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ORIGINAL ARTICLE

Scolicidal effects of *Cassia fistula* and *Urtica dioica* extracts on protoscoleces of hydatid cysts

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Abstract Echinococcosis is among the most underestimated parasitic diseases that have universal distribution. The primary treatment is surgery. Hence, the development of new and more effective scolicidal agents with lower side effects is crucial. This study evaluated the therapeutic effects of *Urtica dioica* and *Cassia fistula* extracts as a scolicidal herbal drug in vitro. Suspension of protoscoleces was obtained from the infected livers of sheep in Khorramabad, Iran. Hydro-alcoholic solution was extracted from the leaves and stems of *Urtica dioica* and the fruit of *Cassia fistula*. *Echinococcus granulosus* protoscoleces were treated with the essential oils at concentrations of 10, 25, 50, and 100 mg/mL for 10, 20, 30, and 60 min and their viability was evaluated by the eosin staining test. The extract of *Urtica dioica* at a concentration of 100 mg/mL killed 90.51% of protoscoleces after 60 min. *Cassia fistula* also killed 67.74% of protoscoleces after 60 min. This study obtained satisfactory results. *Urtica dioica* and *Cassia fistula* extracts are promising protoscolicides and can be used in the treatment of hydatid cysts and pre-surgically to prevent secondary infections.

Keywords *Echinococcus granulosus* · Cystic echinococcus · Protoscolex · Hydatid cyst · Protoscolicidal activity · *Cassia fistula* · *Urtica dioica*

Abbreviations

CE	Cystic echinococcosis
PAIR	Puncture, aspiration, injection, and respiration
RFTA	Radiofrequency thermal ablation

Introduction

Hydatidosis or cystic echinococcosis (CE), caused by *Echinococcus granulosus* tapeworm, is a significant zoonotic infection that harms humans and farm animals in many countries (Possenti et al. 2016). CE affects the host's internal organs, including the liver, lungs, spleen, kidneys, and brain, posing a global health issue, especially in developing countries like Iran. (Barabadi et al. 2017; McManus et al. 2003). Hydatid cysts are also endemic in countries and areas that practice conventional livestock breeding, including the Southwestern United States, South America, Australia, New Zealand, East Africa, India, Eastern Europe, and China. Morbidity and fatality can be high if the condition is left untreated, and the prognosis is poor if cases are managed incorrectly (Bagheri et al. 2009; Zibaei et al. 2012). Even in nations where hydatid disease is not endemic, the migration and importation of infected livestock should be considered as a potential source of new endemic dissemination of the disease (Yones et al. 2011,

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Mahmoudvand et al. 2017b). Currently, the primary method of treating hydatid disease is surgery. Chemotherapy and puncture, aspiration, injection, and respiration (PAIR) are alternative treatments for patients who do not suffer from complex cases of CE (Abdulkareem et al. 2020). Nevertheless, CE and secondary infection is observed to occur in about 9% of patients who have undergone surgical treatment. Hence, it is necessary to use effective scolicidal agents instead of hypertonic saline, silver nitrate, cetrimide, povidone-iodine, albendazole sulfoxide, octenidine hydrochloride, chlorhexidine gluconate, or ethanol. These agents are associated with sclerosing cholangitis, liver necrosis, and methemoglobinemia. Unfortunately, a substantial number of patients do not respond to these drugs (Rajabi, 2009; Mahmoudvand et al. 2016a, b; Hesari et al. 2020). In developing countries, medicinal plants are well-known and widely used because they are safe and cheap (Voon et al. 2012). *Cassia fistula L.* (Fabaceae) is a flowering plant widely grown as an ornamental plant in tropical and subtropical regions. Recent studies have shown that phytochemicals in *C. fistula*, including flavonoid and phenolic metabolites, have antibacterial, antifungal, antiviral, and anthelmintic properties (Rahimi-Esboei et al. 2016; Bahorun et al. 2005; Duraipandiyan and Ignacimuthu 2007). *C. fistula* extract demonstrates significant and valuable pharmaceutical activity and thus is used in traditional South Asian and Middle Eastern pharmacopeia (Siddiqua et al. 2018). Another plant investigated in this article is stinging nettle (*Urtica dioica*). The nettle family (Urticaceae) includes annual and perennial herbs, some of which grow stinging hairs. Stinging nettle is found worldwide and is traditionally used in northern Iran for its medicinal properties (Mzid et al. 2017). Various analyses have shown that the phytochemicals of *U. dioica* have anti-inflammatory, antimicrobial, and, especially, anti-parasitic activities. Compounds such as saponins, phenols, tannins, flavonoids, and alkaloids can have significant scolicidal effects (Kukrić et al. 2012; Gülçin et al. 2004; Gül et al. 2012). This study evaluates the scolicidal effects of *C. fistula* and *U. dioica* extracts on protoscoleces of *E. granulosus* in an in vitro model.

Materials and methods

Collection and identification of the plants and preparation of extracts

The fruits of *C. fistula* and the leaves of *U. dioica* are sources of the active ingredients in these plants. *C. fistula* grows mostly in tropical regions of the world, such as Africa, India, and Southern Iran. In the present study, the specimens were collected from the rural areas of Khorramabad, the capital of the Lorestan province of Iran. *U. dioica* grows mostly in the northern parts of Iran (Javaherdeh) and in northwestern and central Iran. In the current study, the specimens were gathered from the Isfahan province of Iran. A botanist identified the plants. The dried fruits of *C. fistula* and the dried leaves of *U. dioica* were ground mechanically using an electric blender. Then, 150 g of dry powder from each plant were added to 350 mL of 70% ethanol and mixed gently for one hour to obtain the ethanolic extract. The obtained suspension was left at room temperature for 72 h. The solution was stirred every 24 h, then filtered through a sterile filter paper. The crude ethanol extracts were wholly evaporated using a rotary evaporator. The obtained residue was placed in a sterile glass container and stored at 4 °C for subsequent usage.

Collection of protoscoleces and the viability test

Livers of naturally infected sheep containing hydatid cysts were obtained from the slaughterhouse in Khorramabad. The infected livers were immediately placed in an icebox. Appropriate cysts were evaluated, selected, and cleared with 70% ethanol. The fluid from hydatid cysts was aspirated using a syringe and aseptically transferred to 50 mL Falcon tubes without centrifugation. After 30 min, the supernatant was discarded, while the settled protoscoleces were washed three times with PBS (pH 7.2) and collected as previously described by Smyth (1967). The viability of the protoscoleces was determined before the experiments using eosin staining at a concentration of 0.1% (in distilled water), and evaluated by low-power microscopy after 5 min. Stained protoscoleces were considered dead, while unstained protoscoleces were deemed alive (Abdel-Baki et al. 2016; Mahmoudvand et al. 2016c). When the percentage of viable protoscoleces in the sediment was 90% or more, they were regarded appropriate for experiments. The percentage of viable protoscoleces was determined by counting a minimum of 150 protoscoleces (Faizi et al. 2018) (Figs. 1, 2, 3 and 4).

Scolicidal assay

In this study, extracts of *C. fistula* and *U. dioica* were used at 10, 25, 50, and 100 mg/mL concentrations for 10, 20, 30, and 60 min. Then, a drop of protoscolex-rich sediment was added. The contents of the tubes were gently mixed, and the tubes were incubated at 37 °C for 30 min. Protoscoleces were examined under a microscope. Researchers counted about 150 protoscoleces each time to determine the percentages of dead protoscoleces. In order to count precisely, each step of the study was repeated three times. Furthermore, 20% hypertonic saline was used as a positive

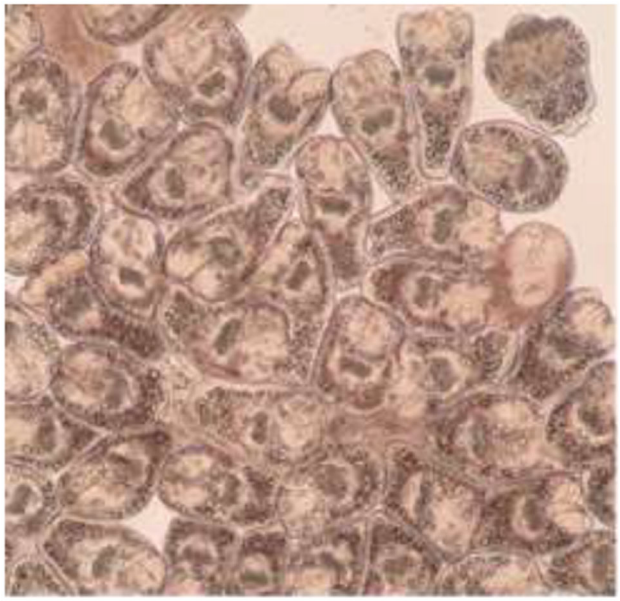

Fig. 1 Unstained hydatid sand of fertile hydatid cyst

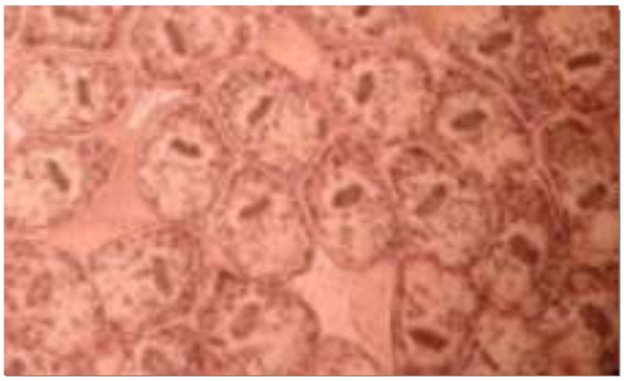

Fig. 2 Live invaginated colorless protoscolices after staining with 0.1% eosin

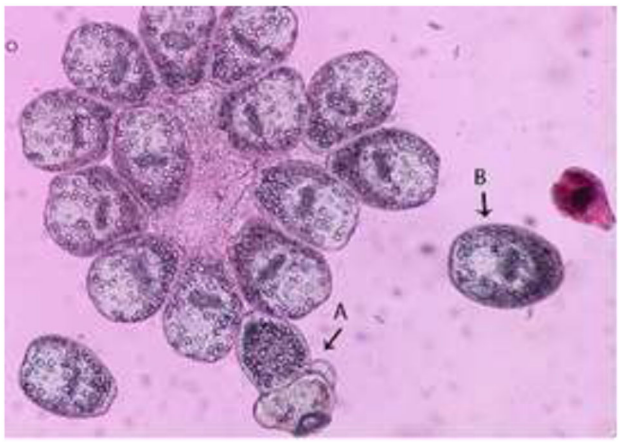

Fig. 3 Live evaginated (**a**) and invaginated (**b**) colorless protoscolices 5 min after staining with 0.1% eosin

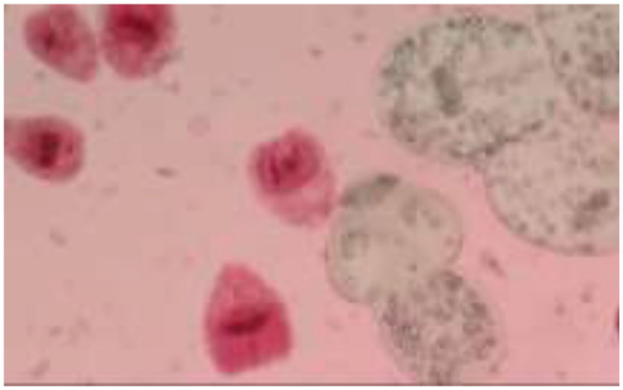

Fig. 4 Live colorless and dead colored invaginated protoscolices after exposure to medical and different concentrations of herbal treatment and staining with 0.1% eosin

control. Non-treated protoscoleces (with plant extracts) were considered the negative control group (Faizei et al. 2015; Mahmoudvand et al. 2019).

Statistical analysis

One-way analysis of variance (ANOVA) was employed to analyze the data using the Statistical Package for Social Sciences (SPSS) software package for Windows. P values of < 0.001 were considered meaningful and significant.

Results

The standard means and deviations of the scolicidal effects of the studied extracts are presented in Figs. 5 and 6 in terms of time and concentration ($P < 0.001$). The findings revealed that the extract of *U. dioica* had stronger

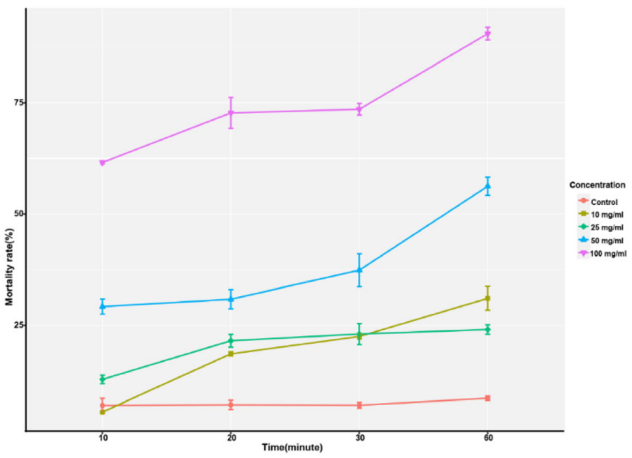

Fig. 5 Relation between mortality rate of protoscoleces of *E. granulosus* and different concentrations of extract of *U. dioica*, in comparison with negative control. Each point represents the mean percentage of dead protoscoleces from different experiments

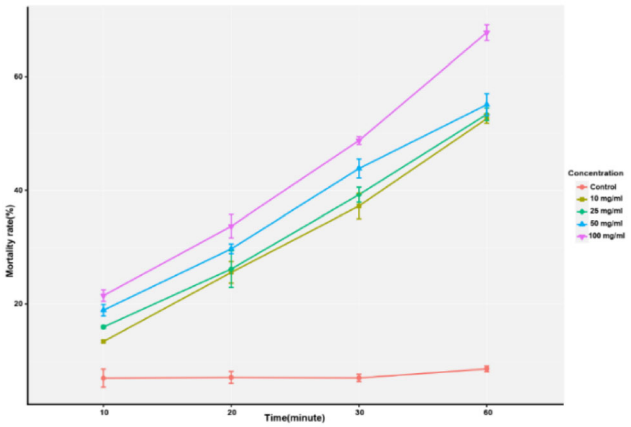

Fig. 6 Relation between mortality rate of protoscolices of *E. granulosus* and different concentrations of extract of *C. fistula*, in comparison with negative control. Each point represents the mean percentage of dead protoscolices from different experiments

scolicidal effects on the protoscoleces of hydatid cysts compared to the extract of *C. fistula*. While the mortality rate of protoscoleces in the negative control group was 8.60% after 60 min of exposure, scolicidal effects (90.51% mortality rate) were observed with the extract of *U. dioica* at a concentration of 100 mg/mL. This is the optimal result and is higher than that of *C. fistula* (67.74%). As shown in Figs. 5 and 6, the slope of the diagram showing the efficacy of *U. dioica* is higher than *C. fistula*. Additionally, the diagram of *C. fistula* does not show much variation between 20 and 30 min at different concentrations. Diagrams of both extracts show dose and time-dependent scolicidal activities. Moreover, the diagram of *C. fistula* is significantly different at the concentration of 100 mg/mL from the rest of the concentrations. In general, as shown in Fig. 7, *U. dioica* has a more scolicidal effect at higher concentrations than *C. Fistula,* but concentrations lower than 50 mg/mL, the effects of *C. fistula* are more evident.

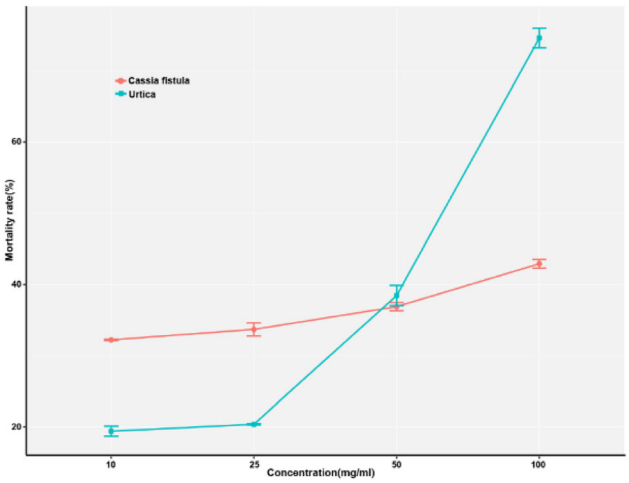

Fig. 7 Comparison of the lethality of *C. fistula* and *U. dioica*

Discussion

Surgical removal is the most effective technique for treating hydatid cysts. The modified surgical technique can be employed to improve cyst drainage. Although, compared to the traditional procedure, this technique provides good results in the hydatid cyst of the liver with lower morbidity, it is still a high-risk operation (Marom et al. 2019; Fattahi et al. 2019). Chemotherapy has numerous side effects, too. Hence, alternative approaches can be used in non-advanced conditions (Ranjan et al. 2015; Navvabi et al. 2019). In some cases, the disease recurs even more severely than before, or secondary infection appears in a different organ from the initial infection site. Under some circumstances, access and operation are difficult because cysts have spread to several organs of the body or if cysts are found in delicate and vulnerable areas. Moreover, cyst rupture and anaphylactic shock are also likely to occur, making the operation even more challenging (Bensghir et al. 2012; Yılmaz et al. 2018). Using plant extracts against protoscoleces of hydatid cyst has received significant attention in recent years. Some experiments have shown that extracts of several plant species belonging to different families may affect the viability of protoscoleces (Zibaei et al. 2016; Navvabi et al. 2019; Mahmoudvand et al. 2017a). As discussed earlier, many protoscolicidal agents used to deactivate protoscoleces may cause adverse effects. Therefore, herbal remedies that have fewer side effects are effective alternatives in this regard. *U. dioica* and *C. fistula* were chosen to be studied in the present research because they have several unique features. A study in 2019 confirmed the high anthelmintic effects of aqueous extracts of *U. dioica* at concentrations of 25 mg/mL and 50 mg/mL against strongyles nematodes (Moussouni et al. 2019; Mahmoudvand et al. 2014b). More recent studies have reported satisfying results in clearing *Leishmania* both in vivo and in vitro. Likewise, the *U. dioica* extract is considered an efficient herbal compound for the treatment of leishmaniasis without toxicity to the host's macrophages (Badirzadeh et al. 2020). Another investigation conducted on 32 male rats revealed the hepatoprotective effects of *U. dioica* (Yıldızhan et al. 2020). Moreover, the antimicrobial activities of *U. dioica* have been examined in different studies (Modarresi-Chahardehi et al. 2012). In some studies, antimicrobial and antiplasmodial activities of *C. fistula* have been observed (Hamad et al. 2017; Grace et al. 2012; Mahmoudvand et al. 2014a). Different studies have been conducted on the efficacy of mixtures of extracts and nanoparticles like silver, selenium,

copper, iron, gold, and zinc. However, the safe application of these extracts requires further research (Rahimi et al. 2015; Norouzi et al. 2019; Esteban-Ballesteros et al. 2019; Norouzi et al. 2020). In a study conducted in 2019, radiofrequency thermal ablation was used for the treatment of hepatic hydatid cysts. After the core temperature of the cyst exceeded 95 °C, the ablation procedure was continued for 3 min in the first group and 4 min in the second group. The cysts were not destroyed to the desired level in the first group. However, in the second group, it was observed that 100% of the protoscoleces died and 100% of the germinative membranes degenerated. This result suggests a promising pathway for the treatment of hydatid cysts that could be considered and studied along with other pathways (Sarıcık et al. 2019). Other species of the genera *Cassia* and *Urtica* can be investigated, as well. For example, *Urtica urens* is another species of this genus with proven antimicrobial and antioxidant effects (Mzid et al. 2017; Maaroufi et al. 2017). These results encouraged us to test the in vitro scolicidal effects of ethanolic extracts of *U. dioica* and *C. fistula*. In the present study, the protoscolicidal effects of the two herbal agents were observed individually. We assessed the protoscolicidal efficacy of both extracts at various concentrations and over different periods. The results showed that the *C. fistula* extract had lower effects on the protoscoleces of hydatid cysts. The highest concentration (100 mg/mL) of both extracts showed the best results. A 100 mg/mL concentration of *C. fistula* showed significant effects at the beginning of the observation period, but other concentrations needed further time to show appropriate effects. Although *C. fistula* is less effective than *U.* dioica, both extracts reached LD_{50} (50% mortality) and are adequate.

In conclusion, these medicinal plants are reliable and demonstrate promising protoscolicidal properties that can be used in the treatment of hydatid cyst and pre-surgically to prevent recurrences. However, the in vivo potency of these extracts has not yet been investigated and thus more studies are required to identify and isolate the active compounds and their roles in the treatment.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Human and animal rights All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies involving human participants performed by any of the authors.

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