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***In-vitro* anthelmintic activity of fenugreek leaves (aqueous extract) in Indian earthworms**

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Abstract

Background- Soil transmitted helminthiasis (STH) is one of the common health problems of under developed countries. Fenugreek is a popular food substitute in Indian kitchen and ayurveda for centuries. Even though a number of the anthelmintic drugs available in the market with considerable side effect, we studied in vitro anthelmintic activity of fenugreek leaves extract in comparison with Albendazole.

Methods- This comparative experimental study was done over the period of 3 weeks in Indian adult earthworm due to its anatomical resemblance with the *Ascaris lumbricoides*. An aqueous extract is prepared from standard method. Albendazole was used as comparator drug; 20 mg/ml concentration was prepared as per the approved method. Each group with six earthworms of equal was released into in each 50 ml of desired concentration of comparator and fenugreek leaves extract in the petridish. 50 ml contained 200 mg/ml of aqueous extract of leaves of fenugreek. Earth worms in the petridish were studied for paralysis /death as end points of our study. The mean time to paralysis/death was recorded and expressed in minutes. The test results were compared with comparator.

Results- Aqueous extract of fenugreek leaves was 144±15 minutes to paralyze and 224±10 minutes to death of the worm, whereas albendazole took 90±12 minutes to paralyze and 155±21 minutes to death of the worms is statistically significant ($P<0.05$). Fenugreek leaves aqueous extract is more potent than control (NS) and lesser anthelmintic activity than Albendazole.

Conclusion- Aqueous extract fenugreek leaves showed noteworthy anthelmintic activity as compared to control (NS) but less potent than albendazole.

Keywords: Soil Transmitted Helminthiasis (STH), Fenugreek leaves aqueous extract, Albendazole, Normal Saline (NS)

Introduction

Soil transmitted helminthiasis (STH) are one of the common health problems affecting developing and under developed countries [1]. Several studies have suggested that mild to moderate intensity of infection may result in deferred physical growth, impaired cognitive functions, absenteeism among school going children [2-4]. Anthelmintics are the agents that expel helminthes from the body either stunning or killing them [5]. There are few plants known to provide a rich source of phyto-anthelmintics in the nature [6-7]. *Trigonella foenum-graecum* belongs to the family Leguminoceae and it is popularly known as Fenugreek (Methi) in India [8]. It is also stated in Ayurveda and Siddha (Indian traditional systems of medicine) that these plants were used to treat different kinds of fever, dysentery and heart diseases, whereas in Unani system, this plant is used as an aphrodisiac, diuretic [9-10]. To this purpose we studied in vitro anthelmintic activity of fenugreek leaves extract in comparison with albendazole.

Methods

This comparative study was done at Pondicherry institute of medical sciences, Puducherry over the period of 3 weeks during in the month of January 2011. The experiment was done in Indian earthworms (*Pheretima posthuma*) adult type due to their anatomical resemblance with the *Ascaris lumbricoides*. The Institute Ethical Committee has approved the study protocol. The earthworms were obtained from moist soil and cleaned with water to remove all dirt. Fresh fenugreek leaves were purchased from the local market then, it was endorsed by certified Taxonomist from Pondicherry University, Puducherry.

The leaves dried under shade and crushed in an electric blender to form powder and subjected Soxhlet extraction using water as solvent. Aqueous extract is prepared from standard methods [11]. Albendazole was used as comparator drug; 20 mg/ml concentration was prepared by as per the approved method. The suspension of aqueous extract of leaves of fenugreek concentration 200 mg/ml was prepared and final volume was made up to 50 ml for relevant concentration.

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Groups of equal size worms consisting of six earthworms individually in each group were released into in each 50 ml of desired concentration of drug and extracts in the petridish. The anthelmintic activity was performed according to standard screening methods [12-14]. Six Indian adult earth worms were placed in petridish containing 50 ml contained 200 mg/ml of aqueous extract of leaves of fenugreek. Earth worm in the petridish was studied for paralysis/death as an end point of our study. The mean time to paralysis/death was recorded and expressed in minutes. The test results were compared with albendazole (20 mg/ml). The procedure continued for 3 times to verify the results.

Statistical Analysis: Mean, SD and percentages were used to describe the results data. Chi-square and unpaired "t" test were used appropriately as inferential tools. P value <0.05 was considered statistically significant.

Results

Aqueous extract of fenugreek leaves was 144±15 minutes to paralyze and 224±10 minutes to death of the worm, whereas Albendazole took 90±12 minutes to paralyze and 155±21 minutes to death of the worm with significant ($P<0.05$) value. Aqueous extract is more potent than control (NS) and lesser anthelmintic activity than Albendazole. Time to paralysis and consequent death were significantly higher in aqueous extract of fenugreek that of Albendazole at same concentrations.

Table 1: Anthelmintic activity of fenugreek leaves

S. No	Anthelmintic Substance	Time taken for Paralysis (Minutes)	Time taken for Death (Minutes)
1	Normal Saline	No action took	No action
2	Aqueous extract of fenugreek	144±15	224±10
3	Albendazole	90±12	155±21
	P-Value	$P<0.05$	$P<0.05$

Discussion

Soil transmitted helminthic infestation is wide spread in rural India. Fenugreek leaves are cheap, easily available in the local market, therefore this can be tried as an alternative remedy to already existing standard anthelmintic drugs. A number of medicinal plants have been used to treat parasitic infections in humans and animals [15]. Anthelmintic derived from plant source can be an answer to this STH problem as they form secure and non-toxic with a modified site of action [16-17]. Chandrashekhar *et al.* [18] and Bhalke *et al.* [19] in their study found that seeds and leaves of *Trigonella Foenum-graecum* showed marked anthelmintic activity.

Limitations of the Study

The bioactivity guided demonstration of anthelmintic activity helps in standardization of its anthelmintic activity.

Conclusion

Aqueous extract fenugreek leaves showed noteworthy anthelmintic activity as compared to normal saline but less potent than albendazole. Additional large studies are warranted using in vivo models to prove fenugreek leaves as better anthelmintic substance.

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Conflict of Interest: None declared

Ethical Approval: None required

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