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RESEARCH ARTICLE

Prosopis farcta Extract Potentiates the Scolicidal Activity Against Protoscolices of Hydatid Cysts

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Abstract This study aimed to assess the efficacy of methanolic extract of Prosopis farcta (*P. farcta*) against protoscolices. In this study, the liver of sheep infected with hydatid cyst was collected from Birjand abattoir and after isolation of hydatid cyst protoscolices, to assess the protoscolicidal effect of *P. farcta*, concentrations of 250, 500, 1000 mg/ml Lit a liter of plant extract with a suspension of protoscolices and their mortality was calculated in 5 to 30 min. The metabolites in the P. farcta extract were characterized by gas chromatography-mass spectrometry (GC–MS). The average of the mortality protoscoleces was 100% after 5 min of incubation with the concentration of 1000 mg/ml of *P. farcta* extract. The mean of the mortality of protoscoleces after 10 min of incubation with the concentration of 500 mg/ml of *P. farcta* extract was 100%. That is, *P. farcta* extract requires a longer length of time to display potent protoscolicidal effects. The results of this investigation revealed that *P. farcta* extract could have a significant scolicidal activity on hydatid cyst protoscoleces. However, further research that must be accomplished particularly in human and animal subjects is required to reach this conclusion.

'Significance' Statement This study aimed to evaluate the efficacy of methanolic extract of Prosopis farcta against protoscolices of hydatid cyst. The results of this investigation revealed that P. farcta extract could have a significant scolicidal activity on hydatid cyst.

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Keywords Prosopis farcta · Hydatid cyst · In vivo · Ex vivo

Introduction

Hydatidosis is one of the main health problems and zoonotic diseases in the world. This disease is caused by larval stage of the tapeworm or (parasite) *Echinococcus granulosus*. Human hydatidosis is endemic in most part of the world [1]. Today, Iran is considered as a hyperendemic country for hydatidosis with several economic loss and health problem [2]. Canine is considered as the final host of the parasite, while human and domestic and wild ruminants are intermediate host. The transmission of hydatidosis takes place by eating of parasite eggs, by drinking water, vegetables and foods contaminated [3]. Although the adult worms of the parasite do not produce any threatening in the final host, hydatid cyst in the intermediate host involved various tissues including liver, lung, brain that may lead to serious illness or even death [4]. The disease has no specific symptoms at the onset, and the clinical symptoms occur depending on the location and size of the cyst. In small and inactive cysts, benzimidazole family drugs are recommended. But in cases of large and active cyst, the best choice is surgical procedure. The *Prosopis farcta* belongs to the Fabaceae family, grown in the tropical area of Asia, Africa, and America. In folk medicine of Iran, the different parts of P. farcta including stems, leaves, and fruits are usually used for rheumatism, diabetic, diarrheal, and even for antimicrobial purposes [5]. The major chemical constituents of *P. farcta* essence are lectins, vitexin, tryptamine, tannin, and apigenin. It is determined that lectins and toxins in the *P. farcta* kill *L. major* promastigotes in sand flies and culture media [6]. While there

is no study to determine which part of *P. farcta* has a most protoscolicidal effect, the present study was made to assess the in vitro and ex vivo protoscolicidal effect and cytotoxicity of hydroalcoholic extract of different parts of *P. farcta* against *E. granulosus* protoscolex pathogenic parasitic strain.

Material and Methods

P. farcta Sample and Extraction

The *P. farcta* samples were obtained from regions of Birjand, east of Iran, from April to June 2020. The percolation method was used for extraction. In brief, the plants were washed, dried, ground, soaked with 70% methanol, and shaken for 48 h at room temperature. The acquired liquid was filtered and dried at 37° C. Different dilutions (0.078–20 mg/ml) were prepared for each extract and placed in 96-well plates.

Preparation of Protoscolices

The liver of sheep infected with hydatid cyst was collected from slaughterhouses in Birjand and then sent to the parasitology laboratory of the medical school. After rinse the cyst surface with alcohol 70% hydatid fluid inside the cyst, which contained the protoscolex by aspirate a 50 cc sterile syringe over several. The step was washed with normal saline solution.

Validity Test

After obtaining live protoscolices, the mortality rate was evaluated by staining with eosin 0.1%. In this method, live protoscolices are transparent, while dead protoscolex is seen reddish.

In vitro Evaluation of Protoscolicidal Effect *P. farcta*

To evaluate the anti-protoscolicidal effect of *P. farcta* in vitro, first concentrations of 250, 500, 1000 mg/ml of *P. farcta* extract with a suspension of protoscolices (1*103 ml) and their mortality rate in 5, 10, 20, 30 min were calculated. Simultaneously, normal sterile saline + Tween 20 and silver nitrate as negative and positive controls, respectively, were used.

Ex vitro Evaluation of Protoscolicidal Effect *P. farcta*

In this study, the liver of sheep that were naturally infected with hydatid cyst was used to evaluate the anti-vascular activity of protoscolex extract in ex vivo. In the first step, more than 50% of the hydatid fluid was aspirated to obtain protoscolices and their viability was confirmed by 0.1% eosin test. Three hydatid cysts were considered for each of the extract concentrations (concentrations of 250, 500, and 1000 mg/ml). Then, the extract was injected to fill the entire inner surface of the cyst. A small amount of cyst fluid was then removed for 5, 10, 20 and 30 min and then combined with 0.1% eosin. The smear was prepared and placed on a glass slide. To determine the viability of protoscolices, they were examined under a light microscope.

Gas Chromatography–mass Spectrometry Analysis (GC–MS)

In this study, GC–MS was used to determine the phytocomponent of *P. farcta* extract. GC–MS was performed at Birjand University of Medical Sciences. Identification of the peaks was based on the previous study [7]. The identification of peak was evaluated by matching their recorded spectra with National Institute of Standards and Technology library and by similarity with those in the literature [8].

Statistical Analysis

All assays were repeated three times, and the results were expressed as the mean ± standard deviation. All data were compared by analysis of variance (one-way ANOVA). The difference was considered significant when $P < 0.05$. Statistical analyses were done by using SPSS version 20.

Results and Discussion

In vitro Effect

Table 1 shows anti-protoscolicidal effects of various concentrations *P. farcta* plant extracts on hydatid cysts protoscolices after 5, 10, 20, 30 min. The results show that *P. farcta* extract in all concentrations showed significant anti-protoscolices effects compared to the control group ($p < 0.001$). The lethality of protoscolices after 5 min of exposure to 1000 mg/mL of *P. farcta* extract was 100%. In addition, after 10 min of exposure to a concentration of 500 mg/mL, the anti-protoscolocidal effects of the plant extract were 100%. Findings show that with increasing exposure time of P. farcta extract, the lethality in all concentrations increased significantly (Fig. 1).

Ex vivo Effect

As shown in Table 2, after injecting different concentrations of *P. farcta* extract directly into hydatid cysts, 100% of protoscolices were killed after 10 and 25 min of

Table 1 Protoscolicidal effects of *P. farcta* essential oil against protoscoleces of hydatid cyst in in vitro

Concentration (μL/mL)	Exposure time (min)	Mean of mortality rate (%)
Normal saline + tween 20	5	0 ± 0.0
	10	0 ± 0.0
	20	2 ± 0.6
	30	5 ± 0.8
Ag-nitrate	5	70 ± 2.8
	10	100 ± 0.0
	20	100 ± 0.0
	30	100 ± 0.0
250	5	32 ± 2.5
	10	57 ± 3.1
	20	80 ± 3.6
	30	95 ± 4.2
500	5	70 ± 3.6
	10	97 ± 4.8
	20	100 ± 0.0
	30	100 ± 0.0
1000	5	100 ± 0.0
	10	100 ± 0.0
	20	100 ± 0.0
	30	100 ± 0.0

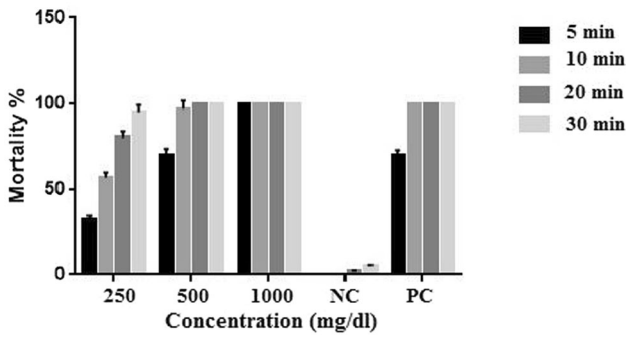

Fig. 1 In vitro scolicidal effects of different concentrations of P. farcta against protoscoleces of E. granulosus over various exposure times (NC: negative control, PC: positive control)

exposure at concentrations of 1000 and 500 mg/ml, respectively. Liters of *P. farcta* extract were lost, indicating that the extract needed more time to show its anti-protoscolicidal effects ex vivo (Fig. 2).

GC–MS Analysis

Twenty-four compounds were identified in the *P. farcta* oil, which constitutes about 97.20% of this oil. The main components were 9,12-octadecadienoic acid, ethyl ester (35.1.1%), palmitic acid (21.4%), cembrene A (3E) (4.7%), and myristic acid (4.4%) (Table 3).

Viability Test

The eosin exclusion test was applied to determine the viability of the protoscoleces. After eosin exposure, the dead protoscoleces absorbed eosin and stained red as in Fig. 3a, whereas colorless protoscoleces without any staining showed muscular body movements and flame cell activity and were considered viable (Fig. 3b).

Discussion

Hydatidosis is one of the most important diseases of zoonotic worldwide which usually causes more than 200 million $ economic loss annually just in Iran [3, 9]. Nowadays surgery has been considered as a suitable method for hydatidosis [10, 11]. However, this invasive method has dangerous risks and complications such as rupture or leakage of cysts and subsequent anaphylactic shock or even recurrence of cysts in the patient's body [4]. Today for prevention of rapture of hydatid cyst during surgery procedure, various chemicals agent such as 20% hypertonic saline solution have been used. However, the use of these chemical agent may have a side effect such as bile duct fibrosis and liver necrosis. In this study, we aimed to investigate the anti-protoscolex effect of P. farcta extract on hydatid cyst; protoscoleces were evaluated in vivo and ex vitro. According to international standards, the main

Table2 Protoscolicidal effects of P. farcta essential oil against protoscoleces of hydatid cyst in ex vivo

Concentration (μL/mL)	Exposure time (min)	Mean of mortality rate (%)
Normal saline + Tween 20	5	0 ± 0.0
	10	0 ± 0.0
	20	3 ± 0.5
	30	7 ± 1.5
Ag-nitrate	5	100 ± 0.0
	10	100 ± 0.0
	20	100 ± 0.0
	30	100 ± 0.0
250	5	16 ± 2.1
	10	20 ± 3.5
	20	40 ± 3.6
	30	68 ± 4.5
500	5	45 ± 3.2
	10	60 ± 4
	20	92 ± 4.5
	30	100 ± 0.0
1000	5	100 ± 0.0
	10	100 ± 0.0
	20	100 ± 0.0
	30	100 ± 0.0

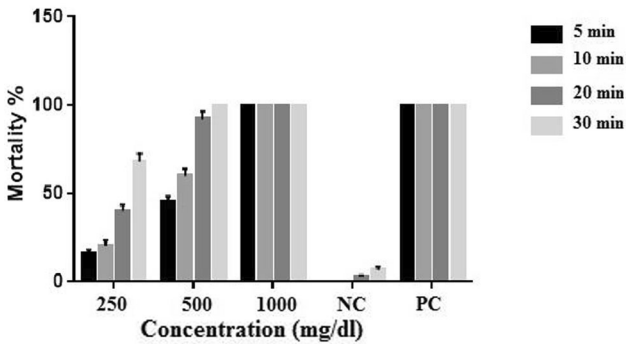

Fig. 2 Ex vivo scolicidal effects of different concentrations of P. farcta against protoscoleces of E. granulosus over various exposure times (NC: negative control, PC: positive control)

feature of a good anti-protoscolices agents is as follows: impact in short periods, high stability in the presence of cyst fluid, high availability and noted low toxicity. The in vitro results of the present study showed that the lethality rate of anti-protoscolices with concentration of 1000 mg/ml of *P. farcta* extract after 5 min of exposure was 100%. In addition, after 10 min of exposure to 500 mg/mL of *P. farcta* extract, the scolicidal activity of the plant extract was 100%. However, in ex vivo results, 100% of protoscolices after 10 and 25 min of exposure to, respectively, with concentrations of 1000 and 500 mg/ml of PE extract were lost, which indicates that extract requires more time to show that ex vivo has its anti-protoscoleces effects in conditions ($p < 0.05$). Based on past studies, the most important plant compounds *P. farcta*, polyphenolic compounds (such as tannins and gallic acid) and flavonoids (such as quercetin) have been reported [12, 13]. Several studies have shown the antimicrobial effects of these compounds such as anti-leishmaniasis, anti-trypanosomal and anti-plasmodium; in the last decade, many studies revealed the high effect of herbal plants on many diseases such as leishmaniasis [14–17]. In addition, Fonseca-Silva et al. in 2011 and Ribeiro et al. in 2015 proved the anti-effects as well as the parasitic quercetin and gallic acid [18]. In connection with the possible mechanisms of these compounds, several studies have shown that phenolic compounds are able to exert their antimicrobial effects by acting on cell membranes and degrading membrane peptidoglycans, as well as disrupting the synthesis of some essential amino acids [19, 20]. Sharafi SM et al. in 2017 proved *Foeniculum vulgare* after 5 min, methanolic extracts of *Allium sativum* and hypertonic saline after 10 min, and warm water after 2 min of killing all alive protoscolices [21]. Based on what has been mentioned, it is inferred that antimicrobial effects of *P. farcta* are probably due to the presence of these compounds in the plant. The results of the present study, like other studies [22, 23], indicate that medicinal plants are valuable and useful for

Table 3 Chemical composition of essential oil of *P. farcta (GC mass)*

Compound	Area %	KI_c	KI_r
1,8-Cineole	0.5	1033	1031
n-Octanol	0.4	1068	1068
Linalool L	0.8	1100	1096
Nonanal	4.7	1106	1100
Decanal	0.7	1206	1201
β-Citronellol	0.9	1228	1225
Linalool acetate	2.2	1257	1254
α-Terpinyl acetate	3.5	1352	1349
Geranyl acetate	0.5	1384	1383
trans-Caryophyllene	0.7	1425	1428
Bicyclogermacrene	1.1	1502	1494
γ-Cadinene	0.5	1519	1513
Δ-Cadinene	1.6	1528	1530
Lauric acid	1.6	1567	1568
(+)-Spathulenol	0.5	1584	1576
Cedrol	1.9	1609	1604
β-Eudesmol	0.4	1657	1654
Neointermedeol	1.4	1662	1658
(Z,Z)-Farnesol	0.7	1697	1698
Myristic acid	4.4	1771	1780
(E,E)-Farnesyl acetate	4	1847	1846
Pentadecanoic acid	1.2	1866	1866
Cyclohexadecane	1	1880	1881
Palmitic acid, methyl ester	0.4	1926	1926
Cembrene A (3E)	4.7	1950	1948
Palmitic acid	21.4	1984	1984
9,12-Octadecadienoic acid, ethyl ester	35.1		

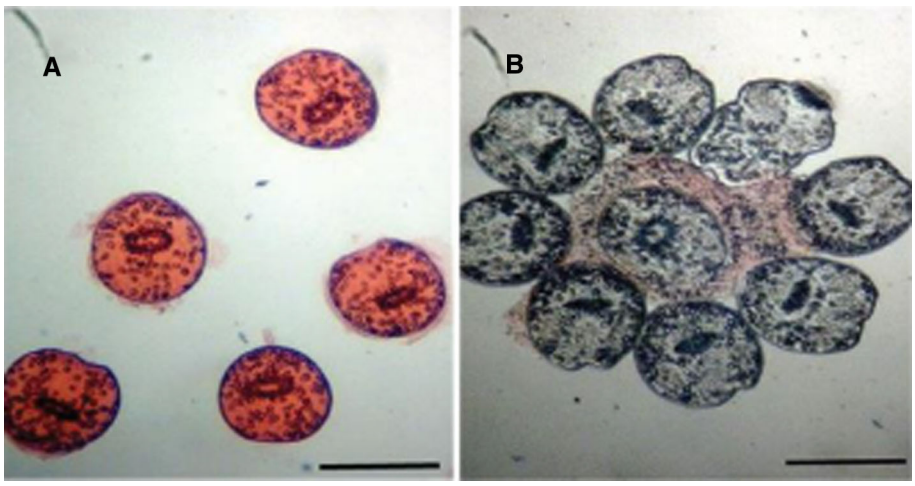

Fig. 3 The dead **a** and live **b** protoscoleces observed following eosin staining

treating a wide range of diseases, including infectious diseases. The present study showed that *P. farcta* extract has significant anti-protoscolices effects, so it can be used as a new alternative drug during hydatid surgery.

Conclusion

To conclude, the findings of this study demonstrated high protoscolicidal activity of *P. farcta* in in vitro and ex vivo model that indicated the potential of *P. farcta* as a medicinal plant for opening a new prospective in the research of new drug with anti-hydatidosis effect. However, further studies will be needed to determine these results by checking the *P. farcta* extracts in animal model.

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Declaration

Conflicts of interest The authors declare that they have no conflicts of interest in this work.

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