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Original Article

NEUROPHARMACOLOGICAL SCREENING AND LACK OF ANTI-DEPRESSANT ACTIVITY OF STANDARDIZED EXTRACT OF *FUMARIA INDICA*: A PRECLINICAL STUDY

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Abstract: ***The standardized 50% ethanolic extract of Fumaria indica (FI) containing 0.45% fumaric acid and 0.35% dimethyl fumarate w/w, was used in this study. Five groups of rats and mice of either sex, each group comprising of six animals, were used (i.e. control, respective standard drug, and 100 mg/kg, 200 mg/kg and 400 mg/kg doses of FI, p.o.). Potentiation of pentobarbital induced sleeping time, locomotor activity, effect on muscle grip performance of mice, maximal electroshock seizures (MES) in rats and pentylenetetrazole (PTZ) induced convulsions in mice were used as behavioural models to evaluate general effects of the FI on central nervous system. Further, antidepressant activity of the extract was also evaluated using validated models of depression in rodents viz. behavioural despair test, learned helplessness test, tail suspension test, reserpine induced hypothermia, 5-Hydroxytryptophan (5-HTP) induced head twitches in mice and L-dopa induced hyperactivity and aggressive behaviour in mice. The animals treated with FI showed significant and dose dependent increase in pentobarbital-induced sleeping time and marked decrease in onset of sleeping time in rats. FI and diazepam have shown significant decrease in locomotor activity. FI did not show any muscle relaxant effect in the rota-rod test in mice while diazepam has shown significant muscle relaxant effect. FI, phenobarbitone and diazepam showed significant anticonvulsant activity in MES in rats and PTZ induced convulsions in mice respectively. However, no antidepressant activity was observed with FI in any of above six validated models of depression. It may be concluded that FI has significant central nervous system depressant activity and lacking antidepressant activity in rodents.***

Key words: *Fumaria indica*, central nervous system, antidepressant

INTRODUCTION

Fumaria indica Linn. (Syn: *Fumaria parviflora*, Fumariaceae) is commonly known as fumitory. It is an annual herb growing wild in plains of India and Pakistan [1]. In traditional medicine the plant is used as antidyspeptic, blood purifier, cholagogue, diaphoretic, diuretic, laxative, stomachic, sedative and tonic [2]. Beneficial effects of the plant in treatment of abdominal cramps [3], diarrhoea and fever [4], jaundice, leprosy and syphilis [5] are also reported. Pharmacological studies on this plant have revealed its anthelmintic [6], antipyretic [7], hepatoprotective [8] and hypoglycaemic [9] properties. Antinociceptive and anti inflamm-atory activities of plant extract [10] have also been reported. Antioxidant activity of two Algerian species of

Fumaria have been investigated [11]. Phytochemical investigations revealed that isoquinoline alkaloids are key chemical constituents of the plant. The main alkaloids of the plant are fuyuziphine [12], narlumicine, narceimine, narlumidine, fumara-mine, fuma-ritine, paprafumicin, paprarine, papracinine, papraline, reddeanine [13], narlumi-cine, narceimine and narlumidine [14]. Besides these alkaloids steroids like ß-sitosterol, stigmas-terol, campesterol, organic acids like, caffeic acid and fumaric acid are also found in the plant [10]. Most of these alkaloids and their salts have been reported to possess central nervous system (CNS) related activities such as CNS stimulant property by protopine nitrate [15], CNS depres-sant, potentiation of pentobarbital hypnosis, anticonvulsant activity by fumariline [16] and antipsychotic activity of l- tetrahydro-coptisine [17].

On the basis of these informations, our objective is to screen the general neuropharmacological properties of the *Fumaria indica*, followed by its evaluation for antidepressant activity in rodents.

MATERIALS AND METHODS

***Animals*:** Adult Charles Foster albino rats (150 ± 10g) and Wistar mice (20 ± 5g), of either sex, were obtained from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University, Varanasi and were randomly distributed into different experimental groups. The rats were housed in groups of six in polypropylene cages at an ambient temperature of 25 ^{o}C ± 1 ^{o}C and 45-55 % relative humidity, with a 12:12 h light/dark cycle. Animals were provided with commercial food pellets and water *ad libitum* unless stated otherwise. Behavioural experiments were conducted between 09.00 and 14.00 h. Animals were acclimatized for at least one week before using them for the experiments. The prior approval of Institutional Animal Ethics Committee (IAEC) of Banaras Hindu University was obtained.

Plant material and extraction: The plant *Fumaria indica* was collected from the local commercial source of Varanasi. The identification of the plant was done by Prof. N. K. Dubey, Department of Botany, Faculty of Science, BHU and herbarium specimen (voucher, JAN- 2009-01) of the plant was preserved in Herbarium of Department of Botany, BHU. for future reference. After shade drying, extraction of the plant was done with soxhlet apparatus using 50% ethanol as solvent [10,18].

Standardization of extract: 50% ethanolic extract of *Fumaria indica* was standardized by High Performance Thin Layer Chromatography (HPTLC) using CAMAG TLC Scanner –III, Camag Linomat applicator IV. Fumaric acid and di methyl fumarate (Sigma-Aldrich, USA) were used as authentic markers. For free fumaric acid, sample and authentic marker (fumaric acid) were dissolved in methanol and for fumaric acid conjugates, the sample and authentic marker (di methyl fumarate) were dissolved in 50 ml of 5N HCL and refluxed for two hours and then dried over water bath and re-dissolved in methanol. The sample and the marker were applied to pre-coated silica gel plate (Merck 60F^{254}), and developed in a solvent system, Formic Acid: Chloroform: Butanol: Heptane (12:16:32:44) up to 90 mm. Developed plates were dried and scanned under the absorbance mode (scanning wavelength λ 260nm) and calculations were done based on the area of peaks of sample and corresponding authentic marker. The sample i.e. 50% ethanolic extract of *Fumaria indica* was found to contain 0.45% w/w of free fumaric acid and 0.35% w/w of fumaric acid conjugates (di-methyl fumarate).

Drug treatments: Ethanolic extract of *Fumaria indica* (FI) was orally administered as 0.3% carboxy methyl cellulose (CMC) suspension, in the doses of 100, 200 and 400 mg/kg, once daily for seven consecutive days [10,18]. Control rats were treated with equal volume of vehicle (0.3% CMC suspension). Experiments were conducted on day 7, one hour after the last dose administration.

(A) GENERAL NEUROPHARMACOLOGICAL SCREENING

(i) Potentiation of pentobarbital-induced sleeping time: Pentobarbital (40 mg/kg, i.p.) was administered to control and drug treated animals. Onset of sleep (loss of righting reflex) was noted and duration of sleep was measured as the period between the loss of righting reflex and its return [19]. FI (100, 200 and 400 mg/kg, p.o.) and diazepam (5 mg/kg, p.o.) were administered 45 min prior to pentobarbital injection, respectively.

(ii) Locomotor activity: The spontaneous locomotor activity was assessed with the help of photoactometer [20]. Each animal was observed for a period of 10 min in a square closed field arena (30 x 30 x 30 cm) equipped with 6 photocells in the outer wall. Interruptions of photocell beams (locomotor activity) were recorded by means of a 6 digits counter.

(iii) Effect on muscle grip performance of mice: Effect on motor co-ordination was examined on rota-rod apparatus. Each animal was placed on a rotating rod (20 rpm) in a pre-test session and only those animals, which stayed on the rod for not less than 3 min, were selected for the test session. The test session was performed on the same day as the pre-test session. Fall-off time (when the mouse falls from the rotating rod) for each animal was noted before and after drug administration [21].FI (100, 200 and 400 mg/kg, p.o) and diazepam (5 mg/kg, p.o.) were administered 45 min before test session, respectively.

(iv) Maximal electroshock (MES) seizures in rats: According to this method, the supramaximal electroshock (150 mA) was given through a pair of corneal electrodes for 0.2 sec duration using a convulsiometer. The hind limb extensor response was taken as the positive end point [22]. Albino rats were prescreened and only those showing positive hind limb tonic extensor response were used after an interval of at least 48 h. FI (100, 200 and 400 mg/kg, p.o.) and phenobarbitone (60 mg/kg, p.o.) were administered 45 min prior to MES challenge.

(v) Pentylenetetrazole (PTZ) induced convulsions in mice: The mice were challenged with pentylenetetrazole (80 mg/kg, i.p.). The number of mice, which exhibited seizures, the latency to first convulsion and percent lethality were recorded [23]. FI (100, 200 and 400 mg/kg, p.o.) and diazepam (10 mg/kg, p.o.), were administered 45 min prior to PTZ challenge.

(B) ANTIDEPRESSANT ACTIVITY

(i) Behavioural despair test: The rat was placed in a cylinder (45 x 20 cm) containing 38 cm water ($25 \pm 2\ ^0C$), so that the rat could not touch the bottom of the cylinder with its hind limb or tail, or climb over the edge of the chamber. Two swim sessions were conducted, an initial 15 min pre-test, followed by a 5 min test 24 h later. Drugs were administered after pre-test. The period of immobility (remained floating in water without struggling and making only those movements necessary to keep its head above water) during 5 min test period were noted [24].

(ii) Learned helplessness test: This model is based on the assumption that, exposure to uncontrollable stress associated with repeated experiences of failure to escape from the stress produces a helpless situation, which results in performance deficits in subsequent learning tasks [25]. A typical experiment involves two parts:

(a) Inescapable shock pretreatment: Electric foot shocks were delivered in 20 x 10 x 10 cm chamber with plexiglass walls and cover. The floor was made of steel grids to deliver electric shock. A constant current shocker was used to deliver 60 scrambled, randomised inescapable shocks (15 s duration, 0.8 mA, every min) to grid floor. Control rats were placed for 1 h in identical chambers but no shocks was administered. Inescapable shock pre-treatment was performed in the morning.

(b) Conditioned avoidance training: In order to evaluate escape and avoidance performance, avoidance training was initiated 48 h after inescapable shock pre-treatment in the jumping box. The jumping box were divided into two equal chambers (27 x 29 x 25 cm) by a plexiglass partition with a gate providing access to the

adjacent compartment through a 14 x 17 cm space. Animals were placed singly in one of the chambers of jumping box and were allowed to habituate to the test environment for 5 min (for the first session only) and then were subjected to 30 avoidance trials (inter-trial intervals being 30 sec). During the first 3 sec of each trial, a light signal (conditioned stimulus) was presenented, allowing the animals to avoid shocks. If a response does not occur within this period, a 0.8 mA shock (3 sec duration) (unconditioned stimulus) was applied via the grid floor. In case no escape response occurs within this period, shock and light conditioned stimulus were terminated. Avoidance sessions performed for 3 consecutive days (days 3-5) in the morning, and the number of escape failures, referred as no crossing response during shock delivery, were recorded.

(iii) Tail suspension test: A mouse was hung on a wire in an upside down posture so that its nostrils just touch the water surface in a container. After initial vigorous movements, the mouse assumes an immobile posture and the period of immobility during a 5-min observation period were noted [25].

(iv) Reserpine induced hypothermia: On the day before testing, rats were dosed with 2 mg/kg reserpine (Sigma, USA) subcutaneously. Rats had free access to food and water. 18 h after reserpine administration, the animals were placed into individual cages. The initial rectal temperature was determined by insertion of digital thermometer to a constant depth of 5 cm. Following administration of FI extract, the rectal temperature was measured again at 60 min interval for 7 h [25].

(v) 5-Hydroxytryptophan (5-HTP) induced head twitches in mice: Mice were treated with 5-HTP (100 mg/kg, i.p.) and the number of head twitches displayed by each mouse was counted by the staggering method using three 2-min periods (19-21 min), (23-25 min) and (27-29 min) after 5-HTP administration.

(vi) L-dopa induced hyperactivity and aggressive behaviour in mice: Mice were treated with L-dopa (100 mg/kg, i.p.). Stages of activity and aggressive behaviour were recorded by a scoring system at every 10 min for 30 min after L-dopa administration by the 'blind observer'. The different parameters of observation were, piloerection, salivation, increase in motor activity, irritability, reactivity, jumping, squeaking and aggressive fighting. The scores were graded in the following manner: 0 = No effect, 1 = Piloerection, slight salivation, slight increase in motor activity, 2 = Piloerection, salivation, marked increase in motor activty and irratibility, 3 = Piloerection, profuse salivation, marked increase in motor activty, reactivity, jumping, squeaking and aggressive fighting.

Statistical analysis: The data are expressed as mean ± SEM for each treatment group. The data obtained from each response measures were subjected to Kruskal-Wallis one way analysis of variance (ANOVA) and inter group comparison was made by Mann-Whitney-*U*-test (two-tailed) for only those responses which yielded significant treatment effects in the ANOVA test.

RESULTS

A. General neuropharmacological screening

(i) Potentiation of pentobarbital-induced sleeping time: The rats treated with FI – 100, 200 and 400 mg/kg showed significant and dose dependent increase in pentobarbital- induced sleeping time and marked decrease in onset of sleeping time. The standard anxiolytic drug diazepam showed similar effect. The results are summarized in figure1a and figure1b.

(ii) Locomotor activity: All the three doses of FI showed significant decrease in locomotor activity in dose dependent manner along with standard drug diazepam. The results are summarized in figure 2.

(iii) Effect on muscle grip performance of mice: The FI did not show any muscle relaxant effect in rota rod test in mice seems to be devoid of any motor incordination effect. However, diazepam showed significant ataxia in mice. The

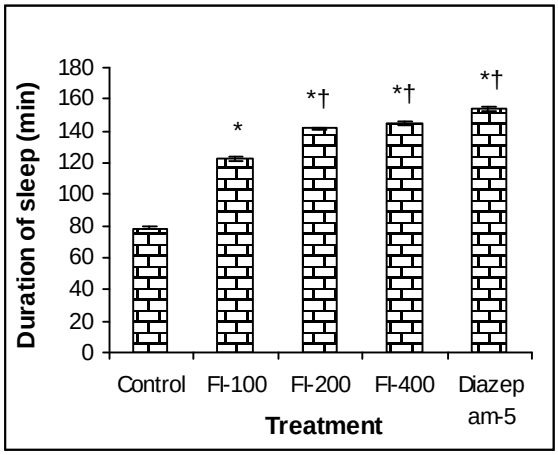

Fig. 1a: Effect of *Fumaria indica* on pentobarbital induced sleeping (duration of sleep) in rats.

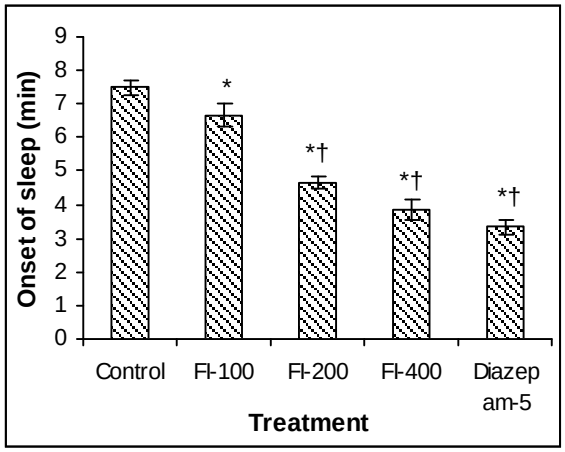

Fig. 1b: Effect of *Fumaria indica* on pentobarbital induced sleeping (onset of sleep) in rats.

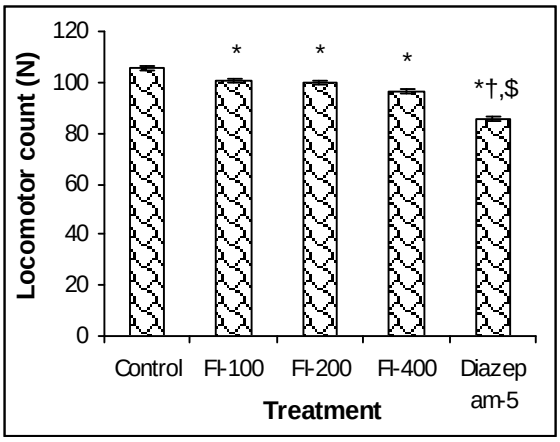

Fig. 2: Effect of *Fumaria indica* on locomotor activity in rats.

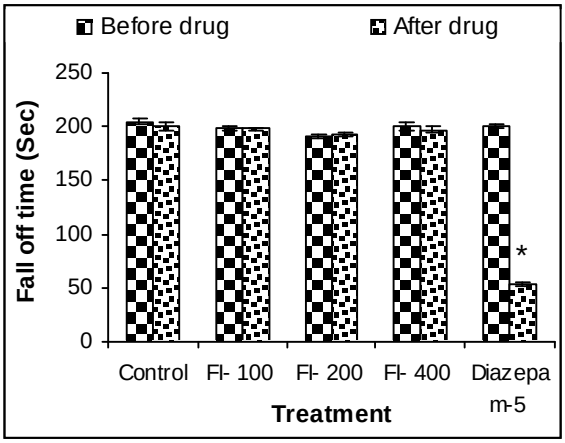

Fig. 3: Effect of *Fumaria indica* on muscle grip performance in mice

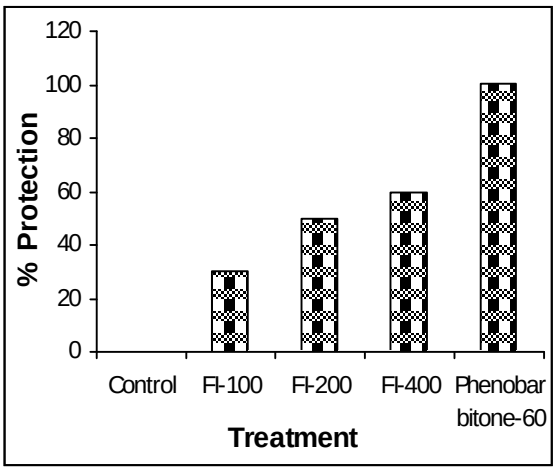

Fig. 4: Effect of *Fumaria indica* on maximal electroshock seizures in rats.

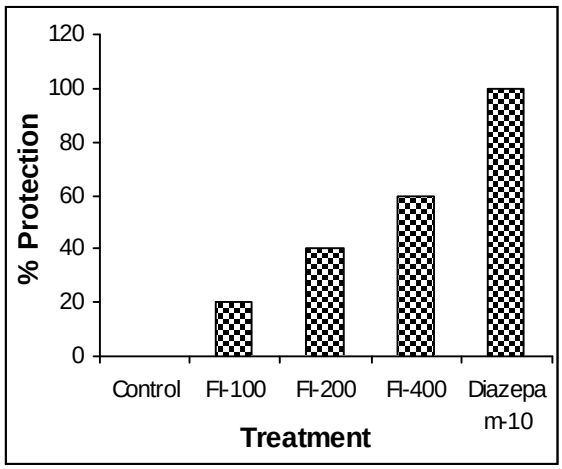

Fig. 5: Effect of *Fumaria indica* on pentylenet-etrazole induced convulsions in mice.

Table 1: Effect of *Fumaria indica* on learned helplessness test in rats. FI= Ethanolic extract of *Fumaria indica*, EF and AR denote Escape Failure and Avoidance Response respectively. n = 6 in each group, * =p<0.001, compared to control.

Treatment	Dose (mg/kg, p.o.)	Day 1		Day 2		Day 3	
		EF	AR	EF	AR	EF	AR
Control	-	25.66±1.63	4.33±1.63	17.33±2.16	12.66±2.16	11.5±2.07	18.5±2.07
FI	100	23.16±1.47	6.83±1.47	16.83±1.60	13.16±1.60	12.3±2.33	17.66±2.33
FI	200	22.5±2.34	8.0±1.67	16.16±1.72	13.83±1.72	12.5±1.64	17.55±1.64
FI	400	23.66±2.50	6.33±2.50	16.83±1.94	13.16±1.94	11.5±2.42	18.5±2.42
Imipramine	15	11.16±1.83*	18.83±1.83*	5.33±1.21*	24.66±01.21*	1.66±0.81*	28.33±0.81*

results are summarized in figure 3.

(iv) Maximal electroshocks (MES) seizures in rats: The FI has got marked dose dependent anticonvulsant property. FI– 100, 200 and 400 mg/kg protected 30 %, 50 % and 60 % rats respectively, from hind limb tonic extensor (HLTE) induced by MES. The standard anticonvulsant drug phenobarbitone (60 mg/kg, p.o.) showed 100 % protection against MES induced HLTE phase. The results are summarized in figure 4.

(v) Pentylenetetrazole (PTZ) induced convulsions in mice: All the three doses of FI- 100, 200 and 400 mg/kg showed dose dependent protection against PTZ- induced tonic-clonic convulsions in mice. The positive control diazepam (10 mg/kg, p.o.) exhibited 100 % protection against tonic clonic convulsions. The results are summarized in figure 5.

B. Antidepressant activity:

(i) Behavioural despair test: The rats treated with FI, did not show any significant reduction in immobility period, in forced swimming model whereas standard antidepressant drug imipramine showed significant reduction in immobility period of the rats. The results are summarized in figure 6.

(ii) Learned helplessness test: The FI treated rats did not show any significant increase in avoidance response and decrease in escape failure in response to shock treatment. But the standard antidepressant drug imipramine showed the significant increase in avoidance response and decrease in escape failure. The results are summarized in table 1.

(iii) Tail suspension test: The FI treated rats did not show any reduction in immobility period in this model, where as imipramine, standard antidepressant drug showed marked reduction in immobility period. The results are summarized in figure 7.

(iv) Reserpine induced hypothermia: The rats treated with FI did not show any significant reversal of hypothermia in comparison to control rats as showed by imipramine. The results are summarized in figure 8.

(v) 5-Hydroxytryptophan (5-HTP) induced head twitches in mice: The mice treated with FI did not show any significant change in number of head twitches, but the standard drug imipramine showed significant increase in number of head twitches. The results are summarized in figure 9.

(vi) L-dopa induced hyperactivity and aggressive behaviour in mice: There was no significant effect of FI on L- dopa induced hyperactivity and aggressive behaviour in mice. The results are summarized in figure 10.

DISCUSSION

The effect of pentobarbitone sodium on righting reflex (hypnosis) is used to elucidate CNS-active properties of drugs [26,27]. The loss of righting reflex is measured as criterion for the duration of pentobarbitone-induced sleeping time. FI produced a dose related potentiation of pentobarbitone hypnosis indicating that the FI has sedative action in the doses used. Interruption of the light beams as lateral movements of rats or mice in a cage has been used by many authors

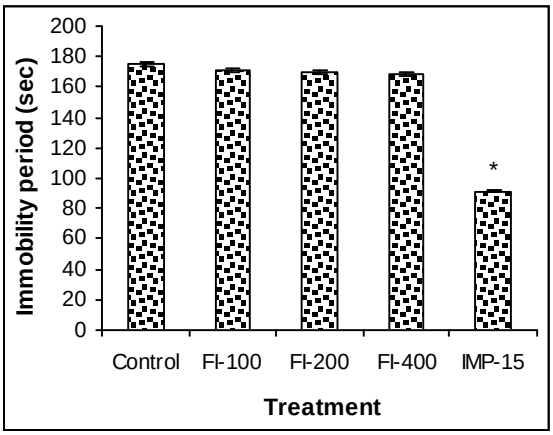

Fig. 6: Effect of *Fumaria indica* on behavioural despair test in rats.

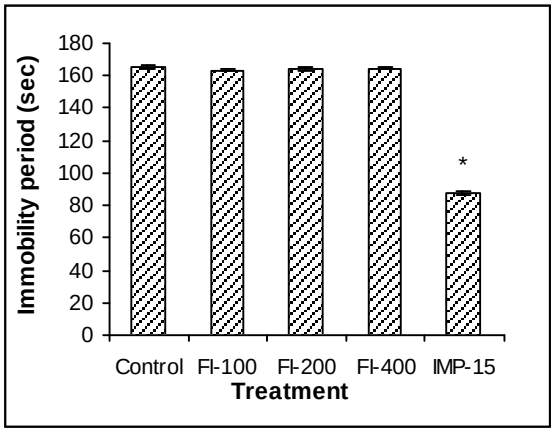

Fig. 7: Effect of **Fumaria indica** on tail suspension test in rats.

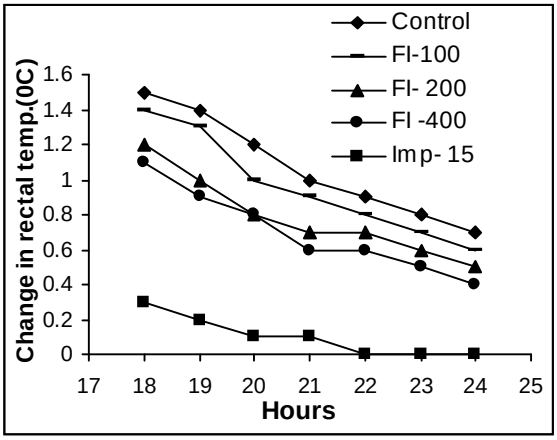

Fig. 8: Effect of *Fumaria indica* on reserpine induced hypothermia in rats.

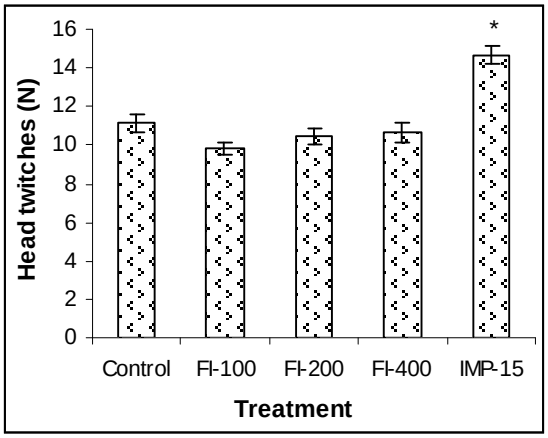

Fig. 9: Effect of *Fumaria indica* on 5- HTP induced head twitches in mice.

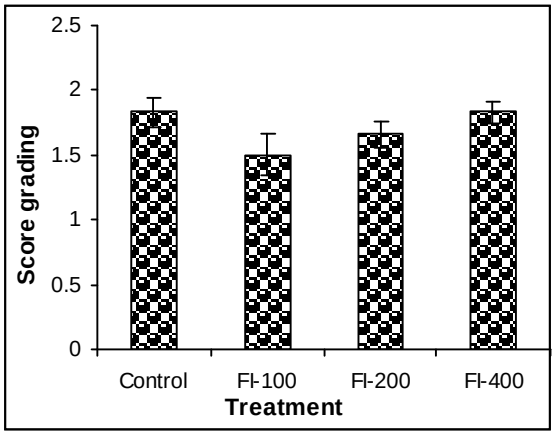

Fig. 10: Effect of *Fumaria indica* on L- dopa induced hyperactivity in mice.

[28-30]. Most of the central nervous system acting drugs influence the locomotor activity in men and animals. The locomotor activity can be an index of wakefulness of mental activity [31]. As FI attenuated motor activity possibly by its central sedative action, it is quite possible that observed antianxiety effect of FI is responsible [32]. The rota-rod test is used to evaluate the activity of drugs interfering with motor coordination. In 1957, Dunham and Miya [33] suggested that skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod. Many central depressive drugs are active in this test. This test does not really differentiate between anxiolytic and neuroleptic but can evaluate the muscle relaxant potency in a series of compounds such as the benzodiazepines. Moreover, this test has been used in toxicology for testing neurotoxicity. The FI extract seems to be devoid of any motor incoordination effect

in the rota-rod test. FI (100, 200 and 400 mg/kg, p.o.) failed to produce muscle relaxant effect, while the benzodiazepine derivative diazepam (5 mg/kg, p.o.) produced significant ataxia due to its significant sedation. Thus FI may have an advantage over benzodiazepines. The maximal electroshock (MES) test in animals is used primarily as an indication for compounds, which are effective in grandmal epilepsy. Tonic hind extensions are evoked by electric stimuli, which are suppressed by anti-epileptics but also by other central active drugs [26]. FI at all doses used in the study has altered the flexor, extensor and clonic phases of MES seizures, and offer complete protection against pentylenetetrazole (PTZ)-induced convulsions. PTZ-induced convulsions has been used primarily to evaluate antiepileptic drugs likely to be used in petitmal epilepsy. However, it has been shown that most anxiolytic agents are also able to prevent or antagonise PTZ-induced convulsions. These methods are widely accepted as screening procedure [26]. FI at all the dose levels i.e. 100, 200 and 400 mg/kg, p.o. has shown dose dependent anticonvulsant activity in MES and PTZ induced convulsions probably by its sedative/anxiolytic action [32].

Amongst a wide variety of proposed and critically assessed *in vivo* models of depression [34,35] the two most commonly used paradigms are behaviour despair forced swim [36], and learned helplessness tests [37,38]. Behavioural despair was proposed as a model to test for antidepressant activity by Porsolt et al.[39] who suggested that mice or rats forced to swim in a restricted space from which they cannot escape, exhibit a characteristic immobility [39]. This behaviour reflects a state of despair, that can be reduced by several agents, which are therapeutically effective in human depression [26]. In our study, FI did not show significant reduction in immobility period indicating lack of antidepressant activity. In learned helplessness test, rodents are exposed to inescapable and unavoidable electric shocks in one situation later fail to escape shock in a different situation when escape is possible [22,40,41]. This phenomenon was evaluated as a potential animal model of depression [42]. A drug is considered to be effective, if the learned helplessness is reduced and the number of failures to escape is decreased [26]. Unlike imipramine, FI at three dose levels did not show any significant decrease in escape failures.

Apart from these two paradigms, the observed results in tail suspension and reserpine induced hypothermia tests, provide additional measures for assessing antidepressant activity. In tail suspension test, the immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempt to escape when suspended by tail [26]. Tail suspension test (TST) is based on the observation that rodents after initial escape-oriented movements develop an immobile posture when placed in an inescapable stressful situation. This condition involves the haemodynamic stress of being hung in an uncontrollable fashion by their tail [43]. In tail suspension test there was no significant reduction in immobility period as of behavioural despair test in FI treated rats. Reserpine induced hypothermia test has been proven as a simple and reliable method to detect antidepressant activity. However, the reversal of hypothermia is not specific for antidepressants.

Amphetamines and some antipsychotic agents (chlorpromazine) can also antagonize the fall in body temperature. The different time course of antidepressants (slow onset of action, long lasting effect) and amphetamines-like drugs (quick onset of action, short lasting effect) allows differentiation between two groups of drugs [26]. Reserpine induced hypothermia is a neurochemical model of depression. Physiological effects of reserpine such as ptosis, hypomotility, diarrhoea, bradycardia and hypothermia are readily observed of which hypothermia is most readily observed and antagonized by tricyclic antidepressant and MAO- inhibitor antidepressants [44]. In this model there was no significant reversal of hypothermia by FI at all three dose levels used

in this study, whereas the standard antidepressant drug imipramine significantly reversed the reserpine induced hypothermia.

The 5-HTP induced head twitch response in mice is indicative of central serotonergic activity [45]. Serotonin is known to be involved in the sleep stages of the sleep wake cycle. The mice treated with FI did not show any significant change in number of head twitches, but the standard antidepressant drug imipramine showed significant increase in number of head twitches. This observation indicates FI is devoid of central serotonergic activity. There was no significant effect of FI on L-dopa induced hyperactivity and aggressive behaviour in mice, indicating absence of effect of FI on dopaminergic system as well.

Depressive disorders are now regarded as a major health problem [46,47]. Despite considerable progress made during the last 5 decades, successful treatment of clinical depression with currently available therapeutic agents can be achieved only in 65-75% of patients, of which only 40-50% achieves complete recovery [48]. Such a situation necessitates the development of more effective antidepressants [49,50]. The first generation of antidepressants, the TCA, discovered only after fortuitous clinical findings with imipramine, are still widely used because of their familiarity and low cost [25].

The introduction of second generation antidepressants may have reduced the risks of adverse effects of the first generation tricyclic antidepressants, but made little impact on improving the effectiveness of treatment [49-51]. The search for new molecules as target for antidepressant drug discovery, therefore, remains a continuing challenge for modern psychiatry. It has been pointed out that, like in various other therapeutic areas [52,53] investigations of traditional herbal products may provide a good chance for novel treatments for affective and other CNS disorders [54,55]. Conversely in our study, FI fails to show antidepressant activity in battery of validated models of depression in rodents confirming lack of antidepressant activity in FI.

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