

Effect of extracts of *Zizphus spina-christi* L and *Olea europaea* L. on the larval stage of *Echinococcus granulosus*

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Citation

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Abstract

Extracts different parts of two medical plants *Zizyphus spina-christi* L. (Fruits, leaves and Christ) and *Olea europaea* L. (Fruit and leaves) were studied in this investigation against the larval stage (protoscolices) of *E. granulosus*. All aqueous extracts have a antimicrobial activity against the Protoscolices viability, but the greater effect was to 1000 mcg/ml for the leaves of *Z. spina-christi* that kill all protoscolices in 72 hrs after treatment, while the lowest effect was to 50 mcg/ml of Christ of the same plant that decreased the viability to zero % in 240 hrs after treatment, and all other extract effects were between these limits. The protoscolices still live in control group in HCF+NS in ratio 4:1 over than 20 days (480) hrs.

INTRODUCTION

Hydatidosis is a hydatid disease of human and domesticated animals caused by infection with the taeniidae metacestode (protoscolices) as a larval stage of *Echinococcus granulosus*. (Mathis , et al., 2005)

Epidemiological data on the distribution of hydration shows that its prevalence remained at nearly the same level during the last several decades. Moreover, the appearance of the disease within recent years in communities previously free of it, has produced an entirely new global situation. (Satoh , et al.,2005)

Hydatid disease proceeds silently and the symptoms are manifested several years after infection. The severity of symptoms depends mainly on the organ affected the results being due mainly to pressure affects. The organ frequently affected by hydatidosis is the right lobe of the liver, but lungs, kidneys, bones and brain may also be affected (Marchiondo, et al. , 1994 and Zhang ,etal.,2003)

No effective chemotherapy is currently available for the medical treatment of cystic and alveolar hydrated disease in humans. Within recent years several drugs, notably mebendazole, albendazole, praziquantel, mitomycinic, isoprinosine and other on the elminthic drugs have shown promising results in the reduction and induction of ultrastructural damage of the larval cystic mass (Sarciron,

et al., 1992 and Satoh , et al.,2005).

However, the treatment of echinococcosis with these compounds has also shown only acytostatic effects as evidenced by the survival and resumption of cystic growth in treated hosts following cessation of drug treatment or after transplantation of cystic material from drug treated hosts into rodent hosts (Novak, 1990).

Limited inconsistent effects of the above, mentioned drags appear to be related directly to the age of infection, dosage of drug, route of administration, and treatment regimens (Andersen, 1995).

The use of antimicrobial active components that were isolated from higher plants has been limited to antiseptics and disinfectants and these had little applications of such ingredients yet reported in systematic therapy (Orjala et al. , 1998, Lim, et al. 1998 and Walker et al., 2004).

As the Iraqi flora is rich in plants the possibility of finding new antimicrobial agents still widely ahead, so, the aims of this study was to study aqueous extracts of *zizyphus spina-christi* l. (leaves, fruits and tree wood) and *olea europaea* L. (fruits and leaves) and determine the exactly effects of these extracts, on the viability of protoscolices of *Echinococcus granulosus* in comparison with a control group.

MATERIAL AND METHODS

PLANTS:

SAMPLING AND EXTRACTION

1: *Zizyphus spina-christi* L. (Family : Rhamnaceae)

Common name (Christ's thorn) Arabic name: Seddar, Nabuq

Studied parts: fruits, leaves and tree woods (Christ).

2: *Olea europaea* L. (Family: Oleaceae)

Common name (Olive) Arabic name: Zayton

Studied parts: fruits and leaves.

These plants were collected from gardens in the Basrah district. Each dried plant parts were grounded to gm of powder and then mixed with 100 ml distilled water. The mixture is to be mixed by magnetic stirrer for 72 hrs in discontinuous period times – at room temperature and heated for 12 hrs at 50-55 Co with stirring. The mixture was put in centrifuge 3000 xg for 1 hr, then made narrow concentrations from crude supernatant extracts (50, 100, 250, 500, 750 , and 1000 mcg/ml) in order to study the influence of these concentrations on viability of protoscolices (Al-Saimary & Benyan, 1997).

HYDATID CYSTS

Protoscolices were collected from hydrated cysts of sheep. The suspension containing the free protoscolices were preserved in preservative solution

Hydrated cyst fluid (HCF) + Normal Saline (N.S.) in ratio

4 : 1

Percentages of protoscolices viability or survival were determined by examining them by compound microscope for permeability of aqueous eosin stain (0.5%) as a vital stain of protoscolices-, Green protoscolices: Viabel, Red protoscolices: Deal. (Smyth and Barrett, 1980).

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

RESULTS

The percentage of protoscolices viability decreased from 98% to 4% in over than 20 days after being preserved in HCF+NS 4:1 without treatment.Fig-1-

Figure 1

Days after treated	Conc. (mcg/ml)	Time after treated																	
		hrs												days					
	0	0.03	1	2	3	6	12	24	48	72	96	120	6 (144)	7 (168)	10 (240)	15 (360)	20 (420)	25 (500)	
(4) Fruits	1000	98	90	82	76	60	52	40	32	26	16	8	Zero						
	750	98	92	86	78	72	60	52	38	30	24	16	10	Zero					
	500	96	90	84	76	70	64	58	50	46	38	28	20	12	Zero				
	250	96	88	80	72	60	58	52	44	38	30	22	14	8	Zero				
	100	98	92	86	78	64	60	48	42	38	32	28	24	20	6	Zero			
(1) Leaves	1000	98	80	72	60	52	44	38	32	18	10	Zero							
	750	96	86	76	66	50	36	30	20	8	2	Zero							
	500	96	90	82	74	60	52	44	32	20	14	6	Zero						
	250	96	90	78	70	64	58	46	30	18	10	4	Zero						
	100	96	90	84	72	62	50	42	36	28	20	14	8	4	Zero				
(3) Tree wood	1000	98	88	80	70	64	54	48	38	30	26	20	18	12	8	Zero			
	750	98	86	78	68	62	58	40	36	22	16	10	6	Zero					
	500	98	84	76	70	68	54	46	38	28	20	12	8	Zero					
	250	98	90	82	78	70	66	58	50	40	34	22	10	4	Zero				
	100	98	88	84	80	76	68	60	52	44	36	30	24	16	10	Zero			
50	98	90	86	84	82	74	66	58	46	38	34	26	20	12	4	Zero			

In general the concentration 1000 mcg/ml showed great effects on protoscolices viability in all of studies plant extracts, while 50 mcg/ml had the lowest effects. The results of effects of plant extracts were arranged according to a great activity.

Leaves extract of *Zizyphus spina-christi* have a great effects on protoscolices in comparison with other extracts of parts. Into inhibited the viability to zero% in (72, 96, 144 and 168) hrs after treated with (1000 & 750, 500 & 250, 1000 and 50) mcg/ml receptively- Fig-3-.

While other antibiotic were decreased the protoscolices viability to zero% as follows

In (72, 96, 120, 168) after treated with (1000, 500 % 750, 250, 100 & 50) mcg/ml of leaves extract of *Olea europaea* respectively. Fig-6-.

In (96, 120, 168 and 240) hrs after treated with (1000 & 750 & 500, 250, 100 and 50) mcg/ml of fruits extracted of *Olea europaea* respectively. Fig -5-

In (96, 120, 144, 168, 240) hrs after treated with (1000, 500, 520, 100 and 50) mcg/ml of fruits extracts of *Zizyphus spina-christi* respectively. Fig. -2-.

And finally in (120, 144, 168 and 240) hrs after treated with (1000 & 750, 500, 100 & 50) mcg/ml of Tree wood (christ) extracts of *Zizyphus spina-christi*.

DISCUSSION

In this investigation we studied the efficiency of different parts of two plants *Zizyphus spina-christi* and *Olea europaea* against protoscolices viability of *Echinococcus granulosus*.

In accidentally infected human, surgical intervention for removal of hydatid cysts is performed but this surgery is not without risks, and in many countries of the world, the

mortality rate ranges between 1-4% and many reach 20% or more in cases of repeat surgery (Walker et al., 2004 and Mathis, et al., 2005).

Some anthelmintic drugs cause glycogen depletion of the parasite and destroy the microtriches of the germinal layer of hydatid cyst. The main function of the microtriches implanted in the laminated layer, is assimilation of nutrients and physiological homeostasis. (Satoh, et al., 2005).

In this study, our results indicated and evidenced the antimicrobial activity of medicinal plants. Leaves of *Z. spina-Christi* decreased the viability of protoscolices in standardized short time (72) hrs for (1000) mcg/ml to the lowest effect of 50 mcg/ml of Christ of the same plant who reached the viability to zero % in 240 hrs after treatment of this can concentration, and all of others concentrations of other plant part distributed between these two limits times.

These parameters found necessary to give the light spot on the action of these plants and open a wide space for more of future studies to find a typical chemotherapeutic agents against many of microbial diseases-In general –or against hydatidosis- in especially.

Our results confirmed the results of previous studies who carried out other anthelmintic drugs that cause a vacuolization and rupture of the germinal membrane along with swelling and rounding of mitochondria that appears to be non specific degeneration effects that have been previously reported following treatment (Richards, et al., 1989, Sareiron, et al., 1992, Marchiondo, et al., 1994 and Zhang, et al., 2003).

We think that the extracts of the medicinal plants decreased the protoscolices viability due to the important role of these extracts in the break down of the biological activities of protoscolices by interference with its metabolism, and may have target sites such as inhibitors of protein and DNA synthesis or within the cytoplasmic components such as - lactam antibiotics. (Chopra and Hacker, 1992, Oliva, et al., 1992, Bennett Chopra, 1993 and Walker et al., 2004).

CONCLUSION & RECOMMENDATION

1. Six concentrations of aqueous extracts of various parts two medicinal plant *Zizyphus spina-Christi* & *Olea europaea* were used in this study and found that the 1000 mcg/ml has greatest activity while 50 mcg/ml has a lowest activity.

2. Each of two above plants affected on the protoscolices viability.
3. In control group, HCF+NS in ration 4:1 keep the protoscolices viable to over than 20 days (480) hrs.
4. We recommend a continuous work about extracted pure compound from these two plants to find another antimicrobial agents against parasites and / or bacteria that not affected by treated with a common therapeutic agents.

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