Original Article

Assessment of antidepressant activity of fenugreek seeds methanol extract

Tahira Assad, Navaid Siddiqui, Muhammad Ali Rajput, Hafiza Touseef Sayyar, Madiha Soban, Taif Hameed

Departments of Pharmacology, Karachi Institute of Medical Sciences, Bahria University Medical and Dental College, Multan Medical and Dental College and Makran Medical College, Quetta, Pakistan

Objective: To assess the effect of fenugreek seeds methanol extract (FS-ME) on depression in mice.

Methodology: The effect of FS-ME was assessed after 14 days of oral feeding. We used two models i.e. forced swimming test (FST) and tail suspension test (TST) at three different doses. Tests were conducted at days 1, 7 and 14.

Results: In both FST and TST, the period of immovability in mice receiving extract dose of 100mg/kg was reduced significantly on day 7th and 14th as compared to control. While the period of immobility in mice received FS-ME at 200 mg/kg was reduced highly noticeably as compared to control and was comparable to reference drug imipramine at 15mg/kg. The decline in immobility time in both tests revealed antidepressant effect.

Conclusion: FS-ME showed noteworthy antidepressant effect in mice but further preclinical and clinical trials are necessary to approve these findings. (Rawal Med J 202;46:236-239).

Keywords: Antidepressant, fenugreek seeds, tail suspension test.

INTRODUCTION

Depression is highly prevailing psychological ailment, characterized by sad mood, lack of interest, absence of vigor, feelings of guilt, disturbed sleep and appetite, decreased energy and reduced centralization.[1] The frequency of depression is about 3-10%, however, it reaches up to 22-46% in patients suffering from chronic ailments.[2] Many plants have documented antidepressant actions like *Hypericum perforatum and Pueraria lobata*.[3] Fenugreek (*Trigonella foenum-graecum*), belonging to family Fabaceae is a renowned prehistoric plant. Different parts of this plant are consumed in diet such as seeds and leaves.[4] Fenugreek seeds have a unique rhomboid shape, distinctive peppery smell and acrimonious taste.[5]

Seeds are usually consumed as spice in diet as well as for the cure of various medical ailments. It contains several bioactive components i.e. carpaine (alkaloids), fenugreekine, diosgenin (saponins), gentianine, trigonelline, flavonoids and 4-Hydroxy isoleucin, arginine (amino acids).[6] Various activities of this plant have been reported such as hypoglycemic, hypolipidemic and antioxidant effects.[7-9] This study was intended to evaluate the effect of FS-ME on depression in mice.

METHODOLOGY

Extract formation: Fenugreek seeds were purchased from local herbal store and identified by Pharmacognosy department (Voucher specimen # FGS-01-14/16). After cleansing, seeds were crushed to fine powder, soaked in methanol (80% 1000ml) in air tight jars for 10 days with every two days stirring. Solvent was filtered by cotton and Whatmann No.1 filter paper. Acquired filtrate was evaporated under reduced pressure on rotary evaporator at 45°C, trailed by freeze-drying at -30°C. Crude extract thus attained was kept at -20°C in Petri dishes.[10]

Animals: This study was performed at Pharmacology department, UOK, after permission from Board of Advanced Study and Research. Male Swiss albino mice, weighing 23-29 g were placed in plastic cages at animal house, 22±2°C temperature and 50-60% humidity were maintained in 12-h light/dark cycle alternatively. Mice were provided standardized diet. National Institute of Health guidelines were followed for experimentation.[11]

Groups: All 35 animals were evenly distributed into 5 groups with seven in each group. Group A served as control and received 1ml/kg body weight

oral distilled water O.D. Group B received Imipramine hydrochloride (Indus Pharma Pvt. Ltd) at 15 mg/kg body weight orally O.D. Group C, D and E served as test groups and received extract in three varying doses i.e. 50, 100, and 200 mg /kg body weight diluted in 1ml distilled water orally O.D. Dosing was continued for 14 days.[12]

Forced swimming test (FST): Transparent Plexiglas cylinder (24 cm height and 13 cm diameter) containing water (22°C, 10cm depth) was used for swim sessions. On day 0, individual mouse was permitted to swim freely in cylinder for 15 min as a pre-test session. No drug or extract was administered. This pre-test session is just a familiarization session and endorses a steady and maximum duration of immobility in the core session. Doses were administered to respective groups orally thrice a day in the following manner: after pre-test session, 4 h and 1 h prior to core session. Core test session was performed 24 hr. after pre-test session for 6 min. Immobility duration was recorded using stopwatch during last 4 min. Water was replaced after each test session.[13]

Tail suspension test (TST): On test day, 40 min after last dosing, each mouse was separately hanged in an upside down position on border of a table 50 cm above the ground using sticky tape sited 1 cm from the tip of the tail. Immobility duration was calculated during the last 4 min of test period. Mice were considered to be immobile when their body was hanging passively in the air without any mobility.[14]

Open field test (OFT): The test was conducted using a wooden box (40cm×40cm×40cm) whose floor was equally divided into 25 squares. Individual mouse were kept in the center of box and permitted to discover it. Number of crossings (number of squares traversed by mouse with four paws) and the number of rearings (standing on the hind leg) were counted for 5 minutes.[15]

Statistical Analysis: We used SPSS version 23. Anova followed by Tukey's post hoc was applied for comparisons with control. $p \leq 0.05$ was considered significant and $p \leq 0.001$ as highly significant.

RESULTS

In both FST and TST model, it can be seen that both at day 7 and 14, immobility period decreased significantly in Group D, while highly significantly in group E and Group B in comparison to control group. (Tables 1 and 2).

Table 1. Effect of FS-ME and Imipramine on immobility period in FST.

Group	Immobility duration (s)		
	Day 1	Day 7	Day 14
Group A Distilled Water 1ml/kg	196.12± 2.03	195.61±1.38	196.32±0.56
Group B Imipramine 15mg/kg	192.31± 1.18	138.83±1.14**	133.61±1.09**
Group C 50mg/kg I	196.55± 3.01	195.49±1.37	193.89±2.01
Group D 100mg/kg	195.06± 1.79	163.19±1.28*	160.17±1.09*
Group E 200mg/kg	195.07± 2.03	146.63±1.61**	142.31±1.54**

n=7, values mentioned as mean ± standard error of mean, *P≤0.05 significant in comparison to control group, **P≤0.001 highly significant in comparison to control group

Table 2. Effect of FS-ME and Imipramine on immobility period in TST.

Group/ Dosage	Immobility duration (sec)		
	Day 1	Day 7	Day 14
Group A Distilled Water 1ml/kg	192.58± 2.20	191.33±0.57	192.47±1.91
Group B Imipramine 15mg/kg	190.07± 1.29	131.71±2.13**	122.53±2.11**
Group C 50mg/kg	191.09± 2.14	189.39±2.27	190.07±1.26
Group D 100mg/kg	190.47± 1.67	163.17±0.77*	161.23±2.42*
Group E 200 mg/kg	190.03± 1.37	155.21±1.91**	137.17±1.82**

Table 3. Effect of FS-ME in OFT.

Group/Dosage	Number of crossings	Number of rearings
Group A Distilled Water 1ml/kg	77±0.6	19± 1.2
Group B Imipramine 15mg/kg	74± 0.3	15±0.5
Group C 50mg/kg	71± 1.5	16± 0.3
Group D 100mg/kg	74± 0.7	15±1.5
Group E 200 mg/kg	76±1.7	16±0.3

In OFT, extract showed non-significant effects at all the three doses as compared to control. (Table 3).

DISCUSSION

Majority of antidepressants take many weeks to show beneficial effects and are associated with side effects.[16] Hence, the development of a new better drug is seriously needed. In both FST and TST models, the immobility period in mice at 100mg/kg dose decreased significantly while highly significantly at 200 mg/kg dosage, as compared to control animals. Decrease in immobility period in both these tests reveals antidepressant action.

Substances that augment locomotor action such as anticholinergics, stimulants or convulsants can give false positive values in either FST or TST.[17] In OFT, the extract demonstrated insignificant effect on number of crossings and rearings in mice at all three dosages compared to control group, hence abolishing doubt of any augmented locomotor activity. Various mechanisms are suggested for antidepressant action of flavonoids such as antioxidant, affecting amines, inhibiting monoamine oxidase enzyme and affecting neuroendocrine system.[18]

Antidepressant effect of Fenugreek seeds could be due to rich amount of Flavonoids in it. Quercetin, a flavonoid found in many plants, has shown antidepressant action due to inhibition of α 2 adrenergic receptors or modification of Brain-derived neurotropic factors.[19,20] The flavonol Rutin showed antidepressant action by augmenting serotonin and norepinephrine levels.[21]

Some other authors have also reported similar findings in recent period similar our results.[22,23] Recently, Fenugreek seeds have shown better outcome in clinical management of type 2 diabetic patients.[24] So they must be studied in patients suffering from depression as well. Present study has provided numerous clues regarding the antidepressant action of Fenugreek seeds. Hence it is must to segregate the active ingredients from plants which may contribute in future discovery of new drugs.

CONCLUSION

FS-ME depicted noteworthy antidepressant effect in mice but further preclinical and clinical trials are compulsory to approve these findings.

Author Contributions:
Conception and Design: Tahira Asad
Collection and Assembly of data: Tahira Asad, Navaid Siddiqui
Analysis and interpretation of data: Muhammad Ali Rajput, Touseef Sayyar, Madeeha Soban
Drafting of the article: Tahira Asad, Navaid Siddiqui
Critical revision of the article for important intellectual content: Tahira Asad, Navaid Siddiqui, Madeeha Soban
Statistical Expertise: Hafiza Tuseef Sayyar, Taif Hameed, Muhammad Ali Rajput
Final approval and guarantor of the article: Tahira Assad
Corresponding author email: Tahira Assad: drtahiraassad@gmail.com
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