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

Fatin Abdul-Jabbar-Mustafa
Department of Biology · College of Education · University of Basrah, Basrah, Iraq.
(Received 12 December 2008, Accepted 23 February 2009)

Key words: Cyperus longous, Protoscolicidal, Anthelmentic.

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 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 ½ an hour and an hour 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 cyclozoontic 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 herbivors.[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) there is a noticeable effect of the drug Tinidazole and Praziquantel on killing the protoscolices.

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.

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

*Cyperus longus* completely inhibited the growth of staphylococcus and Pseudomonas bacteria which cause severe and sometimes fatal infections.

It is also spread on the skin as a bactericide and a fungicide to prevent infection of wounds. A recent Japanese study indicates that cyperus extract act as an anticoagulant by preventing blood platelets from clumping together to form clots.

The Egyptian 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 about effects of these plant extract and the drugs Tinidazole and Praziquantel on the protoscolices of sheep *E. granulosus*, therefore, this investigation was designed to study this subject in vitro.

**MATERIALS AND METHODS**

**Plant extract:** The Rhizomes of plant *Cyperus longus* (Cyperaceae) was used in this study. The plant material have been ground after being dried. The extraction of plant were prepared according to .

**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 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 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>
<thead>
<tr>
<th>متوسط الحسابي لبقاء الروئيات/المدة الزمنية بعد المعاملة بالمستخلص</th>
<th>مدة المعالجة</th>
</tr>
</thead>
<tbody>
<tr>
<td>79</td>
<td>80</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

R.L.S.D =12.38
جدول (2) معدل المتوسط الحسابي لبقاء الرويسات الأولية بعد تعرضها لتراكيز مختلفة من العقار تينيديازول

<table>
<thead>
<tr>
<th>المدة الزمنية (دقيقة)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>11</td>
<td>17</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>2</td>
<td>48</td>
<td>48</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.15</td>
<td>6</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.L.S.D = 14.79</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

جدول (3) معدل المتوسط الحسابي لبقاء الرويسات الأولية بعد تعرضها لتراكيز مختلفة من العقار برازوكينتول

<table>
<thead>
<tr>
<th>المدة الزمنية (دقيقة)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>18</td>
<td>19</td>
<td>6</td>
<td>42</td>
<td>94</td>
</tr>
<tr>
<td>0.2</td>
<td>0</td>
<td>15</td>
<td>37</td>
<td>85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.15</td>
<td>0</td>
<td>6</td>
<td>70</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.L.S.D = 14.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
تأثیر المستخلص المائي والكحولي لنبات البسَد وعقاري (البرازيكويتِل والتينيدازول) على قتل الرؤیسات الأولیة لطفيلي المشوكة الحبيبية في المختبر Echinococcus granulosus

فاطن عبد الجبار مصطفى
قسم علوم الحياة، كلية التربية، جامعة البصرة، البصرة، العراق.

الخلاصة

الهدف الرئیسي لهذه الدراسة هو تقیم فعالیة المستخلص المائي والكحولي لریزومات نبات البسَد Cyperus longous (Cyperaceae) على قتل الطور البرقی (الرؤیسات الأولیة) لطفيلي المشوكة الحبيبية Echinococcus granulosus في المختبر مقارنة مع فعالیة العقارین تینیدازول وبرازیکویتِل ضد الرؤیسات الأولیة وذلك

Cyperus longous (Cyperaceae)
بااستعمال التراكيز (5 , 10 , 15 , 20) % للمستخلص النباتي المائي والكحولي و (25 , 1.2 , 1.8 , 2.5) % (0.2 , 0.15 , 0.15 , 0.1) % للأعفارين تينيدازول وبرازيكوينتيل على التوالي

أظهرت نتائج الدراسة أن المستخلص المائي يتركز 20% يملك أكبر فعالية في قتل الروئيات حيث ماتت جميع الروئيات الأولية في اليوم الأول من المعاملة بينما أظهر التركيز 5% أقل فعالية في قتله للروئيات الأولية وكان في اليوم السادس من بدء المعاملة بينما تراوح زمن قتل الروئيات 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 فле هذا

اعتمد على دراسة المستخلص المائي لقمة تكمفتو ولضمانه.

عقاري التينيدازول و البرازيكوينتيل أظهرت فعالية كبيرة في قتلهم للروئيات الأولية فقد كان زمن قتلهم في الدقيقة 30 والدقيقة 60 عند التراكيز (2.5 , 0.2) % على التوالي ، حافظ المحلول هانك على حيوية الروئيات 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.

REFERENCES


