Vol. 4/1 (2004) 61 - 65

JOURNAL OF NATURAL REMEDIES

Anxiolytic activity of *Trigonella foenum-graecum* seeds

M. Iyer*[1], H. Belapurkar[1], O. Sherikar[1], S. B. Kasture[2]

1. Department of Pharmacology. M.G.V's College of Pharmacy, Panchvati, Nashik - 422 101
2. Department of Pharmacology. N.D.M.V.P Samaj's College of Pharmacy, Nashik - 422 002

Abstract

Objective : To study the anxiolytic activity of *Trigonella foenum–graecum* (Fenugreek) seeds. *Methods:* Methanol extract (ME) and ethyl acetate fraction of methanol extract (EAF) were assessed for their anxiolytic activity. The ME (30,100 mg/kg) and EAF (30, 100mg/kg) administered intra-peritoneally exhibited anxiolytic activity in elevated plus maze (EPM) paradigm, Light/Dark apparatus (LDA) and Open field apparatus (OFA). Meta-chlorophenylpiperazine (m-CPP, 1 mg/kg), a 5-HT_2 receptor agonist, known to induce anxiety was used to study antagonism with different doses of EAF and with diazepam. Results : In EPM, both ME and EAF increased the time spent in open arms and the number of entries in open arms. In Light: Dark apparatus, ME and EAF increased the time spent in lit zone. In OFA, the number of assisted rearing and the number of squares traversed were increased. Different doses of EAF (30,60,120,240,480 mg/kg) also antagonized the effects of m-CPP(1 mg/kg). The ED_{50} value of EAF was found to be 48.97mg/kg. *Conclusion :* Thus, the seeds of *Trigonella foenum graecum* contain bio-active principle(s) which possess anxiolytic activity.

Key words: Anxiolytic, *Trigonella foenum-graecum,* 1,3-Chlorophenylpiperazine.

1. Introduction

Plants have multiple pharmacological actions as they contain numerous constituents of diverse chemical nature. *Trigonella foenum-graecum* L. (Leguminosae) commonly known as fenugreek possesses laxative, expectorant, hypo-cholesterolaemic and hypoglycaemic activity [1,2]. The seeds are hot with a sharp bitter taste, antipyretic, anthelmintic and appetite stimulant [3]. During our preliminary studies on methanol extract of fenugreek seeds, we noted a general depressant activity on the motor activity in mice. In the present study, the methanol extract of fenugreek seeds was studied for its anxiolytic potential, using different animal models of anxiety based on exploratory behaviour.

Corresponding author
E. Mail: mohan_mahalaxmi@indiatimes.com

2. Materials and methods

2.1 Preparation of extract

Fenugreek seeds (1kg), purchased locally were 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 methanol extract was charged into chromatography column of neutral alumina. The column was eluted with ethyl acetate, methanol and dimethyl sulfoxide (DMSO). Each of these fractions was tested for activity.

2.2 Animals

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. Animals were deprived of food but not water 4 h before the experiment. The Institutional Animal Ethical Committee approved the protocol of this study.

2.3 Drugs and Chemicals

Meta-chlorophenylpiperazine (m-CPP, Sigma,U.S.A) and diazepam were used in the study. The drugs were dissolved in the water for injection and were administered orally. The methanol extract of fenugreek seeds and its ethyl acetate fraction were suspended in PEG-400 (just sufficient to dissolve) and administered intraperitoneally. The pH of methanol extract and its ethyl acetate fraction was 6.4 and 7.9 respectively. Ethyl acetate, methanol and DMSO were purchased from Modern Scientific, Nashik.

2.4 Behavioral Studies

2.4.1 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 MF (30 and100 mg/kg) or EAF (30 and 100 mg/kg) 30 min before placing individually in the EPM and the time spent in open arms, entries in open and closed arms were recorded [4].

After each test, the maze was thoroughly cleaned. In another study, different doses of EAF (30, 60, 120, 240, 480 mg/kg) and diazepam (0.5, 1, 2, 4, 8, 16 mg/kg) were administered 30 min after giving m-CPP (1mg/kg) and the same above parameters were noted. Diazepam (1mg/kg) was used as a standard anxiolytic and m-CPP (1mg/kg) was used as a reference anxiogenic drug.

2.4.2 Light/ Dark test

Two equal sized boxes (20 x 20 x 14, one dark and the other lit) were connected with a tunnel (5 x 7 x 10 cm). Mice in groups of five treated with vehicle, EAF (30, 100 mg/kg) or ME (30,100mg/kg) were placed individually in the lit area. The number of transitions in the lit and dark box and the time spent in the lit box were noted in 5 min [5].

2.4.3 Open Field Test

The apparatus consisted of a wooden box (96 x 96 x 5 cm). The floor of the box was divided into 16 (6 x 6cm) squares. Mice divided into groups of 5 each received ME (30,100 mg/kg) or EAF (30,100 mg/kg) or vehicle. After 30 min they were placed individually in one corner of the square. The transfer latency, number of rearing, assisted rearing, and the numbers of squares traversed were counted for five min [6].

3. Results

3.1 Elevated Plus Maze

The vehicle treated mice spent 31.77 ± 6.23 seconds in the open arm, whereas animals treated with ME and EAF spent significantly more time in the open arm. EAF (100 mg/kg) also increased the time spent in open arms,

Table 1
Effect of ME and EAF on time spent in open arms and entries in open and closed arms of the elevated plus maze in mice

SL. No	Treatment (Dose: mg/kg)	Time spent in O.A	Entries in O. A	Entries in C. A
1	Vehicle	31.77±6.23	2.55±0.55	5.55±1.7
2	Diazepam (1)	83.6±13.42*	4.6±0.18	5.2±0.72
3	ME (30)	46.33±5.10	3.33±0.16	3.67±0.19
4	ME (100)	74.4±14.51*	4.4±1.18	5±0.77
5	EAF(30)	83.4±16.39*	5.6±0.79	5.4±0.9
6	EAF(100)	99±9.53*	9±1.05*	11.75±1.5*

n = 5, The observations are mean ± SEM. *P< 0.05,ANOVA followed by Dunnett's test.

Table 2
Reversal of m-CPP by EAF and Diazepam on time spent in open arms and entries in open and closed arms in mice

Sl. No .	Treatment (Dose: mg/kg)	Time spent in open arms	Entries in open arms	Entries in closed arms	No.of head dips
1	m-CPP(1)	10.25±5.04	1.75±0.75	3.75±0.94	2.75±2.13
2	m-CPP + EAF(30)	23.25±3.32	2.35±0.47	5.25±0.85	2.5±0.27
3	m-CPP + EAF(60)	36.5±12.23	3±0.57	9.5±1.5	7.5±5.48
4	m-CPP + EAF(120)	73.25 ±23.44	4.5±0.64	7.25±0.7	9±5.03
5	m-CPP + EAF(240)	79±16.02*	2.16±0.57	5±0.5	19±0.5
6	m-CPP + EAF(480)	61±15.25	3.5±1.25	4±1.35	9.5±1.04
7	EAF(70)	147.16±40.11*	6±2.29	4.66±1.15	9±3.278
8	m-CPP + Diazepam (0.5)	22.25±8.32	2.5±0.95	7.5±0.64	2.25±0.25
9	m-CPP + Diazepam (1)	21.75±4.80	2.25±0.47	4.25±0.85	0.75±0.47
10	m-CPP + Diazepam (2)	36.75±4.80	1.75±0.47	6±1.41	2.75±0.47
11	m-CPP + Diazepam (4)	63.75±18.15	4.75±2.92	5.25±2.65	10.5±5.35
12	m-CPP + Diazepam (8)	147±50.55*	4.5±1.6	5.25±1.79	18.25±6.75
13	m-CPP + Diazepam (16)	51±8.02	4.5±0.64	7.25±1.03	7.25±1.25

The values are mean ± SEM of five observations. *P< 0.05, ANOVA followed by Dunnett's test

entries in both the open and closed arms significantly. The observations are given in Table 1. In another study, EAF (30,60,120,240,480 mg/kg) and Diazepam (1,2,4,8,16 mg/kg) significantly antagonized m-CPP (1 mg/kg) in a dose dependent fashion. The ED_{50} value was found to be 48.97 mg/kg. The observations are given in Table 2.

3.2 Light/Dark test:

The vehicle treated group spent 70.89 ± 10.05 sec in the lit box and showed 7.22 ± 0.74 number of transitions, whereas animals treated with EAF (100 mg/kg) showed a significant increase in the time spent in lit zone. The observations are given in Table 3.

Table 3
Effect of ME and EAF on time spent in lit zone and number of transitions in light/dark apparatus

Sl. No.	Treatment (Dose: mg/kg)	Time spent in lit zone	No. of transitions
1	Vehicle	70.89 ±10.05	7.22 ± 0.74
2	Diazepam (1)	111.8 ±8.06*	9.2 ± 1.04
3	ME (30)	108.33± 4.76	10.0 ± 1.76
4	ME (100)	105.6 ±21.09	6.0 ± 0.0
5	EAF(30)	134.6 ±28.41	8.4 ±0.83
6	EAF(100)	116 ±16.60*	7.25 ±1.31

n= 5, observations are mean ± SEM. *P< 0.05,ANOVA followed by Dunnett's test.

3.2 Open Field Test

The vehicle treated mice reared for 2.28 ± 0.68 times during the test interval of 5 min. The EAF (100mg/kg) significantly increased the number of assisted rearing and the number of squares traversed. The observations are given in Table 4.

4. Discussion

The study observed that alkaloid containing ethyl acetate fraction (EAF) of methanol extract of trigonella foenum-graecum seeds possessed anxiolytic activity.

However researchers have reported different effects of buspirone on EPM i.e anxiolytic [7-11], non-effective [12, 13], anxiogenic [14-19]. A drug may have both anxiolytic and anxiogenic activities and either of the activities may be dependent on experimental conditions [20]. Drugs must be carefully assessed on elevated plus maze test and therefore in the present study EPM is supported by other tests.

In another study using EPM different doses of EAF (30, 60,120,240,480 mg/kg) antagonized the effects of m-CPP (1mg/kg) in a dose dependent fashion. An inverse U dose response relationship was observed. The antagonistic action of EAF against m-CPP substantiates anxiolytic activity. EAF increased occupancy in open arm of the elevated plus maze and also exhibited diminished preference to the closed arm.

The Light/Dark test is based on the natural preference of mice for a dark place rather than a brightly lit area. Anxiolytics increase exploration in the lit area [21].

It is interesting that many studies in the Light/Dark test have been conducted using mice rather than rats. Crawley reported that rats were not respondent to treatment with diazepam in this paradigm [22], and that their exploratory tendencies appeared considerably lower than in mice, suggesting that rats were not useful in this test.

Table 4
Effect of ME and EAF on transfer latency, rearing and locomotion in open field test in mice

SL. NO	Treatment (Dose: mg/kg)	Transfer Latency	Rearing	Assisted Rearing	Squares traversed
1	Vehicle	5.14±0.84	2.28±0.68	26.71±2.75	111.57±5.14
2	Diazepam (1)	1.8±0.28	2.8±1.23	25.2 ±3.08	160.2±16.23*
3	ME (30)	2±0	2±0.88	36.33 ±1.01	109±11.73
4	ME (100)	5.4±1.9	1.8±0.99	31.4 ±3.77	112.8±11.08
5	EAF(30)	2.8±0.36	29±3.48*	8.6 ±2.21*	104.6±11.05
6	EAF(100)	1±0.0	7±0.90	36.75±3.96*	140.75±17.49*

n = 5, values are mean ± SEM. *P< 0.05 ANOVA followed by Dunnett's test.

Anxiolytic benzodiazepines, chlordiazepoxide and clonazepam, increased light-to-dark transitions and total locomotion in mice at non-sedative doses but did not change the time spent. In our study EAF (100 mg/kg) increased the time spent in lit zone but did not change the number of transitions.

In open field test, EAF (100 mg/kg) showed an increased tendency to reach to the walls and rear rather than rearing without support . It also increased the squares traversed. Thus, the observations suggested anxiolytic effect of EAF. Decrease in locomotion is indicative of diminished dopaminergic transmission, which may be secondary to the rise in 5-HT level caused by anxiogenic agents. [23, 24].

Thus, it is concluded that EAF of methanol extract of *Trigonella foenum-graecum* seeds that contain alkaloid possess 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.

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