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Neuropharmacological activity of *Trigonella foenum graecum* Linn. seeds

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Abstract

Objective: To evaluate the neuropharmacological activity of *Trigonella foenum graecum* (Fenugreek) seeds. Methods: Total alcoholic extract (TA), Total aqueous extract (TQ), Petroleum ether extract (PE), Total alkaloidal extract (TK), Total glycosidal extract (TG), Fenugreek oil (FO), Diosgenin (DI) and Trigonelline (TR) were assessed for their neuropharmacological activity in primary screening studies, locomotor activity and phenobarbitone induced sleeping time using albino Wistar rats. The extracts TA, TQ, PE and FO were administered 100 mg/kg b.w. and TK, TG, DI and TR were administered 50 mg/kg b.w. intraperitoneally. Caffeine (CF) (48 mg/kg) and Chlorpromazine hydrochloride (CP) (150 µg/kg) were used as standard drugs. Results: In all the studies, all the extracts and active principles except TQ showed significant central nervous system (CNS) stimulant activity, while TQ alone showed significant CNS depressant activity. Conclusion: Thus from the above studies, the active compounds present in the fenugreek seed extracts possess CNS stimulant and depressant activities.

Keywords: *Trigonella foenum graecum*, locomotor activity, phenobarbitone induced sleeping time.

1. Introduction

During the last few decades, therapeutics with psychoactive synthetic drugs has been recognized as most effective in the management of central nervous system (CNS) related disorders. However, continuous use of these neuropharmacological based drugs will lead to a variety of autonomic, endocrine, allergic, hematopoietic and neurological side effects [1]. To avoid such side effects, currently search for new therapeutic agents with minimum side effects and maximum potency from medicinal plants continues. Generally, plants possess many

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pharmacological actions, since they contain numerous constituents of active chemicals in it. Based on the above criteria, this study is designed to evaluate the activity of different extracts and two phytochemicals from fenugreek seeds on CNS.

Trigonella foenum graecum Linn. is commonly known as fenugreek and belongs to the family leguminosae [2]. Fenugreek is one of the most widely used plants in various indigenous systems of medicine for the treatment of different ailments. The seeds of this plant are known to possess anti-ulcer [3], hypocholesterolemic [4], wound healing [5], Immunomodulatory [6] and anti-inflammatory [7] activities. During the period of our research, we performed and published for the first time, the cardio-tonic activity [8] of fenugreek seeds. Previous phytochemical studies reveals the presence of Diosgenin, Trigonelline, Vicenins 1 and 2, Vitexin, Kaempferol, Luteolin, Quercitin and β-Sitosterol in the seeds [9]. In the light of the above information and folk fore use, the present study was designed to evaluate the CNS activity of fenugreek seeds in experimental models in rats.

2. Materials and methods

2.1 The plant

Fenugreek seeds were purchased locally, authenticated by Prof. Dr. R. Rangasamy, CAS Botany, University of Madras, Chennai, India.

2.2 Preparation of extracts

1500 g of seeds were used for extraction. The seeds were coarsely powdered and shade dried. Total alcoholic extract was obtained by macerating 500 g of powdered seeds in ethanol (95%) for 1 month, then the filtrate was taken and concentrated at 55°C using water bath, the extract concentrate was coded as TA and the yield was 5%. Aqueous extract was obtained by macerating 500 g of seeds in double distilled water for 24 h, then the filtrate was taken and concentrated at 55°C using water bath, coded as TQ, yield was 3.4%. Petroleum ether extract was obtained by macerating 500 g of seeds in petroleum ether solvent (boiling point 65°C) overnight at room temperature, then the filtrate was taken and concentrated at 55°C using water bath, coded as PE and the yield was 3.6% [9]. The total alkaloidal extract (TK) and total glycosidal extract (TG) were obtained using the method of Ferguson [10]. Based on the literature survey and the percentage of phytochemicals present in the seeds [11], Diosgenin (DI) and Trigonelline (TR) were obtained commercially along with fenugreek oil and were used in this study.

2.3 Animals

Albino Wistar rats (150 - 200 g) were obtained from central animal house, University of Madras and used for the studies. Animals were housed in colony cages at an ambient temperature of 25 ± 2°C with free access to food and water *ad libitum*. All the experiments were carried out during the light period (08:00 – 16:00). The Institutional Animal Ethical Committee approved the protocol of this study.

2.4 Drugs and Chemicals

Diosgenin and Trigonelline were obtained from Sigma Aldrich Company; Fenugreek oil was obtained from local essential oil marketing company.

2.5 Preparation of dosage forms

The extracts, principles and drugs such as TA, PE, FO, DI and CP were found to be insoluble in water. To overcome this water insolubility, primary emulsion with 5% gum acacia followed by a suspension in double distilled water was prepared. The required doses were prepared

according to the experiments. The codes TQ, TG, TK, TR and CF were soluble in normal saline. Thus, they were made up for required doses with normal saline. Normal saline (NS) and gum acacia suspension (GA) were run as controls for comparing the activities of the aqueous soluble substances and gum acacia suspensions, respectively.

2.6 CNS studies

2.6.1 Primary screening studies

This study was conducted as described in the techniques proposed by Sheth *et al*., [12] and Vane [13]. This study shows the observatory parameters such as sedative, hypnotic, loss of pinna reflex for CNS depressant activity and increased motor activity, jumping, frequent urination for CNS stimulant activity. Each extract and principle was administered 50 and 100 mg/kg b.w. intraperitoneally, each group consisting of six animals. The animals were observed for first 2 h, then half-hourly once for next 6 h.

2.6.2 Locomotor activity

The locomotor activity was measured using an actophotometer. Movement of the animal cuts off a beam of light falling on the photocell and a count was recorded and displayed digitally. The total readings for 5 minutes gives a measure of the spontaneous motor activity. Animals were selected and divided into 12 groups, each consisting 6 animals. Two control groups were injected with normal saline and 5% gum acacia 10 ml/kg b.w. respectively. Positive control groups were administered with CNS stimulant caffeine (CF) (48 mg/kg b.w.) and a CNS depressant chlorpromazine hydrochloride (CP) (150 µg/kg b.w.). TA, TQ, PE and FO were administered 100 mg/kg b.w. and TK, TG, DI and TR were administered 50 mg/kg b.w. The spontaneous motor activity of the controls and the treated groups were measured after 30 min of administration of extracts and principles [12].

2.6.3 Phenobarbitone induced sleeping time

This test determines the influence of a test drug or extract on the duration of sleep induced by a barbiturate hypnotic such as phenobarbitone sodium in rats (40 mg/kg b.w., i.p) [12]. The animals were divided into 11 groups each consisting of 6 animals. The groups were mentioned and the administration of the drugs, extracts and principles were done as per the procedure mentioned above. The extracts, active principles and drugs were administered 30 min before commencement of the experiment. At the commencement of the experiment, phenobarbitone was injected intraperitoneally at a dose of 40 mg/kg b.w. to all the animals. The animals were placed on their back repeatedly until they fail to regain the normal posture of resting on their ventral side (loss of righting reflex). Once the animals lost their righting reflex, the time was noted until the regaining of their righting reflex.

3. Results

3.1 Primary screening studies

The observations were presented in the Table 1. titled “Primary screening studies”. All the extracts and active principles except TQ produced CNS stimulant effect which was characterized by increased motor activity, jumping and frequent urination. This frequent urination might be due to the consequence of heart stimulation as these extracts also produced significant stimulation of heart in our earlier studies [8]. TQ alone showed CNS depressant effect, which was characterized by prolonged sleep and loss of pinna reflex.

Fig. 1.
Effect of fenugreek seeds on locomotor activity

Statistical significance test for comparison of test with control was done by one way ANOVA followed by Dunnet's *t*-test (n=6).
a - compared with normal saline (NS) control,
b - compared with gum acacia (GA) control
*p<0.05, **p<0.01, ***p<0.001

Fig. 2.
Effect of fenugreek seeds on phenobarbitone induced sleeping time

Statistical significance test for comparison of test with control was done by one way ANOVA followed by Dunnet's *t*-test (n=6).
a - compared with normal saline (NS) control,
b - compared with gum acacia (GA) control
*p<0.05, **p<0.01, ***p<0.001

3.2 Locomotor activity

The results of locomotor activity study were presented in the Fig. 1. titled as "Effect of fenugreek seeds on locomotor activity". All the extracts and active principles except TQ significantly increased the score reading of motor activity. TQ significantly reduced the motor activity. The values obtained (Mean ± SEM of motor scores) with respect to different extracts and active principles were shown in the following ascending rank order.

CP (60.33 ± 4.153) < TQ (148.96 ± 11.00) < NS (208.60 ± 18.63) < GA (214.04 ± 15.99) < PE (259.98 ± 11.47) < TK (260.85 ± 12.48) < TA (265.32 ± 10.69) < TG (271.38 ± 11.48) < FO (279.56 ± 11.62) < DI (280.22 ± 13.75) < TR (283.49 ± 10.59) < CF (328.58 ± 17.49).

3.3 Phenobarbitone induced sleeping time

The results obtained were presented in Fig. 2. titled "Effect of fenugreek seeds on phenobarbitone induced sleeping time". All the extracts and principles except TQ significantly reduced the sleeping time induced by phenobarbitone sodium. The extract TQ alone significantly potentiated the sleeping time induced by phenobarbitone sodium. The values obtained (Mean ± SEM of sleeping time) with respect to different extracts and active principles were shown in the following ascending rank order.

CF (80.74 ± 6.42) < FO (108.92 ± 7.29) < TK (114.68 ± 8.08) < DI (118.92 ± 7.74) < TG (120.37 ± 9.76) < PE (122.19 ± 9.65) < TA (124.66 ± 9.88) < TR (126.08 ± 9.20) < NS (174.52 ± 16.27) < GA (180.36 ± 17.06) < TQ (262.80 ± 21.77).

4. Discussion

The human cerebral cortex serves to control functions such as speech, memory, logical and emotional response, as well as consciousness, interpretation of sensation and voluntary movement [14]. It is generally accepted that the sedative effect of the drugs on CNS can be evaluated by the measurement of spontaneous motor activity and phenobarbitone induced sleeping time in laboratory animal model [15]. Also, Fujimori [16] proposed that the enhancement of barbital hypnosis is a good index of CNS depressant activity. The prolongation of barbital hypnosis might be due to sedative property attributed to the inhibition of barbital metabolism [17] or central mechanisms involved in the regulation of sleep [18].

Table 1. Primary screening studies

Extracts/Active Principles	**Observations**		**Inference**
	50 mg/kg	**100 mg/kg**	
TA-Total alcoholic extract	Increased motor activity	Jumping, frequent urination	CNS Stimulation
TQ- Total aqueous extract	Sedation	Loss of pinna reflex, sleep for 1 hour	CNS Depression
PE-Petroleum ether extract	Hyper activity	Jumping, frequent urination	CNS Stimulation
TG-Total glycosidal extract	Hyper activity	Frequent urination	CNS Stimulation
TK-Total alkaloidal extract	Increased motor activity	Jumping, frequent urination	CNS Stimulation
FO-Fenugreek oil	Hyper activity	Frequent urination	CNS Stimulation
DI-Diosgenin	Hyper activity	Frequent urination	CNS Stimulation
TR-Trigonelline	Increased motor activity	Jumping, frequent urination	CNS Stimulation

The present study reports the neuropharmacological activities of different extracts and active principles of the seeds of *Trigonella foenum graecum* Linn. in rats. The results indicated that all the extracts and active principles except the aqueous extract significantly showed CNS stimulant activity on all the methods. The aqueous extract alone showed CNS depressant activity. The CNS depressant action of TQ might be due to the hypothesis stated by Fujimori and the CNS stimulant activity of rest of the extracts and active principles might be due to the reverse action of the above mentioned hypothesis. The efficacies of the most herbal therapies are attributed to numerous active principles in combination. Plant based natural compounds – flavonoids like Quercitin and Kaempferol were shown to possess CNS stimulant activity [19]. As discussed previously the fenugreek seeds also contains the above mentioned flavonoids [9]. Recent literature survey also revealed that the fenugreek seeds possess nootropic and anxiolytic activity [20]. These informations provides a vital support for this valuable findings of fenugreek and its effect on CNS.

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