Vol. 6/2 (2006) 153 - 156

JOURNAL OF NATURAL REMEDIES

Nootropic and anxiolytic activity of Fenugreek seeds

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Abstract

Objective: To study the nootropic and anxiolytic activity of *Trigonella foenum-graecum* (Fenugreek) seeds. Methods: Acetone soluble fraction (ASF) of methanol extract (ME) of *Trigonella foenum-graecum* (Fenugreek) seeds was assessed for its nootropic and anxiolytic activities. ASF (100,200 and 400 mg/kg) administered intra-peritoneally exhibited nootropic and anxiolytic activity in Passive shock avoidance paradigm and elevated plus maze (EPM) paradigm respectively. Scopolamine (1mg/kg) was used to induce cognitive dysfunction. Mentat (100 mg/kg) and Diazepam (1 mg/kg) served as standard nootropic and anxiolytic drugs respectively. Results: In Passive shock avoidance paradigm, ASF (100,200 and 400 mg/kg) significantly ($P<0.05$) reduced the number of mistakes and increased the time spent in shock free zone compared to vehicle treated group. ASF (400 mg/kg) significantly reversed the effects of Scopolamine(1 mg/kg). In Elevated plus maze ASF 100, 200 and 400 mg/kg) significantly ($P<0.05$) increased the time spent in open arms and the entries in open arms Conclusion: Thus, the seeds of *Trigonella foenum-graecum* contain bio-active principle(s) which possess nootropic and anxiolytic activity.

Key words: Anxiolytic, *Trigonella foenum- graecum*, nootropic

1. Introduction

Plants have multiple pharmacological actions as they contain numerous constituents of diverse chemical nature. Nootropics constitute a class of psychotropic agents with specific effects on intellectual faculties of man especially learning and memory capabilities [1]. A large number of medicinal plants have been reported to impart facilitatory effects on the central nervous system. Nalini *et al* [2] reported nootropic activity of oil obtained from *Celestrus paniculatus*. The alkaloids from *Vinca minor* and *Secale cornutum* [3] and saponins, bacoside A and bacoside B from *Bacopa monnieri* [4] and ginsenoside Rb-1 from *Panax ginseng* [5] and the acetone soluble fraction of pet ether extract of *Lawsonia inermis* [6] are the active principles in enhancing cognitive behaviour in experimental animals. *Trigonella foenum-graecum* L. (Leguminosae) commonly known as fenugreek possesses laxative, expectorant, hypocholesterolaemic and hypoglycaemic activity [7, 8]. The seeds are hot with a sharp

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bitter taste, antipyretic, anthelmintic and appetite stimulant [9]. During our preliminary studies on methanol extract of fenugreek seeds, we noted a general depressant activity in mice. Thus, the current study was undertaken to evaluate the nootropic and anxiolytic activities using various behavioral paradigms.

2. Materials and methods

2.1 Preparation of extract

Fenugreek seeds (1kg), purchased locally were authenticated, crushed to a coarse powder and defatted with petroleum ether (60- 80°C) using Soxhlet's extractor. The marc obtained was subjected to extraction with methanol. The extract was concentrated under vacuum. The methanol extract (5.85% w/w yield) was separated into acetone soluble (0.95% w/w yield) and acetone insoluble (4.90%w/w yield) fractions. The acetone soluble fraction was used for further investigation.

2.2 Animals

Albino rats (150-200 gms) and male albino mice (22-25g) were obtained from Serum Institute, Pune. Animals were housed into groups of five at an ambient temp of 25±1°C. Animals had free access to food (Hindustan Lever, India) and water. They were deprived of food but not water 4hr before the experiment. The experiments were carried out between 9:00 and 14:00 h. The Institutional Animal Ethical Committee approved the protocol of this study.

2.3 Drugs and Chemicals

Mentat Syrup (Himalaya Drug company, Mumbai), Scopolamine (Buscopan-German Remedies) and Diazepam were used in the study. Mentat was dissolved in distilled water and administered orally. The ASF of methanolic extract of fenugreek seeds were suspended in PEG-400 (just sufficient to dissolve) and administered intraperitoneally. Scopolamine and Diazepam were administered intraperitoneally. Pet ether, methanol and acetone were purchased from Modern Scientific, Nashik.

2.4 Behavioral Studies

2.4.1 Passive shock avoidance paradigm

The passive shock avoidance paradigm was used as described earlier [10]. Rats were placed on electric grid and allowed to explore for 1 min. The stimulus (20v) with AC current of 5 mA was then applied. Animals that reached SFZ (shock free zone) in 2 mins in the first trial were selected for the study.

After 1 h of the first trial, each animal was put on the SFZ and number of mistakes (descents) in 10 min and the time spent in SFZ were recorded on Day1 and Day 2. Electric shock was not given on Day 2. Scopolamine (1 mg/kg) was used to induce cognitive dysfunction and Mentat (100 mg/ kg) served as a positive control. The extract and mentat were administered 30 min before scopolamine. All drugs were dissolved in distilled water.

2.4.2 Elevated plus maze (EPM)

The EPM consisted of two open arms (25 x 5 cm) crossed with two closed arms (25 x 5 x 20 cm). The arms were connected together with a central square of 5 x 5 cm. The apparatus was elevated to a height of 25 cm. Mice in groups of 5 were treated with vehicle or ASF (100, 200 and 400 mg/kg) 30 min before placing individually in the EPM and the time spent in open arms, entries in open arms were recorded [11].

3. Results

3.1 Passive shock avoidance paradigm

Rats receiving vehicle when placed on the SFZ committed 7.0 ± 0.57 and 5.33 ± 0.88 mistakes and spent 255 ± 7.63 and 263.33 ± 6.67 seconds

Table 1
Effect of ASF of methanolic extract of Fenugreek seeds on Passive Shock Avoidance Paradigm

Treatment	Number of mistakes in 10 min		Time spent in SFZ (sec)	
(mg/kg)	Day 1	Day 2	Day 1	Day 2
Control	7 ± 0.57	5.33 ± 0.88	255 ± 7.63	263.3 ± 6.67
Mentat(100)	2 ± 0.16*	0.6 ± 0.0*	299 ± 0.58*	298.3 ± 1.66*
Scopolamine(1)	11.75 ± 0.72*	22.33 ± 1.2*	225.5 ± 4.08*	237.66 ± 11.35
ASF (100)	3.5 ± 0.17*	4.66 ± 0.89	295.25 ± 0.72*	280 ± 5.78
ASF (200)	2.5 ± 0.33*	8.5 ± 0.33	297.25 ± 0.87*	291 ± 1.49
ASF (400)	2.66 ± 0.33*	1.33 ± 0.33*	298.25 ± 0.73*	299 ± 1.49*
ASF (400) + Scopolamine(1)	2.5 ± 0.57*	5.5 ± 0.57	277.5 ± 9.01*	250 ± 14.21
F	61.69	75.16	35.63	9.96

n=6, *P<0.05 ANOVA followed by Dunnett's test

on SFZ on day 1 and day 2 respectively. Scopolamine significantly increased the no of mistakes to 11.75 ± 0.72 and 22.33 ± 1.20 and decreased the time spent in SFZ to 225.5 ± 4.08 and 237.66 ± 11.35 on day 1 and day 2 respectively.

On treatment with Mentat the no of mistakes was significantly reduced to 2 ± 0.0 and 0.6± 0.0 and the time spent in SFZ was significantly increased to 299 ± 0.58 and 298.3 ± 1.66 on day 1 and day 2 respectively. AE (400mg/kg) showed the no of mistakes as 2.66 ± 0.33 and 1.33 ± 0.33 and the time spent in SFZ as 298.25 ± 0.72 and 299 ± 0.0 on day 1 and day 2 respectively. AE (400mg/kg) also antagonized the effects of scopolamine.

3.2 Elevated Plus Maze

The vehicle treated mice spent 33.25 ± 2.83 seconds in the open arm, whereas animals treated with ASF 100, 200 and 400 mg/kg) spent significantly more time in the open arm. ASF (400 mg/kg) also increased the time spent in open arms and entries in both the open arms significantly (P<0.05).

4. Discussion

The preliminary phytochemical screening of ASF of methanolic extract of *Trigonella foenum-graecum* (Fenugreek) seeds showed the presence of saponins and alkaloids. The study showed that acetone soluble fraction (ASF) of methanolic extract of *Trigonella foenum-graecum* seeds possessed nootropic and anxiolytic activity.

Trigonella foenum-graecum meets a major criterion for nootropic activity *i.e* improvement of memory in absence of cognitive deficit [12]. The antagonistic effect of ASF of Fenugreek seeds against Scopolamine induced amnesia substantiates nootropic acvtivity. The anxiolytic activity was studied by using elevated plus maze model. ASF increased the open arm occupancy and the number of open arm entries.

A dose dependent increase in effects were observed. In agreement with these results, anxiolytic activity was recently confirmed in our laboratory [13]. Crawley reported that rats were not respondent to treatment with diazepam in this paradigm [14], and that their exploratory

Table 2
Effect of ASF of methanolic extract of Fenugreek seeds on time spent in open arms and entries in open arms in elevated plus maze.

Treatment (mg/kg)	Elevated Plus Maze	
	Time spent in O.A (sec)	Entries in O. A
Vehicle	33.25± 2.83	2.55± 0.55
Diazepam (1)	83.6±9.42*	4.6±0.18
ASF (100)	53.25±7.0	3.25±0.85
ASF (200)	65.27±2.88*	5.75±0.75
ASF (400)	81.25±7.6*	8±1.78*
F	11.17	4.87

n = 5, *P< 0.05, ANOVA followed by Dunnett's test; O.A = open arm

tendencies appeared considerably lower than in mice, suggesting that rats were not useful in this test.

Thus, it is concluded that ASF of methanol extract of *Trigonella foenum-graecum* seeds that contain alkaloid and saponin possess nootropic and anxiolytic activity. The fraction holds potential for further mechanistic studies.

5. Acknowledgement

The authors are grateful to Prin .V. M Aurangabadkar of M.G.V's Pharmacy College for providing necessary laboratory facilities and to Dr.S.B Kasture and Dr.V.S Kasture for their continuous support and encouragement.

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