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IN VITRO STUDY OF THE ANTHELMINTIC ACTION OF TRIGONELLA FOENUM GRAECUM L. GROWN IN IRAN

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ABSTRACT

The water extract of *Trigonella foenum* graecum stem and leaves was tested for lethal activity against *Hymenolepis nana, Syphacia obvelata,* and *Moniezia expansa.* The plant extract demonstrated good anthelmintic activity *in vitro* which is directly related to the concentration of the extract used. The extract also prevents the formation of ascaris larvae from the eggs of ascaris lumbrico - ides (human).

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INTRODUCTION

Trigonella foenum graecum (Fenugreek) seed has been known and valued as a medicine and used for its stimulating effect on the digestive process (1), as a carminative tonic, aphrodisiac and antidiarrheal (2). It is also credited with antidiabetic properties (3). Although this plant is reputed for its anthelmintic property in Africa and the East (2), with reference to different international abstracts, we found no work have been done to assess this anthelimintic effect.

The purpose of the present communication is to investigate the anthelmintic property of water extract of stem and leaves of *trigonella foenum* gracum grown in Iran which is locally known as "Shanbalileh".

MATERIAL AND METHODS

Fresh *Trigonella foenum* graecum stems and leaves were purchased in the local market, air dried at room temperature in the shade (ca 20°C), pulverized in an electric blender and extracted with water for 12 hours at room temperature. The extract was filtered through Whatman No. 1 filter paper. Before use, the volume of the clear filtrate was reduced at 37°C under reduced pressure, to give a final concentration of one gram stem & leaves per ml. of extract.

The helminths used were *hymenolepis* nana (Cestoda) and *Syphacia obvelata* (Nematoda) obtained from the intestine of mice sacrificed 15 days after infection and *Monieza expansa* (Cestoda) collected at the slaughter house from the intestine of sheep. The parasites were carefully taken from their habitat and washed out two to three times with 0.9% NaCl solution at ?°C to remove visible adhering substances from their body. Only those tape worms in which the scolex was intact and were about 6 cm in length were selected for the study. The worms were transferred to petri dishes containing 50 ml. of growth medium 199 (Grand Island Biological Company, New York). To each ml. of the medium, penicillin (1000 units) and streptomycin (1 mg.) were added.

To test the anthelmintic activity of water extract of *Trigonella foenum* graecum, the *in vitro* method described by Mishra et al. (4) was used. Ten cestodes or nematodes were kept in one petri dish and three such petri dishes were used for a single concentration of the extract. The extract was mixed with the growth medium to give a final concentration of plant extract of 10, 20 and 30 per cent, the 4th set of petri dishes were used as control with no extract added. The petri dishes were kept in the incubator, at a temperature of 37.0°C and examined every 30 minutes. The lental times were recorded.

In a second test, ascaris lumbricoide eggs were isolated from human feces and suspended in physiological in the saline. The egg suspension was then diluted in the saline to contain approximately 1000 eggs per ml. To each of 5 test tubes 1 ml. of egg suspension was added and then graded concentrations of 0.25, 0.5, 0.75 and 1 ml. of plant extract was mixed with egg suspensions. The fifth tube served as control and was corrected for the volume with the saline.

The test tubes were kept in an incubator at 22°C and examined for embryonated eggs daily for 15 days in accordance with the method of Cleeland and Laurence (5). The number of embryonated eggs were recorded.

RESULTS

A number of *in vitro* tests employing *H. nanú S. obvelata, M. expansa* and *ascaris larvae* were carried out to test the activity of water extract of *trigonella foenum* graecum in direct contact with the helminths. From the results presented in Table I, the plant extract appeared to have anthelmintic activity on both cestodes and nemathodes tested. The extract at different concentrations was active against the helminths accordingly.

Table 2. shows the effect of plant extract on the embryonation of ascaris lumbricoide eggs. In the presence of 1 ml. of plant extract, there is about 87% inhibition of embryonation. The extract at the lowest concerntration (0.25 ml) in the incubation medium did not affect the embryonation of ascaris eggs.

DISCUSSION

The experimental evaluation of the activity of an anthelmintic agent has been the subject of many studies (4, 6). The *in vivo* methods in small animals are with no doubt convincing, nevertheless, *in vitro* tests are considered essential in preliminary studies (4,6).

The *in vitro* methods used in the present investigation employing readily available helminths has yielded results indicative of a possible usefulness of *Trigonella foenum* graecum. It has been reported that the fenugreek seeds are effective against nemathodes in humans (7). Therefore, it is interesting to note that the extract of the stem and leaves of the Iranian variety of fenugreek is also active against cestodes and nematodes.

Studies are now in progress in our laboratory to separate the responsible compound or compounds for the anthelmintic activity of this vegtable used in Iran.

Table I The effect of Trigonella foenum graecum extract on the helminths

Treatment		100% Lethal times in minutes		
		S. Obvelata	H. Nana	M. expansa
Control [1]		610 - 670	520 - 560	490 - 520
Extract [2]	10%	360 - 480	240 - 360	360 - 480
"	20%	240 - 360	120 - 240	240 - 360
"	30%	120 - 240	100 - 150	120 - 240

1 No extract is added to the medium

2 The water extract was mixed with the medium to give a final concentration of plant extract 10, 20 and 30%.

Table II The effect of plant extract on embryonation of ascaris lumbricoide eggs

Treatment	No of embryonated in 1000 eggs after 15 days [1]	% inhibition of embryonated eggs
Control	820	0
Extract 0.25 ml	820	0
" 0.5 ml	784	4.8
" 0.75 ml	500	39
" 1.0 ml	100	87

1 One of egg suspension in saline contained approximately 1000 eggs.

of atmospheric fluoride. The area under survey contains some 268 brick, lime, and gypsum kilns, this is the largest brick making area in Iran.

The present study examins the distribution of fluoride pollutant through out the area and leaving vegetation uptake and urine analysis for near future.

MATERIALS AND METHODS

Sampling was done using Dorsey and Kemnitz method (9) at about 13 L.p.m. for 9 hours per sample. The probes were fixed at two meters from ground level.

Elfers potentiometric analysis applied for air samples (10) was selected for determination of fluoride in alkaline sample solution, out of several methods (10-15). The collected samples were taken to the laboratory diluted 1:1 by ionic strength adjuster buffer containing CDTA (1,2 Cychlohexylen dinitrilo tetraacetic acid) as complexing agent for prevention of interfering ions.

Fluoride concentration was determined by Orion 94-09 Fluoride Electrode and Orion 901 Ionalyzer.

SAMPLING AREA

Mahmood-Abad area, at 18 kilometers from Tehran, on the South of Tehran-Mashad high way was selected for air sampling. The area contains a population that varies from 1000 to 2000 people depending labor activity seasons. Most people except chidlren are involved in bricks, plaster, and lime making industry. There were 41 bricks, 36 plaster and 3 lime kilns. Each brick kilns has a capacity of about 40000 bricks at one load, and lime and plaster kilns bake 30000 kilograms of product per day. The stack height for brick kilns are 50 to 55 meters, but lime and plaster kilns have no elevated stack.

Five different sites were selected for air sampling in this complex of constructing materials industry. Number of sites were limited due to electricity limitation. However effort was made for covering most possible area; stes 1 & 2 were located in the middle of the complex and the other sampling sites were selected on the border area. In addition five samples were collected outside the complex. These palces were: 2. 5, 4, 8, 9 and 10 kilometers far from Mahmood-Abad border toward Tehran city. This survey was done from April 12 to May 19, 1978.

RESULTS AND DISCUSSION

The current work on measurements of atmospheric fluoride in relation to brickworks of Southern Tehran is summerized in tables 1-6. As can be seen in tables 1 and 2 the average fluoride concentrations range from 3.01 to 4.17 ugm^{-3} as hydrogen fluoride, for days and nights respectively. The maximum measured concentration in these two sites, which were located within the kilns, was as high as 7.80 ugm^{-3}. The fluoride concentrations in other three sites are tabulated in tables 3 to 5. These tables show relatively lower concentrations than the first tow sties. The maximum of these less dense kilns area was found to be 3.92 ugm^{-3}.

Looking at outcome in the distance locations (table 6) the atmospheric fluoride reduces drastically as a function of distance from the sources. The difference in sample means is significant at the 0.5 per cent level of 0.001 P 0.005 (16). At nearly 9 km from the source it was not possible to detect a measurable amount of F^- by applied method. The average concentrations for 2.5, 4, 5 and 8 kms from the sources were 2.16, 1.98, 1.50 and 0.33 micrograms per cubic meter respectively.

It can be concluded from these results that the ambient fluoride dispersion in southeasern aera of Tehran is quantitatively atributable to brick manufacturing. Comparing day and night concentration, the nightly fluoride levels were almost constantly higher than the day time values. This difference may be due to nightly inversion which occurs in the area. The difference tested by pairing the mean difference (16) and this was significant at 0.01 level.

Fluoride concentration was decreased the day after rain, this was not only because of washout, but also extinction of some kilns due to lack of raw bricks.

If it is assumed that the average man with moderately strenous work inhales approximatcly $20m^3$ of air in 24 hour period (17) and the whole measured fluoride is absorable then his daily air borne fluoride intake of a man will be about 0.07 mg. Although this figure is far below the T.L.V. recommended by AGGIH (5-6mg day^{-1}), (18,19) but the continuous long time exposure to it and other air borne gaseous pollutants, may lead to serious health hazards.

Regaridng fluoride effects on vegetations, the amounts found in the area are much higher than the established criteria. According to standards, the average 24 hours gaseous fluorides should not be exceed 1.7 ugm^{-3} and the average of 30 days should be less than 0.5 ugm^{-3} (20). In other words the average fluoride concentration

in the area is two folds greater than the standards established for vegetations.

To conclude discussion, the quantity of fluoride found to be emitted by brick kilns especially in short period are not severely dangerous for human health, but it might be of some concern for those living in the area and it is injurious for vegetations and crope (22, 23, 24), On the other hand the emitted fluoride that may affect crops and forage can be hazardous for animals feeding on those forage.

Table 1,Fluoride concentration at site 1

Sampling date		ug.m^{-3} HF	
		Days	Nighttes
April	12	1.83	3.77
	14	2.26	3.14
	17	1.92	2.65
	19	2.98	3.07
	22	3.60	2.82
	24	3.74	3.84
	27	4.32	7.80
	29	3.35	3.38
May	2	2.68	2.61
	4	3.39	3.77
$\bar{x}$		3.01	3.69
s		0.82	1.52

Table 2, Fluoride concentration at site

Sampling date		ug.m^{-3} HF	
		Days	Nighttes
April	12	3.14	4.07
	15	3.83	4.27
	17	2.93	3.77
	20	3.44	7.02
	22	3.84	4.55
	25	2.72	3.99
	27	4.01	3.75
	30	-	-
May	2	2.01	2.40
	5	3.21	3.73
$\bar{x}$		3.24	4.17
s		0.64	1.22

Table,3. Fluoride concentration at site 3

Sampling date	ug m^{-3} HF	
	Days	Nighttes
April 13	1.83	1.87
15	2.08	2.08
18	2.90	2.23
20	2.12	1.82
23	1.90	2.79
25	2.53	2.93
28	2.15	2.97
30	-	-
May 3	2.96	2.92
5	1.82	2.33
$\bar{x}$		
s		

Table, 4. Fluoride concentration at site 4

Samplind date	ug m^{-3} HF	
	Days	Nighttes
April 13	1.87	2.95
16	1.74	2.00
18	2.01	1.88
21	2.41	2.54
23	3.66	3.43
26	3.08	3.50
28	4.43	2.96
May 1	1.05	1.16
3	3.23	3.53
6	2.11	1.89
$\bar{x}$	2.56	2.58
s	1.02	0.82

Table 5, Fluoride concentration at site 5

Sampling	ug.m^{-3} HF	
	Days	Nightes
April 14	2.01	2.01
16	1.74	2.14
19	2.14	3.48
21	1.93	2.18
24	1.91	2.93
26	3.19	2.93
29	2.30	4.09
May 1	0.94	1.78
4	2.96	3.56
6	1.98	3.92
$\bar{x}$	2.11	2.90
s	.63	.84

Table 6, Fluoride concentration at the distances from the source

distances in kilometers	ug.m^{-3} HF	
	Days	Nightes
2.5	2.16	
4	1.98	
5	1.50	
8	0.33	
9	trace	
10	trace	

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