Effect of some aqueous plants extracts on the viability of protoscolices for *Echinococcus granulosus* in vitro

Received :10/ 2 / 2016 **Accepted : 12 / 4 /2016**

Mohammed Hassen Fleih Al-Kurayshi

Department of Medical Laboratory / Al-Nasiriya Technical Institute/ Southern Technical University

Email: m_alkurayshi@yahoo.com

Abstract

The present study was done to evaluate the effectiveness of the cool aquatic extract of *Spinacia oleracea*, *Ziziphus spina* and *Citrus aurantifolia* on protoscolices viability in vitro. The samples of hydatid cysts collected from sheep, then Calculated the number of protoscolices in one drop. A numbers from concentrations tested per-extract for determined periods. The results of *Citrus aurantifolia* extract study appeared significant decrease in protoscolices viability percentage reached to 0% at concentrations (15, 20, 50 and 100) mg/ ml after (96, 72, 48and 24) hr. respectively, while results of *Ziziphus spina* extract showed decrease in viability percentage reached to 0% at concentrations (15, 20) mg/ ml after 96 hr. , whereas the results of *Spinacia oleracea* extract recorded lower viability percentage (18.35%) at concentration of 20 mg/ ml after 96hr. The results of present study revealed that the aquatic extract of *Citrus aurantifolia* was more effective in inhibiting the viability of protoscolices then *Ziziphus spina* in comparison with control specimen.

Kay words: plant extract, hydatid cyst protoscolices and protoscolices viability

Introduction

Hydatid cyst is zoonotic diseases between human and animal, endemic in the Arabic world and most countries of the world [1]. Larval stage for *Echinococcus* genus parasite to the causes hydatid cyst in human and other hosts (sheep, cows, buffaloes, camels, horses, pigs and other animals), this stage can attacks any organ in intermediate host body [2]. Life cycle of parasite need to intermediate and definitive host (dogs, wolves, hyenas, foxes, and other animals species canis) [3]. Adult worm lives in small intestine for definitive host, attach itself to the intestinal mucosal layer by a row of spicules in the head [4,5]. The eggs can remain for a year capable on infection and resistance to environmental conditions [6]. Intermediate host infects when dealing with eggs, then hexacanth embryo liberate after eggs hatching in the duodenum [7]. penetrate the intestinal mucosa and reach the portal circulation and transfer to entrails, grows to cyst in 4 days, those cysts filled with fluid include protoscolices, all of them have the ability to grow into adult worm when digest by the final host [8]. Echinococcosis does not appear clinical symptoms for several years, but after increasing of the cyst size because the pressure on nearby tissues form cyst [9]. Surgical intervention of the best therapeutic methods thus far despite the difficulty in some cases [10]. There are some side effects for chemical compounds for therapeutic medicines on human or animal which may affect on some of the vital organs such as the liver, kidneys, pancreas and some cells [11]. Medicinal plants plays a role in the treatment of some diseases in humans and animals because they contain influential materials on parasites, bacteria and fungi [12]. Spinach full of vitamins and minerals and a number of anti-oxidants components such as polyphenols, carotenoids and Flavonoids which possess anti-inflammatory effects [13,14].The studies have shown that Zizphus plant contain many acids, alkaloids, saponins and flavonoids isolated from the leaves and fruits of Zizphus [15]. Zizphus plant contains alkaloids that have a pharmacological effect on humans where they work as sedatives and as fatal or retardant to growth of microorganisms and anti-parasitic [16]. In addition to organic acids (high concentrations) especially

citric acid also, lime oil [17]. Lime oil use in treatment of many diseases, antiseptic against viruses, appetitive, germicidal and analgesic of fever [18]. This study aims to knowledge of aqueous extracts effect on protoscolices viability.

Materials and methods

Samples collection and protoscolices isolation:

Samples of hydatid cyst collect from infected sheep livers in massacre of the Nasiriyah city in Dhi-Qar province, the samples transfer cool boxes and It was dealt with during a period not exceeding three hours. Method [19] adopt with protoscolices preparation, surface of the cyst sterilize with a 70% ethanol then the hydatid fluid draw by syringe (10 ml) and was transferred to sterile container (250 ml), the cyst open with scissors and was placed in sterile vessel, cut to small pieces and it is put in bottle with the amount of Kreb's - Ringer's solution (K.R.S.) (solution prepare according to [20]. The bottle shake fully then fluid filtrate by sterile refinery allows the passage of protoscolices. The filtrate washes three times with Kreb's - Ringer's solution then deposit with the centrifuge 1500 r / min for 10 minutes, the sediment save with the Keeper medium (1-4) in a clean container in the incubator at 20-25 °C.

Evaluation of protoscolices viability :

Protoscolices viability evaluate by take 10 µl from the suspension of the protoscolices was mixed together with 10 µl of 0.1% aqueous eosin on glass slide. Calculate living protoscolices numbers that appear by bright green color while dead protoscolices stain by red color for stain leverage inside protoscolices [21].

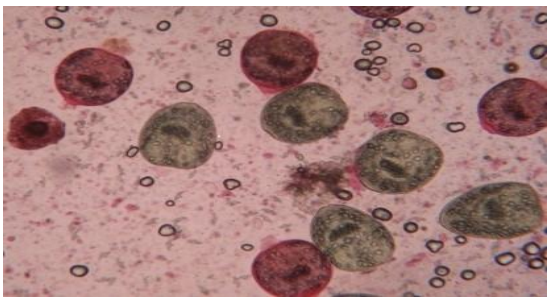

Fig (1) show living and dead protoscolices (Eosin 40X)

Calculate the number of protoscolices :

Number of protoscolices calculate by using the method of constant size transfer by micropipette (10 µl), after shark protoscolices suspension

The average number of protoscolices constant size (10 µl) = 20.25 protoscolice for four replicates (22, 19, 22, 18).

A size of one drop = 50 µl , 20.25 × 50/10 = 101.25 protoscolices .

The number of protoscolices per milliliter = 20.2 × 100 = 2020 protoscolice in one milliliter.

The required approximate number of 2,000 protoscolice is a bright green color and protoscolices stained with red color has been neglected for being dead.

Preparation of the cool aqueous extracts for Spinach, Zizphus and Lime :

It adopted the method [22] for prepare of aqueous extract for leaves of Spinach , Zizphus and Fruits of lime. Spinach, Zizphus and Lime are wish with distilled water and left to several days even completely dry then grind at electric mill. Weigh 15 g from powder of the dry matter per plant and put in a special flask 500 ml and add 100 ml from distilled water for it. The flask leave within shaking incubator for 24hr 37°C. The aqueous extract filtrate by medical gauze to remove large parts of the plant then by using of filter papers. The filtrate put within centrifuge 2500 r / min for 15 minutes then intensify by rotary evaporator until getting on a thick liquid at a temperature of 60°C. The thick liquid within incubator 37°C for 48-72hr for dry powder then put in the refrigerator under 4°C. Effect of the aqueous extract tests for each extract on protoscolices viability.

statistical analysis :

The results were analyzed statistically using by SPSS statistical analysis program, paired-samples T test.

The results

The table (1) shows results of Spinach extract effect on protoscolices viability in vitro by a concentrations of 5, 10, 15, 20 mg/ml in comparison with control specimen. The results recorded decrease in viability percentages extrusive proportion with concentration increase and extract exposure time (82.29% - 18.35%). The study disclose non- presence of significant difference between 5 mg/ml concentration and control specimen while other concentrations recorded significant differences in comparison with control specimen.

Tab (1) Effect of the different concentrations from aqueous extract for *Spinacia oleracea* on protoscolices viability after different periods at P<0.05.

Time / Con. (mg/ml)		No. of protoscolices in one drop (50 µl)			
		24 hr.	48 hr.	72 hr.	96 hr.
Control speci.	Total	99	95	99	100
	Alive	84 a	76.5 a	70.5 a	60 a
Viability percent.		84.85	80.53	71.21	60.00
5	Total	96.5	100	99	97
	Alive	80 a	77 a	70 a	59 a
Viability percent.		82.29	77.00	70.71	60.82
10	Total	95	98	100	96
	Alive	74 b	66.25 b	57.25 b	40 b
Viability percent.		77.89	67.60	57.25	41.67
15	Total	96	94.5	95	95
	Alive	59.25 c	50 c	42.5 c	31 c
Viability percent.		61.72	52.91	44.74	32.63
20	Total	93	95	94	94
	Alive	40 d	33.5 d	21 d	17.25 d
Viability percent.		43.01	35.26	22.34	18.35

Different characters refer to significant differences presence within one column

The table (2) shows results of Zizphus extract effect on protoscolices viability in vitro by a concentrations of 5, 10, 15, 20 mg/ml in comparison with control specimen. The present study showed decrease in percentages of protoscolices viability in 15 mg/ml concentration from 42.71% - 0.00% with exposure time increase. The results showed presence of significant differences between concentrations and control specimen.

Tab (2) Effect of the different concentrations from aqueous extract for *Ziziphus spina Christi* on protoscolices viability after different periods at P<0.05.

Time / Con. (mg/ml)		No. of protoscolices in one drop (50 µl)			
		24 hr.	48 hr.	72 hr.	96 hr.
Control speci.	Total	99	95	99	100
	Alive	84 a	76.5 a	70.5 a	60 a
Viability percent.		84.85	80.53	71.21	60.00
5	Total	99	100	97	95
	Alive	73.5 b	60 b	47.5 b	30 b
Viability percent.		74.42	60.00	48.97	31.58
10	Total	96	95.5	98	97
	Alive	58 c	39 c	20 c	8.5 c
Viability percent.		60.42	40.84	20.41	8.76
15	Total	96	97	96	96
	Alive	41 d	29.75 d	12.25 d	0 d
Viability percent.		42.71	30.67	12.76	0.00
20	Total	95	93.5	93	90
	Alive	29 e	14.5 e	5.25 e	0 d
Viability percent.		30.53	15.51	5.65	0.00

Different characters refer to significant differences presence within one column

The table (3) shows results of lime extract effect on protoscolices viability in vitro by a concentrations of 5, 10, 15, 20, 50, 100 mg/ml in comparison with control specimen. The present study showed decrease in percentages of protoscolices viability in concentration of 15 mg/ml from 33.07% - 0.00% with exposure time increase, while showed concentration of 20 mg/ml from 15.05% - 0.00% and concentration of 50 mg/ml from 6.59% - 0.00% with exposure time increase while 100 mg/ml concentration was the better in protoscolices killing. The results showed presence of significant differences between concentrations and control specimen.

Tab (3) Effect of the different concentrations from aqueous extract for *Citrus aurantifolia* on protoscolices viability after different periods at P<0.05.

Time / Con. (mg/ml)		No. of protoscolices in one drop (50 µl)			
		24 hr.	48 hr.	72 hr.	96 hr.
Control specie.	Total	99	95	99	100
	Alive	84 a	76.5 a	70.5 a	60 a
Viability percent.		84.85	80.53	71.21	60.00
5	Total	98	97	101	97
	Alive	73.5 b	62.75 b	50 b	30.5 b
Viability percent.		80.61	64.69	49.50	31.44
10	Total	95	97	98	97
	Alive	50 c	42 c	30.25 c	5 c
Viability percent.		52.63	43.30	30.87	5.15
15	Total	96	96.5	94.75	97
	Alive	31.75d	22 d	11.25 d	0 d
Viability percent.		33.07	22.80	11.87	0.00
20	Total	93	93.5	92.25	92
	Alive	14 e	9 e	0 e	0 d
Viability percent.		15.05	9.68	0.00	0.00
50	Total	91	90	89	90
	Alive	6 f	0 f	0 e	0 d
Viability percent.		6.59	0.00	0.00	0.00
100	Total	86.75	88	86	85.5
	Alive	0 g	0 f	0 e	0 d
Viability percent.		0.00	0.00	0.00	0.00

Different characters refer to significant differences presence within one column

Discussion

The results of present study showed that plants extracts have inhibitive efficiency against protoscolices but by different degrees. It adopted eosin stain permeation for measure of protoscolices viability without depending on movement or evagination, due to the difficulty of distinguishing between static and motile protoscolices, while evagination method does not give accurate results because some of alive protoscolices are unable on evagination for physiological causes and calculated within dead protoscolices

whereas eosin stain permeation is physical process related to the permeability of the biosphere, the dead protoscolices stain by red color while alive protoscolices remain with normal green color because non-permeation of the eosin stain [23]. The study results showed that exposure of protoscolices to different concentrations from spinach extract, a significant decrease of protoscolices viability rate, extrusive proportion with concentration increase and extract exposure time. Spinach extract does not show high inhibitive efficiency in protoscolices killing. This study recorded results higher from results of [24] which used the aqueous extract of *Thymbra spicata* and *Prosopis fracta* and less from Citrullus aqueous extract. The zizphus aqueous extract showed clear significant differences between different concentrations compared with control specimen, the best concentration in protoscolices killing was 15 mg/ml in four day, this result approach to [25] who recorded reducing of protoscolices mean from 87 to 9 at the sixth day of the experiment with the (10 %) concentration and from 89 to zero at the second day with the (30 %) concentration from cool aqueous zizphus extract. A extract efficiency in protoscolices viability reduction may be due that extract contains flavonoids which consider one of the main components for zizphus plant [15]. Also contains saponins that play major role in parasite proteins metabolism, its ability to destroy the cell membrane also the effect on mitochondria, thus effect on respiration mechanism then parasite death [26]. Flavonoids and saponins effect on protoscolices viability by interference these materials with enzymes, proteins and carbohydrates of protoscolex. The lime aqueous extract revealed presence of significant differences between different concentrations compared with control specimen, the concentration of 15 mg/ml was mean of alive protoscolices 0.00 in four day, while 20 mg/ml in three day, 50 mg/ml in two day, finally 100 mg/ml in one day was 0.00 alive protoscolices. The present study agree with result of [24] which showed that alcoholic extract of *Prosopis fracta* has achieved full killing for protoscolices. The inhibitive efficiency may be due to contain it citric acid, oil, flavonoids, nitrogenous compounds, vitamin and potassium, these are effect on protoscolex osmotic or inhibition of protoscolice metabolism or

analysis of part from cell membrane where coverts from optional of permeability to full permeability [27]. Citric acid and potassium may be effect on permeability of plasma membrane, also nitrogenous compounds may effect on protoscolex metabolism.

References

1-**Salih, S.S., (2001).** Effect of praziquantel and fenbendazole on hydatid protoscolices of *Echinococcus granulosus In vitro* and in vivo. Kufa.Med.J., 4(1): 34-38.

2- Kismet, K. ; Kilicoglu, S. S. ; Kilicoglu, B. ; Erel, S. ; Gencay, O. ; Sorkun, K. ; Erdemli, E. ; Alkhan, O. ; Akkus, M. A. and Sayek, I. (2008). The Effect of scolicidal Agent propolis on liver and biliary tree. J. Gastroenterol. Surg. , 12: 1406-1411.

3- Abu-Esbeih, T.S. (2001). Anaphylaxis during hydatid liver disease surgery. J. The Arab. Bor. Med. Special., 3(2): 51-52.

4- Roberts , L . and Janovy , J . (2000). Schmidt and Roberts foundations of parasitology sixth Edition . Mc Graw Hill Book Co ., NewYork :670.

5- Nepalia , S . ; Joshi , A . ; Shende , A . and Sharma , S . S .(2006) .Management of Echinococcosis .Department of Gastroenterology ,SMS Medical College and Hospital ,Jaipur .JAPI.Vol.54

6- Ammann , R . and Eckert , J.(1996). Cestodes , *Echinococcus* Gastroenterol .Clin . North America. 25:655- 89.

7- WHO . (2001). WHO/OIE Manual on Echinococcosis in Humans and Animals ,a public Health problem of Global Concern .Eckert , J. Gemmel, MA. Meslin,FX. Pawlowski ,ZS. eds.Geneva :World Health Organization .

8- Thompson , R . C . A . (1995). Biology and systematic of *Echinococcus* .In:Thompson ,R.C.A. Lymbery ,A. J .(eds) .*Echinococcus* and hydatid disease .Wallingford :CAB International.1-50.

9- Dvorak, G.; Rovid-spickler, A. and Roth, J. A. (2008). Hand book for Zoonotic disease of Companion Animal. Center for Food Security and Public Health. Pp120-121.

10- Mentes, A.; Yalaz, S.; Killi, R. and Altintas, N. (2000). Radical treatment for hepatic echinococcosis. HPB. 2(1): 49-54.

11- Hamim, S. S. (2002). The effectiveness of some plant extracts against common disease in bacterial skin infections. Thesis. Coll. Ed. Univ. Basrah, PP: 67.

12- Mansour, A. T. (2005). The Complete Guide to Herbal medicinal plants. second edit. Alahlih of the publication and distribution.

13- Boivin, D.; Lamy, S.; Lord-Dufour, S.; Jackson, J.; Beaulieu, E. ; Cote, M.; Moghrabi, A.; Barrette, S.; Ginras, D. and Beliveau, R. (2009). Antiproliferative and antioxidant activities of common vegetables: Acomparative study. Food Chem.; 112: 374–380.

14- Hait-Darshan, R.; Grossman, S.; Bergman, M.; Deutsch, M. and Zurgil, N. (2009). Synergistic activity between a spinach-derived natural antioxidant (NAO) and commercial antioxidants in a variety of oxidation systems. Food. Res. Intel. 2009; 42: 246–253.

15- Lee, T. C.; Logendra, L.; Pyosy, H. and Rosen, R.T. (2004). Purification of saponins. Food chem. J ; 58:19-26.

16- Nawash, O. S. and Al-Horani, A. S. (2011). The most important medicinal plants in Wadi Araba desert in South West Jordan: A review article. Adv. Environ. Biol., 5(2): 418-425.

17- Sasson, A. and Monselise (1977). Organic acid composition of Shamouti oranges of harvest and during prolonged postharvest storage. J. Am. Soc. Hortic. Sci., 120: 331-336.

18- Sinclair, W. B. (1984). The Biochemistry and Physiology of the Lemon and other *Citrus fruits*. University of California Press, Oakland, California, USA.

19- Smyth, J. D. (1985). *In vitro* culture of *Echinococcus* spp. Proc. 13th Int. Cong. Hydatidol., Madrid: 84-89.

20- Al-hamiary, A. K. A. (2010). Evaluation of the Activity Datura stramonium Seeds Extracts on Growth and Development of Hydatid Cysts for Echinococcus granulosus in White Mice Balb/c (Therapeutic , Histologic and Immunologic Study). M. Sc. Thesis, Coll. sci. Univ. Kufa, PP: 167.

21- Smyth, J. D. & Barrett, N. J. (1980). Procedures for testing the viability of human hydatid cysts following surgical removal, especially after chemotherapy. Trans. Roy. Soc. Trop. Med. Hyg., 74(5): 649-652.

22- Anesini, C. and Perez, C. (1993). Screening of plant used in Argentine folk medicine for antimicrobial activity. J. Ethnopharmacol., 39 (2): 119-128.

23- Risan,F. A. (1994). A study of the possibility of attenuation of protoscolices of *E. granulosus* using different types of Laser rays. M.Sc. Thesis, Coll. Sci. Univ. Baghdad.

24- Kareem, A. A. (1999). Effect of Some Plant Extracts on The Viability of Protoscolices of *Echinococcus Granulosus* That Causes Hydatid Cyst Disease. M. Sc. Thesis, Coll. Sci. Univ. Mustansiriyah.

25- Al-Musawi, S. A. (2014). Effect of Zizphus Plant Extract on The Viability of Hydatid Cysts Protoscolecies In Vitro. Kufa. J. Nursing. Sci., 4(3).

26- Badaraco, J. L.; Ayala, F. J.; Bart, J. M.; Gottstein, B. and Haag, K. L. (2008). Using mitochondrial and nuclear markers to evaluate the degree of genetic cohesion among *Echinococcus populations*. Exp.Parasitol.;119(4):453-9.

27- AL-Kennany, E. R. and Ali, F. F. (2004). A comparative study of the effect of aqueous extract Solanum *nigrum* & albendazole on the viability of protoscolices in vivo & in vitro. AL-Qadisiya J. Vet. Med. Sci. 3(1): 64-69.