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In Vitro Effects of Ethanolic Extract and Crude Alkaloids of *Prosopis farcta* Leaves on the Viability of *Echinococcus granulosus* Protoscolices in Comparison to Mebendazole

Marua H. Hammoshi *Department of Pharmacology College of Pharmacy Musol University*

Adeeba Y. Shareef **Ahmed A. Al-Daoody** *Department of Biology College of Science Musol University*

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ABSTRACT

The scolicidal effects of crude ethanolic extract and crude alkaloids of *Prosopis farcta* leaves on protoscolices of *Echinococcus granulosus* were appraised *in vitro*, in comparison with mebendazole (the drug of choice in the treatment of echinococcosis) at $37C^0$ and $4C^0$ and at different time intervals.

Both extracts exerted higher effect at $37C^0$, and the crude alkaloids were more effective than ethanolic extract as it gave a scolicidal effect especially at the lower concentrations used (62.5, 31.2 mg/ml) within shorter periods (3 days and 13 days at $37C^0$ and $4C^0$ respectively)

دراسة في الزجاج لتأثير المستخلص الكحولي والقلويدات الخام لأوراق نبات البجنجل *Prosopis farcta* في حيوية الرؤيسات الأولية للمشوكة الحبيبية *Echinococcus granulosus* مقارنة بالميبندازول

الملخص

تم في هذا البحث تحديد التأثير القاتل لكل من المستخلص الأيثـانولي والقلويـدات الخـام لأوراق نبـات البجنجـل *Prosopis farcta* علـى الرؤيـسات الأوليـة لطفيلـي المـشوكة الحبيبيـة *Echinococcus granulosus* في الزجاج بالمقارنة مع عقار الميبندازول (العقار المتـداول فـي عـلاج مرض الأكياس المائية) في الدرجتين الحراريتين 37 و 4 $م^0$. وضمن فترات زمنية مختلفة.

اظهر كلا المستخلصين تأثيرا قاتلا قويا في درجة 37 $م^0$، كما تبين ان مستخلص القلويدات الخـام لأوراق البجنجل له تأثيرا اقوى من المستخلص الكحولي لتلك الاوراق في قتل الرؤيسات الاولية، حيث ان تأثيرها القاتل للرؤيسات الاولية في التراكيز الدنيا (31.2، 62.5) ملغم/مل حصل خلال مدة قصيرة نسبياً (3 و 13 يوم، في الدرجتين الحراريتين 37 و 4 $م^0$) على التوالي.

INTRODUCTION

Hydatidosis is a parasitic infection caused by larval stage of the tapeworm *EchinococclIS granuloslls*. Adult worms inhabit the small intestine of carnivorcs, larval stage (hydatid cyst) Inhabits tissues of herbivores and omnivores host including human.

Inlectivc stagc to man is the eggs (Zeibig, 1997). Although surgery is still the treatment of choice for operable cystic disease due to *Echinococcus gramulosus ,* chemotherapy with behzimidazole, such as mebendazole and albcndazole, which may be of value prior to surgery and in inoperable cases (WHO, 1996).

Success of treatment with mebendazole depends highly on the local ization of hydatid cyst. Akhan et al. (1994) showed that thc cure pcrcentage of pulmonary cysts using mebendazole was 2\ %, while it was 7% in case of hepatic hydatidosis. (AIdan and Yalin, 1996) reported that 35<% of desiccated hydatid cysts from patients who were pretreated with mcbendazolc wcre non-fertile. Kuro et al. (1997) recorded those 30% cure perccntage in-patients with hepatic hydatidosis who were treated with albendazole and nlcbendazole. lIence, great efforts are being spent to find an effective medical cure for the disease.

Rccently, many workers are focusing their researches toward the use of natural products in the treatmcnt of discase including echinococcosis1\1 . Ilammo (2002) reported that the aqueous extracts of *Matricaria camomilla* and *Cyperus rotundus* possess an inhibitory effect on the viability of *Echinococclis granu!oslus* protoscolices. Mahmoud (2002) studied the effect of three plants on thc viability of *Echinococclis granulosus* Protoscolices, which are *Thymlls vlligaris*, *Cyperus rutundus* and *Peganum harmala,* respcctivcly. She showed that the aqueous, ethanolic and alkaloid extracts of thcse plants have a good inhibitory effect on the viability of Protoscolices. Thus, the present work aims at studying the effects of ethanolic and alkaloidal cxtracts *of Prosopis farcta* leaves on thc viability of *Echinococcus grnulosus* Protoscolices.

Prosopis fracta is a member.of Memosaseae family. It is a struggling shrub with 30-100cm height, grows abundantly in Iraq (Rechinger, 1964). The genus *Prosopis* has been used in folk medicine as an astringent in rheumatic disorders and as a remedy against scorpion sting and snakebite. The Icaves of *Prosopis spp.* were reported to relief irritated conjunctiva as used by Indians in South Western U.S. and in Mexico (Usmanghani et aI., 1982,.and Aqeel et al.,1991) *Prosopis spp .*were also known to have medicinal propcrties. It was proved to have cytotoxic effects in its fruits, which exhibit significant activity against lung carcinoma (Ahmad and Sultana, 1989).

Juliforin, which is the main alkaloid of Prosopis julifora, has been shown to possess antidermatophytic and antibacterial activities (Al- Shaikh Hamed and AI-.Jammas,1999). Usmanghani et al. (1982) isolated juliprosopin alkaloid from *Prosopis farcta* leaves, and then she studied its activity against some species of bacteria.

MATERIALS AND METHODS

Plant Materials:

Lcaves of *Prosopis farcta* were collected from Mosul and it's surrounding ficlds during June and: August 200 I. The plant was air-dried and 'the dried leaves were used. Biology department, College of Science, Mosul University, authenticated this plant.

Preparation of Ethanolic Extract:

The dried leaves were ground into coarse powder. Then extracted with 80% ethanol and filtered .The filtrate was evaporated using rotary vacuum evaporator. It was then lyophilized to get dry powder of the extract (Verport et al.,1988)

Preparation of Crude Alkaloids:

Thc powdered leaves were extracted with acid alcohol (I N HCl in 80(% ethanol) then pigments and unwanted materials were removcd by shaking with petroleum ether. The water layer was alkalinizcd with ammonium hydroxide. The precipitated alkaloids then was separated by Ii Itration (Evans, 1997).

Protoscolices Collection and Suspension:

In this study, hepatic hydatid cysts of infected sheep were obtained 11'om the municipal abattoir in Mosul City, Iraq, washed in several changes of steriIe phosphate buffer saline (pH 7.2). Protoscolices were then suspended in stcrile hydatid fluid ultrafilterated using 0.4 millipore filters), 2% of dimethyle sulphoxide was added to the huid as organic solvent (Farjou and AI-Hussainawi, 1984). Two ml of the suspension containing 500 Protoscolices wcre transferred into each siliconized test tube.

The viability of the Protoscolices used was ≥ 90 % This was determined by peristaltic movement of protoscolices and negative staining with 0.1 (Yo aqueous eosin and flame cell movements (Smyth and Barrett, 1980).

The Effect of Ethanolic Extract on the Viability of the Protoscolices:

Two groups of Protoscolices suspensions were used .Each group consists of nine test tubes; the first was considered as control, the second was treatcd with O.I-mg mebendazole/ml. of the suspension. The remaining tubes (3-9) were treated with different concentrations of the ethanolic extract (500,250,125,62.5,31.2) mg of the extract/ml of the protoscolices suspension, respectively.

The first group was incubated at 37Co and the sccond group was incubated at 4Co. The viability of Protoscolices was exam i ned for each group aller each exposure period (each treatment was carried out in triplicates).

Thc effect of Crude Alkaloids on the Viability of the Protoscolices:

To prepare di ITerent concentrations of crude alkaloids extract, the S_lmc proccdmc in preparing cthanol ic extract treatments was used as mcntioncd before.

Statistical Analysis used in this study includes F-test using analysis of variance (ANOV A table) ami Dunce's multiple range tests. The results were evaluated at $p<0.05$ and $p<O.O$ I (AI-Rawi and Kalaf Allah, 1980).

RCSULTS AND DISCUSSION

The antiparasitic activity of ethanolic extract and crude alkaloids of medicinal plant *Prosopis lareta* has been tested against *Echinococcus granulosus* Protoscolices *in vitro,* the study was carried out at37Coand 4 Co respectively (table I and table2). ANOV A table (Table3) showed that there are no signilicant dinerences among the inhibitory effect of all treatments (A) which are used in this study, but the interaction between treatments and exposure periods (A*P) showed high significant differences among viability percentage of the Protoscolices. This result means that the inhibitory effect of both extracts at both temperatures was weak (non-significant) and it got stronger (significant) with process of time. The delayed inhibitory effect of this plant may due to the presence of small amounts of active constitutes, i.e. scolicidal materials in the crude extracts which is used in the present study.

Table (3) also showed that there are high significant differences in the inhibitory effect of the extracts between the two temperatures used (B). Furthermore, interaction between the effect of temperatures and exposure periods (B*P) showed a high significant differences between viability percentclge of Protoscolices. Comparing the mean viability percentages of Protoscolices at 37Co(table I) and at *4*Co (table 2) it was evident that the inhibitory cried of both extracts used was higher at 37Co than at 4Co, and this result became more clear with the passage of time. This result is coincided with our fcml1er study in which the scolicidal effect *of Peganum harma!a* seeds against *Echinococcus granll!oslIs* protoscolices was stronger at 37Co than at 4Co (Hammoshi *et a*l.,2002) This may be due to the fact that living cells are metabolicaly more active at 37Co (AI-Habib, 1991) and many of the inhibitory substances are more effective at this temperature.

Simultaneously, thc smvival period for Protoscolices incubated at 37Co is shorter than those incubated at 4 Co, because of the faster autolytic activity of Protoscolices at 37Co (Andersen and Loveless, 1978; Mahmood and AI-Hannon, 1981). I-Iammoshi and Rahemo 2002 showed that incubation of *Echinococcus gronu!osus* protoscolices at 4o *in vitro* keeps viability of protoscolices up to 18 days, while incubation of protoscol ices at 37 Co leads to destruction of thc protoscolices within 3 days. They also demonstrated that vesiculation and evagination of protoscolices occurred within few hours aller incubation at 37Co while they occurred at a much lower level after two clays of incubation at 4 Co. Thus, metabol ic activities of protoscolices are increased at 37 Co including the autolytic activity) Icading to faster destruction of the protoscolices, but bettcr reaction with metronidazole and other tested extracts and *vice-versa* when the protoscoliccs arc incubated _lt 4Co(Table 1 and Table 2).

Table 1 : The effect of ethanolic extracts and crude alkaloids of *Prosopis farcta* leaves on the viability % of *Echinococcus granulosus* protoscolices *in vitro*, 37 C^0 in comparison with mebendazole and control groups.

Treatments	Concentration	Mean viability% of Protoscolices after…day			
	Mg/ml	1st	2nd	3rd	5th
*Control	0	99	91	85	3
Mebendazole	0.1	9	0	0	0
Ethanolic extract	500	75	37	0	0
	250	88	67	0	0
	125	93	66	10	0
	62.5	95	68	9	0
	31.2	95	81	10	0
Crude alkaloids	500	43	26	0	0
	250	56	38	0	0
	125	81	33	0	0
	62.5	82	64	0	0
	31.2	92	58	0	0

N: 3 replicates

*Control group=500 potoscolices/ml of hydatid fluid.
Percentage at zero time considered as 100%

Table 2 : The effect of ethanolic extracts and crude alkaloids of *Prosopis farcta* leaves on The viability % of *Echinococcus granulosus* protoscolices *in vitro*, 37 C^0 in comparison with mebendazole and control groups.

Treatments	Concentration	Mean viability% of Protoscolices after…day								
	mg/ml	1st	2nd	3rd	5th	7th	9th	11th	13th	15th
*Control	0	98	90	81	73	62	44	30	7	2
Mebendazole	0.1	20	3	0	0	0	0	0	0	0
Ethanolic extract	500	70	53	21	0	0	0	0	0	0
	250	85	77	62	50	33	10	2	0	0
	125	92	72	58	56	52	35	26	7	0
	62.5	96	91	82	78	70	37	28	8	0
	31.2	95	88	88	80	67	40	33	12	0
Crude alkaloids	500	46	25	14	0	0	0	0	0	0
	250	82	67	45	24	10	3	0	0	0
	125	90	78	72	70	52	34	20	0	0
	62.5	97	91	90	75	50	32	25	2	0
	31.2	92	92	90	83	64	45	31	1	0

N: 3 replicates

*Control group=500 potoscolices/ml of hydatid fluid.

Percentage at zero time considered as 100%.

Table 3 : Combined ANOVA table for the effect of treatment (A), temperature (B) and exposure times (P) on the viability of *Echinococcus granulosus* protoscolices *in vitro.*

Source of variance	Degree of freedom	Sum of square	Mean square	Calculated F
A	11	63068.27	5733.48	0.78
B	1	61302.707	61302.707	**8.11
AxB	11	1939.583	1763.05	0.24
Error a	46	335317.17	7289.503	
P	8	270779.24	33847.405	**631.8
AP	88	384255.12	4366.54	**81.51
BP	8	8404.16	1051.02	**19.62
AbP	88	3685.767	41.88	0.78
Error b	384	20571.27	53.57	
Total	647	527044.61		

**P<0.01

1n table 4, the results were arranged from highest to lowest inhibitory effect on the viability percentage of the Protoscolices at 37Co (using capital letters). Table 1 shows that the Proto scolices treated with both extracts giving a progressive decline in survival means and this is marked in case of ethanolic extract since the first two days of incubation, especially at the high concentrations *500* and 250mg/ml, while inhibitory effect *of* the crude alkaloids was evident from the highest *to* the lowest concentration used in this research. Hence all Protoscolices were killed before the third day *of* incubation.

Pursuing levels of effects for each treatment (Table 4) show that both ethanolic extract and crude alkaloids exhibit less inhibitory effect than mebcndazolc. But gencrally the scolicidal effect of crude alkaloids was stronger than that *of* ethanolic extract.

Observing mean viability percentage *of* Protoscolices at 4CO (Table 2) shows that both ethanolic and alkaloidal extract possessed low scolieidal effect in comparing with mebendazole. They also exhibit weak or no inhibitory effect in the low concentrations (G2.5,31.2mg/ml). But when you follow up levels of inhibitory effect of each treatment at this temperature (Table 5), it seams that the crude alkaloids have more potent scolicidal effect than ethanolic extract. It appears that the alkaloids used in the present study rather have a good scolieidal efTect, even present at *low* concentrations in the crude extract *of prosopisfarcta* leaves.

The use of some drugs is probably limited due *to* the risk of their side effects and toxicity .Plants are relatively safe and rich source of therapeutic compound, and alkaloids in certain respect rank among the most significant naturally occurring substances (Aqeel et al. 1991) Typical alkaloids were derived from plant source sharing an important location in chemotherapy world; Alkaloids founel in all parts of the plant or concentrated in certain parts (Kotb, 1981).

Table 4 : Leaves of the inhibitory effects of ethanolic and alkaloidal extracts on the viability of *Echinococcus granulosus* protoscolices at $37C^0$ in comparing with mebendazole and control group (Duncan's test at P>0.05)

at P>0.05).

Treatments	Concentration Mg/ml	Viability mean of Protoscolices	Level of effect
Control	0	694.86	H
Mebendazole	0.1	58.38	A
Ethanolic extract	500	297.66	BC
	250	376.68	CDE
	125	448.62	EFG
	62.5	454.77	FG
	31.2	483.54	H
Crude alkaloids	500	221.995	B
	250	264.43	BC
	125	302.91	CD
	62.5	393.54	DEF
	31.2	374.76	CDE

*Each value represents the mean of three replicates. *There are no significant differences between the values that subscripted by one litter or more.

Table 5 : Leaves of the inhibitory effects of ethanolic and alkaloidal extracts on the viability of *Echinococcus granulosus* protoscolices at $4C^0$ in comparing with mebendazole and control group (Duncan's test at P>0.05)

at P>0.05).

Treatments	Concentration Mg/ml	Viability mean of Protoscolices	Level of effect
Control	0	1255	HI
Mebendazole	0.1	114.87	A
Ethanolic extract	500	396.87	C
	250	862.86	E
	125	1074.02	F
	62.5	1262.04	G
	31.2	1284	HI
Crude alkaloids	500	288.54	B
	250	660.74	D
	125	1071.88	F
	62.5	1205.81	GH
	31.2	1296.35	I

*Each value represents the mean of three replicates. *There are no significant differences between the values that subscripted by one litter or more.

Mahmoud, (2002) reported that the crude alkaloids of *Thymus vulgaris* leaves, *Cypenls rotundus* tubercles and *Peganum hannala* seeds, exhibited the best scolicidal effect in comparison with aqueous and ethanolic extracts of the same plants. Hammoshi et a1. (2002). also showed that the crude alkaloids of *Peganllln hannala* seeds were more effective than the ethelllolic extract against *Echchyinococcus granulous* protoscolices.

Several alkaloids have been derived from leaves of *Prosopis* spp like spcigerine julillorine, jllliflоricine, jlllilloridine and juliprisopine that were reported to have medicined value (Ahmad et a1., 1979 ; Ahmad and Qazi, 1985). Ahmad and Sultana (1989) showed that the aqueous and alco_1O1ic extracts of *Prosopisjuli./dra* have an antibacterial activity. Usmanghani et al., (1982).Isolated two alkaloids from shade dried leaves of *Prosopis glandulosa* and *Prosopis.!arcta,* and characterized them as juliforine and juliprosopine. They also found that the alcoholic extract and isolated juliprosopine showed antibacterial activity against *Bacillus subtilis, Bacillus magatherium* and *Sarcinnia lulea* .Juliflorine had been shown to possess antibacterial, antidermatophytic and non-mutagenic activity (Aqeel et al., 1991*)* .

However, further investigations are needed to isolate and purify the different types of' alkaloids from the crude alkaloid of *Prosopis fareta* leaves and then identify the scolicidal efTect of each.

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