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Pharmacological Study

Effects of ethanolic extract of *Fumaria indica* L. on rat cognitive dysfunctions

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Abstract

Fumaria indica L. in Ayurveda is known as *Parpat* and traditionally used to calm the brain. Due to lack of scientific validation, 50% ethanolic extract of *F. indica* L. (FI) was evaluated for putative cognitive function modulating effects. Suspension of FI in 0.3% carboxymethyl cellulose (CMC) was orally administered to rats during the entire experimental period of 16 days at dose levels of 100, 200, and 400 mg/kg/day. Piracetam was used as standard nootropic. Behavioral models of learning and memory used were modified elevated plus-maze (M-EPM) and passive avoidance (PA) tests. Scopolamine (1 mg/kg, s.c.), sodium nitrite (25 mg/kg, i.p.), and electroconvulsive shock (150 mA, 0.2 sec) were used to induce amnesia**.** Acetylcholinesterase (AChE) activity, muscarinic receptor density, oxidative status, and cytokine expressions [tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-10] were also assessed. Piracetam (500 mg/kg/day)-like memory-enhancing and anti-amnesic activity of the extract was observed. FI showed dose-dependent decrease in brain AChE activity and increase in muscarinic receptor density, and such was also the case for its observed beneficial effects on the brain antioxidative status. FI also inhibited the scopolamine-induced overexpression of the three tested cytokines observed in rat's brain. FI possesses nootropic-like beneficial effects on cognitive functions.

Key words: Acetylcholinesterase, cytokines, *Fumaria indica*, memory, muscarinic receptors, *Parpata*

Introduction

Fumaria indica L. (FI) is one of the more commonly used medicinal herbs in Ayurveda and other traditionally known health care systems of India. Diverse, therapeutically interesting neuro and psychopharmacological properties of many FI alkaloids have been described, and suggestions have also been made that it could be pharmacologically classified as a neuroleptic herb.[1] This was based on older pharmacological observations revealing some chlorpromazine-like bioactivities of two of the many alkaloids found in the plant.[2] Since FI extracts contain several psychoactive alkaloids and other bioactive molecules, efforts are now being made in our laboratories to properly define the psychopharmacological activity profile of a hydro-alcoholic extract of the plant.[3]

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Initially, our interest in defining psychopharmacological activity profile of a 50% ethanolic extract of FI was triggered by a report revealing antidepressant-like therapeutic potential of protopine isolated from Chinese medicinal plants.[4] This alkaloid is quantitatively one of the major psychoactive alkaloids of *F. indica*,[5] and since a long time, protopine has also been known to be a bioactive secondary metabolite of several other medicinal plants commonly used in many other traditionally known healthcare systems currently popular in many Asian and European countries. However, observations made in our laboratories have revealed that FI does not possess antidepressant-like activities[6] and that the extract could be a functionally novel class of anti-anxiety agent[7] with therapeutically interesting effects against mental stress-triggered pathologies.[8] Subsequent behavioral and mechanistic studies indicated benzodiazepine-like anxiolytic activity profile of FI in a battery of animal models, and demonstrated that repeated daily oral doses of the extract is necessary for obtaining its therapeutically interesting bioactivities in animal models (manuscript under preparation). It is now well recognized that anxiety state dictates cognitive functions,[9] and that benzodiazepines and other anxiolytic drugs adversely affect such functions. Observations made during our efforts to test the

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effects of repeated daily FI treatment on the cognitive functions suggested that FI could as well be a nootropic-like cognitive function improving agent.[10] Consequently, further studies were conducted to clarify possible neurological and biochemical processes involved in its cognitive function improving effects and to test its anti-amnesiac activities. Observations made to date during these efforts will be described and discussed in this communication.

Materials and Methods

Animals

Adult Charles Foster albino rats (150 ± 10 g) of either sex were obtained from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, and were randomly distributed into different experimental groups. The rats were housed in polypropylene cages at an ambient temperature of 25 ± 1°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. They were acclimatized to laboratory conditions for at least 1 week before using them for the experiments. Principles of laboratory animal care (NIH publication number 85-23, revised in 1985) guidelines were followed. Prior approval of Institutional Animal Ethics Committee (Ref no. Dean/2009-10/694) of Banaras Hindu University was obtained.

Plant material and extraction

The whole plant *F. indica* was acquired from a local commercial source in Varanasi. The identification of the plant was done at, Department of Botany, Faculty of Science, Banaras Hindu University, and herbarium specimen (specimen voucher, JAN-2009-01) of the plant was preserved. After shade drying, extraction of the whole plant was done with Soxhlet apparatus using 50% ethanol as solvent. Extraction process was conducted for continuous 72 h. After extraction, the solvent was evaporated using rotary evaporator to get a semi-solid mass. The yield of the crude extract was found to be 10.17% w/w of the dried plant.

Analytical characterization of the extract

The tested *F. indica* extract was analytically characterized by its conjugated and free fumaric acid contents. Hereupon, high-performance thin layer chromatography (HPTLC), CAMAG TLC Scanner III, and Camag Linomat applicator IV were used. Commercially available fumaric acid and dimethyl fumarate (Sigma-Aldrich, USA) served as authentic analytical markers. For free fumaric acid quantification, the test sample and fumaric acid were dissolved in methanol, and for estimation of fumaric acid conjugates, test sample and dimethyl fumarate were dissolved in 50 ml of 5 N HCl, refluxed for 2 h, 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. The developed plates were dried and scanned under absorbance mode (scanning wavelength λ 260 nm), and calculations were based on the area of peaks of the sample and the corresponding authentic marker. The tested 50% ethanolic extract (FI) contains 0.45% w/w of free fumaric acid and 0.35% w/w of fumaric acid conjugates (calculated as dimethyl fumarate).

Drug treatment

FI was orally administered as 0.3% carboxymethyl cellulose (CMC) suspension in doses of 100, 200, and 400 mg/kg once daily. Rats in control groups were treated with equal volume of the vehicle (0.3% CMC suspension). Piracetam (500 mg/kg, p.o.) was used as reference nootropic drug, and all treatments were continued for the entire 16 days of the study period. One hour after drug treatment on the 7th day (i.e. on the training day considered as day 1 of the behavioral test), the rats were exposed to the learning trial on elevated plus maze or on passive avoidance test box. Retention tests were similarly conducted on the day following and 8 days (i.e. on the 2nd and 9th behavioral test days) after the training day. For testing anti-amnesic potential, the same drug treatment regimen was used whereupon scopolamine, sodium nitrite, and electroconvulsive shock were administered 1 h after the learning trial on day 1 of the tests. Choice of the 16-day treatment regimen was based on the observations made during our earlier dose finding and other studies.[6-8]

Modified elevated plus maze test

The modified version of the elevated plus maze (M-EPM) test, useful for quantifying memory acquisition and retrieval in experimental animals and standardized for rats under our laboratory conditions, was used.[11,12] In short, the plus maze consisted of two opposite open arms crossed with two enclosed arms of the same dimensions (50 × 10 cm), with walls 40 cm high. The arms were connected with a central square (10 × 10 cm) to give the apparatus a plus sign appearance. The maze was kept in a dimly lit room, elevated 50 cm above the floor level. On day 1, a rat was individually placed on the far end of one of the open arms, facing away from the center, and the time taken by the animal to enter one of the closed arms (transfer latency day 1; i.e. TL1) was recorded with the help of a stop watch. The rat was left in the enclosed arm for 15 sec and returned to its home cage. On day 2, the procedure was repeated and the day 2 transfer latency (TL2) was recorded. Similarly, after an interval of 1 week, on day 9, the transfer latency (TL9) was again recorded.

Passive avoidance test

Standard procedures in earlier studies were used in the current experiment.[12-13] Briefly, on day 1, a rat was placed in a white box and the time taken to enter into the dark box was noted [step through latency (STL) 1]. As soon as the rat entered the dark box, the guillotine door was closed and foot electric shock (0.5 mA, 3 sec) was delivered. The rat was then replaced to its home cage. On the following day (24 h retention interval), each rat was again placed in the white box and given a 5-min habituation period. Latency to step through to the dark chamber was recorded on day 2 and day 9 (STL9) without delivering electric shock.

Amnesia models

The effects of FI and piracetam treatments on experimentally induced amnesia were tested using the described M-EPM and PA test procedures. The three stimuli used for amnesia induction were scopolamine hydrobromide (1 mg/kg, s.c.), sodium nitrite (25 mg/kg, i.p.), and electroconvulsive shock (150 mA, 0.2 sec). These amnesic treatments were administered immediately after the end of the learning trials on day 1 of the behavioral tests.

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Brain acetylcholinesterase (AChE) activity

Effects of seven daily doses of FI, or of piracetam, on brain acetylcholinesterase (AChE) activity[14] and Protein were estimated using the standard method.[15]

Quinuclidinyl binding study

The hippocampus regions of brain of the rats were removed after 45 min of the final administration of the FI and the crude synaptic membranes were prepared as described earlier.[16] Briefly, isolated brain region hippocampus was homogenized in 19 volumes of 0.32 M sucrose and centrifuged at 50,000 × *g* for 10 min. The resulting pellets were homogenized in distilled water and recentrifuged at the same speed. The final pellets were suspended in 40 mM Tris-HCl buffer, pH 7.4, at a concentration representing 50 mg of the original tissue/ml.

Brain oxidative status

Effects of seven daily FI doses (100, 200, and 400 mg/kg/day) on the brain oxidative status were assessed by sacrificing the rats by decapitating them 1 h after the last oral dose. Brains were quickly removed, weighed, and homogenized in ice-cold 50 mM potassium phosphate buffer (pH 7.4) containing 1 mM ethylenediaminetetraacetic acid (EDTA). Ratio of brain weight to homogenate volume was always 1:10, and this homogenate was always used in all assays used for assessing oxidative status of the brain by the methods given below.

Lipid peroxides (LPO): Malondialdehyde (MDA) concentrations were used as an index for lipid peroxidation levels in the incubated homogenates. They were assessed by using the standard method described.[17] *Superoxide dismutase* (SOD), *Catalase* (CAT) activity and *Reduced glutathione* (GSH) were also assessed using the standard method. [18-20]

Brain cytokine expressions: In order to assess the effect of scopolamine and FI treatment on the expression of cytokines in brain, rats were divided into three groups, viz. control without FI treatment, scopolamine-treated rats, and scopolamine-treated rats with prior daily FI (100 mg/kg) treatment for seven consecutive days. After 24 h of the last treatment, rats were sacrificed by spinal cord dislocation, brain was removed, and brain tissues were washed with phosphate-buffered saline (PBS) and lysed in TRI reagent™ (Sigma-Aldrich, St Louis, MO, 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 was quantified using Nano Drop Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and equal amount of total RNA from each rat was pooled in each study group.

Reverse transcription-polymerase chain reaction

(a) *cDNA preparation*: In order to avoid amplification of contaminating genomic DNA, total RNA was treated with RNase-free DNase (New England Biolabs, Ipswich, MA USA). DNase-treated RNA (2 μg) was reverse transcribed by using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA USA). Briefly, 10 μl of nuclease-free water containing 2 μg of RNA was added to 10 μl of RT mix containing 2 μl of RT random primer (10X), 0.8 μl of dNTPs mix (100 mM), 2 μl of RT buffer (10X), 1 μl of MultiScribe™ reverse transcriptase (50 U/μl), and 1 μl of human placental ribonuclease inhibitor (10 U/μl). The samples were then incubated at 25°C for 10 min, followed by incubation at 37°C for 2 h. The reverse transcriptase was then inactivated by heating the reaction mixture at 85°C for 5 min.

(b) *Polymerase chain reaction* (PCR): All mRNA sequences were retrieved from NCBI database. The gene-specific primers were either designed using primer 3 software and synthesized at Integrated DNA Technologies (Coralville, IA, USA) or were purchased from New England Biolabs (Ipswich, MA, USA). Primer sequences and corresponding product size are given in Table 1. The PCR was carried out in a thermal cycler (Applied Biosystems, USA) in a 15-μl final volume containing 0.5 μl of cDNA, 1.5 μ1of PCR buffer (10X), 0.15 μl of Taq polymerase (5 U/μl), 0.9 μl of $MgCl_2$ (25 mM), and 0.3 μl of each forward and reverse primer (10 pM). After an initial denaturation step at 94°C for 5 min, temperature cycling was initiated. Each cycle consisted of denaturation step at 94°C for 30 sec, annealing at primer-specific temperatures for 30 sec, and extension at 72°C for 30 sec. After 35 cycles, a final extension at 72°C for 7 min was done. PCR products were separated on 2% agarose gel and visualized using ethidium bromide staining. The density of each band was measured using densitometric software provided with Alpha Imager gel documentation system (Alpha Imager, Bangalore, India). The cytokine mRNA expressions were expressed as a ratio of cytokine band intensity to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping control.

Statistical analysis

The data are expressed as Mean ± SD for each treatment group. Ratios of TL, or STL, in the M-EPM, or PA, tests on day 1 to those observed on test days 2 and 9 (i.e. TL2/TL1 and TL9/TL1, or STL2/STL1 and STL9/STL1) for each individual animal were used to judge the anti-amnesic effects of the extract. The data obtained from each response measure 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. Tukey–Kramer multiple comparison test was used for P value correction. Differences were considered as statistically significant when $P < 0.05$.

Results

Learning and memory tests

Results of the experiments conducted to test the effects of FI on memory acquisition, consolidation, and retrieval are presented in Figures 1 and 2. In comparison to control on day 2, FI 100 ($P < 0.05$), FI 200 ($P < 0.05$), FI 400 ($P < 0.05$), and PIRA 500 ($P < 0.01$), respectively, showed significant differences. Similarly, on 9th day, in comparison to control, FI 100 ($P < 0.05$), FI 200 ($P < 0.05$), FI 400 ($P < 0.05$), and PIRA 500 ($P < 0.01$), respectively, showed significant differences. In comparison to day 1, FI 100 ($P < 0.05$), FI 200 ($P < 0.05$), FI 400 ($P < 0.05$), and PIRA 500 ($P < 0.01$), showed significant differences on day 2. Again on day 9, FI 100 ($P < 0.05$), FI 200 ($P < 0.05$), FI 400 ($P < 0.05$), and PIRA 500 ($P < 0.01$) showed significant differences when compared to day 1 [Figure 1]. In comparison to day 1, FI 100 ($P < 0.05$), FI 200 ($P < 0.05$), FI 400 ($P < 0.05$), and PIRA 500 ($P < 0.05$) showed significant

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Table 1: Primers used for RT-PCR analysis

Gene	NCBI sequence ID	Primer sequence	Product size (bp)	Annealing temperature (°C)
TNF-α	NM_012675.3	F: 5′ TCTCAAAACTCGAGTGACAAGC 3′ R: 5′ GGTTGTCTTTGAGATCCATGC 3′	127	60
IL-10	NM_012854.2	F: 5′ GAGAGAAGCTGAAGACCCTCTG 3′ R: 5′ TCATTCATGGCCTTGTAGACAC 3′	142	54
IL-1β	NM_031512.2	F: 5′ AAATGCCTCGTGCTGTCTGACC 3′ R: 5′ CTGCTTGAGAGGTGCTGATGTACC 3′	337	64
GAPDH	NM_017008.3	Proprietary sequence	327	55

NCBI: National Center for Biotechnology Information, RT-PCR: Reverse transcription-polymerase chain reaction, TNF: Tumor necrosis factor, LI: Interleukin, GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

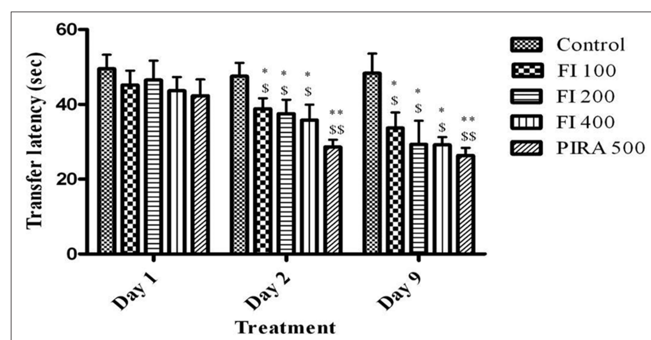

Figure 1: Effects of the *Fumaria indica* extract (FI) on rat transfer latencies in elevated plus maze test. *n* = 6; Data, Mean ± SD; FI, standardized 50% ethanolic extract of Fumaria indica; FI 100, Ethanolic extract of *Fumaria indica* 100 mg/kg; FI 200, Ethanolic extract of *Fumaria indica* 200 mg/kg; FI 400, Ethanolic extract of Fumaria indica 400 mg/kg; PIRA 500, Piracitam 500 mg/kg; *In comparison to control on day 2 and 9, *P* < 0.05; **In comparison to control on day 2 and 9, *P* < 0.01; $In comparison to day 1 with day 2 and 9, *P* < 0.05; $$In comparison to day 1 with day 2 and 9, *P* < 0.01

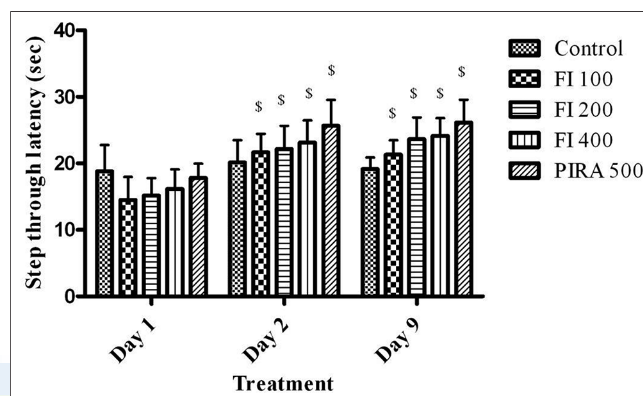

Figure 2: Effects of the *Fumaria indica* extract (FI) on rat transfer latencies in elevated plus maze test. *n* = 6; Data, Mean ± SD; FI, standardized 50% ethanolic extract of Fumaria indica; FI 100, Ethanolic extract of *Fumaria indica* 100 mg/kg; FI 200, Ethanolic extract of *Fumaria indica* 200 mg/kg; FI 400, Ethanolic extract of *Fumaria indica* 400 mg/kg; PIRA 500, Piracitam 500 mg/kg; $In comparison to day 1 with day 2 and 9, *P* < 0.05

differences on day 2. Again on day 9, FI 100 ($P < 0.05$), FI 200 ($P < 0.05$), FI 400 ($P < 0.05$), and PIRA 500 ($P < 0.05$) showed significant differences when compared to day 1 [Figure 2]. As shown in the figures, the transfer and step through latencies in the M-EPM and PA tests on the training day 1 of the control group were not significantly different from those on the retest days 2 and 9. Such was not the case for the FI- or piracetam-treated groups. Although these latencies of all the three FI-treated groups were statistically not different from those of the control group on the training day 1, these values altered significantly for the extract-treated groups on the retest days 2 and 9. Analogous was the case for the piracetam-treated group. The observed effects of FI on memory retrieval on both these days increased somewhat with its increasing doses, but there were no significant differences between the mean values of the three groups on both the retest days. The observed effects of piracetam in the retrieval tests on the days 2 and 9 were quantitatively higher than those of the FI-treated ones. These observations indicate that FI could as well have piracetam-like beneficial effects on memory acquisition, consolidation, and retrieval, and that these effects of the extract increase with increasing number of treatment days. However, quantitatively, the efficacy of FI on cognitive functions seems to be somewhat lower than that of the reference nootropic piracetam.

Amnesia tests

One unexpected observation of the learning and memory tests was that no significant effects on memory acquisition and retrieval could be observed in the control group treated daily with CMC. These observations suggest that either daily handling or CMC treatment adversely affects learning and/or memory functions of the rats. As the effects of daily handling on rodent behavior in EPM and PA tests have been known since a long time,[21] the observed lack of learning in the control group could as well be due to the daily handling procedures used in our experiments. However, the amnesic effects of scopolamine, sodium nitrite treatment, and electroconvulsive shock in the CMC-treated control rats were apparent in the M-EPM as well as PA tests. The ratios of the mean TL of different experimental groups in the M-EPM test observed on days 2 and 9 to that observed on day 1 (i.e. TL2/TL1 and TL9/TL1, respectively) are summarized in Table 2, and the observed ratios of STL in the PA test (i.e., STL2/STL1 and STL9/STL1) are given in Table 3. These results demonstrate piracetam-like anti-amnesic effects of FI against all the three amnesic agents in both the behavioral tests used. Like in the learning and memory tests, such efficacies of FI in all anti-amnesic tests were always quantitatively lower than that of the reference nootropic. However, even after the lowest

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Table 2: Ratio of transfer latencies in elevated plus maze test

Treatment	Dose (mg/kg)	Transfer latency ratio	
		TL2/TL1	TL9/TL1
Control		0.96±0.12	0.98±0.15
SCP	1	1.27±0.18*	1.17±0.13
FI+SCP	100+1	0.87±0.11###	0.78±0.01###
FI+SCP	200+1	0.76±0.11###	0.60±0.10###
FI+SCP	400+1	0.75±0.10###	0.62±0.10###
PIRA+SCP	500+1	0.68±0.08###	0.69±0.08###
Sod. nit	25	1.46±0.22**	1.31±0.16**
FI+sod. nit	100+25	0.78±0.08^^^	0.62±0.17^^^
FI+sod. nit	200+25	0.68±0.12^^^	0.54±0.1^^^
FI+sod. nit	400+25	0.67±0.10^^^	0.53±0.12^^^
PIRA+sod. nit	500+25	0.60±0.06^^^	0.45±0.09^^^
ECS	-	1.63±0.22***	1.50±0.17***
FI+ECS	100+ECS	0.74±0.09†††	0.69±0.14†††
FI+ECS	200+ECS	0.70±0.16†††	0.57±0.10†††
FI+ECS	400+ECS	0.68±0.11†††	0.54±0.10†††
PIRA+ECS	25+ECS	0.62±0.06†††	0.47±0.08†††

FI: Standardized 50% ethanolic extract of *Fumaria indica*; PIRA: Piracetam, SCP: Scopolamine, sod. nit: Sodium nitrite, ECS: Electroconvulsive shock, TL: Transfer latency ratio, *n*: 6 animals in each group, values are Mean±SD, **P*<0.05, ***P*<0.01, and ****P*<0.001 in comparison to control, ###*P*<0.001 in comparison to SCP, ^^^*P*<0.001 in comparison to sod. nit., †††*P*<0.001 in comparison to ECS

Table 3: Ratio of step through latencies in passive avoidance test

Treatment	Dose (mg/kg)	Step through latency	
		STL2/STL1	STL9/STL1
Control		1.03±0.36	1.06±0.29
SCP	1	0.60±0.21*	0.64±0.18*
FI+SCP	100+1	1.39±0.39##	1.44±0.36###
FI+SCP	200+1	1.46±0.34##	1.44±0.30###
FI+SCP	400+1	1.43±0.50##	1.47±0.31###
PIRA+SCP	500+1	1.54±0.33##	1.58±0.48###
Sod. nit	25	0.64±0.15*	0.70±0.19*
FI+sod. nit	100+25	1.42±0.28^^	1.34±0.28^^
FI+sod. nit	200+25	1.44±0.40^^	1.52±0.21^^^
FI+sod. nit	400+25	1.49±0.53 ^^	1.50±0.36^^^
PIRA+sod. nit	500+25	1.49±0.25^^	1.48±0.28^^^
ECS	-	0.65±0.27*	0.67±0.15*
FI+ECS	100+ECS	1.50±0.44††	1.37±0.36††
FI+ECS	200+ECS	1.43±0.39†	1.44±0.21†††
FI+ECS	400+ECS	1.48±0.43††	1.40±0.36††
PIRA+ECS	25+ECS	1.59±0.39††	1.53±0.51†††

FI: Standardized 50% ethanolic extract of *Fumaria indica*; PIRA: Piracetam, SCP: Scopolamine, sod. nit.: Sodium nitrite; ECS: Electroconvulsive shock, *n*: 6 animals in each group, values are Mean ± SD, STL: Step through latency, **P* < 0.05 in comparison to control, ##*P* < 0.01 and ###*P* < 0.001 in comparison to SCP, ^^*P* < 0.01 and ^^^*P* < 0.001 in comparison to sod. nit., †*P* < 0.05, ††*P* < 0.01, and †††*P* < 0.001 in comparison to ECS

FI dose tested (100 mg/kg/day), significant beneficial effects of the extract were observed in all tests. Quantitatively, the effects of the extract increased only marginally after its higher doses.

Brain cholinergic mechanisms

The mean values of AChE activity in brain prefrontal cortex were significantly lower in all FI-treated groups. These effects after the higher two tested doses (200 and 400 mg/kg/day) of the extract were not statistically significantly different from each other, and that in the lowest dose group (100 mg/kg/day) was significantly lower than that of the highest one. Significant effects of FI on AChE activity in the other brain regions studied were not dose dependant [Table 4]. These observations reveal that 100 mg/kg/day dose of the extract is high enough for lowering the enzyme activity in the brain, and indicate that central cholinergic mechanisms are involved in the observed cognitive function improving effects of FI. Clear dose-dependant increase in the muscarinic receptor density in the hippocampus of FI-treated rats [Table 5] is in agreement with this inference. All the three tested brain regions are profusely endowed with cholinergic neurons and are also considered to be those involved in processing of memory.[22]

Brain oxidative status

Results summarized in Table 6 reveal that seven daily oral doses of FI increased the levels of SOD, CAT, and GSH and decreased the formation of MDA in rat brain. Inhibitory effects of FI on MDA production in the incubated brain homogenates were dose dependant, whereas its effects on the brain levels of GSH and the two antioxidative enzymes studied did not significantly increase further with its increasing doses. These observations are analogous to those reported earlier.[8] in mentally stressed rats treated daily with FI for 15 days. Thus, it seems certain that maximally obtainable antioxidative effects of FI are already achieved by seven daily treatments with 100 mg/kg dose of the extract.

Brain cytokine expressions

Daily oral 100 mg/kg/day dose of FI efficiently inhibited the induction of brain cytokine expressions in rats treated with the anticholinergic amnesic agent scopolamine. In comparison to the vehicle-treated control group, expressions of all the three cytokines studied, i.e. tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-10, were elevated in the brains of the scopolamine challenged one. As can be seen from the results presented in Figure 3, these effects observed 24 h after scopolamine challenge were more pronounced for TNF-α and IL-10 expressions. No such effect of scopolamine challenge was observed in the FI (100 mg/kg/day) treated group. These observations are analogous to those made in our earlier studies using rats subjected to unavoidable mental stress,[8] or with prior experience on EPM (as yet unpublished observations in our laboratories). Therefore, these observations add further experimental evidence to the conviction that suppression of exaggerated cytokine production triggered by noxious stimuli is involved in the observed anti-amnesic and other therapeutically interesting bioactivities of FI.

Discussion

Results of the reported behavioral experiments suggest that repeated daily oral doses of FI can actually improve cognitive functions. The TL in the M-EPM test of the FI-treated groups on days 2 and 9 were always significantly lower than those of the control group, and in the PA test, the STL of the three extract-treated groups on these two days were significantly

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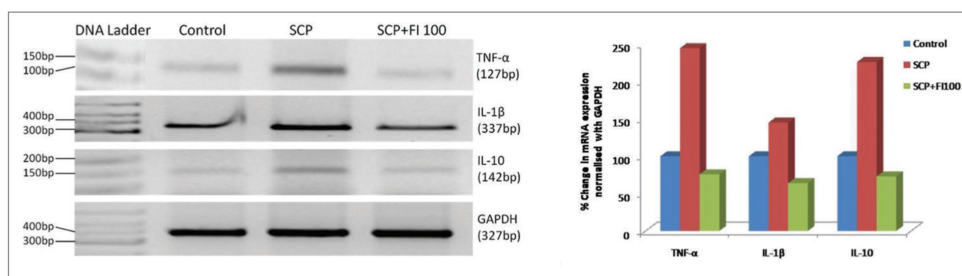

Figure 3: Effect of *Fumaria indica* on scopolamine-induced expressions of cytokines in rat brain

Table 4: Effect of *Fumaria indica* on acetylcholine esterase activity in different brain regions of rat

Treatment	Dose (mg/kg, p.o.)	*n* Moles of substrate hydrolyzed/min/mg protein		
		Prefrontal cortex	Hippocampus	Hypothalamus
Control	-	8.27±0.711	9.47±1.65	11.06±1.56
FI	100	5.22±0.56***	6.79±0.98**	7.67±1.28**
FI	200	4.10±0.52***,$	6.42±0.81***	7.23±1.39***
FI	400	3.98±0.48***,$$	5.55±0.79***	5.95±0.69***

FI: Standardized 50% ethanolic extract of *Fumaria indica*, *n*: 6 animals in each group, values are Mean±SD, ***P*<0.01 compared to control, ****P*<0.001 compared to control, $*P*<0.05 and $$*P*<0.01 compared to FI-100

Table 5: Effect of *Fumaria indica* on high-affinity muscarinic receptor density in rat hippocampus

Brain region	Receptor (radioligand)	Treatment (mg/kg, p.o.)	Pmoles bound/g protein
Hippocampusia	M (^{3}[H] QNB)	Control	309.33±8.93
		FI (100)	348.66±7.99***
		FI (200)	390.16±13.96***,$$$
		FI (400)	456.83±9.49***,$$$,###

FI: Standardized 50% ethanolic extract of Fumaria indica, *n*: 6 animals in each group, values are Mean±SD, ****P*<0.001 compared to control, $$$*P*<0.001 compared to FI-100, and ### *P*<0.001 compared to FI-200

higher than those observed on day 1 [Figures 1 and 2]. These observations indicate that the extract facilitates acquisition, and/or retrieval, of the memory of the learned task on day 1, and that its effects persist after prolonged treatments. In this respect, the observed activity profile of FI in both the experimental paradigms used was analogous to that of the reference neuroactive drug piracetam. Furthermore, the observed activity profile of FI in all anti-amnesic tests was always qualitatively analogous to that of the standard nootropic piracetam, which also possesses anxiolytic-like efficacy.[23,24] Indeed, it has been reported that in animal models, anxiolytic-like activities of piracetam can be observed only after its repeated daily doses, and that its memory-enhancing effects are sensitive to corticosteroid status of the experimental animals.[25] Since our earlier observations have revealed that analogous is also the case for FI, it would seem reasonable to pharmacologically classify FI as a nootropic herbal extract.[8]

During the past few decades, numerous Ayurvedic and other plants have been identified as cognitive function improving, or nootropic, herbs. Also, it has been pointed out that identification of their active constituents and modes of actions are essential prerequisites for obtaining better standardized phytotherapeutics.[26] Observations reported in this communication strongly suggest that like some other cognitive function improving herbs, *F. indica* also improves cognitive functions by facilitating central cholinergic functions. Not only AChE activities in different brain regions of the FI-treated animals were lower than those of the control animals [Table 4], but also muscarinic receptor density in the hippocampus was increased by the extract [Table 5]. It must be noted though, that after the doses and treatment regimen used in this study, FI not only counteracted amnesia induced by four different challenges [Tables 2 and 3] but also enhanced the brain antioxidative status of the treated animals [Table 6]. Taken together, these observations strongly suggest that cellular oxidative mechanisms involved in the regulation of cognitive functions could as well be the primary targets of bioactive constituents of FI.[27,28]

Cytokine expression is regulated by oxidative processes,[29] and involvement of cytokines in psychopathologies has also been implicated.[30-33] Results of the experiment conducted to experimentally verify the possibility that modulation of cytokine expression is involved in the mode of action of FI indicate that it could indeed be the case [Figure 3]. Oral 100 mg/kg/day dose regimen of FI was high enough for completely suppressing scopolamine-triggered elevated expressions of all the three cytokines studied. These observations are in agreement with a recent report revealing enhanced TNF-α and IL-1β expressions in the brains of scopolamine-treated rats, which could be suppressed by a plant saponin with anti-amnesic properties.[34] Moreover, analogous efforts made during our earlier studies also revealed that 100 mg/kg/day dose regimen of the extract was high enough for completely suppressing cytokine expressions in the whole blood of stressed rats.[8] Therefore, it seems reasonable to assume that bioactive component(s) of FI, modulating peripheral as well as central cytokine expressions could be involved in its observed nootropic-like effects. Fumaric acid and its conjugates are some of the known bioactive secondary metabolites of *F. indica*, which are known to possess modulating effects on cytokine expression and CNS function

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Table 6: Effect of *Fumaria indica* on the antioxidant status of rat brain

Treatment	Dose (mg/kg, p.o.)	MDA (n mole/mg protein)	SOD (U/mg protein)	CAT (U/min/mg protein)	GSH (μ mole/mg protein)
Control		9.33±1.09	4.55±0.27	45.27±3.34	3.20±0.21
FI	100	7.39±1.32**	6.60±0.95**	54.74±3.14***	4.47±0.39*
FI	200	6.58±1.12***	6.95±0.68***	55.89±2.44***	4.76±0.96**
FI	400	5.40±0.66***,$	7.19±1.24***	56.67±4.9***	5.26±0.77***

FI: Standardized 50% ethanolic extract of *Fumaria indica*, *n*: 6 animals in each group, values are Mean±SD. **P*<0.05, ***P*<0.01, ****P*<0.001 in comparison to control, $*P*<0.05 compared to FI-100

modulating activities.[35,36] Available quantitative data on the phytochemical constituents of hydro-alcoholic *F. indica* extract indicate that they could as well be quantitatively the major bio-active constituents of FI. Since preliminary observations made during efforts to experimentally verify this possibility are encouraging, efforts are now being made to clarify the role of fumarates and cytokines in the observed therapeutically interesting psychopharmacological activity profile of FI.

It must be mentioned though, that apart from the observed effects of FI on cholinergic biomarkers, other diverse neurotransmitter systems are also modulated by repeated daily doses of FI (Singh w*wet al*. and other as yet unpublished recent observations in our laboratories).[8] Moreover, results of the biochemical experiments reported in this communication reconfirm that those oxidative and other processes commonly known as hallmarks of inflammation are also involved in the mode of action of the extract. These observations reveal high efficacy of FI in regulating the cellular processes involved in adaptive functions of the brain. Since involvement of these biochemical processes in the control of numerous metabolic processes is now well established, it could as well be that such central metabolic process are also involved in the observed effects of FI on cognitive functions and neurotransmitter systems.

In any case, the reported observations add further experimental evidences to the conviction that Ayurvedic recommendations, and current widespread medicinal uses of *F. indica*, could as well be due to the presence of a unique combination of psychoactive secondary metabolites in the plant. Moreover, they further justify our working hypothesis that fumaric acid, or its conjugates, is quantitatively the major extractable bioactive secondary metabolite of the plant. Although monomethyl fumarate has been suggested to be a hepatoprotective secondary metabolite of *F. indica*, till date, little concentrated efforts have been made to understand the role of fumarates in the traditionally known medicinal uses of the plant.[37] It has since long been known though, that fumaric acid esters possess antiprutitic activities.[38-40] During more recent years, other diverse therapeutic potentials of fumaric acid and its esters have been identified.[35,36] Critical analysis of the available information on such molecules, and on their natural abundance in *F. indica* and other traditionally known medicinal plants, have led us to the working hypothesis that fumaric acid could as well represent a common pharmacophore of psychoactive components of many traditionally known medicinal herbs. Our observations reveal that the concentrations of fumaric acid and its conjugates in FI (ca. 0.8% in total) are much higher than those of other known bioactive constituents of such extracts. Moreover, antioxidative and anti-inflammatory effects of such molecules are now well established.[35] Therefore, it seems reasonable to assume that apart from protopine and other psychoactive alkaloids, fumarates could also be the CNS function modulating component of the extract.

Cognitive disturbances are not only the hallmarks of dementia but also often encountered in patients suffering from many medical and surgical conditions.[41,42] Most antidepressants, anxiolytics, and other psychotherapeutics currently widely prescribed for combating cognitive function associated psychopathologies do not properly meet the therapeutic demands, and adverse effects of such psychotherapeutics on cognitive functions are well known. Moreover, during more recent decades, modern drug discovery strategies have consistently failed to identify novel psychotherapeutic leads or pharmacological targets potentially useful for helping patients with cognitive function associated comorbidities and other neurological disorders.[43] In view of the situation, the observed antioxidative and cytokine production modulating actions of FI could be of considerable interest for drug discovery purposes. Although the involvement of these processes in the etiology and pathogenesis of psychopathologies is now well recognized,[44] due to lack of pharmacological tools, as yet little progress has been made to obtain therapeutic leads from this knowledge.[45] Efforts to identify CNS function modulating components of FI could be a feasible means for obtaining such tools, and could eventually lead to novel structural leads and pharmacological targets urgently needed for drug discovery and development purposes.

Plants of the Fumariaceae family are just a few examples of a class of psychoactive plants commonly used by almost all practitioners of every traditional known medical system. However, till now, little efforts have been made to properly exploit such commonalities for more rationally integrating herbal remedies with the modern medicine evolving from reductionist concepts of western medicine. The observations made to date with FI became possible only by the use of a holistic pharmacological approach based on postmodern concepts of psychobiology.[46] The pharmacological activity profile of FI revealed by our efforts is quite analogous to that described for *F. indica* by Ayurvedic practitioners. Ayurvedic practitioners consider *F. indica* as a pacifier of *Kapha* (somatic energy) and *Pitta* (energy regulator).[3] Observations reported in this communication reaffirm that cognitive function modulating effects of FI are associated with its effects on inflammatory and metabolic processes, and we have recently reported pacifying effects of FI against diverse psychopathologies and metabolic abnormalities associated with mental stress. Since fumarates, protopine, and other alkaloids, and other diverse bioactive secondary metabolites of *F. indica* are also encountered in numerous other medicinal plants, the observations made with FI could as well be useful for better pharmacological characterization of herbal remedies

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containing such bioactive secondary plant metabolites. Thus, it seems reasonable to suggest that behavioral studies with other plant extracts known to contain bioactive molecules encountered in *F. indica* are a feasible, and cost-effective, alternative means not only for obtaining therapeutic leads urgently needed for combating comorbid mental health problems associated with metabolic disorders but also for making more rational progress toward modern science based integrative medicine already widely practiced in India, China, and also in many other parts of the world.

Conclusions

The reported observations reveal nootropic-like activities of FI, and indicate that its diverse therapeutically interesting pharmacological activities could be due its beneficial effects on cognitive functions. Furthermore, they suggest that inhibition of exaggerated brain cytokine production and oxidative metabolic processes are involved in its modes of action. These observations can be useful for more rational exploitation of other medicinal herbs known to produce bioactive plant metabolites encountered in *F. indica*.

Acknowledgments

The authors are thankful for the financial assistance provided by the Indian Council of Medical Research, Government of India, New Delhi. They are also thankful to the R and D Centre, Indian Herbs Research and Supply Co. Ltd., Saharanpur, for carrying out the chemical standardization of the plant extract used in this study.

References

1. Dandiya PC. Psycopharmacology, Herbal Drugs; CINP and Myself. In: Ban TA, Healy D, Shorter E, editors. Reflexions on Twentieth-Century Psychopharmacology. 2nd ed. Scotland, UK: CINP; 2010. p. 212-6.
2. Kumar A, Pandey VB, Seth KK, Dasgupta B, Bhattacharya SK. Pharmacological actions of Fumariline isolated from *Fumaria indica* seeds. Planta Med 1986;4:324-5.
3. Williamson EM, editor. Philadelphia (US): Churchill Livingstone; 2002. Major herbs of Ayurveda; pp. 150–3.
4. Xu LF, Chu WJ, Qing XY, Li S, Wang XS, Qing GW, *et al.* Protopine inhibits serotonin transporter and noradrenaline transporter and has the antidepressant-like effect in the mice models. Neuropharmacology 2006; 50: 934-40
5. Tripathi YC, Rathore M, Kumar H. On the variation of alkaloid contents of *Fumaria indica* at different stages of life span. Anc Sci Life 1994;13:271-3.
6. Singh GK, Kumar V. Neuropharmacological screening and lack of antidepressant activity of standardized extract of *Fumaria indica*: A preclinical study. Electronic J Pharmacol Ther 2010;3:19-28.
7. Singh GK, Kumar V. Anxiolytic activity of standardized extract of *Fumaria indica.* Ann Neurosci 2009;16 Suppl: S81.
8. Singh GK, Rai G, Chatterjee SS, Kumar V. Beneficial effects of *Fumaria indica* on chronic stress-induced neurobehavioral and biochemical perturbations in rats. Chin Med 2012;3:49-60.
9. Mathews A. Why worry? The cognitive function of anxiety. Behav Res Ther 1990;28:455-68.
10. Singh GK, Kumar V. Memory enhancing and anti-amnestic activity of standardized extract of *Fumaria indica*: An *in-vivo* study. International Symposium on Brain Aging and Dementia: Basic and Translational Aspects, organized by Banaras Hindu University, Varanasi, India, November 29-30, 2010a.
11. Itoh J, Nabeshima T, Kameyama T. Utility of an elevated plus maze for the evaluation of memory in mice: Effects of nootropics, scopolamine and electroconvulsive shock. Psychopharmacology 1990;101:27-33.
12. Kumar V, Khanna VK, Seth PK, Singh PN, Bhattacharya SK. Brain neurotransmitter receptor binding and nootropic studies on Indian *Hypericum perforatum* Linn. Phytother Res 2002;16:210-6.
13. King RA, Glasser RL. Duration of electroconvulsive shock-induced retrograde amnesia in rats. Physiol Behav 1970;5:335-9.
14. Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88-95.
15. Lowry OH, Rosenborough NJ, Farr AL, Randal RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951;193:265-75.
16. Seth PK, Alleva FR, Balaz T. Alteration of high affinity binding sites of neurotransmitter receptors in rats after neonatal exposure to streptomycin. Neurotoxicology 1982;3:13-20.
17. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;351-8.
18. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of super oxide dismutase. Indian J Biochem Biophys 1984;21:130-2.
19. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972;47:384-9.
20. Jollow DJ, Mitchell JR, Zampaghone N, Gillete JR. Bromobenzene induced oxide as the hepatotoxic intermediate. Pharmacology 1974;11:161-9.
21. Claassen V. Neglected factors in pharmacology and neuroscience research. Elsevier; 1994. p. 422-59.
22. Ojha R, Sahu AN, Muruganandam AV, Singh GK, Sairam K. *Asparagus recemosus* enhances memory and protects against amnesia in rodent models. Brain Cogn 2010;74:1-9.
23. Leuner K, Kurz C, Guidetti G, Orgogozo JM, Müller WE. Improved mitochondrial function in brain aging and Alzheimer disease – The new mechanism of action of the old metabolic enhancer piracetam. Front Neurosci 2010;4.pii: 44.
24. Malykh AG, Sadaie MR. Piracetam and Piracetam-like Drugs: From basic science to novel clinical application on CNS disorders. Drugs 2010;70:287-312.
25. Mondadori C. In search of the mechanism of action of the nootropics: New insights and potential clinical implications. Life Sci 1994;55:2171-8.
26. Pattewar AV, Katedeshmukh RG, Vyawahare NS, Kagathara VG. Phytomedicine and cognition. Int J Pharm Sci Res 2010;2:778-91.
27. Gackowski D, Rozalski R, Siomek A, Dziaman T, Nicpon K, Klimarczyk M, *et al.* Oxidative stress and oxidative DNA damage is characteristic for mixed Alzheimer disease/vascular dementia. J Neurosci 2008;266:57-62.
28. Giridharan VV, Thandavarayan RA, Sato S, Ko KM, Konishi T. Prevention of scopolamine-induced memory deficits by schisandrin B, an antioxidant lignan from *Schisandra chinensis* in mice. Free Radic Res 2011;45:950-8.
29. Chen CY, Huang YL, Lin TH. Association between oxidative stress and cytokine production in nickel-treated rats. Arch Biochem Biophys 1998;356:127-32.
30. Connor TJ, Leonard BE. Depression, stress and immunological activation: The role of cytokines in depressive disorders. Life Sci 1998;62:583-606.
31. Cragnolini AB, Schiöth HB, Scimonelli TN. Anxiety-like behavior induced by IL-1β is modulated by α-MSH through central melanocortin-4 receptors. Peptides 2006;27:1451-6.
32. Zalcman SS, Siegel A. The neurobiology of aggression and rage: Role of cytokines. Brain Behav Immun 2006;20:507-14.
33. McAfoose J, Baune BT. Evidence for a cytokine model of cognitive function. Neurosci Biobehav Rev 2009;33:355-66.
34. Lee Bi, Jung K, Kim DH. Timosaponin AIII, a saponin isolated from Anemarrhena asphodeloides, ameliorates learning and memory deficits in mice. Pharmacol Biochem Behav 2009;93:121-7.
35. Gold R, Link RA, Stangel M. Fumaric acid and its esters: An emerging treatment for multiple sclerosis with antioxidative mechanism of action. Clin Immunol 2012;142:44-8.
36. Ellrichmann G, Petrasch-Parwez E, Lee D, Reick C, Arning L, Saft C, *et al.* Efficacy of fumaric acid esters in the R6/2 and YAC128 models of Huntington's disease. PLoS One 2011;6:e16172.
37. Rao KS, Mishra SH. Antihepatotoxic activity of monomethyl fumarate isolated from *Fumaria indica*. J Ethnopharmacol 1998;60:207-23.
38. Thio HB, Zomerdijk TP, Oudshoorn C, Kempenaar J, Nibbering PH, van

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der Schroeff JG, *et al*. Fumaric acid derivatives evoke a transient increase in intracellular free calcium concentration and inhibit the proliferation of human keratinocytes. Br J Dermatol 1994;131:856-81.
39. De Jong R, Bezemer AC, Zomerdijk TP, Van de Pouw Kraan T, Ottenhoff TH, Nibbering, PH. Selective stimulation of T helper 2 cytokine responses by the anti-psoriasis agent monomethylfumarate. Eur J Immunol 1996;26:2067-74.
40. Nibbering PH, Thio B, Zomerdijk TP, Bezemer AC, Beijersbergen RL, Van Rurth R. Effects of monomethylfumarate on human granulocytes. J Invest Dermatol 1993;101:37-42.
41. Sareen J, Jacobi F, Cox BJ, Belik SL, Clara I, Stein MB. Disability and poor quality of life associated with comorbid anxiety disorders and physical conditions. Arch Intern Med 2006;166:2109-16.
42. Kroenke K, Spiltzer RL, Williams JB, Monahan PO, Löwe B. Anxiety disordes in primary care: Prevalence, impairment, comorbidity, and detection. Ann Intern Med 2007;146:317-25.
43. Schoepp DD. Where will new neuroscience therapy come from? Nat Rev 2011;10:715-6.
44. Debnath M, Doyel KM, Langan C, McDonald C, Leonard B, Cannon DM. Recent advances in psychoneuroimmunology: Inflammation in Psychiatric disorders. Transl Neurosci 2011;2:121-37.
45. Zidek Z, Anzenbacher P, Kmonickova E. Current status and challenges of cytokine pharmacology. Br J Pharmacol 2009;157:342-61.
46. Hamilton-West K. Psychobiological processes in health and illness. London: Sage Publication Ltd.; 2011.

हिन्दी सारांश

पर्पट के इथेनॉलिक सत्त्व का चूहों के संज्ञानात्मक प्रक्रियाओं पर अध्ययन

गिरीशकुमार सिंह, गिता राय, श्याम सुंदर चटर्जी, विकास कुमार

फ्युमारिया इंडिका को आयुर्वेद में पर्पट नाम से जाना जाता है तथा परम्परागत रुप से मस्तिष्क को शांत करने के लिए प्रयोग किया जाता है । वैज्ञानिक परीक्षणों के अभाव के कारण पर्पट का मूल्यांकन इसके संभावित संज्ञानात्मक प्रक्रिया के नियामक प्रभाव के लिए किया गया। पर्पट को सम्पूर्ण १६ दिनों के प्रयोगात्मक अवधि के दौरान कार्बोक्सी मेथाइल सेल्युलोज (सी.एम.सी.) के ०.३% सस्पेन्शन के राशप में चुहों को १००, २०० और ४०० मि.ग्रा./कि.ग्रा./दिन मुख द्वारा दिया गया । पाईरासिटाम का उपयोग मानक स्मृति वर्द्धक के रुप में किया गया । परिवर्तित एलिवेटेड प्लस मेज (एम–इपीएम) और पैसिव अवॉइडेन्स टेस्ट (पी.ए.टी.) का प्रयोग सीखने और स्मृति के व्यवहार संबंधी मॉडल के रुप में किया गया । स्कोपोलामीन (१ मि.ग्रा./ कि.ग्रा./अंतः पेरिटोनियल) और विद्युत आपेक्षित आघात (१५० मि.लि. एम्पियर, ०.२ सेकंड) का उपयोग स्मृति हानि प्रेरित करने के लिए किया गया । एसिटाइल कॉलिन एस्टेरेज (ए.सीं.एचइ) गतिविधि, मस्कारेनिक रिसेप्टर घनत्व, ऑक्सीडेटिव अवस्था व साइटोकाइन्स (टीएनएफ–अल्का, आई. एल.–वन बीटा और (आई.एल.–टेन.) की अभिव्यक्ति का भी आँकलन किया गया । पर्पट में पाईरासिटाम के समान स्मृति वर्द्धक एवं ह्रास रोकने की क्षमता है । पर्पट से मस्तिष्क के ए.सी.एचइ गतिविधि में कमी, मस्कारेनिक रिसेप्टर के घनत्व में वृद्धि एवं मस्तिष्क के ऑक्सीडेटिव अवस्था पर लाभदायक प्रभाव प्रदर्शित किया । पर्पट ने स्कोपोलामीन द्वारा प्रेरित तीनों साइटोकाइन्स के (चूहों के मस्तिष्क में) अत्याधिक अभिव्यक्ति का भी निषेध किया । पर्पट के इथेनॉलिक सत्त्व का स्मृतिवर्द्धकों के समान संज्ञानात्मक प्रक्रियाओं पर लाभदायक प्रभाव है ।