Potential antianxiety activity of *Fumaria indica*: A preclinical study

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ABSTRACT

Background: In the view of diverse CNS modulating properties of *Fumaria indica*, present study was planned to evaluate its putative anxiolytic activity in behavioural models of rats, followed by elucidation of mechanism of observed activity through biochemical estimations. **Materials and Methods:** Effects of seven daily 100, 200 and 400 mg/kg oral doses of a *Fumaria indica* extract (FI) was compared with those of an acute oral dose (5 mg/kg) of lorazepam in a battery of rat models consisting of open-field, elevated plus and zero maze, social interaction, and novelty induced feeding tests. **Results:** Dose dependant antianxiety effects of FI observed in all tests were qualitatively similar to those of the reference anxiolytic drug. Although FI treatments did not alter the concentrations of noradrenaline and serotonin in hippocampus and hypothalamus, concentrations of both these monoamines were dose dependently elevated in prefrontal cortex of FI treated animals. Flunitrazepam binding in brain frontal cortex was also elevated by the extract. Moreover, higher levels of brain expressions of the cytokines TNF-α, IL-1β, and IL-10 observed in animals with prior experience on elevated plus maze were almost completely reversed by the lowest dose of FI tested in the behavioral models. **Conclusion:** Taken together, these observations strongly suggest that FI is a functionally novel type of antianxiety agent, and that inhibition of cytokine expressions in the brain could be involved in its mode of action.

Key words: Antianxiety activity, behavioral psychopharmacology, cytokines, *Fumaria indica,* flunitrazepam binding, monoamines.

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INTRODUCTION

Diverse medicinal uses of *Fumaria indica* Linn. (Fumariaceae; Syn. *F. parviflora* Lamk., *F. vallantii* Loisel.) have been known to Ayurvedic practitioners since ages[1] and numerous recent reports describe a broad spectrum of therapeutically interesting bio-activities of its extracts.[2] However, little concentrated efforts have yet been made to define the psychopharmacological activity profiles of diverse types of *Fumaria indica* extracts commonly used in numerous commercialized Ayurvedic poly-herbal formulations. This is surprising not only because mental health problems are given due attention in the Ayurvedic health care system, but also numerous psychoactive alkaloids are known to occur in the plant.[3] Moreover, central nervous system (CNS) function modulating activities of other structurally diverse secondary metabolites of the herb have become apparent during more recent years. Available information on extractable bioactive components of the plant indicates that fumaric acid and its conjugates could as well be quantitatively the major bioactive constituents of its extracts. Antioxidative activity of fumaric acid has been known since long, and during more recent years modulating effects of its conjugates on cytokine production and immunological functions have also been described. Consequently, such molecules are now attracting attention of drug discoverers for treatment of neurodegenerative diseases.[4-6]

Methyl fumarate is one of the two hepatoprotective constituents of hydro-alcoholic *Fumaria indica* extracts identified to date.[7] The other one being protopine[8] which is pharmacologically more well studied alkaloid of the plant.[9] Our interest in testing the efficacy of a *Fumaria indica* extract (hereafter referred to as FI) in a

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battery of animal models for antidepressants was triggered by a report suggesting antidepressant like activities of protopine,[10] i.e. quantitatively one of the major psychoactive alkaloid of the plant.[3] Although we could not detect any antidepressants like activity of the extract, our psychopharmacological screening studies revealed diverse other therapeutic potentials of the extract.[11] Consequently, efforts were made to more precisely define the neuro-psycho-pharmacological activity profile of the extract, and to understand its mode(s) of action(s). Results of experiments dealing with anxiolytics like activity of FI are described in this communication. Observations made to date with this extract add further experimental evidences to the conviction that some traditionally known medicinal uses of the plant could as well be due to its effects on the CNS, and suggest that FI could be a potential candidate for developing a pharmacologically and phytochemicallly better standardized phyto-therapeutic potentially useful for helping patients suffering from anxiety disorders.

MATERIALS AND METHODS

***Animals*:** Adult Charles Foster albino rats (150 ± 10g) 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 polypropylene cages at an ambient temperature of 25°C ± 1°C and 45-55% relative humidity, with a 12:12h light/dark cycle. Unless stated otherwise, animals were always provided *ad libitum* with commercial food pellets and drinking tap water. They were acclimatized for at least one week before using them for the experiments. Principles of laboratory animal care (NIH publication number # 85-23, revised in 1985) guidelines were followed, and prior approval of Institutional Animal Ethical Committee (IAEC) of Banaras Hindu University was obtained.

***Plant material and extraction*:** *Fumaria indica* is a small, scandent, branched annual herb up to 30 cm height, with grooved branchlets. The leaves are pale green, 2-3 pinnatisect, 5-7 cm long. Flowers are asymmetrical, pale pink or white with purple tips, in terminal or leaf-opposed racemes, with a filiform style and a two-lobed stigma. A batch of whole *Fumaria indica* plant was acquired from a commercial source in Varanasi. Its botanical identification was done by Prof. N. K. Dubey, Department of Botany, Faculty of Science, Banaras Hindu University, and a herbarium specimen (specimen voucher, Jan 2009-10) of the plant has been preserved. After shade drying, exhaustive extraction of the plant was done with soxhlet apparatus using 50% ethanol as solvent.

Extract characterization: The *Fumaria indica* extract (FI) was analytically characterized by its conjugated and free fumaric acid contents using High Performance Thin Layer Chromatography (HPTLC), CAMAG TLC Scanner –III, and Camag Linomat applicator IV. Commercially available fumaric acid and di-methyl fumarate (Sigma-Aldrich, USA) were used as authentic markers. For free fumaric acid quantification, the test sample and fumaric acid were dissolved in methanol. For estimation of fumaric acid conjugates present in FI, the test sample and 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 samples and the marker fumaric acid were applied to pre-coated silica gel plate (Merck $60F_{254}$), and developed in the solvent system Formic Acid: Chloroform: Butanol: Heptane (12:16:32:44) up to 90 mm. Developed plates were dried and scanned under absorbance mode (scanning wavelength λ 260nm), and calculations were based on the area of peaks of sample and corresponding authentic marker. FI was found to contain 0.45% w/w of free fumaric acid and 0.35% w/w of fumaric acid conjugates (calculated as di-methyl fumarate).

Drug treatments: FI was suspended in 0.3% carboxymethyl cellulose (CMC), and its 100, 200 and 400mg/kg/day doses were orally administered once daily for seven consecutive days before the experiments. The last dose was given 1 hour before subjecting them to experimental procedures. Control rats were treated with equal volume of the vehicle (0.3% CMC suspension). For comparison sake, a group of animals treated once (1 hour before the start of the observations) with the anxiolytic drug lorazepam (5mg/kg, p.o.) were always run parallel in all behavioral experiments. Choices of doses and treatment regimen were based on the observations made during our earlier studies with FI.[12,13]

Behavioral tests: Well standardized open-field, elevated plus-maze (EPM), elevated zero-maze, social interaction, and novelty induced suppressed feeding tests commonly used for identifying and characterizing benzodiazepine like anxiolytics were used. Detailed descriptions of apparatus and procedures used for such purposes have been described in a previous report from our laboratories.[14] All experimental groups in each test consisted of 6 animals, and all tests were started one hour after the last oral dose of the extract. The reference anxiolytic lorazepam was always tested after its single oral dose. All observations were made by a neutral observer not aware of the treatment regimen.

Brain monoamines levels: Serotonin and norepinephrine contents in the prefrontal cortex, hypothalamus and hippocampus of FI (100 and 400mg/kg/day for 7 days) treated and control rats were quantified. For such purposes the brain regions were dissected out after the animals were

decapitated. They were weighed and homogenized in 1.5ml ice cold 0.01N HCl. The homogenate was quantitatively transferred to a glass stoppered bottle containing 4g NaCl with the help of 25ml n-butanol. Monoamine contents of the n-butanol phase were assayed by the method described by Welch and Welch using Shimadzu RF-1501 spectrofluorometer. The results were expressed as ng/g of the wet brain region.[15]

Flunitrazepam binding: Rat brains were removed 60 min after the final administration of FI and the crude synaptic membranes from the prefrontal cortex were prepared as described earlier.[16] Briefly, the brain region was homogenized in 19 volumes of 0.32 M sucrose and centrifuged at 50,000xg for 10 min. The resulting pellets were homogenized in distilled water and re-centrifuged at the same speed. The final pellets were suspended in 40 mM Tris-HCl buffer, pH 7.4, at a concentration representing 50mg of the original tissue/ml.

Binding incubations were carried out in triplicate in a final volume of 1 ml containing 40 mM Tris-HCl buffer (pH7.4) and methyl-^{3}[H] flunitrazepam. At the end of 15-min incubation period at 37^{0}C, the samples were filtered on glass fiber discs (25-mm diameter, 0.3-μm-pore size, Gelman Inc., Ann Arbor, MI) and rapidly washed twice with 5 ml of the Tris buffer. Filter discs were dried and counted in 5 ml of scintillation mixture using a Tricarb 2660 scintillation counter (Packard Instrument Co., Downers Grove, IL) at an efficiency of 38-43%. Control incubations, containing unlabeled competing ligand diazepam, were carried out simultaneously with the experimental series to determine the extent of nonspecific binding.

Cytokine expression: Expressions levels of the three cytokines TNF-α, IL-1β and IL-10 in the prefrontal cortex of rat were quantified in three groups of animals. One of them was not exposed to EPM and was not treated with FI (control group). The two others were exposed to EPM for five minutes, whereupon one of them was treated with FI (100 mg/kg/day for seven consecutive days) and the other not. All animals were sacrificed 24hrs after the EPM exposures (or at corresponding time point for the control group). Prefrontal cortex of the brain was dissected out after the animals were sacrificed by spinal cord dislocation. The dissected brain region was washed with phosphate buffer saline (PBS) and lysed in TRI Reagent™ (Sigma-Aldrich, USA). The total RNA was isolated from this lysate according to manufacturer's instructions. The quality of RNA was assessed by running the RNA samples on 1% denaturating formaldehyde-agarose gel. The RNA contents of the samples were quantified using Nano Drop Spectrophotometer (Thermo Scientific, USA), and thereafter equal amount of RNA from each sample of a study group were pooled.

Reverse Transcription-Polymerase Chain Reaction: The validated methods for cDNA preparation and polymerase chain reaction (PCR) were followed. Detailed description of apparatus and procedures used for such purposes, and for quantification of cytokine expressions, has been described in details in a previous report from our laboratories.[13]

Statistical analysis: Except for the cytokine expression experiment (where pooled samples were used), all data are expressed as means ± SD 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 comparisons was made by Mann-Whitney-U-test (two-tailed) for only those responses which yielded significant treatment effects in the ANOVA test.

RESULTS

Open field test: This paradigm was used for assessing the exploratory and anxiety like behavior of rats in a novel, or challenging, environment. In this test advantage is taken of the fact that in general rats avoid the central arena. Treatments with anxiogenic agents make them stay close to the enclosed wall and decrease their exploratory behaviors and increase defecation. Mean values of diverse parameters quantified in our study are summarized in Table 1. In comparison to the vehicle treated group, ambulation, rearing, self grooming, and activity in the center of the cage of the lorazepam and FI treated groups were higher, and

Table 1: Effects of the *Fumaria indica* extract (FI) in open field test observed in rats

Treatment	Dose (mg/kg, p.o.)	Ambulation (N)	Rearing (N)	Self grooming (N)	Activity in centre (N)	Fecal droppings (N)
Vehicle	-	29.50 ± 3.39	6.81 ± 1.72	5.66 ± 1.07	2.91 ± 0.99	2.02 ± 0.60
FI	100	44.16 ± 1.16*	10.87 ± 1.47*	7.50 ± 1.04	3.66 ± 0.81*	1.80 ± 0.75
FI	200	63.16 ± 3.18*,†	12.83 ± 1.40*	10.66 ± 0.81*,†	4.66 ± 0.81*	1.66 ± 1.03
FI	400	73.16 ± 3.18*,†,$	15.66 ± 1.36*,†,$	10.50 ± 1.04*,†	7.00 ± 1.78*,†,$	1.50 ± 0.83
Lorazepam	5	66.00 ± 4.00*	14.16 ± 1.72*	11.50 ± 1.51*	6.00 ± 1.78*	1.33 ± 0.51

Each observed value represent the mean (± SD) numbers of recorded events (N) in a group of six animals, and their statistical significance are given by: *, † and $ representing P<0.05 as compared to vehicle, FI-100 and FI-200 groups respectively

the observed effects of the extract were dose dependant. However, no significant effects of FI, or of lorazepam, on defecation were observed. Since after the same doses of FI tested in this study, the extract significantly and dose dependently reduces locomotion in motility tests,[11] the observed increased activities in the extract treated groups can be interpreted in terms of its antianxiety activity. The observed dose dependent antianxiety effects of FI in this test were always qualitatively analogous to that of the reference anxiolytic lorazepam. Choices of FI dose range in other well known behavioral tests for anxiolytics were based on these and other observations made during our earlier dose finding experiments.[12]

Elevated plus maze (EPM) test: This paradigm is one of the more frequently used one for pharmacological screening and characterization of anxiolytics, and is considered to be a suitable one for assessing unconditioned anxiety state of rodents. Avoidance of the open arm by the animals placed on the central open elevated space of the maze is interpreted as anxiety. Treatments with FI, or with lorazepam, increased the number of entries and time spent on the open arm, and decreased their entries and duration of stay in the closed arm [Table 2]. The observed effects of FI were dose dependant and statistically significant effects of the extract on quantified parameters were observed even after its lowest dose tested (100mg/kg/day). Our earlier dose finding studies[12] have revealed that repeated daily oral doses FI is necessary for observing its antianxiety activity in animal models, and that no such efficacy of the extract is observed after its daily doses below 100mg/kg/day. Since anxiolytic activities of benzodiazepines and other psychoactive drugs in this and other tests are observed even after their single doses, molecular mechanism(s) of action(s) of FI must not necessarily be identical to those of benzodiazepines, and other psychoactive drugs currently often prescribed for anxiety disorders.

Elevated zero maze test: Elevated zero maze offers a better animal model to study antianxiety activity, when compared with elevated plus maze as there is no ambiguity in the interpretation of the time spent in the central square of the traditional design and allowing uninterrupted exploration.[17] This test is a modified version of the EPM test, in which some additional anxiety state associated behavioral parameters can be conveniently assessed. Results of the observations made in this test are summarized in Table 3. Although the mean values of numbers of head dips and stretched postures in the FI (100 mg/kg/day) treated animals were somewhat higher than those of the vehicle treated control group, these differences were statistically not significant. Otherwise, observed dose dependant effects of the extract on all four quantified parameters was apparent also in this test. Qualitatively the observed effects of the extract were again quite analogous to the standard anxiolytic lorazepam.

Social interaction test: Unlike in other behavioral test used in this study, the rats were housed individually during the daily treatment periods, and one day prior to the test day (7th day) they were also acclimatized with the test box. Anxiety state of the treated rats were judged by the

Table 2: Observed effects of the *Fumaria indica* extract in elevated plus maze test

Treatment	Dose (mg/kg, p.o.)	Time spent on (sec)		Entries on (N)	
		Enclosed arm	Open arm	Enclosed arm	Open arm
Vehicle	_	257.00 ± 5.19	40.50 ± 5.16	11.41 ± 1.92	4.41 ± 1.78
FI	100	213.33 ± 3.98**	55.50 ± 4.63**	7.16 ± 1.69**	7.16 ± 1.46**
FI	200	203.33 ± 4.71**,††	66.50 ± 4.50**,††	5.16 ± 0.75**	9.83 ± 2.40**
FI	400	192.50 ± 4.88**, ††, $$	78.16 ± 4.91**, ††, $$	4.66 ± 1.96**	11.50 ± 1.04**, †
Lorazepam	5	187.00 ± 4.96**	83.00 ± 3.89**	3.33 ± 1.63**	9.50 ± 2.16**

Statistical significance of the observed values is given by: **, †† and $$ representing $P<0.01$ as compared to the corresponding value of the vehicle, FI-100 and FI-200 groups respectively. For other details see Table 1.

Table 3: Observed effect of the *Fumaria indica* extract in elevated zero maze test

Treatment	Dose (mg/kg, p.o.)	Time spent on open arms (sec)	Head dips (N)	Stretched attend posture (N)	Entries in open arms (N)
Vehicle	-	83.41 ± 3.20	9.58 ± 1.24	5.83 ± 1.26	7.66 ± 2.14
FI	100	111.50 ± 2.25**	11.83 ± 1.72	7.33 ± 1.21	9.33 ± 1.21**
FI	200	122.16 ± 2.63**, †	13.33 ± 2.65**	8.16 ± 1.80**	10.16 ± 1.83**
FI	400	129.50 ± 3.50**, ††, $$	17.66 ± 1.63**,††, $$	8.66 ± 1.96**	11.33 ± 1.21**
Lorazepam	5	119.66 ± 3.30**	17.16 ± 1.60**	8.33 ± 1.63**	11.66 ± 1.36**

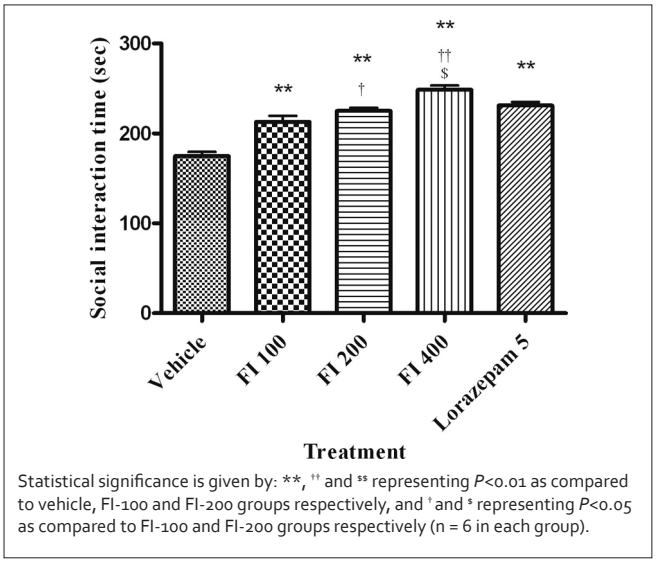
For other details see table 2

total time (social interaction time) they spent in sniffing, following, grooming, kicking boxing, biting and crawling under or over an untreated a naïve rat of a the same sex and age placed in the test box together with the treated animal on the 7th day. In comparison to the vehicle treated group, this social interaction time in the FI treated and lorazepam treated ones were significantly higher. As can be seen from the results summarized in Figure 1, the mean values of the FI treated groups increased dose dependently in magnitude with its increasing doses, and its observed activity profile was qualitatively quite similar to that of lorazepam (results not shown). These observations suggest that FI could as well be a therapeutic alternative for social phobias often encountered in patients with anxiety disorders.

Novelty induced suppressed feeding test: The novelty induced suppressed feeding latency test is a conflict based model wherein conflict of approach and avoidance lies between desire to consume food and defensive behavior in response to novel arena.[18] Importance of proper eating behavior in maintaining proper health is well recognized by Ayurvedic as well as modern health care systems. Since Ayurvedic practitioners often use *Fumaria indica* for diverse medical conditions, this feeding behavior test for anxiolytics was also included in the test battery used to test whether its antianxiety activity could lead to altered feeding behavior as well. In this test rats were deprived of food for two days before placing them in the novel chamber containing food pellets used for their maintenance. As can be seen from the results summarized in Figure 2, mean feeding latencies of FI or of lorazepam treated groups were significantly lower than that of the vehicle treated control group. Like in other tests for anxiolytics, the observed effects of FI were dose dependent, and such effects of its lowest tested were also statistically highly significant. Since antianxiety effects of 100mg/kg/day dose of FI was consistently observed in all the five behavioral tests, this treatment regimen was considered appropriate for some later biochemical studies conducted to gain information on the mode(s) of actions(s) of the extract.

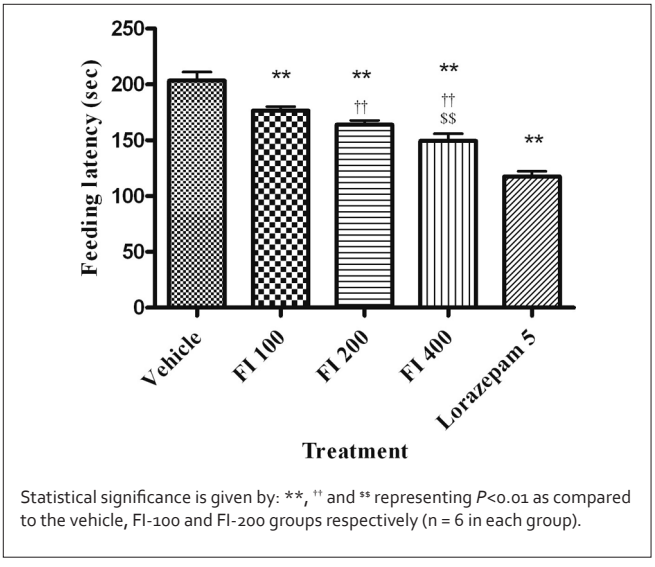
Brain monoamine levels: Antianxiety activities of diverse psychotherapeutics acting on the central monoaminergic system are often observed after their repeated daily doses only.[19,20] Since such is also the case for FI, it was of interest to test its effects on the brain serotonin and noradrenaline levels. Results summarized in Table 4, revealed that daily oral doses of FI increased the levels of both the neurotransmitters in the prefrontal cortex, and had no effects on their levels in hippocampus or hypothalamus. Observed effects of 100mg/kg/day dose of the extract were statistically not different from those of its higher one (400mg/kg/day) tested. These observations indicate that the effects of FI on brain serotoninergic and noradrenergic systems are maximal after its tested lower dose, and that one of its brain targets of action(s) is located in the prefrontal cortex, i.e. the brain region involved in emotional and cognitive functions.

Flunitrazepam binding: Except for benzodiazepines, antianxiety activities of other therapeutically used psychoactive drugs are not always detectable in all the five behavioral tests used in this study.[21] Moreover, benzodiazepines like anticonvulsant and sedative activities of FI were also detected in our earlier study.[11] It has been known since long that conflict situations causing anxiety decrease benzodiazepine binding in the cortex, and that treatment with diazepam increases such binding in specific



Statistical significance is given by: **, †† and $$ representing $P<0.01$ as compared to vehicle, FI-100 and FI-200 groups respectively, and † and $ representing $P<0.05$ as compared to FI-100 and FI-200 groups respectively (n = 6 in each group).

Figure 1: Effect of the *Fumaria indica* extract observed in rat social interaction test. For details see text



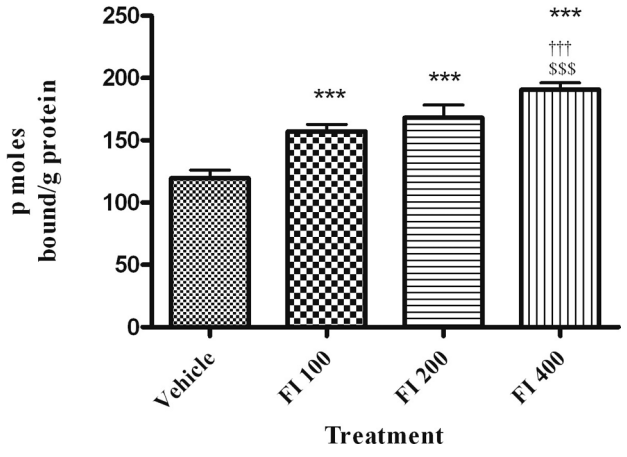
Statistical significance is given by: **, †† and $$ representing $P<0.01$ as compared to the vehicle, FI-100 and FI-200 groups respectively (n = 6 in each group).

Figure 2: Effect of the *Fumaria indica* extract observed on novelty induced suppressed feeding lat ency in rats

Table 4: Observed effects of the *Fumaria indica* extract on serotonin (5-HT) and norepinephrine (NE) concentrations in three brain regions of rats

Treatment	Dose (mg/kg, p.o.)	Concentration of 5-HT (ng/g of tissue)		
		Prefrontal cortex	Hippocampus	Hypothalamus
Vehicle	-	1.91 ± 0.11	2.24 ± 0.16	2.58 ± 0.16
FI	100	3.00 ± 0.49*	2.02 ± 0.15	2.64 ± 0.23
FI	400	3.66 ± 0.27**	1.97 ± 0.08	2.86 ± 0.56
Concentration of NE (ng/g of tissue)				
Vehicle	-	2.93 ± 0.19	2.58 ± 0.43	2.73 ± 0.21
FI	100	4.02 ± 0.27**	2.61 ± 0.15	2.81 ± 0.21
FI	400	4.39 ± 0.27***	2.71 ± 0.03	2.85 ± 0.35

Each value represents the mean (± SD) of six independent observations, and their statistical significance is given by: *, ** and *** representing $P<0.05$, $P<0.01$ and $P<0.001$ as compared to the corresponding value of the vehicle treated group.



(***, ††† and $$$ representing $P<0.001$ as compared to the vehicle, FI-100 and FI-200 groups respectively, n= 6/group).

Figure 3: Effect of the *Fumaria indica* extract (FI) on high affinity flunitrazepam binding in prefrontal cortex of rats

brain regions. The results summarized in Figure 3 reveals that FI dose dependently increased specific flunitrazepam binding in prefrontal cortex, and this observed effect of the extract was statistically highly significant even after its lowest anxiolytic dose tested, i.e. 100 mg/kg/day.

Brain cytokines expression: It is now becoming increasingly apparent that inflammatory processes are intrinsically involved in CNS functions and that brain cytokine levels could be involved in diverse behavioral processes.[22] Since brain prefrontal cortex was suspected to be one of the brain regions involved in the observed effects of FI, it was of interest to test its potential effects on cytokine expressions in this brain region. As shown in Figure 4, in comparison to the values observed in the control group not exposed to EPM, the expressions of all three cytokines studied (TNF-α, IL-1β and IL-10) in the prefrontal cortex of untreated rats exposed to EPM were much higher. Such was not the case in the FI (100mg/kg/day) treated and EPM exposed group. These observations indicate that apart from monoamines and benzodiazepine receptors, brain cytokines could also be involved in the observed antianxiety effect of FI in behavioral models.

DISCUSSION

Anxiety disorders are cognitive function associated psychopathologies almost-inevitably encountered in many medical and surgical conditions.[23,24] Currently available psychoactive drugs, mainly anxiolytics and antidepressants,[25] do not often properly meet the therapeutic demands of patients suffering comorbid psychiatric conditions, and the drawbacks of such drugs in terms of side effects and costs are also well known. Lack of appropriate pharmacological models necessary for identifying urgently needed pharmacotherapies against comorbid anxiety disorders is one of the major hurdles for discovering novel therapeutic leads suitable for further development,[21,26] and by far a vast majority of currently available animal models are suitable only for identifying potential anxiolytics acting either on GABAA, or on 5-HT1A, receptors.[27] The activity profiles of FI in all the five tests used in our study were always qualitative analogous to that of the benzodiazepine lorazepam [Tables 1-3 and Figures 1 and 2], and benzodiazepine receptor density in the prefrontal cortex of FI treated rats were also dose dependently elevated after repeated daily anxiolytic doses of FI [Figure 3]. Therefore it seems reasonable to assume that the benzodiazepine site of the GABAA receptor is involved in the putative antianxiety effect of FI.

However, unlike for therapeutically used benzodiazepines and other psychoactive drugs acting on GABAA or 5-HT1A receptors, repeated doses of FI is necessary to observe its anxiolytic like effects.[13] In addition, since

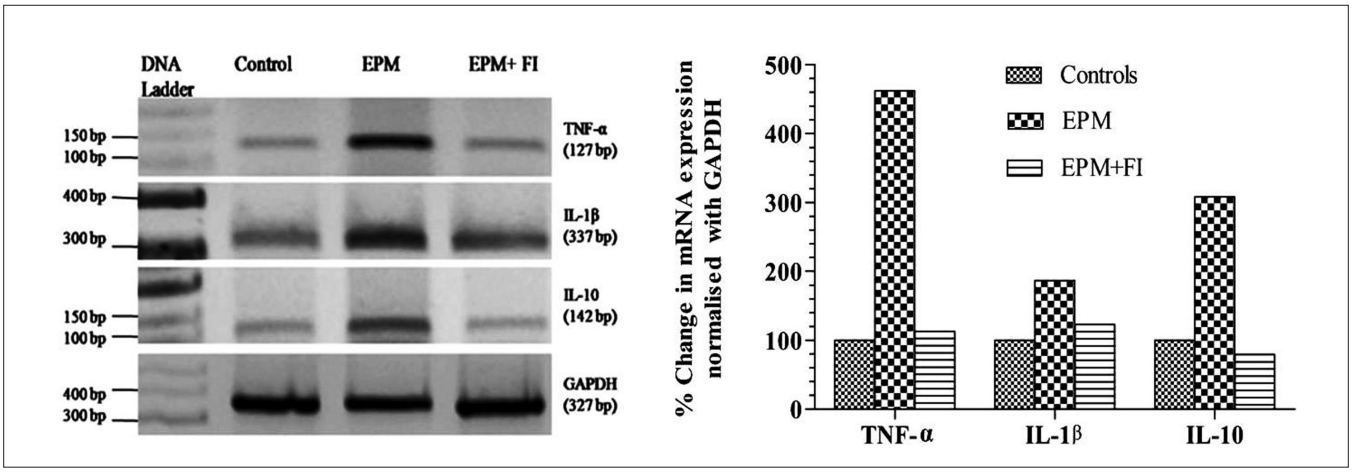


Figure 4: Expression of various cytokines and the housekeeping gene GAPDH in the brains of rats under different experimental conditions. For details see text

unlike benzodiazepines and other sedatives, no muscle relaxant, memory function deteriorating, and other well known side effects of such agents were observed in our earlier pharmacological and toxicological studies,[11,28] it was of interest to test whether neuronal mechanisms involving monoamines are also involved in the behavioral activity profile of FI. Observed increases of serotonin and noradrenaline concentrations in prefrontal cortex of FI treated rats [Table 4] suggest that such could indeed be the case. Since these neurotransmitters are known to be involved in modulating effects of drugs on emotional, cognitive and other functions involved in behavioral manifestations of anxiety,[29] it seems reasonable to assume also that FI posses therapeutically interesting beneficial effects on such mental functions via modulations of the monoaminergic functions of the central nervous system. Protopine is one of the known secondary *Fumaria indica* metabolite possessing modulatory actions on the synaptic uptakes of serotonin and noradrenaline.[10] However, it cannot be overemphasized that numerous other psychoactive alkaloids and other constituents are also encountered in its extracts. Consequently, the questions concerning the role of protopine and other alkaloids in the observed behavioral activity profile of FI can be properly answered only after the results of appropriate analytical and pharmacological studies are available.

Efforts to rationally answer such question can be successful only when special analytical methods appropriate to solve complex problems arising from physicochemical properties of protopine and other psychoactive *Fumaria indica* alkaloids are available. It is known since long that protopine and other alkaloids exist in plants as their salts, and that depending on the acid component of the salt they can exist in diverse tautomeric and/or isomeric forms.[9,30] Depending on the tautomeric or isomeric form of such bioactive constituents of *Fumaria indica* extracts, their bioactivity profiles can vary considerably. Currently available analytical methods are not suitable for resolving such complex problems arising from these facts. Consequently, for practical purposes, quantification of the acidic components of FI was considered to be a more feasible means for provisional analytical characterization of FI. Moreover, with the assumption that that fumaric acid and methyl fumarate are quantitative the major bioactive organic acids present in FI, till now we have concentrated our efforts in understanding the possible roles of these acidic molecules in dictating the pharmacology and medicinal phytochemistry of FI. Although antioxidant activity of fumaric acid has been known since long, and monomethyl fumarate has been suggested to be a hepatoprotective constituent of *Fumaria indica* reports on quantitative data on the contents of fumarates and other bioactive constituents of FI like extracts are very rare.[7] It has been reported though,[8] that protopine content of a FI like hydro-alcoholic *Fumaria indica* extracts is low (0.08 ± 0.03 mg/g; i.e. ca. 0.008%), which is a ca. 10 fold lower than those of the fumarates quantified in FI (ca. 0.8%).

Several reports during more recent years have pointed out the therapeutic benefits obtainable from fumaric acid esters and others suggest that modulation of cytokine synthesis could be involved in their observable anti-inflammatory effects.[5] It is now becoming increasingly apparent that oxidative stress triggering such mechanisms and processes are also involved in pathologies caused by, or associated with, anxiety disorders.[31] Our efforts to quantify cytokine expressions in the brain tissue of animals subjected to the elevated plus maze conditions revealed that expressions of all the three cytokines quantified, i.e. TNF-α, IL-1β and IL10, were several folds higher in untreated animals with prior experience on the maze [Figure 4]. Hereupon the effects on TNF-α and IL-10 expressions were more prominent. These observations indicate that their excessive expressions might as well be longer lasting consequences of prior experiences of a conflict situation. Thus, they could

be considered as further experimental evidences in favor of the current concepts that inflammatory processes are intrinsically involved in psychiatric disorders.[22] Although IL-10 was initially considered to be an anti-inflammatory cytokine, more recent findings strongly suggest its involvement in diverse behavioral and cognitive processes, and that it could be an important link between the changes in hormonal and cytokine milieus of relevance to mental health conditions.[32,33] Since even the lowest anxiolytic FI dose (100 mg/kg/day) almost completely reversed the elevated cytokine expressions in the brain, it could be a useful tool for more precise characterization of the roles of these cytokines in behavioral processes.

In any case, it remains certain that FI is an orally active and novel type of psychoactive agent with therapeutic potential for helping patients diagnosed with anxiety disorders. Since diverse types of *Fumaria indica* preparations have been used safely for ages, and no muscle relaxant, sedative, and other side effects commonly encountered in psychoactive drugs could not be detected in our earlier studies, it seems to be an economically more affordable herbal alternative for therapeutic purposes. However, since qualitatively as well as quantitatively the contents of the extractable bio-active constituents of the herb vary considerably proper selection of appropriate plant material, and/or of processing steps is necessary for obtaining FI like therapeutically interesting extracts.[3] More precise knowledge of the active constituents of FI is an essential prerequisite for such purposes, and such knowledge can become available only after convenient bioassay models necessary for identifying them are available. Therefore, further efforts based on the observations reported in this communication are now being made in our laboratories to solve some such problems. Hereupon our immediate goal is to generate more convincing evidences on the role of fumarates in the observed therapeutically interesting psychopharmacological properties of FI.

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