

**IN VITRO EFFECTS OF ALCOHOL GARLIC EXTRACTION ,  
FORMALIN AND GLUCOSE ON PROTOSCOLICES OF  
*ECHINOCOCCUS GRANULOSUS*.**

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**ABSTRACT**

This study to evaluate the protoscolicidal effects of various concentration of alcohol garlic extraction ,formalin and glucose .Liver and lung protoscolices of sheep were exposed to 100ul,200 ul,500 ul & 1000 ul of alcohol garlic extraction ;100ul ,500 ul & 1000 ul of formalin; 10%,20% ,30% &50% of glucose were used as the positive control ,while physiological saline was used as negative control. Viability of protoscolices was determined using 1% of aqueous eosin stain method. The percentage ( 100%) of mean of dead protoscolices after 1 hr ,2.5 hr , 3 hr& 5 hr of 1000 ul, 500 ul ,200 ul & 100 ul of alcohol garlic extraction respectively . The statistical analysis ( T- test ) showed that was significant differences between the means protoscolicidal effects of alcohol garlic extraction & physiological saline ( $P<0.01$ ).

The percentage average of dead protoscolices (100%) in ( 10,15,20 ) min after exposure with ( 1000 ,500 &100 ) ul of formalin respectively. The statistical analysis showed that there was significant difference between the protoscolicidal effect of formalin & physiological saline (  $P < 001$ ).

The percentage average of dead protoscolices (100 %) was noticed with 50 % of glucose after exposure 10 min &3 hr,4 hr after treated with 30 % & 20 % of glucose

respectively. Significant difference was observed between the means protosclidal effect of glucose & physiological saline ( $P < 0.01$ ).

### Introduction

*Echinococcus granulosus*, which is the causative agent of cystic hydatid disease (or cystic echinococcosis, CE); Infections with *Echinococcus granulosus* occur worldwide, predominantly in countries of South and Central America, the European and African part of the Mediterranean area, the Middle East and some sub-Saharan countries, Russia, China, Turkey, Iran & Iraq [1,2, 3]. The annual incidence rates of diagnosed human cases/100,000 inhabitants vary widely, for example 13 in Greece, 143 in some provinces of Argentina, 197 in the Hingang province of China and 220 in the Turkana district of Kenya. Most cases observed in Europe and the USA are associated with immigrants from highly endemic areas [4,5].

Various strains of *E. granulosus* have been described, and differ especially in their infectivity for intermediate hosts such as humans. The most important strains for human infection include sheep and cattle as intermediate host [4].

The aim of the present work to evaluate the protosclidal effects of various concentration of alcohol garlic extraction, formalin & glucose.

## MATERIALS AND METHODS

100ul, 200 ul, 500 ul 1000 & ul of alcohol garlic extraction [6], 100 ul, 500 ul & 1000 ul of formalin, 10% 20% 50% & 30% of concentration of glucose & 1ml of physiological saline (0.9%) of sodium chloride as control group were used in this study. Sheep liver & lung having hydatid cyst were transferred to parasitology lab. of medical technology department of Basrah Technical Institute within an hour after slaughter in Basrah abattoir. Cyst surface were sterilized by heat and the cyst content

were evacuated completely and isolated hydatid sand fluid and germinal layer were cut into small pieces and washed in hank s balanced salt solution [ 7]. The protoscolices were precipitated& separated [ 7 ].

In order to determine the viability \ vitality of protoscolices,0.01 ml of pool protoscolices was transferred over slide and mixed by 0,01ml of 1% aqueous eosin stain and was examined by low power of microscope.Dead protoscolices absorbed eosin and colored red but alive protoscolices remain green or colorless.

At least (150-200) protoscolices were counted for each experiment .Each experiment was repeated 5 times.

The statistical analysis ( T- test ) was used for comparing the mean dead or viability protoscolices for different materials.

## **RESULTS**

The results of effected alcohol garlic extraction on protoscolces of *Echinoicoccus granulosus* .The mean percentage of dead protoscolices and this agent used to the time exposure were summarized in table(1). protoscolices were killed (100%) in1 hour,2.5 hr,3hr &5 hr of 1000 ul ,500ul,200 ul&1 00ul respectively .

In control group (physiological saline),the viability of protoscolices preserve. The percentage of mean dead protoscolices were 6%,4.5%,4%,3%,2.5%2.2%,2%, 1.5%&1 % according to the time of exposure 6 hr,5 hr,4 hr,3 hr,2.5 hr,2 r,1hr,30 min 15&min respectively.

Statistical analysis ( T- test ) showed that there was high significant difference between the means protoscolicidal effect of alcohol garlic extraction& physiological saline (  $P < 0.01$  ).

In present work refers the formalin has a great effect on protoscolices viability in comparison with other protoscolicidal. The percentage average of dead protoscolices (100%) in (10,15,20) min after treated with ( 1000,500,100)ul of the formalin respectively Table ( 2 ).

The statistical analysis referred that there was high significant difference between the means protoscolicidal effect of formalin & physiological saline (  $P < 0.01$  ).

Hypotonic glucose in present study (10,20,30)% concentration had negligible protoscolicidal effects even 10 min of exposure while in 50% of glucose concentration had higher protoscolicidal effect (100%) while (3,4) hr after treated with 30% & 20% of glucose concentration respectively to kill all protoscolices Table (3) .

Significant difference was observed between the means protoscolicidal effect of glucose & physiological saline (  $P < 0.01$  ).

Table (1) : protoscolicidal effects of different alcohol garlic extraction according to the time of exposure

Average of dead protoscolices %												
Alcohol garlic extraction	No. Of tests	5 min	10 min	15 min	30 min	1 hr	2 hr	2.5 hr	3 hr	4 hr	5 hr	6 hr
100 ul	5	18	23	32	50	60	70	80	82	93	100	
200 ul	5	21	25	35	51	65	80	92	100			
500 ul	5	22	32	38	60	70	89	100				
1000 ul	5	25	35	44	70	100						
Physiological saline	5	0	0	1	1.5	2	2.2	2.5	3	4	4.5	6

Table (2) : : protoscolicidal effects of different concentration of formalin according to the time of exposure

Average of dead protoscolices %

Formalin concentration	No. of tests	5 min	10 min	15 min	20 min
100 ul	5	75	81	92	100
500 ul	5	90	95	100	
1000 ul	5	98	100		
Physiological saline	5	0	0	1	1.1

Table (3) : protoscolicidal effects of different concentration of glucose according to the time of exposure

Average of dead protoscolices %

Glucose concentration	No Of tests	5 min	10 min	15 min	30 min	1 hr	1.5 hr	2 hr	2.5 hr	3 hr	3.5 hr	4 hr	5 hr
10 %	5	1	2	6	8	10	15	22	30	35	40	45	55
20 %	5	4	10	15	40	55	60	70	73	84	90	100	
30 %	5	10	15	40	55	66	73	80	92	100			
50 %	5	90	100										
Physiological saline	5	0	0	1	1.5	2	2.1	2.2	2.5	3	3.5	4	4.5

## DISCUSSION

In present study the chemotherapeutic efficiency of alcohol garlic extraction ,formalin & glucose solution on viability of protoscolices of *Echinococcus granulosus* were isolated from liver & lung from Basrah abattoir . No data are available in pervious studies detailed this subject in Iraq.

The best treatment is considered surgery .During the surgery and before evacuation of cyst ,protoscolicidal agents are used to be injection into the cyst in order to prevent secondary cyst formation [8 ] .The most common protoscolicidal agents used are cetrimide ,hypertonic saline, silver nitrate and formalin each has a variety of dangerous complication such as biliary tract fibrosis and liver necrosis [9, 10, 11,12] . In this regard ,World Health Organization (WHO) purposed an urgent need to find new protoscolicidal agents which are effective and with less complications [13 ] .

In present study recorded good protoscolicidal of alcohol garlic extraction ,this results of the present study were confirmed with study of [14 ] . The garlic derives were used as parasitocidal against of other parasites as *Ascaris lumbricoides*, *Hymenolepis nana* ,*Giardia lamblia*, *Plasmodium bergdei*,*Trypanosoma brucei* & *Entamoeba histolytica* [ 15,16, 17, 18, 19 ]. Chemical composition of garlic and its derives as antioxidative ,Allicine,Ajoene (an organosulfur compound derived from garlic) and others [6,20]. The main active ingredient in garlic is Alliin. Allicin is the active compound that gives garlic its characteristic odor and many of its healing benefits. Other sulphur compounds are thiosulfinates, gamma glutamylcysteine peptides and various Cu-peptides, 2 mercapto-L-cysteins, anthocyanins, glycosides of kaempferol and quercetin, polysaccharides, allinase, sterols, hydrocarbons, sativin I & II, scordinines A & B [6,20,21].

In present work refers the formalin has a great effect on protoscolices viability in comparison with other protoscolicidal . The percentage average of dead protoscolices (100%) in (10,15,20) min after treated with ( 1000,500,100)ul of the formalin respectively.

A direct communication between the hydatid cyst and the biliary tree may contraindicate the use of protoscolicidal solutions, which can cause chemical cholangitis leading to sclerosing cholangitis. Formalin should not be used for this reason[ 7,23 ]. Effective protoscolicidal with a relatively low risk of toxicity are 70–95% ethanol or 15–0% hypertonic saline solution [4] . Surgery remains the mainstay in the treatment of hepatic hydatid disease. Cystectomy and pericystectomy offer a good chance for cure and should be undertaken wherever possible. Occasionally, formal hepatic resection will be required. Radical surgery – either pericystectomy or resection – is possible in 50–85% of cases. In the absence of complications this can be achieved with little mortality and an acceptable morbidity. Recently, laparoscopic pericystectomy has been demonstrated to be as safe and effective as open laparotomy in selected cases with hepatic and/or splenic involvement[ 22] .

Hypotonic glucose in present study (10,20,30)% concentration had negligible protoscolicidal effects even 10 min of exposure while in 50% concentration had higher protoscolicidal effect (100%). Hypertonic glucose was reported to be a successful protoscolicidal agent for pericardial hydatid cyst [ 23 ]. Results of glucose in present work were confirmed result of others as[ 5,7 , ]. Therefore the strain of *E. granulosus* were isolated from Basrah abattoirs \Iraq in the present study closely similar that in others countries as Iran ,Italia , in spit of there are at least nine strains of *E. granulosus* that have adapted to different hosts and in most cases occupy a wide geographical area [4].

As the viability protoscolices with the criteria used in vitro studies is doubtful ,it should be reminded that exposed protoscolices have to be injected into the mice peritoneum for confirmation of its protoscolicidal effects [ 9] . In addition before usage of 50% glucose as protoscolicidal agent ,its probable complication over the internal body organs and biliary system should be evaluated.

## **تأثير مستخلص الثوم الكحولي والفورمالين وسكر الكلوكوز على الرؤيسات الأولية لطفيلي الاكياس العذرية خارج الجسم الحي**

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### **الخلاصة**

هذه الدراسة لتقييم تراكيز مختلفة من مستخلص الثوم الكحولي ،الفورمالين وسكر الكلوكوز على الرؤيسات الأولية لطفيلي الاكياس العذرية لأكباد وراثت الأغنام ،عوملت هذه الرؤيسات ب 100 مايكروليتر ، 200 مايكرو ليتر 500 مايكرو ليتر و 1000 مايكرو ليتر من مستخلص الثوم الكحولي ، 100 مايكرو ليتر ، 500 مايكرو ليتر و 1000 مايكرو ليتر من الفورمالين ، و 10%، 20% ، 30% و 50% من الكلوكوز وقورنت بمحلول الملح الفسلولوجي . استخدمت تقنية التصبيغ بمحلول الايوسين المائي ( 1% ) لتقييم حيوية الرؤيسات الأولية . بلغت نسبة موت الرؤيسات الأولية ( 100% ) بعد ساعة ، ساعتين ونصف ساعة ، ثلاث ساعة و خمس ساعات لكل من 1000 مايكرو ليتر ، 500 مايكرو ليتر 200 مايكرو ليتر و 100 مايكروليتر من مستخلص الثوم الكحولي على التوالي . وجدت فروق معنوية بين التراكيز المختلفة لمستخلص الثوم الكحولي ومحلول الملح الفسيولوجي (  $0.01 <$  )

أظهرت نسبة موت الرئيسات الأولية ( 100% ) بعد تعرضها ( 2, 10, 15, 20 دقيقة للتراكيز ) 100 ، 500 ، 1000 مايكروليتر على التوالي من الفورمالين. إحصائيا وجود أن هناك فروق معنوية بين الفورمالين ومحلول الملح الفسيولوجي (  $0.01 <$  )

بلغت نسبة موت الرؤيسات الأولية ( 100% ) بعد تعرضها ل 50% من سكر الكلوكوز بعد 10 دقيقة و 3 و 4 ساعة بعد تعاملها بتراكيز ( 20%، 30% ) على التوالي . وجدت فروق معنوية بين الكلوكوز ومحلول الفسيولوجي (  $0.01 <$  )



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