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

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ABSTRACT

The main objective of this study was to evaluate the activity of aqueous and alcoholic extracts of *Cyperus longous* (Rhizomes) on killing the (larval stage) 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 showen 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 6^{th} day , while the time of killing protoscolices was in the 3^{rd} day and 2^{nd} 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 showen the great activity on killing the protoscolices in the ½ an hour and an hour at (2.5 , 0.2) % respectively.

The preservative solution (Hankó 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 worldó 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 this 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 : The Rhizomes of plant *Cyperus longous* (Cyperaceae) was used in this study . the plant material have been ground after being dried . the extraction of plant were prepared according to .[10]

Antibiotics : Two commercial antibiotics were used in this study. Tinidazole , Praziquantel .

Experimental design: Protoscolices were collected from sheep hydatid cysts. The protoscolices from the hydatid cysts fluid and germinal layer preserved in preservative solution [5] was divided into 15 groups , each group contain six laboratory mice.

Eight groups were treated with each of live concentrations (5 , 10 , 15 , 20) % for both aqueous and alcoholic extract of the plant. Six groups treated with the concentrations (1.25 , 1.8 , 2.5)% of the drug Tinidazole and (0.1 , 0.15 , 0.2)% of the drug Praziquantel and the last was control group without treatment .

Percentages of protoscolices killing were determined by examining them with dissecting microscope for permeability of aqueous eosin stain (1%) evagination, motility .[11]

The viability for each of treated and control group were examined in serial period times until dead all protoscolices.

Complety 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 longous* 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% showed great effects on killing protoscolices in the two drugs studied Tinidazole and Praziquantel respectively . Table (2 , 3)

In control group the time, the viability of protoscolices preserved in Hankó 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.

جدول (1) معدل المتوسط الحسابي لبقاء الرؤيسات الأولية بعد تعريضها لتراكيز مختلفة من المستخلص الفينولي لثمار نبات البلوط وغلافها

مدة المعاملة	معدل المتوسط الحسابي لبقاء الرؤيسات/ المدة الزمنية بعد المعاملة بالمستخلص						
التركيز	1 يوم	2 يوم	3 يوم	4 يوم	5 يوم	6 يوم	7 يوم
% 5	73	46	29	25	13	0	0
% 10	68	19	0	0	0	0	0
% 15	51	0	0	0	0	0	0
% 20	0	0	0	0	0	0	0
Control	100	99	98	98	87	80	79

R.L.S.D =12.38

جدول (2) معدل المتوسط الحسابي لبقاء الرؤيسات الأولية بعد تعريضها لتراكيز مختلفة من العقار تينيدازول

مدة المعاملة / التركيز	معدل المتوسط الحسابي لبقاء الرؤيسات/ المدة الزمنية بعد المعاملة بالعقار					
	0 دقيقة	30 دقيقة	60 دقيقة	90 دقيقة	120 دقيقة	150 دقيقة
1.25	100	17	11	9	0	0
1.8	89	2	0	0	0	0
2.5	48	0	0	0	0	0
Control	100	100	100	100	100	100

R.L.S.D =14.79

جدول (3) معدل المتوسط الحسابي لبقاء الرؤيسات الأولية بعد تعريضها لتراكيز مختلفة من العقار برازيكوينتل

مدة المعاملة / التركيز	معدل المتوسط الحسابي لبقاء الرؤيسات/ المدة الزمنية بعد المعاملة بالعقار					
	0 دقيقة	30 دقيقة	60 دقيقة	90 دقيقة	120 دقيقة	150 دقيقة
0.1	94	42	6	19	18	0
0.15	85	37	15	0	0	0
0.2	70	6	0	0	0	0
Control	100	100	100	100	100	100

R.L.S.D =14.1

Cyperus longous (Rhizomes)

تأثير المستخلص المائي والكحولي لنبات السِعد *Cyperus longous* (Cyperaceae) وعقاري (البرازيكوينتل والتينيدازول) على قتل الرؤيسات الأولية لطفيلي المشوكة الحبيبية *Echinococcus granulosus* في المختبر

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الخلاصة

الهدف الرئيسي لهذه الدراسة هو تقييم فعالية المستخلص المائي والكحولي لريزومات نبات السِعد *Cyperus longous* (Cyperaceae) على قتل الطور اليرقي (الرؤيسات الأولية) لطفيلي المشوكة الحبيبية *Echinococcus granulosus* في المختبر مقارنة مع فعالية العقارين تينيدازول وبرازيكوينتل ضد الرؤيسات الأولية وذلك

باستعمال التراكيز (5 , 10 , 15 , 20) % للمستخلص النباتي المائي والكحولي و (1.25 , 1.8 , 2.5)% ، (0.2 , 0.15 , 0.1)% للعقارين تينيدازول وبرازيكوينتل على التوالي .

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

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

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

DISCUSSION

There is no drugs 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 .[12]

The use of this medicinal plant *Cyperus longous* extracts was found in our experiments previous studies indicated the role of extracts of *Cyperus longous* as antibacterial agents against various bacterial types such as *Staphylococcus sp*. & *Pseudomonas sp.* , and also as fungicide to prevent infection of wounds .[8]

In two studies , one done in Thailand and the other in Tanzania , compound found in extracts from the root of *Cyperus longous* were isolated and several were found to have antimalarial properties.

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 *Echinococcus granulosus* collected from sheep specimens by low concentrations of drugs and short time of treatment .

The activity of this plant on killing the protoscolices in 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 . [13]

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 .[14]

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.

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