Effects of Methanolic Extract of *Ficus carica* Leaves on Cystic Echinococcosis

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ABSTRACT

Background and objective: Surgery is the gold standard treatment for hydatidosis. Scolicidal agents could be used during surgery to kill protoscoleces and prevent cyst recurrence after rupturing of main lesion. Non-chemical agents with sufficient protoscolex-killing activity are known to be fully effective in this regard with fewer side effects. Fig tree is an Iranian native plant, which has been used for medicinal purposes in traditional medicine.

Methods: After obtaining infected hydatid cyst of the liver from a slaughterhouse in Babol (Iran), the percentage of live protoscoleces was calculated by critical staining with 0.1 % eosin. Then, the protoscolex-killing activity of methanolic extract of fig leaves was evaluated at concentrations of 2.5, 5, 10, 15 and 20 mg/ml in 2, 4, 8, 12, and 16 minutes exposure times. Statistical analysis was performed using SPSS software (version 22).

Results: Concentration of 20 mg/ml of the extract showed 100% protoscolex-killing activity within the first 2 minutes of exposure. In addition, the lowest protoscolex-killing activity (45%) was observed at concentration of 2.5 mg/ml after 2 minutes. The results also showed that the protoscolex-killing activity of the extract increases significantly in a concentration- and time-dependent manner ($P < 0.001$).

Conclusion: The methanolic extract of fig leaf at concentration of 20 mg/ml could exert significant scolicidal effect within 2 minutes of exposure. Therefore, complementary experiments should be performed on animal models to further assess the efficiency of the extract for killing protoscoleces of hydatid cyst during surgery.

Keywords: *Echinococcus granulosus*, methanolic extract of *Ficus carica*, broth dilution.

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INTRODUCTION

Hydatidosis is one of the most important zoonotic diseases caused by the larval stage of dog tapeworm belonging to the genus *Echinococcus*.

Dominant species of the parasite; *Echinococcus granulosus*, causes single-alveolar hydatid cysts in the internal organs of herbivores and humans. The disease is endemic in some parts of the world including Australia, North Africa, the Far East, and the Middle East, and is still considered a global health problem (1, 2).

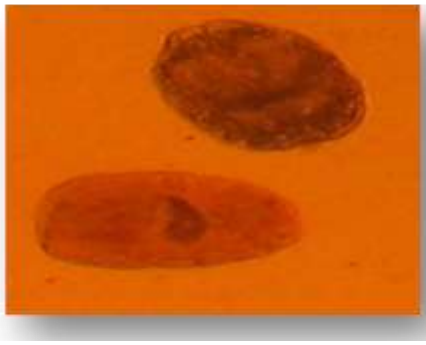
Puncture-aspiration-injection-reaspiration (PAIR) is the main method of treatment. When hydatid cyst are under pressure, rupture and release of hydatid fluid containing protoscolex during surgery is not an unusual phenomenon, which is the most important reason for the recurrence of the disease. Hence, it is necessary to use scolicidal agents during surgery for killing the protoscoleces (3). Hypertonic salt solution, silver nitrate, cetrimide and formaldehyde are the most commonly used scolicidal agents for killing protoscoleces. However, each of these agents has some side effects such as bile duct fibrosis and liver necrosis (4-6).

Primary sclerosing cholangitis is an uncommon disease characterized by diffuse inflammation of bile ducts, leading to fibrosis and stenosis of the biliary system. This serious side effect could be caused after surgery and following the passage of scolicidal solutions through bile ducts. There is currently no fully effective agent without side effect that could be used during surgery. Therefore, the World Health Organization (WHO) has announced an urgent need for a new scolicidal agent to kill protoscoleces more effectively and with fewer side effects. Use of medicinal herbs with healing properties has a long history (7-8). Among the medicinal plants extract used for killing protoscoleces in different investigations, blueberry, garlic, elderberry, pistachio, thyme, savory, nettle, and *Paganum harmala* seed are proven to have protoscolexcidal activity (9-16). The active ingredients of medicinal plants have been used as useful sources of new therapeutic agents (17). The present study aimed to investigate the effect of methanolic extract of fig leaf on *E. granulosus* protoscoleces.

MATERIAL AND METHODS

This experimental study was conducted in 2014. Liver of sheep infected with *E. granulosus* was obtained from Bandpey slaughterhouse in Babol, and transferred to the laboratory of parasitology and mycology at Babol University of Medical Sciences, Iran. After washing the samples with sterile normal saline, the hydatid fluid inside the cyst was transferred to a sterile glass using a 20 ml syringe. The fluid was later poured into a 50 ml falcon tube and placed diagonally in sterile condition for 30 minutes to allow precipitation of protoscoleces. After accumulation of the protoscoleces at the bottom of the falcon tube, the supernatant was aspirated. The protoscoleces were dyed with 0.1% eosin to calculate the percentage of live protoscoleces. Live protoscoleces are colorless and transparent under light microscope, while the dead protoscoleces appear red due to the penetration of eosin (Figure 1). Live protoscoleces were kept in a dark sterile container containing normal saline at 4 ºC for future use (11).

Figure 1- Image of a live protoscolex (above) and a dead protoscolex (bottom)



Caspian fig leaves were collected from *Ficus Carica* tree at the university's campus. Specimens were deposited in the herbarium (No: mu1132) of Department of Plant Sciences, Faculty of Sciences, Tehran University, Iran.

The leaves were dried at room temperature away from direct sunlight. The samples were then powdered by an electric crusher (9-16).

The most suitable solvents for plant extraction are methanol and ethanol because they can solve 80% of the plant's components. However, the starch content of a plant cannot be extracted by ethanol. Therefore, methanol was used as solvent for extraction in this study due to its high permeability and preserving property for active ingredients (18, 19). For this purpose, 100 g of the dried leaf powder was uniformly mixed with 400 ml of methanol on a rotary (electric) mixer for 1 hour. The mixture was then kept at room temperature for 24 hours. The solution was mixed again, and then passed through a paper filter to remove pulp. The resulting solution was placed at room temperature to be evaporated. Lid of the extract's container was sealed with parafilm, and then container was kept at room temperature for 2-7 days. In the meantime, the content of the container was stirred occasionally, and stored in a sterilized dark glass at 4 °C for future use (10). A 40mg/ml solution was prepared by dissolving 400 mg of the extract in 10 ml of dimethyl sulfoxide (DMSO). The solution obtained was placed on a shaker for 10 minutes. The prepared mixture was kept in refrigerator at 4℃ for future use.

According to the results of similar studies on fig plant, concentrations of 2.5, 5, 10, 15, and 20 mg/ml were used in the present study. The mentioned concentrations were prepared by diluting the stock solution of the extract with 10% DMSO. Effect of the five concentrations of the methanolic extract of fig leaf on protoscolex was assessed using a positive control (saturated saline) and a negative control (normal saline) in five different periods. A unit (200 µl) of the protoscolex suspension and a unit (200 µl) of the methanolic extract of fig leaf were placed in separate tubes (this way, our concentrations remained constant). Then, two drops of 0.1% eosin were added to each tube. The impact of the different concentrations of the extract was recorded after 2, 4, 8, 12, and 16 minutes by counting the number of live protoscoleces. For better accuracy, 100 protoscoleces were counted each time in triplicate, and then the mean values were calculated and presented in percentage (9-11).

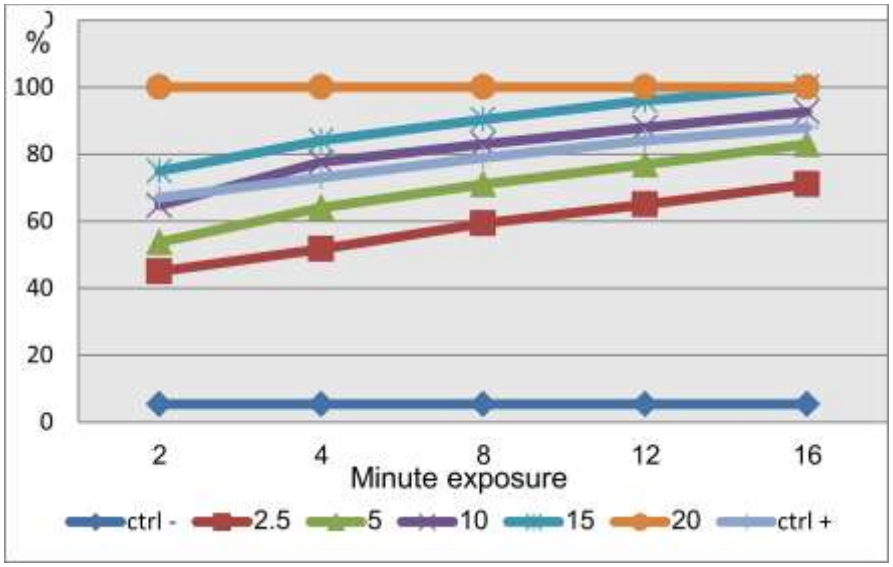
To analyze the data, the number of live protoscoleces were calculated and then compared with the number of protoscoleces before treatment with the extract. Data analysis was done using descriptive statistics including mean and standard deviation (SD) in SPSS software (version 22).

The data obtained from the study was statistically analyzed using one way analysis of variance (ANOVA) and t-test. P-values less than 0.05 were considered as statistically significant.

RESULTS

The results showed that all concentrations of the extract tested had protoscolex-killing activity (Table 1).

Figure 1- Scolicidal effect of different concentrations of the methanolic extract of fig leaves in



As shown in figure 1, the protoscolex-killing activity of the positive control increases with time. In addition, 20 mg/ml of the extract destroyed 100% of protoscoleces at all time intervals tested.

Although concentration of 15 mg/ml of the extract showed less scolicidal activity in comparison with 20 mg/ml, it had 100% protoscolex-killing effect after 16 minutes. The results show that the protoscolex-killing activity of the extract increased in a time- and concentration-dependent manner. The lowest protoscolex-killing activity was observed at concentration of 2.5 mg/ml, which was significantly lower compared to other groups ($P < 0.05$).

Table 1- Scolicidal effect of different concentrations of the methanolic extract of fig leaves in different exposure times

Solutions	Exposure time (min)	Repeat	Mortality rate ± SD
Negative control (Normal saline)	2.00	3	5.33±0.57
	4.00	3	5.33±0.57
	8.00	3	5.33±0.57
	12.00	3	5.33±0.57
	16.00	3	5.33±0.57
	Total	15	5.33±0.48
Extract at a concentration of 2.5 mg / ml	2.00	3	45±1
	4.00	3	51.66±0.57
	8.00	3	59.33±0.57
	12.00	3	65±1
	16.00	3	71±1
	Total	15	58.4±9.6
Extract at a concentration of 5 mg / ml	2.00	3	53.66±0.57
	4.00	3	64±1
	8.00	3	71±1
	12.00	3	77±1
	16.00	3	83±1
	Total	15	69.73±10.59
Extract at a concentration of 10 mg / ml	2.00	3	64.66±0.57
	4.00	3	77.66±0.57
	8.00	3	83±1
	12.00	3	88±1
	16.00	3	92.66±1.15
	Total	15	81.2±10
Extract at a concentration of 15 mg / ml	2.00	3	75±1
	4.00	3	84±1
	8.00	3	90.33±0.57
	12.00	3	96±1
	16.00	3	100±0.0
	Total	15	89.06±9.19
Extract at a concentration of 20 mg / ml	2.00	3	100±0.0
	4.00	3	100±0.0
	8.00	3	100±0.0
	12.00	3	100±0.0
	16.00	3	100±0.0
	Total	15	100±0.0
Positive control (saturated saline)	2.00	3	67±1
	4.00	3	73±1
	8.00	3	79±1
	12.00	3	84±1
	16.00	3	88±1
	Total	15	78.20±7.83

According to the results of two-way ANOVA, increasing the adjoining concentrations significantly increases the protoscolex-killing property of the extract ($P < 0.001$).

DISCUSSION

In this study, the fig leaf extract was used as a scolicidal agent for killing protoscoleces. Different parts of the plant such as bark, leaves, tender roots, fruit, seeds, and vegetable juice (sap) are of importance in herbal medicine (20, 21). According to previous studies, fig is a medicinal plant with antioxidant, antibacterial, antifungal, and antiparasitic properties (22-26).

Surgery is the treatment of choice for liver hydatid cyst, although medication and PAIR are considered as the second-line therapy, especially in patients who have had surgical problems (27, 28). One of the most important complications of the hydatid cyst surgery is cyst rupture and spread of protoscolex in the affected organ. In addition, remaining of germinal layer after partial removal is seen in nearly 10% of the patients (29).

Different scolicidal agents have been used to kill protoscoleces and sterilize the content of the cyst. Hypertonic salt solution is one of the most commonly used agents for this purpose (30). Our results indicated that silver nitrate, dextrose-aminomix-1 solution, and mannitol-salt solution could kill 20% (20 min), 50% (30 min), and 20% (45 min) of protoscoleces, respectively. Moreover, 20 mg/ml of albendazole could kill 65% and 70% of protoscoleces within the first 5 minutes and 60 minutes of exposure, respectively. All these scolicidal agents are effective in killing protoscoleces when compared with controls. In addition, most of these agents do not have the common side effects including sclerosing cholangitis, burning, stenosis of the bile ducts, and increasing blood sodium level (31).

A low dose of an ideal scolicidal agent should be stable in hydatid fluid, and maintain its protoscolex-killing property when diluted with the fluid. In addition, this non-toxic, easily prepared, quickly available, and inexpensive agent should be effective on daughter cysts with minimal local and systemic side effects. Considering these characteristics, no ideal agent or solution has been yet identified (32). Recent studies have suggested the use of antimicrobial compounds derived from medicinal plants as natural alternative to chemical antibiotics (33). In this study, 20 mg/ml of the fig leaf methanolic extract destroyed all protoscoleces within 2 minutes. According to the results, all concentrations of the extract tested showed protoscolex-killing activity, which increased in a concentration- and time-dependent manner.

Study of Salehi et al. showed that 2 mg/ml of the alcoholic extract of *Berberis vulgaris* fruit has 100% protoscolex-killing activity after 5 minutes, while the methanolic extract of the fig leaf in our study could exert the same effect more quickly (2 minutes) (9). Another study on different concentrations of garlic extract showed that concentration of 10% of the extract could only destroy 16.8% of protoscoleces after 60 minutes (10). Study of Taran et al. reported that pistachio extract has no significant anti-helminthic activity at concentrations of 128, 256 and 512 mg/ml (12). Moreover, study of Luqman Omar on the effects of nettle on protoscolex showed that concentration of 4 mg/ml is able to destroy 97% of protoscoleces after 30 minutes (15).

CONCLUSION

According to our results, 20 mg/ml of the fig leaf extract could kill all protoscoleces within the first 2 minutes of exposure in laboratory conditions. However, the volume of fluid in the cyst must be determined prior to surgery so that the final concentration of the extract reaches 20 mg/ml after exposure to the fluid. It is suggested to conduct in vivo studies to further assess the protoscolex-killing activity of the fig extract.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

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