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Anxiolytic activity of ethanolic extract of *Trigonella foenum-graecum* seeds

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ABSTRACT

The purpose of this study was to characterize the putative anxiolytic-like activity of an ethanolic extract prepared from the seeds of Trigonella foenum-graecum (TFG) using an elevated plus-maze (EPM) and light dark transition model in mice. In the EPM test, TFG seeds extract (100 and 200 mg/kg) significantly ($p<0.01$) increased the percentage time spent and number of entries in the open arm. The effect was comparable to that of references compound, diazepam (2 mg/kg). In light dark transition model, diazepam-treated rats significantly increased the time spent in light zone and decreased the duration of immobility, while TFG treated mice also showed a significant ($p<0.01$) increase in the time spent in light zone. Diazepam and the TFG extracts do not produced any overt motor dysfunction. These results indicate that TFG is an effective anxiolytic agent and could be useful in alternative treatment.

Keywords: *Trigonella foenum-graecum*, Anxiolytic, Elevated plus-maze, Light dark transition

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INTRODUCTION

India is a country of vast biodiversity and traditional knowledge for using herbal medicines to cure many ailments in various cultures and tribes [1]. Anxiety is an exaggerated feeling of apprehension, uncertainty, uneasiness and dread. Anxiety causes various disorders such as generalized anxiety, panic, obsessive-compulsion, phobias or post traumatic stress. Excessive anxiety can weaken and damage the quality of life [2]. In the clinical care of anxiety benzodiazepines, $GABA_A$ receptor agonist and buspirone, $5\text{-}HT_{1A}$ receptor agonist, are chiefly prescribed as first choice treatment [3]. Chronic administration of benzodiazepines causes physical dependence such as sedation, myelorelaxation, ataxia, amnesia and pharmacological

dependence. Furthermore, buspirone also results in dizziness, headache, nervousness, paresthesia, diarrhea, excitation and sweating as adverse effects [4,5].

Therefore, research has been conducted to investigate natural anxiolytic agents using ethanolic extract prepared from the seeds of *Trigonella foenum-graecum* (TFG, Papilionaceae). TFG commonly known as Fenugreek is an aromatic, 30-60 cm tall, annual herb, cultivated throughout the India. In Indian traditional system of medicines, seeds of this plant are widely used as antipyretic, anthelmentic, astringent, cure leprosy, emetic, anti-inflammatory, anti-arthritis, emmenagogues and also useful in heart disorders [6]. Fenugreek can be taken along with Gurmar, goat's rue and neem leaves for the treatment of diabetes [7]. Earlier workers have reported to possess antipyretic, antidiabetic, wound healing activities [8-10].

MATERIAL AND METHODS

Plant Material: TFG seeds were collected from the local market, during the month of July 2004. The seeds were authenticated by Dr. P. Channabasappa, Department of Botany, Sree Siddaganga College of Pharmacy (SSCP), Tumkur, Karnataka. A voucher specimen No. PP-679 has been deposited in the herbarium of SSCP.

Drugs and Chemicals: Ethanol LR grade were employed for the extraction of the plant material. Diazepam ampoule was procured from Sigma, India and was used as standard drugs. Two different concentrations (100 and 200 mg/kg) of the TFG seeds extract were prepared by dissolving the extracts in control (distilled water).

Preparation of ethanolic TFG seeds extract: The fresh TFG seeds (250 g) were washed with tap water and shade dried at room temperature (28±2°C). The dried seeds were powdered by electrical blender and extracted with ethanol (8 h) in soxhlet apparatus following the standard procedure. The solvent was removed at the reduced pressure with the help of rotary vacuum evaporator to yield a syrupy extract (10.5 g) and was used for phytochemical investigation.

Animals: Swiss albino mice between 6 and 12 weeks old and weighing 22–34 g were procured from animal house, SSCP. Mice were housed in cages of five at 20±1 °C in a 12 h light/dark cycle. The mice were allowed standard food pellets (Hindustan lever, Bangalore) and tap water *ad libitum*. Animals were habituated to laboratory environment for 48 h prior to experimental steps to minimize non-specific stress. Groups of five mice were used in entire sets of experiments. The Institutional Animal Ethical Committee approved the protocol of this study.

Acute Toxicity Studies: TFG ethanolic extract at different doses (50-2000 mg/kg) was administered orally to mice. During the 24 hours after the drug administration, the animals were observed for gross behavioral changes such as hyperactivity, grooming, convulsions, sedation, hypothermia, and mortality were observed and doses selected were 100 mg/kg and 200 mg/kg, body weight.

Assessment of Anxiolytic Activity: The anxiolytic activity of TFG was examined using the elevated plus maze (EPM) and Light dark transition model in mice. The animals were divided into four groups, consisting of five mice per group. Group 1 received vehicle (distilled water);

Groups 2 and 3 received TFG 100 and 200 mg/kg, respectively; Group 4 received diazepam 2 mg/kg.

Elevated plus-maze model: The plus-maze apparatus consisting of two open arms (16×5 cm) and two closed arms (16×5×12 cm) having an open roof, with the plus-maze elevated (25 cm) from the floor was used to detect anxiolytic behavior in mice. The animals were deprived of food but not water 18 h prior to the experiment. The dose administration schedule was so adjusted that each mice was having its turn on the elevated plus-maze apparatus 45 min after the administration of the dose. Mice were placed at the center of the elevated plus-maze with its head facing the open arms. During this 5 min experiment procedure, the behavior of the mouse was recorded as: preference of the mouse for its first entry into the open or closed arms, number of entries into the open or closed arms, average time spent by the mouse in each of the arms (average time=total duration in the arms/number of entries). Ethanolic extracts of TFG seeds (100 and 200 mg/kg) were administered orally using a tuberculin syringe fitted with oral canula. During the entire experiment, the mice were allowed to socialize. Every precaution was undertaken to ensure that no external stimuli, except the height of the plus maze could invoke anxiety in mice. Likewise observations were reported for the standard group (Diazepam 2 mg/kg) as well as the vehicle group [11].

Light dark transition model: The apparatus used was open top wooden box. The box was divided by a barrier possessing a doorway (7.5 x 5 cm), which mice could cross in two chambers of measures (20 x 30 x 35 cm) painted black and illuminated with dimmed red light and a bright chamber (30 x 30 x 35 cm) painted white and illuminated with 100-W white light source. Mice was placed individually in the center of the light zone and observed for the next 5 minutes for the number of crossing between two compartments and time spent in the light and dark arena. Diazepam dose of 2 mg/kg, i.p. was used as a standard compound [12].

Statistical analysis: All data are given as mean ± SEM and analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's test. The groups treated with TFG extracts were compared with the respective vehicle group. The diazepam-treated group was compared with vehicle. P values <0.01 were considered statistically significant.

RESULTS

Elevated plus-maze model: Oral administration of 100 and 200 mg/kg of TFG produced a significant ($p<0.01$, ANOVA followed by Dunnet's test) increase in time spent and total arm entries in the open arms of the maze, as compared to control, suggesting an anxiolytic effect of this extract. Animals treated with diazepam (2 mg/kg) showed more pronounced anxiolytic effect (Table 1).

Light and Dark Box model: Diazepam-treated rats significantly ($p<0.01$) increased the time spent in light zone and decreased the duration of immobility. TFG-treated animals also showed a significant ($p<0.01$) increase in the time spent (100 and 200 mg/kg) in light zone. TFG seed extracts reduced the duration of immobility at the highest dose (200 mg/kg). An increase in the number of entries into light arena was not significant (Table 2).

Table 1: Effects of TFG seeds extract on following parameters in EPM paradigm

Treatment	Dose (mg/kg)	No. of entries (n)		Time spent in open arm (sec)	Motor activity
		Open arm	Closed arm		
Control	-	1.9 ±0.3148	16.9±0.6013	29.1±1.981	131.23±2.191
TFG extract	100	8.1±0.6832	12.2±0.5811	39.2±1.439**	119.72±3.134**
	200	8.4±0.6598	11.5±0.5873	46.7±1.863**	125.87±2.98**
Diazepam	2	7.6±0.5671	11.7±0.5729	115±1.971**	79.61±2.379**

*Data expressed as mean ±S.E.M (n=5); **p<0.01 compared with vehicle treated*

Table 2: Effects of TFG seeds extract on following parameters in light dark transition model

Treatment	Dose (mg/kg)	No. of entries (n) in the light compartment	Time spent in the light compartments (sec)	Motor activity
Control	-	10.1±0.8711	25.1±1.304	131.23±2.191
TFG extract	100	13.5±0.7874	44.2±1.3923**	119.72±3.134**
	200	17.5±0.8341	76.3±1.1704**	125.87±2.98**
Diazepam	2	16.2±0.7413	103±1.971**	79.61±2.379**

*Data expressed as mean ±S.E.M (n=5); **p<0.01 compared with vehicle treated*

DISCUSSION

There has been a considerable popular interest in the use of herbal products, to treat anxiety reaction. *Hypericum perforatum* is the most well-known herbal product available over the counter. Recently, several herbal constituents have been reported to possess anxiolytic effects through animal models of anxiety [13].

More recently, it has been laid out that the incorporation of a range of ethological parameters may enhance the utility of this paradigm [14]. Thus, we chose and their ability to facilitate exploratory activity in the light/dark paradigm in mice [15].

The EPM is the first choice model for anxiolytic drugs and has been validated for both rats and mice [16,17]. Transitions have been described to be an index of activity exploration because of habituation over time and the time spent in each zone to be a reflection of aversion [18]. It has been concluded that measurement of the time spent in the light zone, but not the number of transfers was the most reproducible and useful parameter for assessing anxiolytic activity [19]. These data seem to be in good agreement with our results. The present data showed that TFG seed extracts increase the time spent in the light zone, suggesting again these extracts possesses anxiolytic properties. Results of this study indicated that the ethanolic seed extracts of TFG had central anxiolytic effects. Future prospects of this work include the identification of the active constituents present in TFG seed extracts along with the pharmacological mechanisms underlying the anxiolytic activity.

CONCLUSION

In conclusion, the action of extracts upon the anxiety models tested are in accordance with the traditional use of TFG. Since there is a need for new anxiolytic compounds with less side effects

compared to synthetic medication, Herbal products could be an inspiration of new prototypes for drug development.

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REFERENCES

[1] D. Bhowmik, Chiranji, P. Tiwari, K.K. Tripathi, K.P.S. Kumar, *Annals of Biological Research*, **2010**, 1(1), 41-46.
[2] J.J. Seo, S.H. Lee, Y.S. Lee, B.M. Kwon, Y. Ma, B.Y. Hwang, J.T. Hong, K.W. Oh, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **2007**, 31, 1363–1369.
[3] M. Lader, S. Morton, *Br. J. Addict*., **1991**, 86, 823–828.
[4] A.D. Jordan, C.P. Kordik, A.B. Reitz, P.J. Sanfillipo, *Expert Opin. Ther. Pat*., **1996**, 6, 1047–1060.
[5] K. Rickels, E. Schweizer, **1997**, *J. Clin. Psychiatry*, 58, 4–9.
[6] K.R. Kirtikar, B.D. Basu, Indian Medicinal Plants, 2nd Edn., International Book Distributors, Rajpur road, Dehradun, India, **1993.**
[7] A. Saneja, C. Sharma, K.R. Aneja, R. Pahwa, *Der Pharmacia Lettre*, **2010**, 2(1), 275-284.
[8] B.N. Shah, A.K. Seth, *Archives of Applied Science Research*, **2010**, 2(3), 188-195.
[9] A. Chauhan, P.K. Sharma, P. Srivastava, N. Kumar, R. Dudhe, *Der Pharmacia Lettre*, **2010**, 2(3), 369-387.
[10] A. Mallik, D. Goupale, H. Dhongade, S. Nayak, *Der Pharmacia Lettre*, **2010**, 2(2), 457-463.
[11] S. Pellow, S.E. File, *Pharmacol. Biochem. Behav*., **1986**, 24: 524–529.
[12] B. Costall, B.J. Jones, M.E. Kelly, R.J. Naylor, D.M. Tomkins, *Pharmacol. Biochem. Behav*., **1989**, 32, 777–785.
[13] G. Beaubrum, G.E. Gray, *Psychiatr. Serv*., **2000**, 51, 1130–1134.
[14] R.J. Rodgers, N.J.T. Johnson, *Pharmacol. Biochem. Behav*., **1995**, 52, 297–303.
[15] J.N. Crawley, F.K. Goodwin, *Pharmacol. Biochem. Behav*., **1980**, 13, 167–170.
[16] S. Pellow, P. Chopin, S.E. File, M. Briley, *J. Neurosci. Methods*, **1985**, 14, 149–167.
[17] R.G. Lister, *Psychopharmacol*., **1987**, 92, 180–185.
[18] C. Belzung, R. Misslin, E. Vogel, R.H. Dodd, G. Chapouthier, *Pharmacol. Biochem. Behav*., **1987**, 28, 29–33.
[19] R. Young, D.N. Johnson, *Pharmacol. Biochem. Behav*., **1991**, 40, 739–743.