

THE EFFECT OF AQUEOUS AND ALCOHOLIC EXTRACT OF *CYPERUS LONGOUS* (CYPERACEAE) AND TWO DRUGS (TINIDAZOLE AND PRAZIQUANTEL) ON KILLING THE PROTOSCOLICES OF *ECHINOCOCCUS GRANULOSUS* IN VITRO.

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ABSTRACT

The protoscolices of *Echinococcus granulosus in vitro* compared with the two drugs Tinidazole and Praziquantel using the concentrations (5 , 10 , 15 , 20) % of the plant extracts and (1.25 , 1.8 , 2.5) % for Tinidazole and (0.1 , 0.15 , 0.2) % for Praziquantel respectively. The study shows that the aqueous extract at 20% have shown highest protoscolicidal activity. All the protoscolices were killed in the first day after treatment. While the concentration 5% shows the lowest activity in killing the protoscolices which was in the 6th day , while the time of killing protoscolices was in the 3rd day and 2nd day at the concentration (10 , 15)% respectively.

There is no significant differences between aqueous and alcoholic extract of *Cyperus longous* $P < 0.01$, so aqueous extract used in our study because of its lower price and its safety.

Tinidazole and Praziquantel have shown the great activity on killing the protoscolices in the 30 & 60 minutes at (2.5 , 0.2) % respectively.

The preservative solution (Hank's solution). Keep the protoscolices viable 59% to 21 days.

INTRODUCTION

The hydatid disease known as hydatidosis or echinococcosis as a cyclozoonotic disease. Its one of the world's most widely spreaded disease resulting from the development of larval hydatid cyst stage in the viscera and other organs of human and herbivorus .[1]

No effective chemotherapy is currently available for the medical treatment of cystic and alveolar hydatid disease in human .[2] with recent years in addition to several anthelmintic drugs (which have shown promising results in the reduction to the larval cystic mass) [3] , there is a noticeable effect of the drug Tinidazole and Praziquantel on killing the protoscolices .[4] &[5]

Various medical plants have been used for years in daily life to treat disease all over the world. According to a study performed by the WHO based on publications on Pharmacopoeias and medical plants in 91 countries . The number of medicinal plants is nearly 20.000 [6].

Traditional medical treatments in daily life are now being used with empiric methods. *Cyperus longous* :(Cyperaceae) refers to a family of marsh-dwelling grass-like plants known as sedges. Perhaps the best known member of the family is the reed, which ancient Egyptians used to make papyrus. Mant other as food and medicine.[7]

Cyperus longous completely inhibited the growth of staphylococcus and Pseudomonas bacteria which cause severe and sometimes fatal infections.[8]

It is also spread on the skin as a bactericide and a fungicide to prevent infection of wounds.[8] Arecent Japanese study indicates that cyperus extract act as an anticoagulant by preventing blood platlets from clumping together to from clots .[9]

The Egyptain researchers found that cyperus extract has moderate level of estrogenic activity.

But these properties have not been studied extensively in the laboratory, and there is no any information available a bout effects of these plant extract and the drugs Tinidazole and Praziquantel on the protoscolices of sheep *E. granulosus* , there for , this investigation was designed to study this subject *in vitro*.

MATERIALS AND METHODS

Plant extract:

1. Aqueous extract :

Distilled water used in the extraction of the cyperus according to [10] with some Modifications. 25 gm of the plant powder was added to 100 ml of the solvent (Distilled water) then the solution left for 24 hr. on the magnetic stirrer. The filtration of the extract was done by vaccum pump using filter paper whatmon No-1. The Filter at was condensed using Rotary vaccum evaporator. The extract was put in Steriled bottoles after drying until using .

2. Alcoholic extract 70 % Ethanol :

The extraction with Ethanol 70 % was done as the same in the previous paragraph Exchanging distilled water with 70 % Ethanol.

Protoscolicidal activity :

The aqueous and alcoholic extract with concentration (5, 10, 15, 20)% of the plant was

Evaluated on killing the protoscolices of *Echinococcus granulosus* as follows:

a. Collection of hydatid cyst.

Hydatid cyst was collected from infected sheep with hydatid cyst from central slaughter in Basrah and carried in cooled boxes to the laboratory to deal with it in the same day.

b. Opening of the cyst and removing of protoscolices.

All the tissue coverd the hydatid cyst was removed and then the external surface was sterilled many times with 70 % Ethanol. Plastic steriled cyring of 10 ml was used in dropping the remain liquid containing a lot of protoscolices and put it in a glass steriled beaker , the germinal layer was removed by seizer and tongs and put it in big steriled Petri dish , and cut it in to many pieces and washed with Hank's balanced salt solution contain two antibiotic penicillin and streptomycin made by dissolving 0.1 gm of each of them in 100 ml distilled water [5].

The mixture was filtered through sterilized funnels and filter papers their pores allow only the passing of protoscolices collected in sterilized glass beaker and then the protoscolices was washed 3-5 times with Hank's solution each time was turned and left for 15 minutes to let the protoscolices to precipitate and the filtrate was removed [5].

Hank's solution pH= 7.2 was prepared from dissolving the follows materials:-

0.800 gm NaCl

0.400 gm KCl

0.100 gm MgSO₄.7H₂O

0.048 gm Na₂HPO₄

0.018 gm CaCl₂.2H₂O

0.350 gm NaHCO₃

0.060 gm KH₂PO₄

0.100 gm MgCl₂.6H₂O

0.100 gm C₆H₁₂O₆

The volume was completed to a liter of water and the solution was filtered by using filter paper 0.45 micron.

c. Examination of protoscolices viability by two ways :

1. Direct microscopical examination.

A little quantity of Hydatid cyst liquid containing the protoscolices put on the slide, examined under a microscope, the shaking movement of scolices was recognized using 40X [11].

2. Using Aqueous eosin dye 1 % .

A little quantity of scolices put on a slide, two drops of eosin dye was added and tested microscopically. The green scolices took green light while the dead ones took red one because the dye was separated through their

Membrans [12].

3. Calculation of the protoscolices members.

The members of the protoscolices was calculated by using the procedure of Transferring fixed volume with micropipette 10 ml by using dissecting microscope With average 3 times.

The number of protoscolices was calculated in on milliliter as follows:-

Number of scolices in one ml = its number in (10 µl) X 100

4. Experimental design of the protoscolicidal activity of the extract:

1. Measurement of the activity and effect of the plant *Cyperus longus* on killing the protoscolices in vitro. After collection of protoscolices and measurement its viability, 1 ml of scolices suspension was removed containing 1500 scolices washed with Hank's solution to sterilled test tubes treated with different concentrations of the extract which are (5,10,15,20) % using three times and three test tubes as control and the average percentage of protoscolices viability was taken after treatment and for different periods of times.
2. Effect of the two drugs Tinidazole and Praziquantel on killing the protoscolices. The protoscolices treated with drugs Tinidazole and Praziquantel (2.5, 1.8, 1.25) % and (0.2, 0.15, 0.1)% respectively.
3. Statistical Analysis. Completely Randomized Design (C.R.D.) was used for statistical analysis using two factors (concentrations and periods of time). The results were tested by using SPSS program with Revised Least Significant Differences (R.L.S.D.) on the level 0.05 [5].

RESULTS

Figure (1) summarize the result of comparative trials of the aqueous rhizomus extract of *Cyperus longus* against the viability of protoscolices in comparison with control group .

In general the concentration 20% showed the great effects on killing the protoscolices in aqueous and alcoholic extracts of the plant, while 5% has lowest effects. Table (1)

The concentration 2.5% and 0.2% of Tinidazole and Praziquantel showed great effects on killing protoscolices . Table (2 , 3)

In control group the time, the viability of protoscolices preserved in Hank's solution decreased from 100 in zero time to 79 in 7th days and to zero % in 21 days after preserved without treatment with the extract & drugs.

Table-1- Average percentage of protoscolices viability after treatment with different plant concentrations

Average percentage for the viability of scolices period of time after treatment with extract							Time of treatment
7 day	6 day	5 day	4 day	3 day	2 day	1 day	concentration
0	0	13	25	29	46	73	% 5
0	0	0	0	0	19	68	% 10
0	0	0	0	0	0	51	% 15
0	0	0	0	0	0	0	% 20
79	80	87	98	98	99	100	Control

R.L.S.D =12.38

Table-2- Average percentage of protoscolidal viability after treatment with different concentration of Tinidazol

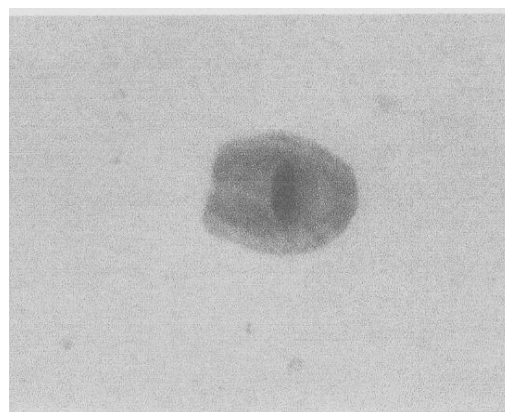
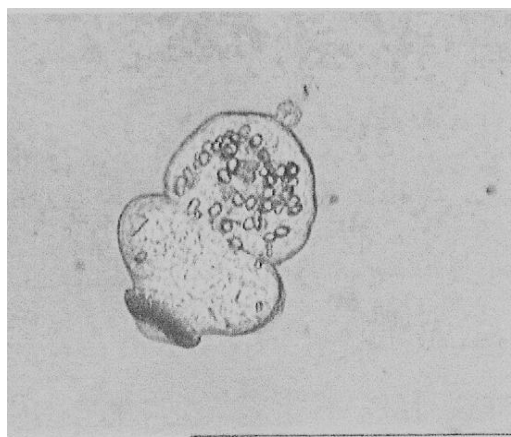
Average percentage for the viability of scolices period of time after treatment with extract						Time of treatment
150 minute	120 minute	90 minute	60 minute	30 minute	0 minute	concentration
0	0	9	11	17	100	1.25
0	0	0	0	2	89	1.8
0	0	0	0	0	48	2.5
100	100	100	100	100	100	Control

R.L.S.D =14.79

Table-3- Average percentage of protoscolidal viability after treatment with different concentration of Praziquantel .

Average percentage for the viability of scolices period of time after treatment with extract						Time of treatment
150 minute	120 minute	90 minute	60 minute	30 minute	0 minute	concentration
0	18	19	6	42	94	0.1
0	0	0	15	37	85	0.15
0	0	0	0	6	70	0.2
100	100	100	100	100	100	Control

R.L.S.D =14.1



Protoscolices of *Echinococcus granulosus*

DISCUSSION

There is no drug is known which is lethal to the cystic larval stage in accidentally infected human, surgical intervention for removal of hydatid cyst generally must follow. This surgery is not without risk., and in many countries of the world the mortality ranges between 1-4 % and many reach 20% or more in cases of repeat surgery .[13]

In two studies , one done in Thailand and the other in Tanzania , compounds found in extracts from the root of *Cyperus longous* were isolated and found to have antimalarial properties.[14] previous studies indicated the role of extracts of *Cyperus longous* as antibacterial agents against various bacterial types such as *Staphylococcus sp.* and *Pseudomonas sp.* , and also as fungicide to prevent infection of wounds .[8]

A recent Korean report on several new compounds isolated from *Cyperus longous* however, indicates that it inhibits the action of benzodiazepine tranquilizer and modifies the effectiveness of several neurotransmitters in central nervous system.[9]

No previous scientific study reported the action and/or role of this plants as protoscolicidal agent, therefore. This problem may become light spot and/or primary step for more of our investigations in near future for this unstudied plants as antihelminthes . In conclusion, we confirmed by the results of the present study the great effects of new plant extracts to *in vitro* killing the protoscolices of hydatid cyst collected from sheep specimens by low concentrations of extract and short time of treatment .

The activity of this plant on killing the protoscolices is due to the active compounds found in this plant (poly phenols) which have the mechanism of this compounds is breaking the cellular membrane of the parasite and the protein and lipids which it contain because of the ability of this poly phenols to precipitate the proteins by making hydrogen bonds between hydroxyl group and nitrogenous compounds and proteins so it depressed enzymes which is necessary for the living organism leading to its death . [14]

In the present study Praziquantel has been shown to be effective against protoscolices of *Echinococcus granulosus in vitro* ,because of the enhance of cell membrane permeability for the worms, because this drug is a derivative of isoquinoline .[15]

The ability of Tinidazole (substance which found in the drug Tinidazole) for rupture the helix structure of DNA and prevent building of nuclear acid leads to destroy the cells and the parasite .[15]

The result is this study resemble what [4] and [5] found in their studies about treatment protoscolices with Tinidazole & Praziquantel.

تأثير المستخلص المائي والكحولي لنبات السعد

وعقاري (التينيدازول و البرازيكوينتل) على قتل *Cyperus longous* (Cyperaceae) في المختبر *Echinococcus granulosus* الرؤيسات الأولية لطفيلي المشوكة الحبيبية

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الخلاصة

والمتواجد في محافظة البصرة لغرض تقييم فعالية *Cyperus longous* (Cyperaceae) أختير نبات السعد *Echinococcus granulosus* مستخلصه المائي والكحولي ضد حيوية الرؤيسات الأولية لطفيلي المشوكة الحبيبية (% 5 , 10 , 15 , 20 في المختبر مقارنة مع فعالية العقارين تينيدازول و برازيكوينتل وذلك باستعمال التراكيز (% للعقارين تينيدازول 0.1 , 0.15 , 0.2) ، (1.25 , 1.8 , 2.5 للمستخلص النباتي المائي والكحولي و) وبرازيكوينتل على التوالي .

% يملك أكبر فعالية في قتل الرؤيسات حيث قتلت جميع 20 أظهرت نتائج الدراسة أن المستخلص المائي بتركيز % أقل فعالية في قتله للرؤيسات الأولية وكان في 5 الرؤيسات الأولية في اليوم الأول من المعاملة بينما أظهر التركيز ، 15 اليوم السادس من بدء المعاملة بينما تراوح زمن قتل الرؤيسات في اليومين الثالث والثاني عند استعمال التراكيز (% على التوالي 10 .

$P < 0.01$ ولا توجد فروق معنوية عند استعمال المستخلص الكحولي للنبات ولنفس التراكيز أعلاه عند المستوى فلهذا اعتمد على دراسة المستخلص المائي لقلته تكلفته ولضمانته .

30 عقاري التينيدازول و البرازيكوينتل أظهرها فعالية كبيرة في قتلها للرؤيسات الأولية فقد كان زمن قتلها في الدقيقة % (59) على التوالي ، حافظ المحلول هناك على حيوية الرؤيسات بنسبة 2.5 ، 0.2 عند التراكيز (60 والدقيقة 21 . لغاية اليوم

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