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Antiamnesic potentials of *Foeniculum vulgare* Linn. in mice

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SUMMARY

Alzheimer's disease is a neurodegenerative disorder associated with a decline in cognitive abilities. Dementia is one of the aged related mental problems and a characteristic symptom of Alzheimer's disease. Nootropic agents like piracetam and cholinesterase inhibitors like Donepezil® are used in situations where there is organic disorder in learning abilities, but the resulting side-effects associated with these agents have limited their utility. *Foeniculum (F.) vulgare* Linn. is widely used in Indian traditional systems of medicines and also as a house remedy for nervous debility. The present work was undertaken to assess the potential of *F. vulgare* as a nootropic and anti-cholinesterase agent in mice. Exteroceptive behavioral models such as Elevated plus maze and Passive avoidance paradigm were employed to assess short term and long term memory in mice. To delineate the possible mechanism through which *F. vulgare* elicits the anti-amnesic effects, its influence on central cholinergic activity was studied by estimating the whole brain acetylcholinesterase activity. Pretreatment of methanolic extract of fruits of *F. vulgare* Linn. for 8 successive days, ameliorated the amnesic effect of scopolamine (0.4 mg/kg) and aging induced memory deficits in mice. *F. vulgare* extract significantly decreased transfer latencies of young mice and aged mice, increased step down latency and exhibited significant anti-acetyl cholinesterase effects, when compared to piracetam, scopolamine and control groups of mice. *F. vulgare* might prove to be a useful memory restorative agent in the treatment of dementia seen in the elderly.

Key words: Acetylcholine; *Foeniculum vulgare*; Memory; Piracetam; Scopolamine

INTRODUCTION

Alzheimer's disease is a progressive neurodegenerative brain disorder that is slow in onset but leads to dementia, unusual behavior, personality changes and ultimately death (Jewart *et al.*, 2005). The personality distortions interfere with the patient's professional life, social activities and relationships (Katzman *et al.*, 1998). Oxygen free radicals, the harmful byproducts of oxidative metabolism are known to cause organic damage to the living system, which may be responsible for the development of Alzheimer's disease in elderly (Smith and Luo, 2003). Nootropic agents such as piracetam, aniracetam and choline esterase inhibitors like donepezil® are being used for improving memory, mood and behavior (Bhattacharya, 1993), but the resulting side effects (Rogers, 1998) associated with these agents have made their applicability limited. Indian system of medicine emphasizes use of herbs, nutraceuticals or life style changes for controlling age related neurodegenerative disorders.

Foeniculum (F.) vulgare (Umbelliferae), commonly known as fennel fruits have been used as a

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traditional herbal medicine in India, Europe and China since centuries. For the treatment of infants suffering from dyspeptic disorders, fennel tea is the drug of first choice. The *svarasa* (soft drink) containing *F. vulgare*, *Carum carvi*, *Gycyrrhiza glabra and* sugar with water are beneficial for nervous debility, headache and depression due to *vata* (Govindadasa, 1884). It is reported to possess anti-dysmenorrhea (Namavar *et al.,* 2003), anti microbial (Aridogan *et al.,* 2002; Kwon *et al.,* 2002; Mimica-Dukic *et al.,* 2003; Lee, 2004), analgesic-anti inflammatory (Choi and Hwang, 2004), NO scavenging effect (Baliga *et al.,* 2003), hepatoprotective (Ozbek *et al.,* 2003), hypotensive (El Bardai *et al.,* 2001), oestrogenic (Albert-Puleo, 1980; Malini *et al.,* 1985) and antioxidant (Ruberto *et al.,* 2000; Satyanarayana *et al.,* 2004) activity. In the present study, the nootropic potential of methanolic extract of *F. vulgare* Linn. was investigated by employing both exteroceptive and interoceptive models. The stimulus lies outside the body in exteroceptive behavioral models, whereas, it lies within the body in case of interoceptive behavioral models. Elevated plus maze is a neutral exteroceptive model used to assess short-term memory whereas, passive avoidance apparatus is a punishment based exteroceptive model used to test long-term memory (Parle *et al.,* 2004a). Interoceptive behavioural models such as scopolamine and natural aging induced amnesia are widely cited as models simulating human dementia in general and Alzheimer's disease in particular.

MATERIALS AND METHODS

Plant material

Dried fruit of *F. vulgare* Linn. (Umbelliferae) was collected from the local herbal market of Dharwad and was identified and authenticated at Department of Pharmacognosy, SET's College of pharmacy, Dharwad, Karnataka, India. A voucher specimen (OT/FV-236) has been deposited in the department. One kg fruit of *F. vulgare* was extracted using methanol (90%) by simple maceration process. The crude extract was filtered and concentrated by rotavapour flash evaporator. The yield was 11% w/w. The fennel extract was suspended in a mixture of Tween 80 distilled water in a ratio of 2:8. The suspension was orally administered to animals. The volume of administration was 1 ml/100 g, body weight of mice.

Drugs

Scopolamine hydro bromide (Sigma Aldrich, U.S.A.), piracetam (Nootropil®, UCB India Pvt. Ltd., Vapi, Gujarat), diazepam (Calmpose®, Ranbaxy, India) and phenytoin (Dilantin® suspension, Parke Davis) were diluted in normal saline. Volume of oral and i.p. administration was 1 ml/100 g.

Animals

Swiss mice of either sex weighing around 18 g (younger ones, aged 8 weeks) and 25 g (older ones, aged 28 weeks) were used in present study. Animals were procured from disease free animal house of CCS Haryana Agriculture University, Hisar (Haryana, India). They were acclimatized to the laboratory conditions for 5 days before behavioral studies. The animals had free access to food and water and were maintained under 12:12 h light and dark cycles. All the readings were taken during same time of the day i.e. between 8 - 11 AM. Institutional Animals Ethics Committee (IAEC) had approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals), Animal welfare division, Ministry of Environment and Forests, Govt. of India.

Elevated plus maze

The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The apparatus consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 12 cm). The arms extended from a central platform

(5 cm × 5 cm), and maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by mouse to move into one of the covered arm with all its four legs. TL was recorded on the first day. If the animal did not enter into one of the covered arm within 90 s, it was gently pushed into one of the two covered arms and the TL was assigned as 90 s. The mouse was allowed to explore the maze for 10 s and then returned to its home cage. Memory retention was examined 24 h after the first day trial on the second day (Itoh *et al.,* 1990; Parle *et al.,* 2004b; Joshi *et al.,* 2005).

Group I: Represented Control group for young mice (n = 6). 10 ml/kg Distilled water (DW), p.o., was administered for 8 days. TL was noted after 45 min of administration on 8th day and after 24 h i.e. on 9th day.

Group X: Served as Control group for aged mice. 10 ml/kg DW, p.o., was administered for 8 days. TL was noted after 45 min of administration on 8th day and after 24 h i.e. on 9th day.

Group III: Diazepam, 1 mg/kg, i.p., was administered to young mice and TL was noted after 45 min of injection on 8th day and after 24 h i.e. on 9th day.

Group IV: Scopolamine (0.4 mg/kg, i.p.) was administered to young mice and TL was noted after 45 min of injection on 8th day and after 24 h i.e. on 9th day.

Group II and XIV: Piracetam, 200 mg/kg, i.p. was injected to both young and aged mice respectively. TL was noted after 45 min of injection and after 24 h.

Group V and VI: *F. vulgare* extract (FV), 50 mg/kg and 100 mg/kg, was administered orally to young mice for 8 days. The last dose was given 45 min before subjecting the animals to elevated plus maze test. TL was noted on 8th day and again after 24 h.

Group XI and XII: FV, 50 mg/kg and 100 mg/kg, was administered orally to aged mice for 8 days respectively. The last dose was given 45 min before noting TL on 8th day.

Group VII and XIII: FV, 200 mg/kg, p.o., was administered to young and aged mice for 8 days. TL was noted on 8th day and again after 24 h i.e. on 9th day.

Group VIII: FV (200 mg/kg, p.o.) was administered to young mice for 8 days. After 60 min of administration of the last dose on 8th day, diazepam (1 mg/kg, i.p.) was administered. TL was noted after 45 min of administration of diazepam and after 24 h that is on the 9th day.

Group IX: FV (200 mg/kg, p.o.) was administered to young mice for 8 days. Ater 45 min of administration of the last dose on 8th day, scopolamine hydro bromide (0.4 mg/kg, i.p.) was administered. TL was noted after 45 min of administration of diazepam and after 24 h that is on the 9th day.

Passive shock avoidance paradigm

Passive avoidance behavior based on negative reinforcement was used to examine the long-term memory. The apparatus consisted of a box (27 cm × 27 cm × 27 cm) having three walls of wood and one wall of Plexiglas, featuring a grid floor (3 mm stainless steel rods set 8 mm apart), with a wooden platform (10 cm × 7 cm × 1.7 cm) in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock (20V AC) was delivered to the grid floor. Training was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the center of the grid floor. When the mouse stepped down and placed all its paws on the grid floor, shocks were delivered for 15 s and the step-down latency (SDL) was recorded (Parle *et al.,* 2004b). SDL was defined as the time taken by the mouse to step down from wooden platform to grid floor with its entire paw on the grid floor. Animals showing SDL in the range (2 - 15 s) during the first test were used for the second session and the retention test. The second-session was carried out 90 min after the first test. When the animals stepped down before 60 s, electric shocks were delivered for 15 s. During the sond test, animals were

removed from shock free zone if they did not step down for a period of 60 s. Retention was tested after 24 h in a similar manner, except that the electric shocks were not applied to the grid floor. Each mouse was again placed on the platform, and the SDL was recorded, with an upper cut-off time of 300 s (Joshi *et al.,* 2006).

Group I: Control group for young mice (n = 6). Distilled water (1 ml/100 g) was administered p.o. for 8 days. After 90 min of administration on 8th day, SDL was recorded. Retention was examined after 24 h.

Group II, III and IV (n = 5 each): *F. vulgare* (FV) extract (50, 100 and 200 mg/kg respectively) orally for 8 days to young mice. SDL was recorded after 90 min of administration on 8th day and after 24 h.

Group V: Scopolamine hydro bromide (0.4 mg/kg) was administered i.p. to young mice after training on the 8th day and SDL was recorded at 45 min after injection.

Group VI: FV (200 mg/kg, p.o.) was administered to young mice for 8 days. After 45 min of administration of the last dose on 8th day, scopolamine hydro bromide (0.4 mg/kg, i.p.) was administered. SDL was recorded after 90 min of administration on 8th day and after 24 h.

Group VII: Control group for aged mice (n = 6). Distilled water (1 ml/100 g) was administered p.o. for 8 days to aged mice. After 90 min of administration on 8th day, SDL was recorded. Retention was examined after 24 h.

Group VIII, IX and X: FV (50, 100 and 200 mg/kg respectively) orally for 8 days to aged mice. SDL was recorded after 90 min of administration on 8th day and after 24 h.

Locomotor function

Locomotor activity of control and drug-treated animals was measured with the help of Photoactometer (INCO, Ambala, India). Photoactomter operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the mice, a count is recorded (Kulkarni, 1987).

Estimation of brain acetyl cholinesterase activity

The time frame of cholinesterase activity estimation was similar to behavioral tests i.e. 8 - 11 AM on each day. On the 9th day the animals were euthanized by cervical dislocation carefully to avoid any injuries to the tissue. The whole brain acetyl cholinesterase (AChE) activity was measured using the Ellman method (Ellman *et al.,* 1961). The end point was the formation of yellow color due to the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The sample was first treated with 5,5′-dithionitrobenzoic acid (DTNB) and the optical density (OD) of the yellow color compound formed during the reaction at 412 nm every minute for a period of three minutes was measured. Protein estimation was done using Folin's method. AChE activity was calculated using the following formula:

$$R = \frac{\delta O.D \times \text{volume of assay (3 ml)}}{E \times \text{mg of protein}}$$

Where R = rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed/min/mg protein δ O.D. = Change in absorbance/min, E = Extinction coefficient = 13,600/M/cm

Group I: served as control and treated with saline water

Group II: treated with phenytoin (12 mg/kg, p.o.)

Group III: treated with piracetam (200 mg/kg, p.o.)

Group IV, V and Group VI: treated with FV (50, 100 and 200 mg/kg, p.o.) respectively for 8 days and acetyl cholinesterase levels were determined.

Statistical analysis

The data were expressed as mean ± SEM. The normally distributed data were subjected to one-way ANOVA followed unpaired '*t*' test. Kruskal Wallis one-way ANOVA followed by multiple range tests was used for the analysis of non-normally distributed data. $P < 0.05$ considered significant.

RESULTS

Effect on locomotor activity

In the present study, FV (50, 100 and 200 mg/kg) did not show any significant change in the locomotor function of animals (score 222.6 ± 8, 218 ± 2 and 211 ± 15) when tested on photoactometer (INCO, Ambala, India) as compared to control group (score 216.4 ± 12) when tested using a photoactometer.

Effect on transfer latency (TL) using elevated plus maze

Aged mice showed higher transfer latency (TL) values on first day and on second day (after 24 h) as compared to young mice, indicating impairment in learning and memory (i.e. ageing-induced amnesia). Piracetam (200 mg/kg, i.p.) pretreatment for 8 days decreased transfer latency on 8th day and after 24 h i.e. on 9th day as compared to Distilled water treated group, indicating improvement in both learning and memory. Scopolamine (0.4 mg/kg) and Diazepam (1 mg/kg) increased TL significantly ($P < 0.05$) in young mice on first and second day as compared to control, indicating impairment of memory.

FV (50 mg/kg and 100 mg/kg, p.o.) decreased the TL on 8th day and 9th day in young and aged mice ($P < 0.05$) when compared to control groups. Higher dose of FV (200 mg/kg, p.o.) more significantly enhanced the learning and memory of aged animals rather than the young mice as reflected by marked decrease in TL on 8t^{h} day and 9th day when subjected to elevated plus maze tests (Tables 1 and 2). The higher dose of FV pretreatment for 8 days successively to young mice protected them ($P < 0.05$) against

Table 1. Effect of FV on transfer latencies of young mice by elevated plus maze

Group	Treatment	Dose (mg/kg)	TLT (8th day)	TLT (9th day)
I	Control	10	21.6 ± 30.3	19.34 ± 1.28
II	Piracetam	200	18.04 ± 1.04*	16.46 ± 1.01*
III	Diazepam	1	28.02 ± 8.05*	31.66 ± 8.63*
IV	Scopolamine	0.4	46.4 ± 9.86*	38.61 ± 2.41*
V	FV	50	21.80 ± 2.92	17.95 ± 1.85
VI	FV	100	17.22 ± 1.76*	16.52 ± 0.52*
VII	FV	200	12.34 ± 1.15*	10.13 ± 1.36*
VIII	FV	100	15.3 ± 1.21$^{\#}$	9.64 ± 1.11$^{\#}$
	Diazepam	1		
IX	FV	100	17.54 ± 1.81^{a}	11.46 ± 1.04^{a}
	Scopolamine	0.4		

Each group consists of 5 animals each except group I (n = 6). Values are mean ± SEM; $^{*}P < 0.05$ compared to control, $^{\#}P < 0.05$ compared to control (diazepam treated), $^{a}P < 0.05$ compared to control (scopolamine treated).

Table 2. Effect of FV on transfer latencies of aged mice by elevated plus maze

Group	Treatment	Dose (mg/kg)	TLT (8th day)	TLT (9th day)
I	Control (Y)	10	21.63 ± 0.3	19.34 ± 1.28
X	Control (A)	10	36.97 ± 1.4*	32.11 ± 1.81*
XI	FV	50	24.82 ± 3.13*	18.02 ± 1.59*
XII	FV	100	18.20 ± 1.42^{a}	10.71 ± 1.29^{a}
XIII	FV	200	16.43 ± 1.32^{a}	9.12 ± 1.78^{a}
XIV	Piracetam	200	18.4 ± 1.1*	12.3 ± 1.9*

Each group consists of 5 animals each except group I and X (n = 6).Values are mean ± SEM; $^{*}P < 0.05$ compared to control (Young), $^{a}P < 0.05$ compared to control (Aged).

Table 3. Effect of FV on SDL using Passive-avoidance apparatus

Group	Mice	Treatment	Dose (mg/kg)	SDL after 24 h (s)
I	Young	Control (DW)	10	112 ± 13.2
II	Young	FV	50	248 ± 16.4*
III	Young	FV	100	191 ± 2.36*
IV	Young	FV	200	284 ± 24.62*
V	Young	Scopolamine	0.4	16.2 ± 2.19*
VI	Young	FV Scopolamine	200 0.4	253.6 ± 23.21*a
VII	Aged	Control (DW)	10	42.46 ± 6.31*
VIII	Aged	FV	50	48.18 ± 6.29b
IX	Aged	FV	100	62.51 ± 4.31b
X	Aged	FV	200	98.19 ± 1.96b

Values are each mean ± SEM; *indicates $P < 0.05$ compared to Control (for young mice); aindicates $P < 0.05$ compared to Scopolamine treated; bindicates $P < 0.05$ compared to Control (aged mice)

scopolamine, diazepam and ageing induced amnesia.

Effect on SDL using passive avoidance apparatus

FV (200 mg/kg, p.o.) profoundly increased SDL significantly as compared to control group on second day indicating improvement in memory of young mice. Scopolamine hydro bromide (0.4 mg/kg, i.p.) significantly decreased SDL on second day after training, indicating impairment of memory. *F. vulgare* (200 mg/kg, p.o.) administered orally for 8 days significantly ($P < 0.05$) reversed amnesia induced by scopolamine and natural aging (Table 3).

Whole brain AChE activity

The whole brain AChE activity with phenytoin (12 mg/kg, p.o.) demonstrated significant rise in AChE activity as compared to control. piracetam (200 mg/kg, p.o.) and FV (50, 100 and 200 mg/kg, p.o.) significantly ($P < 0.05$) lowered AChE activity (Table 4).

Table 4. Effect of *F. vulgare* and piracetam on AChE activity in aged mice

Treatment	Dose (mg/kg, p.o.)	AChE (mM)
Control	10	118.45 ± 6.20
Phenytoin	12	192.2 ± 11.84*
Piracetam	200	90.55 ± 8.68*
FV	50	111.23 ± 6.21*
FV	100	93.27 ± 8.52*
FV	200	79.71 ± 8.10*

Values are mean ± SEM; AChE-whole brain AChE activity, *$P < 0.05$ vs. control (multiple range test), H = 16.67; df = 5; $P < 0.05$.

DISCUSSION

Alzheimer's disease (AD), a chronic, progressive disabling organic brain disorder characterized by disturbance of multiple cortical functions, including memory, judgment, orientation, comprehension, learning capacity and language (Jay, 2005; Joshi *et al.*, 2006). The National Institute of Health predicts, if the current trend continues, there will be more than 8.5 million AD patients by the year 2030 in U.S.A. alone (Anonymous, 2000).The present study indicates that *F. vulgare* is a potential anti-cholinesterase agent. It also possesses nootropic activity in view of its facilitatory effect on retention of acquired learning. *F. vulgare* decreased transfer latencies, increases SDL in mice when subjected to passive avoidance paradigm, indicating its potent antiamnesic activity.

Central cholinergic system plays an important role in learning and memory (Biegon, 1986; Perry, 1994). Phenytoin is known to reduce hippocampal ACh concentration (Agarwal *et al.*, 1964; Sudha *et al.*, 2001; Vohora *et al.*, 2004) and causes cognitive impairment (Aldenkamp *et al.*, 1994). In our study,

phenytoin per se (12 mg/kg, p.o.) significantly elevated brain AChE activity. Piracetam (250 mg/kg, p.o.) and FV (50, 100 and 200 mg/kg, p.o.), on the other hand significantly ($P < 0.05$) lowered this activity indicating the counteracting action of the two drugs on the cholinergic system. The precise mechanism by which piracetam exerts its nootropic effects is not known, but multiple mechanisms have been suggested such as enhancement of oxidative glycolysis (Verbnyi *et al.,* 1996), an effect on the Ca^{2+} channels (McGeer *et al.,* 1999) and an effect on the cholinergic system (Verbnyi *et al.,* 1996). Both piracetam and *F. vulgare* meets major criteria for nootropic activity, namely improvement of memory in absence of cognitive deficit and anticholinesterase effects. Furthermore, *F. vulgare* also reversed the scopolamine-induced impairment in learning and memory, when assessed on passive avoidance paradigm. Therefore, it seems that *F. vulgare* improved learning and memory probably due to its effect on cholinergic transmission.

Immunohistochemical studies suggested the existence of chronic inflammation in certain regions of the brain in Alzheimer's disease patients. Since inflammation can be damaging to host tissue, it was hypothesized that anti-inflammatory drugs might be inhibiting both the onset and the progression of Alzheimer's disease. This hypothesis is supported by the observation that indomethacin (NSAID) halted the progressive memory loss seen in Alzheimer's disease patients (Rao *et al.,* 2002). Moreover, it has also been observed that elderly patients suffering from Alzheimer's disease showed reduction in symptoms of Alzheimer's disease upon chronic use of anti-inflammatory drugs. Indomethacin, a non-steroidal anti-inflammatory drug exhibited a memory protective effect against electro convulsive shock induced retrograde amnesia and also against amyloid deposits in the brain (Stephen *et al.,* 2003). Anti-inflammatory action of *F. vulgare* might also be contributing to the observed memory-enhancing activity (Choi and Hwang, 2004).

Numerous epidemiologic studies have indicated that individuals who consume diets containing large amounts of fruits, vegetables may be at a reduced risk of developing age related diseases like Alzheimer's disease (Joseph *et al.,* 2005). A cognitive impairment has been associated with lower vitamin intakes, fruits and vegetables have been demonstrated to have protective effects against stroke and vascular dementia (Gillman *et al.,* 1995). Many free radical scavengers are present in food, particularly in fruits, vegetables and grains and the regular consumption of these nutritive substances may therefore have a beneficial impact. Oxygen free radicals, the harmful byproducts of oxidative metabolism are known to cause organic damage to the living system, which may be responsible for the development of Alzheimer's disease in elderly (Nagy, 2001). *F. vulgare* is reported to possess antioxidant (Ruberto *et al.,* 2000; Choi and Hwang, 2004) activity.

Therefore, the memory improving activity of *F. vulgare* may be attributed to its antioxidant, anti-inflammatory, neuroprotective, pro-cholinergic and anti-acetylcholinesterase properties of *F. vulgare* and hence may be of enormous use in delaying the onset and reducing the severity of Alzheimer's disease. However, further investigations are warranted to explore the possible involvement of other neurotransmitters like glutamate, GABA, catecholamines etc. (Parle *et al.,* 2004c) responsible for memory improving property of *F. vulgare.*

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