</td>
<td>1,033</td>
<td>70.8</td>
<td></td>
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<tr>
<td><strong>30 mg/ml</strong></td>
<td>1</td>
<td>500</td>
<td>270</td>
<td>54</td>
<td>490</td>
<td>433</td>
<td>88.4</td>
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<td>52</td>
<td>460</td>
<td>399</td>
<td>86.7</td>
<td>480</td>
<td>480</td>
<td>100</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1,460</td>
<td>773</td>
<td>52.9</td>
<td>1,450</td>
<td>1,257</td>
<td>86.7</td>
<td>1,480</td>
<td>1,480</td>
<td>100</td>
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<tr>
<td><strong>50 mg/ml</strong></td>
<td>1</td>
<td>500</td>
<td>403</td>
<td>80.6</td>
<td>480</td>
<td>490</td>
<td>100</td>
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<tr>
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<td>100</td>
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<td>100</td>
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<tr>
<td></td>
<td>3</td>
<td>470</td>
<td>383</td>
<td>81.5</td>
<td>500</td>
<td>500</td>
<td>100</td>
<td>480</td>
<td>480</td>
<td>100</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1,470</td>
<td>1,196</td>
<td>81.4</td>
<td>1,470</td>
<td>1,470</td>
<td>100</td>
<td>1,480</td>
<td>1,480</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>1</td>
<td>500</td>
<td>26</td>
<td>5.2</td>
<td>480</td>
<td>41</td>
<td>8.5</td>
<td>500</td>
<td>60</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>2</td>
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<td>5.4</td>
<td>500</td>
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<td>8.8</td>
<td>460</td>
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<td>3</td>
<td>500</td>
<td>25</td>
<td>5</td>
<td>500</td>
<td>39</td>
<td>7.8</td>
<td>480</td>
<td>53</td>
<td>11</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1,460</td>
<td>76</td>
<td>5.2</td>
<td>1,480</td>
<td>124</td>
<td>8.4</td>
<td>1,440</td>
<td>165</td>
<td>11.5</td>
<td></td>
</tr>
</tbody>
</table>

**Trypan blue dye exclusion test**

The test was performed following the protocol of Siddiqui et al. [16]. In brief, HepG2 cells were exposed to various concentrations (10, 30, 50 mg/ml) of ethanol extracts of *S. persica* for different time intervals (10, 20, and 30 min). After the respective exposure, cell suspensions were aspirated, centrifuged at 2,000 rpm for 3 min and washed with sterile PBS twice immediately after the incubation. The cells were stained with trypan blue (0.4% solution at a ratio of 1:5, dye: cell suspension) and counted using a hemocytometer. The counting for live (unstained) and dead (blue stained) cells was made at 100× magnification under a phase-contrast inverted microscope. The untreated cells were also run simultaneously under the identical conditions and served as the control. Each experiment was performed in triplicate.
significant ($P < 0.001$) scolicidal effects against protoscolices of *E. granulosus* for all of the various concentrations of *S. persica* ethanolic extract, compared to the control results, both within the same period of time and different periods as shown in Table 1. While the maximum death rate in the control group was 11.5%, scolicidal activity of *S. persica* extract at a concentration of 10 mg/ml was 36.3%, 50.3%, and 70.8% after application for 10, 20, and 30 min, respectively. The scolicidal effect of the extract at a concentration of 30 mg/ml was 52.9%, 86.7%, and 100% after application for 10, 20, and 30 min, respectively. *S. persica* extract at a concentration of 50 mg/ml, meanwhile, killed 81.4%, 100%, and 100% of protoscolices after 10, 20, and 30 min, respectively.

The results of the cytotoxicity assay using trypan blue exclusion test are shown in Fig. 2. The cell viability was 100%, 99%, and 98% after exposure to 10 mg/ml for 10, 20, and 30 min, respectively, while it was 98%, 96%, and 95% after exposure to 30 mg/ml for the same time intervals. Application of 50 mg/ml extract for 10, 20, and 30 min showed cell viabilities of 96%, 94%, and 90%, respectively.

**DISCUSSION**

Hydatid disease (hydatidosis) is a parasitic disease that remains a clinical problem worldwide, especially in areas where animal husbandry and subsistence farming form an integral part of community life [17]. Seventy-five percent of all hydatid cysts are found in the liver [18], and these are usually managed either by surgery or medicinal treatment [19]. Surgery is associated with considerable mortality, morbidity, and recurrence rate. It is also expensive, and, clearly, needs expertise [20]. Chemotherapy can be used as treatment for patients considered not fit for surgery, or as an adjuvant to surgical treatment preoperatively or postoperatively, or both [19]. Various degrees of success have been claimed for many drugs that are now being used in hydatidosis treatment [21]. However, the metabolites of these drugs, including albendazole, albendazole sulfoxide, benzimidazole, and mebendazole, are potentially toxic in some subjects and are associated with severe hepatobiliary complications [22]. Natural scolicidal agents offer a safe alternative with no adverse associated effects [9].

Researchers around the world have begun to explore the real effect of many plants traditionally used as medicines which have not hitherto been validated by rigorous scientific experimentation. In this context, recently, many plants have been screened for their in vitro scolicidal activities, including *Ocimum bacleicum* and *Allium cepa* [6], *Sambucus ebulus* [9], *Trachyspermum ammi* [10], *Allium sativum* [14], *Zingiber officinale* [23], and *Zataria multiflora* [24].

With respect to *S. persica*, Reuben et al. [25] confirmed very high anthelmintic effects (86.7% and 98.9%) for aqueous extracts of *S. persica* at concentrations of 25 mg/ml and 50 mg/ml, respectively, against strongylid nematodes. These results urged us to test the in vitro scolicidal effect of ethanolic extract of *S. persica*. It was shown in this study that the ethanolic extract of *S. persica* exhibited the strongest scolicidal effect (100% and 100%) after 20 min at a concentration of 50 mg/ml and after 30 min at a concentration of 30 mg/ml. The ethanolic extract of *S. persica* is reported to contain a number of important phytoconstituents, such as indole alkaloids (e.g., salvadoricine), flavonoids (e.g., quercitin), the sulphur-containing compound tropeaoidin, triterpenes, phytosterols, and isothiocyanates (e.g., benzyl isothiocyanate) [26]. Sofrata et al. [26] proved that *S. persica* root sticks consist of more than 98% benzyl isothiocyanate, while another report has confirmed that benzyl isothiocyanate is the predominant or sole anthelmintic agent in papaya seed extracts against *Caenorhabditis elegans* [27]. Our results showed a high scolicidal effect of *S. persica* root extracts against protoscolices of *E. granulosus*, which might be due to the presence of anthelmintic isothiocyanates. The present study proved that the ethanolic extract of *S. persica* have no cytotoxic effect on HepG2 cells. Similarly, previous studies have found the ethanol extract of this plant to be completely devoid of cytotoxic effects on HGFs and on L929 consistently in 3 cytotoxic assays [28]. Studies have also shown that the extract is safe.
in respect to liver and kidney functions and hematological parameters of rats [29]. Also, Ahmed et al. [30] confirmed that the ethanolic extract of \textit{S. persica} did not show any untoward effect up to a dose of 5,000 mg/kg body weight and did not cause death in any tested albino mice.

In conclusion, the root ethanolic extract of \textit{S. persica} is a safe and potent protoscolicidal with the potential to be used in hydatid cyst treatment and pre-surgery to prevent secondary cyst recurrence. However, in vivo efficacy of this extract remains to be explored, and further studies are necessary to identify and isolate the active compounds. This is the first report on the scolicidal activity of \textit{S. persica}.

ACKNOWLEDGMENTS

We extend our appreciation to the Dean of Scientific Research, King Saud University, for funding the work through the research group project no. RG-004.

CONFLICT OF INTEREST

The authors declare that the present study followed the ethical standards and the ethical rules applicable for this journal and no conflict of interest.

REFERENCES

17. Shaw JM, Bormman PC, Krije JEJ. Hydatid disease of the liver. SAJS 2006; 44: 70-77.
24. Reuben DK, Aji SB, Andrew W, Abdulrahman Fl. Preliminary


