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Investigation of in vitro Anti Protoscoleces of *Echinococcus granulosus* activity for *Cyperus rotundus* plant extract

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Abstract

Hydatid cyst is a continuing health problem in various parts of the world that is not linked to the economic level of countries but rather linked to adherence to health conditions. Therefore, both animals and humans are still at risk of infection. The study was conducted to evaluate the effect of the boiled water extract of *Cyperus rotundus* on the viability of *Echinococcus granulosus* protoscoleces in vitro as an environmentally friendly means of controlling the disease. Livers infected with hydatid cysts were collected from the slaughtered animals (sheep and cows) from different areas, including butchers' Baladruz and Baquba in Diyala Province. The concentrated suspension of protoscoleces (1000-1500 protoscoleces per ml) was added to different concentrations of the boiled water extract of *Cyperus rotundus* root at different concentrations (100, 50, 25, 12.5 mg/ml), and for different time periods (30, 60 120, 180, 240, 300, 360, 420, 480 and 540 min)) and the viability of these protoscoleces was measured using 0.1% aqueous eosin stain. The results of the current study showed that the mortality rate of protoscoleces was associated with an increase in the concentration of the plant extract and the exposure time. The highest mortality rate (100%) was at the highest concentration of 100 mg/ml within 4 hours, while the lowest mortality rate (34.54%) was at the concentration of 12.5 mg/ml within half an hour (30 minutes). The differences in mortality rates of protoscoleces at different

concentrations and different exposure times were significant at (P<0.05).In conclusion, the extract has a potential therapeutic ability and can be used to treat cysts.

Key words: *Echinococcus granulosus*, Protoscoleces, *Cyperus rotundus*, plant extract.

Introduction

Hydatid cystic disease is a common disease resulting from infected with the larva of *Echinococcus granulosus* distributed in various parts of the world. It is endemic in Middle Eastern countries, parts of America, Australia, the Mediterranean, Central Asia, and Central and Eastern Europe [1]. Being a zoonotic disease, the hydatid cyst becomes more seriousness since humans can easily get infected when in contact with their domestic animals [2]. Due to the difficulty an early diagnosis, the condition is prevalent and difficult to treat. Hydatid cystic disease represents an endemic huge health problem in Iraq, it is a difficult-to-treat disease due to the difficulty an early diagnosis and the fact that the infection is not detected until a long time has passed [3].

Medicinal plants are preferred over other treatments for two reasons: first, they are a repository of medicinal compounds and can be tested on humans without side effects; second, plants are available in large quantities in most countries of the world [4]. Among these medicinal plants is *Cyperus rotundus*, a distinguished medicinal plant with therapeutic benefits. It was used in in Egypt and Iraq since the ancient Age, and its tubers are used to treat various diseases due to the concentration of active ingredients [5]. This plant was effective against many disease and microbes because it contains compounds that have a therapeutic effect. It was used to treat diabetes and joint pain, allergic reaction, hypertension, inflammatory, and it was also used for weight loss. Its therapeutic efficacy has also been tested for many types of microorganisms including bacteria, fungi and parasites [6]. The study was conducted to evaluate the effect of the boiled water extract of *Cyperus rotundus* on the viability of *Echinococcus granulosus* protoscoleces in vitro as an environmentally friendly means of controlling the disease.

Materials and Methods

Collection of protoscoleces and test viability: The livers were collected from the slaughtered animals at the butchers' shops (cows and sheep) infected with hydatid cysts from different areas,

including butchers' Baladruz and Baquba in Diyala province during period from September to February 2024. The livers were transported to plastic bags placed inside a container containing ice and they were directly washed with distilled water, placed in a sterilized dish, and sterilized with 70% ethyl alcohol. The largest possible amount of hydatid fluid containing the protoscoleces were aspirated and were placed in test tubes to centrifuge. The concentrated suspension of protoscoleces (1000-1500 protoscoleces per ml) were collected to determine the viability using eosin (1%) (and was used to estimate the effect of extract). The percentage of protoscoleces viability was calculated by dividing the number of live protoscoleces in the sample counted on the slide by the total number of protoscoleces counted (the average of three replicates were determined, which was 92.73%). This process was repeated after each exposure.

Collection of plant and preparation of extract: *Cyperus rotundus* roots (tubers) were collected from agricultural land and home gardens in Baladruz District, Diyala Province. It was classified at the Natural History Museum and Research Center of Baghdad University. The roots were washed with water to remove soil. They were dried at room temperature. The dried roots were ground, and the powdered roots were kept in an airtight glass bottle away from light and heat. For extract preparation, 4000 mg of the plant powder was prepared in 40 ml of boiled deionized water. The mixture was then left to soak in water for 72 hours and filtered. The resulting solution was evaporated at 70 °C to obtain the concentrated powder and kept at -20 °C. The concentrations (12,5, 25, 50, 100 mg/ml) were prepared and used in experiments.

Estimate the effect of the extract on protoscoleces: One ml of the aqueous extract (in different concentrations for different exposure times) and concentrated protoscoleces fluid were placed in a 5 ml test tubes. Then, 100 microliters of the mixture were examined under a light microscope after adding the eosin stain. The desired exposure period (30, 60 120, 180, 240, 300, 360, 420, 480 and 540 min) was determined using a digital watch. The number of hydatid cysts was counted, (dead protoscoleces were stained with eosin while a live were not stained). The percentage of mortality was then calculated (Fig1). The same steps were done for both negative and positive control (deionized distilled water and albendazole, respectively). The concentrations 10, 50, and 100µg per ml were used for positive control. The percentage of mortality was calculated by following equation [7]:

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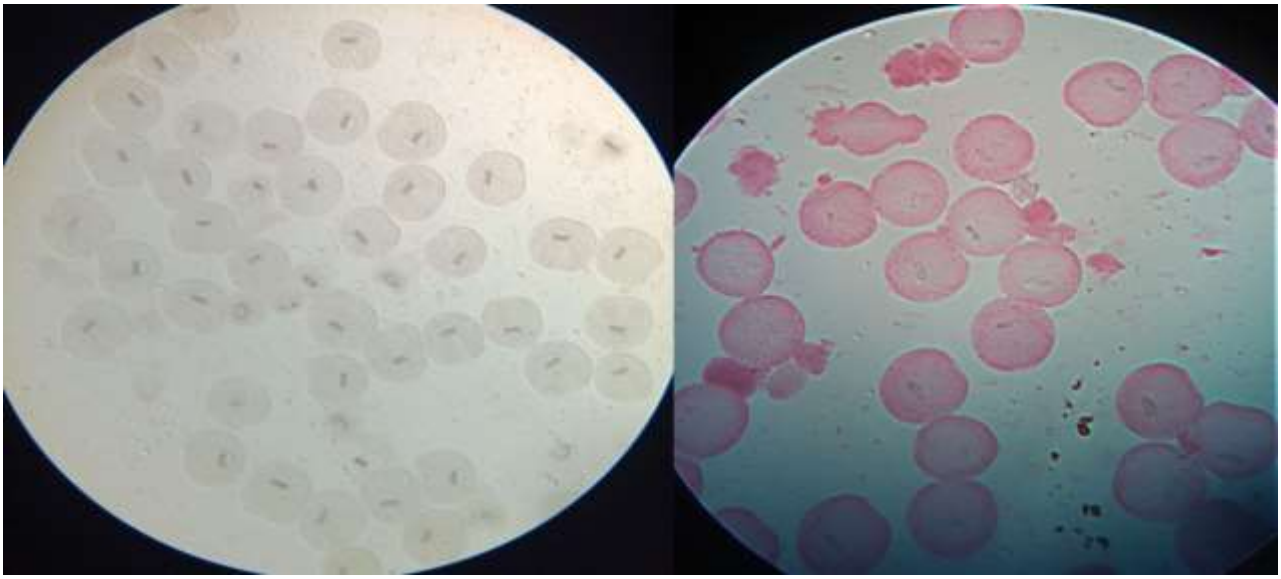
Percentage mortality (%) = [(A - B)/A] × 100

Where A is the number of dead protoscoleces in the untreated (control) and B is the number of dead protoscoleces in the treated groups.

Statistical Analysis: The data of the current study were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 25 and Excel version 2013. The data were described as percentages and the groups were compared using the chi-square test at a significance level of $P<0.05$.



A **B**

Figure 1: Protoscoleces: (A) alive, not stained with eosin stain, (B) dead, stained with eosin stain.40X**.**

Results

The results of the current study showed the therapeutic effect of *Cyperus rotundus* at different concentrations and exposure times on the protoscoleces, and the mortality rate of protoscoleces was associated with an increase in the concentration of the plant extract and the exposure time (Fig 2).

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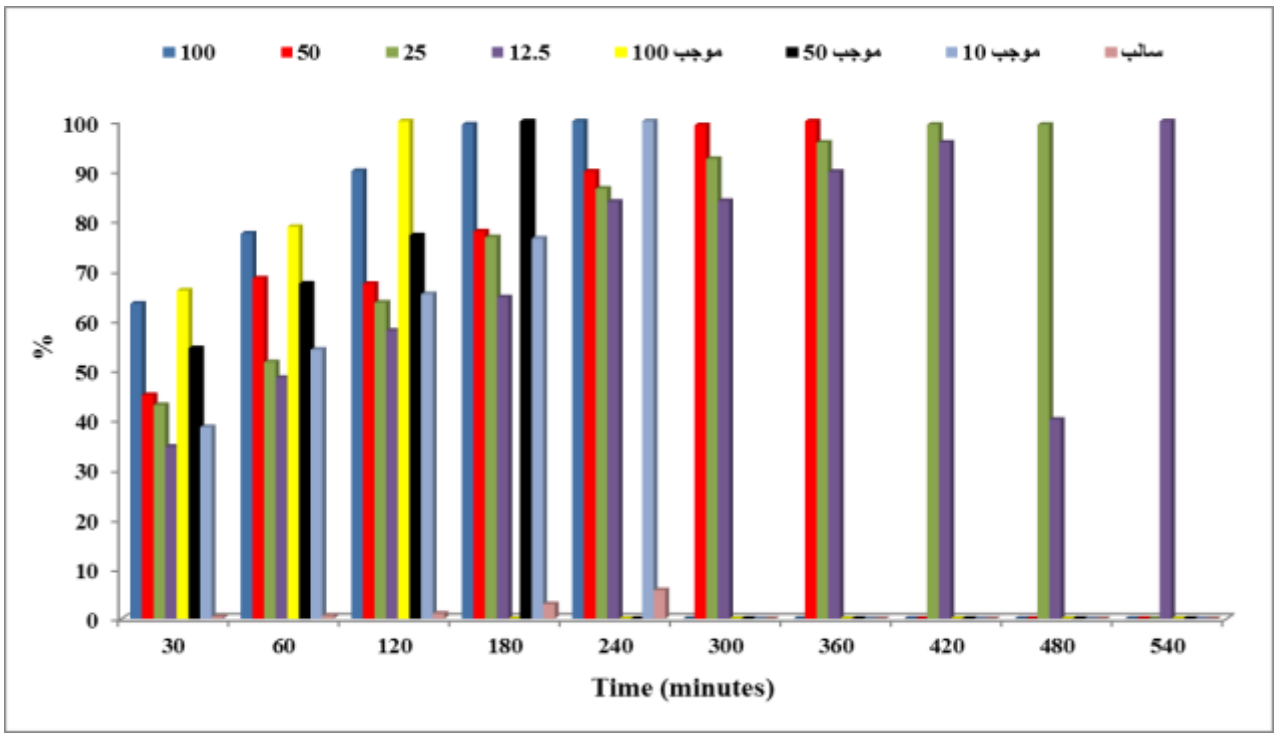

Figure 2: Percentage mortality of *Echinococcus granulosus* protoscoleces after treatment with extracts *Cypress rotunda* plant, negative deionized distilled water and positive control albendazole

It was seen that the concentration 100 mg/ml of *Cyperus rotundus* extract recorded the highest mortality rates at 120 minutes 90%, 180 minutes 99.34%, and 240 minutes 100% compared to the positive control, which recorded the highest mortality rate at 120 minutes 100%, and the negative control, which recorded mortality rates at 120 minutes 1.03%, 180 minutes2.92%, and 240 minutes 0.00%, respectively. In comparison, the concentration 12.5 mg/ml recorded mortality rates of 57.92% at 120 minutes, 64.63% at 180 minutes, and 83.80% at 240 minutes, and the positive control at the concentration 10 mg/ml) recorded mortality rates of 65.26% at 120 minutes, 76.44% at 180 minutes, and 100% at 240 minutes, and the negative control recorded mortality rates of 1.03% at 120 minutes, 2.92% at 180 minutes, and 5.71% at 240 minutes, with statistically significant differences ($P<0.05$). The differences in mortality rates of hydatid cysts at different concentrations and times were significant ($P<0.05$), as shown in Table (1).

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Table 1: Therapeutic effect of *Cyperus rotundus* on the protoscoleces

Time	Concentration									P value
		Extract (mg ml^{-1})				Positive control (mg ml^{-1})			Negative control	
		100	50	25	12.5	100	50	10		
30	Mean %	63.34	44.95	42.94	34.54	65.97	54.37	38.52	0.40	P<0.001***
	SEM %	1.47	1.80	1.54	0.93	2.46	1.78	2.36	0.09	
60	Mean %	77.42	68.43	51.59	48.41	78.78	67.34	54.09	0.60	P<0.001***
	SEM %	7.53	0.05	5.79	1.06	0.79	1.08	2.90	0.11	
120	Mean %	90.00	67.32	63.56	57.92	100.00	77.07	65.26	1.03	P<0.001***
	SEM %	0.87	7.47	12.07	6.03	0.00	0.67	2.40	0.19	
180	Mean %	99.34	77.84	76.68	64.63	0.00	100.00	76.44	2.92	P<0.001***
	SEM %	0.24	7.35	4.55	11.62	0.00	0.00	1.00	0.21	
240	Mean %	100,0	89.89	86.45	83.80	0.00	0.00	100.00	5.71	P<0.01**
	SEM %	0.00	0.88	1.59	5.20	0.00	0.00	0.00	0.91	
300	Mean %	0.00	99.21	92.45	83.98	0.00	0.00	0.00	0.00	P<0.05*
	SEM %	0.00	0.14	2.95	1.63	0.00	0.00	0.00	0.00	
360	Mean %	0.00	100.00	95.72	89.82	0.00	0.00	0.00	0.00	P>0.05
	SEM %	0.00	0.00	2.56	0.88	0.00	0.00	0.00	0.00	
420	Mean %	0.00	0.00	99.31	95.72	0.00	0.00	0.00	0.00	P>0.05
	SEM %	0.00	0.00	0.19	2.56	0.00	0.00	0.00	0.00	
480	Mean %	0.00	0.00	99.31	40.00	0.00	0.00	0.00	0.00	P<0.001***
	SEM %	0.00	0.00	2.00	1.19	0.00	0.00	0.00	0.00	
540	Mean %	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	1.00
	SEM %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
P value	P<0.05 *		P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.01 **	P<0.05 *	P<0.01 **	P<0.001 ***	

Discussion

High concentrations of plant extracts have a great effect on reducing the viability of protoscoleces compared to other concentrations. This is what was proven in the current study and is consistent with the results of [8], in her study of the aqueous extract of the *Cyperus rotundus* rhizomes and the leaves of fenugreek, she referred to the ability of both extracts to homicide the protoscolces with tend the percentage of mortality for both extracts to depended on the increase in time and concentration. The present research was carried out using the rhizome extract of the *Cyperus rotundus* plant. Deionized boiling water was utilized as a solvent during the extraction process to manage the disease in an eco-friendly manner, as it has no impact on the potency of the plant compounds being extracted. Unlike organic solvents that can interact with extracted compounds either positively or negatively, it does not impact the compounds[10].

As previously observed by [10], [11]), and [12], the biological activity of *Cyperus rotundus* extract is ascribed to the presence of active chemical components, including alkaloids, phenols, glycosides, tannins, saponins, and volatile oils. The ability of alkaloids to break down the parasite's cell wall and its contents of fats and proteins, as well as their interference with a series of protein metabolism reactions crucial to the parasite's viability, have been explained as the reasons for their inhibitory effect [13]. Meanwhile, phenols are antioxidants that interfere with the synthesis of microorganisms' cell walls, DNA replication, or the production of enzymes, and they can target multiple sites in these organisms, ultimately resulting in their death[14]. Glycosides are known for their potent anti-parasitic effects against helminths. Cardenolide works by blocking the passage of sodium and potassium ions into helminths, resulting in their death. Saponins consist of sugar chains and contain triterpene or occasionally, steroidal-aglycone. Saponins demonstrate their anthelmintic properties by inhibiting acetylcholinesterase, leading to worm paralysis and ultimately causing death. Tannins are water-soluble polyphenolic compounds that help in killing nematodes by either blocking the worms from taking in nutrients from the host cell or by attaching to the intestinal mucosa of the parasitic worms when the larvae ingest condensed tannins [15].

Increasing the exposure time had a significant effect on the viability of protoscoleces. This is consistent with the results of [16], who found that the effect of both aqueous and alcoholic *Cyperus* extract on the viability of primary hydatid cysts increases with increasing exposure time. The reason for this may be that increasing the exposure time leads to increased penetration of the active substances into the parasite's membranes, leading to their breakdown or weakening of the parasite [17].

Conclusion

In conclusion, the current study found that the boiled aqueous extract of the rhizomes of the *Cyperus rotundus* plant has a significant effect on the protoscoleces due to the presence of active compounds depended on the duration of exposure and the concentration of the aqueous extract. The extract has a potential therapeutic ability and can be used to treat and treat cysts.

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Conflict of interest: The authors declare that there is no conflict of interest.

Ethical clearance: The samples were gained according to Local Research Ethics Committee approval in the College of Education for Pure Sciences, University of Diyala, No. CEPEC/02 in 4th October 2022.

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