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Preventive and therapeutic effects of *Zataria multiflora* methanolic extract on hydatid cyst: An *in vivo* study

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ABSTRACT

The phenolic compounds of *Zataria multiflora* extract, were identified by HPLC analysis. Gallic acid, catechin, caffeic acid, and quercetin were found to be the major phenolic compounds. Eighty healthy laboratory Balb/C mice were infected intraperitoneally by injection of 1500 viable protoscoleces and were divided into prevention (40 mice) and therapeutic (40 mice) groups. To prove the preventive effect of *Z. multiflora* extract on development of hydatid cyst, the 40 infected animals were allocated into three treatment groups including *Z. multiflora* (4 g/l in drinking water for 8 months), albendazole (150 mg/kg BW/day for 10 days) and untreated (control) group. To estimate the therapeutic effect of *Z. multiflora* extract on the hydatid cyst, after 8 months of infection, the infected mice were allocated into three experimental treatment groups including *Z. multiflora* (8 g/l in drinking water for 30 days), albendazole (300 mg/kg BW/day for 20 days) and untreated (control) group. At the end of the treatment period, all mice were euthanized and necropsied, the hydatid cysts were carefully removed, weighed and their size were recorded. Weight and size of the hydatid cysts significantly decreased ($p < 0.05$) upon the treatment with *Z. multiflora* extract in both prevention and therapeutic groups. The germinal layer of the hydatid cysts recovered from the treated mice, either from the prevention or therapeutic group, were completely damaged at ultrastructural level by scanning electron microscopy.

1. Introduction

Echinococcosis/hydatidosis is a zoonotic disease that occurs throughout the world and causes considerable economic losses and public health problems in many countries (Dalimi et al., 2002). Echinococcosis is mostly prevalent in Australia, South America, Middle East, South Africa, Eastern Europe, and the Mediterranean region (Zulfikaroglu et al., 2008) and immigration between continents, has led to the disease being prevalent in other countries as well (Sayek and Onat, 2001). Cystic echinococcosis (CE), which is considered as an emerging disease in various regions, is economically important and constitutes a threat to public health in many countries (Dinkela et al., 2004). Human infection with *Echinococcus granulosus* typically results in a slowly growing parasitic cystic disease most frequently seen in the liver. The cysts may be asymptomatic for many years, and occasionally spontaneous regression has been noted. More commonly the disease is slowly progressive, and symptoms and complications eventually arise. The

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symptoms include pain from expansion or rupture, fever from pyogenic infection due to intrabiliary rupture and jaundice, or anaphylaxis from intrabiliary or extrahepatic rupture (Sielaff et al., 2001).

Currently, there are three treatment options for hydatid disease of the liver. These treatment strategies include: surgery, percutaneous aspiration and medical treatment from which surgery has been known as the most efficient treatment option in humans (Adas et al., 2009). Chemotherapy is the preferred treatment where, surgeons are not available or the cysts are too numerous, and in inoperable cases, "chemotherapy" is the only option. Chemotherapy has also been used as an adjunct to surgery for prophylaxis against spillage of the cyst contents (Blanton et al., 1998; Spicher et al., 2008b). Few chemotherapeutic agents are available for the medical management of hydatid disease (Blanton et al., 1998). Benzimidazole carbamate derivatives, such as mebendazole and albendazole, are currently used for chemotherapeutic treatment of cystic echinococcosis. In human patients, benzimidazoles are commonly applied in high doses and for extended periods of time; these frequently results in adverse side effects (Walker et al., 2004). Therefore, a new effective alternative treatment regime is extremely important in today's climate, where species are becoming resistant, and there is resurgence in the use of natural alternative therapies, instead of synthetic pharmaceuticals that often have severe side effects (Harris et al., 2000).

It has been shown that *Zataria multiflora* (Lamiaceae) has antibacterial (Sharififar et al., 2007; Misaghi and Akhondzadeh Basti, 2007), antifungal (Gandomi et al., 2009), antiprotozoal (Abdollahy et al., 2004) and scolicidal (Moazeni and Roozitalab, 2012) properties. The aim of the current experimental work was to evaluate the efficacy of the methanolic extract of *Z. multiflora* on the prevention and treatment of hydatid cyst in a murine model.

2. Materials and methods

2.1. Extraction

Methanolic extract of *Z. multiflora* was prepared as described by Moazeni and Roozitalab (2012) with some modifications. Briefly, the leaves of *Z. multiflora* were dried under shade and powdered mechanically, using a commercial electric blender. A total of 2800 g of dried powder was extracted. One hundred grams of dry powder was added to 400 ml of pure methanol and gently mixed, using a shaking incubator, at 30–37 °C for 1 h at 120–130 rpm. The obtained solution was left at room temperature for 24 h. The solution was stirred again and filtered, and the solvent was removed by evaporation in a rotary evaporator. The remaining semisolid material was then left at room temperature for 4 h and then freeze dried. The collected residue was transferred to a sterile glass container and stored in the refrigerator at 4 °C before being used. 119 g of the dried extract was obtained from 2800 g of the dried powder of *Z. multiflora*.

2.2. Identification of phenolic compounds

For identification of the phenolic compounds from the *Z. multiflora* extract, HPLC analysis was carried out on a Agilent 1200 series (USA), equipped with a Zorbax Eclipse XDB-C18 column (10 cm × 5 μm i.d.; ×150 mm film thickness, RP), and a photodiode array detector (PAD). For preparing the injectable extract, 0.02 g of the dried residue of the plant extract was dissolved in 2 ml of methanol–acetic acid (85:15) and the aliquots was filtered through a 0.2 μm membrane millipore chromatographic filter and injected into the HPLC system. The elution was monitored at 280 and 320 nm. Gradient elution was selected to achieve the maximum separation and sensitivity. The elution was performed by varying the proportion of solvent A (formic acid 1% in deionized water) to solvent B (methanol (v/v)) as follows: methanol:formic acid 1% (10:90), at 0 min; methanol:formic acid 1% (25:75), at 10 min; methanol:formic acid 1% (60:40), at 20 min and finally, methanol:formic acid 1% (70:30), at 30 min. The total running time was 30 min. The column temperature was 30 °C.

2.3. Collection of protoscoleces

Protoscoleces of *E. granulosus* were collected aseptically from the hydatid cysts of the liver of the naturally infected sheep, slaughtered at Shiraz Slaughterhouse, Shiraz, southern Iran. The hydatid fluid of the cysts was aseptically transferred into glass cylinders and left to set for 30 min. The protoscoleces were settled at the bottom of the cylinders. The supernatant was then removed, and the yielded protoscoleces were washed several times with sterile 0.85% NaCl and stored in RPMI 1640 medium overnight at 37 °C. The viability of the protoscoleces was confirmed from their motility characteristics under an ordinary light microscope after 0.1% eosin staining. They were finally transferred into a dark container containing normal saline and stored at 4 °C for further use.

2.4. Infection of mice

Eighty healthy BALB/c male mice weighing 22–24 g were used in this study. All the mice were infected intraperitoneally by injection of 1500 protoscoleces per animal, dissolved in 0.5 ml of RPMI 1640 medium. The infected animals were allocated into two experimental treatment groups: (a) Prevention group, (b) Therapy group. All the animals in the study were fed *ad libitum*, and kept at 24–25 °C.

2.5. Preventive trials

To prove the preventive effect of *Z. multiflora* extract on the formation of hydatid cysts, the 40 infected animals were allocated into three experimental treatment groups: (a) *Z. multiflora* treatment group which were drunk with *Z. multiflora* extract (4 g/l) for 8 months (15 mice), (b) albendazole treatment group which received 150 mg/kg BW/day albendazole for 10 days (10 mice) and (c) untreated control group (15 mice). The remaining 25 mice were euthanized 8