

Antidepressant effect and categorization of Inhibitory activity of monoamine oxidase type A and B of ethanolic extract of seeds of *Trigonella foenum graecum* Linn

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Abstract: *Trigonella foenum-graecum* Linn (*Fabaceae*) is an annual aromatic herb and now it is cultivated globally like in Pakistan, Egypt, India, Middle East etc. Traditionally it was used in anorexia, as febrifuge, to soothe gastritis and gastric ulcers, as a galactagogue and as condiment, hypoglycemic agent and employed in various nervous disorders. The study aimed to investigate the antidepressant effect of ethanolic extract of seeds of *Trigonella foenum graecum* and underlying mechanism of action. For assessment of antidepressant activity Forced Swimming Test (FST), Tail Suspension Test (TST), Monoamine (MAO) Assay and Locomotor Activity Test were studied. Acute toxicity, Rota Rod and Grip Strength Tests were also performed. The significant declining in immobility time as compared to control was shown in Forced swimming test as compared to tail suspension test. Considerable change was not found in open field test (OFT). EtOH extract of seeds of fenugreek represent maximum significant reduction which was 30 and 24.65% in MAO- A and B activity respectively in the rat's whole brain as compared to control animals in Monoamine oxidase (MAO) assay. All tested doses were found ineffective in impairment of muscle coordination in Rota rod and in grip strength related to muscle relaxant property. According to experimental findings it is revealed that ethanolic extract of seeds of *Trigonella foenum graecum* showed antidepressant effect by inhibiting the activity of MAO-A and B.

Keywords: *Trigonella foenum graecum* Linn., antidepressant, monoamine oxidase assay, neurodegenerative diseases.

INTRODUCTION

Depression is a varied disorder that affects person's mood, physical fitness and behavior and considered as a serious health care problem worldwide. Major depression has symptoms that produce changes in brain monoamine neurotransmitters mostly serotonin, nor epinephrine, and dopamine at synapses (Schildkraut, 1965). As per world health information, around 450 million people go through mental or behavioral disorders (WHO, 2001). This counts to 12.3% of the global burden of disease, and will mount to 15% by 2020 (Reynolds, 2003). MAO is a flavoenzyme responsible for the catalyzation of the oxidative deamination of biogenic amines and is sited on the surface of mitochondrial membrane (Zatta, 1999). It is alienated into types A and B according to substrate specificity and sensitivity to particular inhibitors (Cesura, 1992; Birkmayer & Birkmayer, 1989). MAO-A inhibitors are responsible for the treatment of depression while inhibition of MAO-B activity provides better quality of life of the elderly because the activity of MAO-B rises with age and is particularly increased in certain neurodegenerative diseases (Castagnoli *et al.*, 2003; Magyar and Szende, 2004). Drugs (TCAs, MAOI etc) that improve the intensity of neurotransmitters (serotonin, nor

epinephrine and dopamine) in the Central Nervous System (CNS) showed antidepressant activity (Delgado, 2000) but their side effects limits their uses. Therefore due to high frequency of depression and a fact that no presently marketed antidepressants produce a sound effect against depression in a considerable proportion of persons will support the need for new therapeutics from medicinal plants to treat depression having fewer side effects.

Herbal remedies still largely provide unexplored source for the development of potent new and safe antidepressant drugs so. There is a need to screen these plants in order to authenticate their utilization and to reveal their chief constituents by isolation and characterization (Mei *et al.*, 2001; Linde *et al.*, 1996). In neurological disorders extensive range of herbal drugs being used such as *Piper methysticum* (Kava) and *Hypericum perforatum* (St. John's wort) are the general examples. They have demonstrated effectiveness and protection not only in animal models but also in clinical trials (Wong *et al.*, 1998).

Trigonella foenum graecum Linn (*Fabaceae*) is an annual aromatic herb commonly known as fenugreek. It is indigenous of the waste grounds of the Mediterranean basin, North-eastern Africa and Western Asia (Ben-Erik, 2004; Chevallier, 1996). It is cultivated worldwide like in

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Pakistan, Egypt, India, Middle East etc. It is the oldest known medicinal plant and is used both as a spice and herb (Lust, 1986). In ancient time fenugreek was used to treat burns, aid labour, delivery (Chevallier, 1996; Yoshikawa *et al.*, 1997) and in gynaecological problems (Chevallier, 1996). This medicinal plant is also used in the treatment of diabetes, dyspepsia, rickets, as a tonic, to treat haemorrhoids and chronic cough (Braun, 2007). Traditionally it is used to treat rheumatism (Kirtikar and Basu, 1991). The steroidal saponins in fenugreek restrain cholesterol absorption and synthesis; produce hypoglycemic effect (Sauvaire *et al.*, 1998) used in nervous disorders (Nadkarni, 1954), imparts immunity (Nadkarni, 1954; Bin-Hafeez *et al.*, 2003) and also possesses significant acute and chronic anti-inflammatory activity (Subhashini, 2011).

The study was conducted to evaluate the antidepressant potential of EtOH extract of *Trigonella foenum graecum* L. in the predictive models of antidepressant action, and underlying mechanism of action because no scientific work has been reported in the literature up till now on antidepressant activity of *Trigonella foenum graecum* L.

Ethical approval

The use of animals for study was approved from Ethical Committee on animals of Pakistan Council of Scientific and Industrial Research (PCSIR) having reference no 45/ET/10.

MATERIAL AND METHODS

Extraction and Isolation of plant material

Trigonella foenum graecum (Tfg) seeds were soaked in a container by weighing 2.0 kg of plant material with 3 liter of ethanol (Merck). After keeping fifteen days the plant was percolated through Whatmann filter paper No.1. Filtrate was evaporated to semisolid residue on a rotary evaporator (BÜTCHI Rota Vapour R-200, Switzerland) at 35°C±5 with controlled pressure. The plant was identified by Prof. Dr. Ghazala H. Rizwani, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi and the Voucher specimen (00132) was deposited in the Herbal Museum of the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi.

Animals

Male Wistar albino rats (160-200g) and Swiss albinomice (22-28g) were grouped into 6-8 per cage and kept in a plastic cage having 43cm length and 19cm height. They were kept under standard conditions with normal light cycle under controlled temperature (22±1°C) with free access to food and water.

Drugs and treatments

Phenelzine sulphate, Flouxetine hydrochloride, Imipramine hydrochloride and Diazepam (Sigma Chemical Companies, USA) were used as standard during

study. The ethanolic (EtOH) extract of Tfg was dissolved in 10% Dimethyl sulphoxide (DMSO), whereas other drugs were dissolved in saline solution (0.9% NaCl) instantly before use. The treatments were administered via intraperitoneal (i.p). Control animals received appropriate vehicles. The animals were divided into different groups, each containing 10 animals, according to different tests.

Acute toxicity test

The EtOH extract of Tfg was administered in male mice with different doses ranges from 100-2000mg/kg (Sukumar *et al.*, 1991). The behavioural responses and mortality were observed for 24 hours and according to preliminary screening 5 doses were selected (100-500 mg/kg) for further experiments.

Forced swim test (FST)

In pretest session rats were individually forced to swim in a glass container having 45 cm height and 17cm width for 15 minutes. It was filled with water up to 15cm and maintained the temperature at 24±1°C. Animals showing wound or nose bleeding or dropped during 1st trial were rejected (Porsolt *et al.*, 1978). In 2nd trial after 1hr of treatment such rat was again forced to swim in the similar container under identical conditions. The duration of immobility was recorded for a period of 5 mins.

Tail suspension test (TST)

In TST after 1 hr administration of treatment such mouse was suspended above 35 cm from the floor on the edge of the tabletop by adhesive tape place around a position of 1 cm from the edge of its tail. The immobility time was recorded for a period of 6 minutes (Steru *et al.*, 1985).

Open field test (OFT)

In this test mobility of animals were evaluated with respect to effect of administration. The behavior of each mouse was evaluated for a period of 5 mins by placing it in the open field (Walsh & Cummins, 1976). Peripheral locomotion (No of squares adjacent to the walls crossed), Central locomotion (No of inner squares crossed), Total locomotion (Peripheral and Central locomotion), Leanings (one or two paws in contact with walls), Rearings (standing of mouse on its hind paws without touching the walls), Grooming (paw licking, face cleaning, head scraping, fur licking and rubbing) and Defecations were observed. The open field apparatus was washed with a detergent solution before each evaluation to eradicate possible odor clues left by former subjects.

Rotarod test

This test was conducted as described by Dunham and Miya, 1957 for assessment of neurological deficit in mice treated with treatments. Mice were placed on a 32 mm diameter rod, which is driven by a motor and rotational speed and was maintained at 5 rpm. At an interval of 0, 30, 60, 90 and 120mins each animal was placed on the rod and the mice capable of remaining on the top for 3

mins. or more, in three consecutive evaluations were chosen for the experiment.

Grip strength

This test was used to assess the muscles strength according to procedure as reported by (Nevins *et al.*, 1993). In apparatus a bar connected to a force transducer measures the animal's forelimbs grip strength in grams. Each group of animal was placed over a plate in front of grasping trapezene, which is connected to peak amplifier. The animal grasps the trapezene when they were pulled by the tail. After the animal loses its grip on the grasping trapezene, the peak preamplifier automatically store the peaks drag force.

Monoamine oxidase (MAO) assay

Preparation of enzyme liquid

Male rat's brains were quickly removed on ice plate after quick decapitation and washed instantly with 0.32 ice cold sucrose to eliminate blood. The process of brain separation was completed within five minutes (Schurr and Livne, 1976; Pan *et al.*, 2005). Then the brains were weighed and suspended in 1:10 volume of cold sodium phosphate buffer (0.2M, Ph 7.4) and mingled twice (20s each with alteration of 30s) with ultrasonic (59 KHz). The pellets were discarded after the mixture was centrifuged at 1000×g for 10 min at 0°C. The consequential supernatant was centrifuged at 17000×g for 30 min at 4°C and the pellets were re-suspended and stored at -30°C for MAO analysis within 3 days (Veronika *et al.*, 2003).

Determination of the concentration of protein

Bovine serum albumin was used as standard for determination of protein content by-Lowry method (Lowry *et al.*, 1951).

MAO assay

Monoamine oxidase (MAO) assay was evaluated spectrophotometrically as described previously by (Charles and McEwen, 1977; Yu and Kong, 2002). The mixture of assay contained 0.3ml 4mM 5-HTas specific substrates for MAO A and 8 m Benzyl amine for MAO B, 0.5ml solution of the mitochondrial fraction and 0.2 M sodium phosphate buffer (pH 7.4) up to 3ml. The reaction was allowed to carry on at 37 to 40°C for 1h and stopped by addition of 1M HCl (0.6 ml) and then the reaction product was extracted with 4ml of Butyl acetate or Cyclohexane for MAO A and MAO-B respectively. At wavelength of 280 n the organic phase was measured with the help of spectrophotometer. Blank samples were prepared by adding 0.3ml 4mM 5-HT and 8 m Benzylamine after the reaction and worked up consequently in the similar manner.

STATISTICAL ANALYSIS

The results were expressed as Mean ±SEM. The data were analyzed by ANOVA (version 15.00) followed by

Tukeypost hoc test. $p < 0.05$ was considered as statistically significant.

RESULTS

Acute toxicity

Trigonella foenum graecum (EtOH) extract was found nontoxic till the dose of 2000 mg/kg and increased abdominal movements were observed.

Forced swim test (FST)

All doses of Tfg showed significant reduction in the immobility time as compared to control animals. Maximum reduction ($p < 0.001$) was attained at 400mg/kg that was 61.01% followed by reversed pattern at doses of 200 and 500mg/kg that was 21.21% and 45.45% respectively. All treatments of standard doses showed significant reduction ($p < 0.001$) in immobility time as compared to control except dose of 15mg/kg of imipramine, which did not show reduction in immobility time (Fig 1). Maximum reductions were shown by phenelzine at dose of 10 mg/kg, which was 62.49

Tail suspension test (TST)

The effects of EtOH extract of seeds of Tfg on active behaviours in the TST of mice are shown in (Fig 2). All treatments showed result in non-specific pattern and produces some reduction in immobility time that is not statistically significant. The positive control imipramine causes significant reduction ($p < 0.05$ and $p < 0.001$) in a dose dependent manner in immobility time of mice as compared to control animal i.e.31.76% and 72.56% at 15 and 30mg/kg respectively.

Open field test (OFT)

The results revealed that no significant change was found in EtOH extract of Tfg except dose of 250 mg/kg which showed significant reduction in total ($p < 0.001$) and peripheral locomotion ($p < 0.01$) (table 1). Imipramine (30 mg/kg) showed significant difference on total ($p < 0.001$) central ($P < 0.05$) and peripheral locomotion ($p < 0.01$) as compared to control animals, which were 42.3, 67.4 and 37% respectively.

Rotarod test (RR)

The EtOH extract (100 and 200mg/kg) of seeds showed no effect in muscle coordination as animals maintained their balance on the rotarod bar. Diazepam showed significant ($p < 0.01$) impaired performance at 30mins after administration of drug (table 2).

Grip strength (GS)

The grip strength is a valuable assessment for evaluation of muscle relaxant property (fig. 3). EtOH extract of seeds (100 and 200mg/kg) showed increase in grip strength at time interval of 90 and 120 mins that was not up to significant level. Diazepam (5mg/kg) showed maximum muscles relaxant property at a time interval of 60 mins as compared to control, which was 25%.

Table 1: Effects of EtOH extract of seeds of *Trigonella foenum graecum* OFT.

Treatments	TL	PL	CL	Leaning	Rearing	Grooming	Defecation
Flouxetine 20 (mg/kg)	160.33±2.20	128.33 ± 4.40	32±2.30	10.33±0.9	0.66±0.33	0.66±0.33	0 ± 0
Imipramine 30 (mg/kg)	96.66±4.37***	86.66±3.52**	10±1.15*	7.66±0.33**	0±0	0.33±0.33	0 ± 0
Tfg100 (mg/kg)	135.33±5.5	102±9. 23	28.33±0.88	17.33±0.33	0±0	1.66±1.66	0 ± 0
Tfg250 (mg/kg)	87.66±10.68***	72.66±7.21**	15±3.46	12.66±2.18	0±0	2±0.57	0 ± 0
Tfg500 (mg/kg)	142±5.0	104±3.05	16±2.30	16.33±2.02	0±0	0.66±0.66	0 ± 0
Control 10 (ml/kg)	167.66±11.34	137±14.57	30.66±6.83	16.66±1.76	2.33±1.20	1.33±1.33	0 ± 0

TL: Total Locomotion, CL: Central Locomotion and PL: Peripheral Locomotion. Asterisks indicate significant percent reduction ($p < 0.05^*$, $p < 0.01^{**}$ and $p < 0.001^{***}$) as compared to control. Results are shown as Mean \pm SEM (n=10).

Table 2: Effects of EtOH extract of seeds of *Trigonella foenum graecum* motor coordination in mice.

Treatments	Time spent on the Rota rod at				
	0mins	30mins	60mins	90mins	120mins
Tfg (100mg/kg)	180±0	180±0	180±0	180±0	180±0
Tfg (200mg/kg)	180±0	180±0	180±0	180±0	180±0
Diazepam (5mg/kg)	180±0	126.33±17.32**	163.33±8.81	143.33±6.00	180±0
Control (10ml/kg)	180±0	180±0	180±0	180±0	180±0

Values are mean time in seconds spent on the rotarod \pm SEM (n=10). Asterisk indicate significant percent reduction ($P < 0.05^*$) as compared to control.

Monoamine (MAO) Assay

The EtOH extract showed significant inhibition ($p < 0.05^*$, $p < 0.01^{**}$ and $p < 0.001^{***}$) of MAO-A activity at all tested doses as compared to control group except 300 mg/kg. Greatest reduction was attained at dose of 400 mg/kg which was 30% followed by upturned pattern at dose of 500 mg/kg that was 24%. The extract also showed significant ($p < 0.001^{***}$) reduction of MAO- B activity at only two doses i.e. 300 and 400 mg/kg as compared to control group. Maximum inhibition was achieved at dose of 300mg/kg which was 24.65% followed by reversed pattern at dose of 400mg/kg which was 11.28% (fig. 4). Flouxetine showed dose dependent inhibition of rat's brain MAO- A activity. Maximum inhibition was achieved at 19mg/kg i.e. 43.58%. The IC_{40} value was 18.4±0.68 mg/kg. The positive control also showed greatest MAO- B inhibitory activity 28.63% at 15mg/kg and followed by reverse pattern at 20mg/kg i.e. 15%. The IC_{20} value was 4.9±0.09 mg/kg (fig. 5).

DISCUSSION

Animal models provide a means for psycho pharmacologist in their attempts to develop new medicines for psychiatric disorders and also to investigate the mechanisms of new drugs (Elinat and Belmaker 2001). The forced swimming and tail suspension tests are two models, which are commonly used for screening of new antidepressant drugs (Porsolt *et al.*, 1978; Steru *et al.*,

1985). The tests are quite sensitive and relatively specific to all main classes of antidepressants drugs (TCA_s, SSRIs and MAOIs) (Porsolt *et al.*, 1978; Detke *et al.*, 1995). Acute toxicity test was performed to determine the appropriate doses and then the chief tests were performed. The results showed that EtOH extract of Tfg showed antidepressant effects by reducing immobility time both in FST and TST.

In this study the behavioral effects of the EtOH extract of seeds of *Trigonella foenum graecum* (Tfg) and their underlying mechanism of action was evaluated in rodents. Crude EtOH extract showed significant reduction in the immobility time as compared to control animals in FST. Higher dose represent greatest declining in the immobility time at 400 mg/kg that was 61.01%. The IC_{20} and IC_{30} values were 200±16 and 330±40 mg/kg respectively. Whilst in TST of extract showed 11.18%, 16.24 and 16.60% reduction at 100, 300 and 400mg/kg in immobility time as compared to control. Doses, which produced antidepressant effects are quite common in both FST and TST. Tfg contain L-tryptophan (Jayaweera, 1981; Yoshikawa *et al.*, 1997) which is an amino acid, utilized as a biochemical precursor for a neurotransmitter i.e. Serotonin, used in the management of depression (Fateme, 2006; Biruk Sintayehu *et al.*, 2012). Therefore, it may be possible that it is responsible for the antidepressant activity of EtOH extract of Tfg.

It is vital to verify that the effect in the FST could not be due to nonspecific stimulant effects of the test compounds, which would be reflected as an enhancement in locomotor activity. Doses of FST and TST produced reduction in immobility time of animals did not cause any significant change in an open field test.

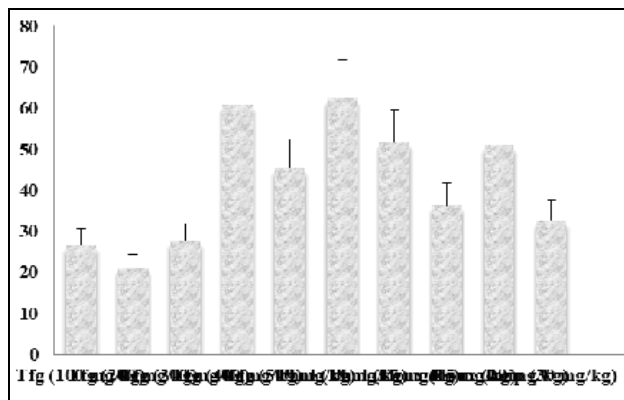


Fig. 1: Effects of EtOH extract of seeds of *Trigonella foenum graecum* and standard drugs on immobility time in forced swimming test. Each column represents mean \pm S.E.M. $p < 0.05^*$, $p < 0.01^{**}$ and $p < 0.001^{***}$ compared with control.

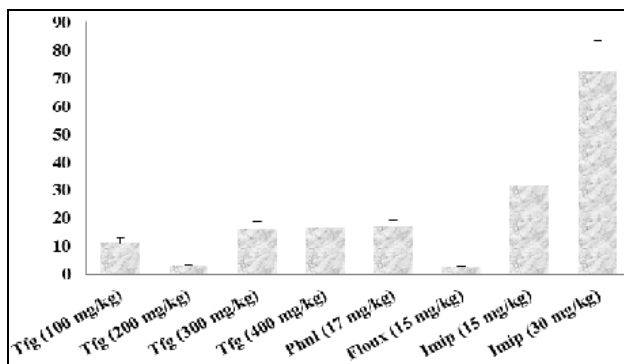


Fig. 2: Effects of EtOH extract of seeds of *Trigonella foenum graecum* and standard drugs on immobility time in tail suspension test. Each column represents mean \pm S.E.M. $p < 0.05^*$, $p < 0.01^{**}$ and $p < 0.001^{***}$ compared with control.

Maximum reduction in immobility time as compared to control in FST was shown by Monoamine oxidase inhibitors (MAOIs) i.e. Phenzelzine in FST. On the basis of IC_{50} value Phenzelzine (16.4 ± 1.73 mg/kg) was found to be more potent than Flouxetine (19.60 ± 1.62 mg/kg). Imipramine was found to be more effective than Phenzelzine and Flouxetine in TST. These findings are similar with the previous findings of many antidepressant drugs (Porsolt *et al.*, 1978). The IC_{50} value of phenzelzine was 16.4 ± 1.73 mg/kg.

Monoamine oxidase (MAO) is a family of mitochondrial enzymes that catalyze the oxidative deamination of monoamines and abnormal levels of these enzymes have

been reported in a number of psychological disorders including depression, Alzheimer's and Parkinson's disease (Fowler *et al.*, 1982). Tfg represented significant reduction in MAO-A activity in the rat's whole brain as compared to control animals. Maximum reduction was attained at 400mg/kg that was 30% and overturned pattern was found in lower (100mg/kg) and higher dose 500 mg/kg i.e. 17.68% and 24% respectively. The outcomes showed that differences in the effective doses of extract are to some extent common in association with FST. EtOH extract showed marked reduction 24.65 and 11.28 % in the activity of MAO - B at the doses of 300 and 400mg/kg. This inhibitory activity of extract may be due to the presence of quercetin in it (Jayaweera, 1981; Yoshikawa *et al.*, 1997) as previously it was reported that quercetin possess significant MAO-B inhibitory activity (Mason *et al.*, 2000).

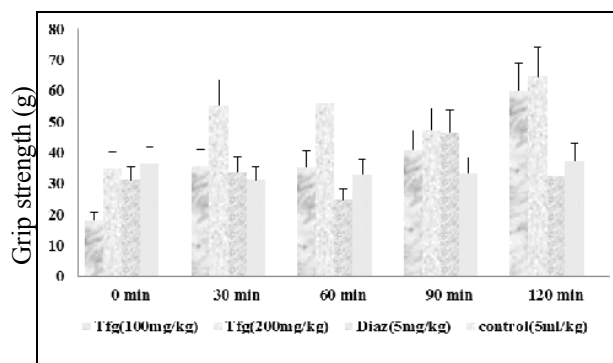


Fig. 3: Myorelaxant effect of EtOH extract of seeds of *Trigonella foenum graecum*

Tfg=*Trigonella foenum graecum*; Diaz= Diazepam

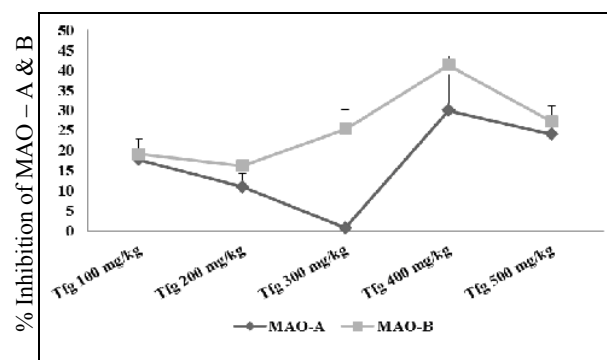


Fig. 4: Effect of ethanolic extract of seeds of *Trigonella foenum graecum* on MAO - A & B activity

Rotarod is used for the evaluation of drug for their interference in motor co-ordination (Gerhard, 2006). Tfg did not cause any interruption in muscles coordination as the animals were able to maintain their balance more than 180mins, which was taken as the cut off time for each recording at 30, 60, 90 and 120 min for the duration of the experiment. Whereas diazepam (positive control) showed sedative effect thus shortening the time spent on rotarod bar.

Grip strength is a valuable assessment for evaluation of muscle relaxant properties of substances (Gerhard, 2006). The 200mg/kg of Tfg extract represent 60% increased in grip strength, which was lasted for 120 min, showed muscles stimulant effect. Diazepam showed my relaxant effect as it reduces muscle strength as compared to control.

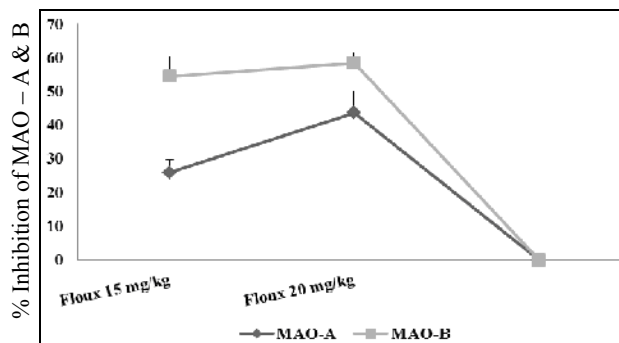


Fig. 5: Effect of ethanolic extract of seeds of standard drug Flouxetine on MAO-A & B activity

CONCLUSION

This is the first report providing evidence at mechanism level that the EtOH extract of *Trigonella foenum graecum* L. showed significant antidepressant effect by inhibiting MAO-A. At the same time it also showed considerable activity against MAO-B but there is further need to explore chemical evaluation, isolation and characterization of active principles from the extract of this plant accordingly. The results emphasized the claim in ethno medicine that this plant could be consumed as an alternative alone or in combined form remedy for curing depression and age related neurodegenerative diseases possibly due to having rich mucopolysaccharides and hormonal substances alongwith ABC vitamins.

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