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Original Research Article

Foeniculum vulgare Mill (Umbelliferae) Attenuates Stress and Improves Memory in Wister Rats

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Abstract

Purpose: *To evaluate the anti-stress and memory-enhancing properties of F. vulgare extract in experimental rats.*
Methods: *F. vulgare plant extract was obtained using Soxhlet extraction technique. The extract, at doses of 50, 100 and 200 mg/kg body weight, was administered orally with an orogastric tube. Urinary levels of vanillylmandelic acid (VMA) and ascorbic acid in rats were used to evaluate anti-stress activity. Conditioned avoidance response was measured in normal and scopolamine-induced amnesic rats to study the memory-enhancing effects. Lipid peroxidation inhibition assay in liver and brain homogenates of rats was used to evaluate antioxidant activity.*
Results: *Daily administration of F. vulgare extract (50, 100 and 200 mg/kg body weight) 1 h prior to induction of stress significantly ($p < 0.05$) altered the stress-induced urinary biochemical levels of VMA from 395.79 ± 11.23 to 347.12 ± 12.28, 311.21 ± 12.48 and 258.86 ± 10.26 µg/kg, respectively, in 24 h, as well as ascorbic acid excretion levels from 65.74 ± 9.42 to 78.59 ± 8.44, 108.41 ± 15.62 and 125.82 ± 16.94 µg/kg also within the same period, respectively. These changes occurred in a dose-dependent fashion, and the levels in the control groups were unchanged within the same period. The memory deficits induced by scopolamine (1mg/kg, i.p.) in rats was reversed by F. vulgare dose-dependently. The extract also exhibited potent antioxidant effect by inhibition of lipid peroxidation in both rat liver and brain homogenates to a greater extent than the standard antioxidant, ascorbic acid.*
Conclusion: *F. vulgare may be useful in the management of stress and stress-related disorders on account of its multiple actions such as anti-stress, memory-enhancing and antioxidant effects.*

Keywords: *Foeniculum vulgare, Stress, Vanillyl mandelate, Memory, Antioxidant, Ascorbic acid*

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INTRODUCTION

Every individual experiences stress, a pattern of metabolic and behavioral reactions in response to external and internal stimuli. Extreme stress may cause threatened homeostasis leading to the pathogenesis of a variety of disease conditions including depression, anxiety, immunosuppression, endocrine disorders, cognitive dysfunction, peptic ulcer, hypertension and reproductive dysfunctions [1]. Stress effects brain function, impairs memory and disrupts cognitive skills leading to the pathogenesis of various central nervous system diseases [2]. Extreme stress builds up free radicals and induces potential damage to neuronal receptors and a variety of other tissues [3]. Therefore agents that inhibit free radicals may have great potential in mitigation of stress and stress-induced diseases.

Drug and food from natural origin play a significant role in the public healthcare systems and are being investigated as remedies for number of stress-related disorders [4]. Spices

and condiments used in culinary practices to impart flavor, color and nutritional values in food are also being used to treat various diseases in traditional medicines [5]. In particular, spices from Umbelliferae family have been reported to possess immense pharmacological benefits [6-8]. *Foeniculum vulgare* Mill. (Umbelliferae, *F. vulgare*), commonly known as fennel is a well known medicinal and aromatic plant widely used as carminative, digestive, lactogogue, diuretic and in treating respiratory and gastrointestinal disorders [9]. Pharmacologically, *F. vulgare* has been shown to possess anti-inflammatory [10], anti-diabetic [6], anti-bacterial [11], anti-fungal [12], anti-oxidant [13], analgesic [14], estrogenic [15], hepatoprotective [16], and anti-tumor activities [17].

The major chemical components of *F. vulgare* are flavonoids, polyphenols, carotenoids, minerals and vitamins. The fruit of *F. vulgare* contains components like estragole, fenchone, alpha-phellandrene and aglycons [18,19].

Despite the potential biological activities presented by *F. vulgare*, the stress-relieving and memory-enhancing effect shave not been studied. In the present study, we evaluated the anti-stress and memory-enhancing activities of *F. vulgare* extract in experimental rat models. In addition, we evaluated the antioxidant defense potential of *F. vulgare* extract to see if it correlates with its anti-stress and memory enhancing effects in rat liver and brain homogenates.

EXPERIMENTAL

Chemicals

Vanillylmandelic acid (VMA) and scopolamine butyl bromide (SBB) were purchased form Sigma Chemical Company, St. Louis, MO, USA. Ascorbic acid was purchased from Loba Chemie, Mumbai, India. All other reagents used were of analytical grade. Stock solutions of all chemicals were prepared in distilled water and the dilutions were made fresh on the day of the experiment.

Preparation of extract

Dried fruit material of *F. vulgare* (1 kg) was obtained from a local market in Vijayawada, India. Dr K Hemadri, a taxonomist at Regional Research Institute, India, confirmed the authenticity of the fruit material and a voucher specimen (FV-2004) was kept in our department herbarium for future reference. The material was powdered and extracted with boiling water (5 L) for 30 min by Soxhlet technique. The filtrate was evaporated at < 70 ^{0}C in a vacuum dryer to give a final yield of 98.74 g. *F. Vulgare* was completely solubilized in distilled water for use in *in vivo* and *in vitro* experiments respectively

Animals

Wistar rats of either sex, obtained from Ghosh Enterprises, Kolkata were housed in an air-conditioned animal room at 23±2°C with 12/12 h light/ dark photoperiod, and free access to water and laboratory rat chow. The animals were kept for seven days in laboratory for habituation. All animal experiments were performed in accordance and approval with our Institutional Animal Ethics Committee (Regd. No. 516/01/A/CPCSEA) following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and the International Guidelines for Handling of Laboratory Animals [20].

Evaluation of anti-stress activity

Induction of stress was carried out using the forced swimming test in rats [21]. The animals were divided into four groups, each consisting of six animals of either sex, in metabolic cages that were designed to facilitate the collection of urine. 24 h urine samples from each group were collected into two different beakers using an inverted 'Y' tube fixed at the bottom of each metabolic cage. One end of the tube received urine into a beaker containing 5 ml of 10 % oxalic acid and then passed on for spectrophotometric determination of ascorbic acid at 550 nm. The other end of the tube received and moved urine into a beaker containing 0.5 ml of 6N hydrochloric acid for determination of vanillylmandellic acid (VMA) spectrophoto-metrically at 360 nm. The experimental protocol followed was as described previously [8].

Evaluation of memory enhancing activity

This experiment was evaluated by conditioned avoidance response (CAR) technique in rats using Cook's pole climbing apparatus (Techno, India). Briefly, rats were divided into 4 groups of 5 animals each. Groups II, III and IV were administered orally with 50, 100 and 200 mg/kg, respectively of *F. vulgare* extract (dissolved in distilled water) while animals in group I were served as control and received only distilled water. After 90 minutes, all the animals were subjected to a training schedule individually by placing them inside the Perspex chamber of the apparatus. After an acclimatization period of 5 min in the chamber, a buzzer was given followed

by a shock through the grid floor. The rat had to jump on to the pole (shock-free zone) to avoid foot shock. Jumping on the pole functionally terminates the shock and this was classified as an escape while such jumping prior to the onset of the shock was considered as avoidance. The session was terminated after completion of 60 trials with an interval of 20-30 seconds between trials. This procedure was repeated at 24 h intervals until all groups reach 95 to 99 % avoidance. Following the attainment of complete training for a particular group, the animals were treated with a single dose of scopolamine butyl bromide (1 mg/kg, i.p.) to induce amnesia, 30 min before the next day dosing with the extract. The training schedule was continued further with the daily doses of the extract and vehicle until the rats returned to normal level from scopolamine-induced amnesia.

Determination of thiobarbituric acid-reactive substances (TBARS)

Rats weighing between 150 - 200 g were sacrificed by spinal traction. Whole liver and brain were excised and washed in ice-cold Tris-HCl buffer (0.1M, pH 7.4), and cytosolic samples of liver and brain homogenate were prepared separately by using a tissue grinder (Thomas Scientific, NJ, USA) and centrifuging at 10,000 rpm for 30 min at 4 °C. The TBARS assay was performed as described previously [8]. Briefly, reaction mixture (0.5 ml) containing rat liver homogenate (0.1 ml, 25% w/v) in Tris-HCl buffer (40 mM, pH 7.0), KCl (30 mM), ferrous ion (0.16 mM), and ascorbic acid (0.06 mM) were incubated for 1 h at 37 °C in the presence and absence of various concentrations of *F. vulgare* extract. The incubation mixtures (0.4 ml) were treated with sodium dodecyl sulfate (8.1%, 0.2 ml), thiobarbituric acid (0.8%, 1.5 ml), and acetic acid (20%, 1.5 ml, pH 3.5). The total volume was then made up to 4ml with distilled water and kept in a water bath at 100 ℃ for 1 h. On cooling, 1ml of distilled water and 5 ml of a mixture of n-butanol and pyridine (15:1 v/v) were added and vortexed. After centrifugation, the absorbance of the organic layer was measured at 532 nm. The percentage inhibition of lipid peroxidation was determined by comparing the results of the test compound with those of the control not treated with the extract. The same procedure was followed with rat whole brain homogenate. The 50 % inhibition values were derived from a plot of quantity (µg) against absorbance.

Statistical analysis

The results are expressed as mean ± SEM. Student's paired *t*-test using GraphPad software was used for statistical analysis. In all cases, $p < 0.05$ was considered statistically significant.

RESULTS

***F. vulgare* extract inhibits stress-induced urinary biochemical changes in rats**

As shown in Figs 1 and 2, a significant increase in urinary levels of VMA ($p < 0.001$), and decrease in ascorbic acid excretion levels ($p < 0.001$), was observed in animals induced by forced swim stress when compared to the normal basal levels in control animals. The altered levels returned to normal basal levels three to four days after withdrawal of stress. The extract treated groups under normal condition produced no significant change in the excretion of VMA and ascorbic acid compared to basal levels. Treatment with extract (50, 100 and 200 mg/kg) one hour prior to the induction of stress significantly inhibited ($p<0.05$) the increase in urinary VMA levels to 347.12 ± 12.28, 311.21 ± 12.48 and 258.86 ± 10.26 µg/kg/24 h, respectively, in a dose dependent fashion (Fig 1). In contrast, administration of *F. vulgare* extract (50, 100 and 200 mg/kg) one hour prior to the induction of stress significantly ($p < 0.05$) and dose dependently inhibited the decrease in ascorbic acid urinary levels to 78.59 ± 8.44, 108.41 ± 15.62 and 125.82 ± 16.94 µg/kg/24 h, respectively (Fig 2).

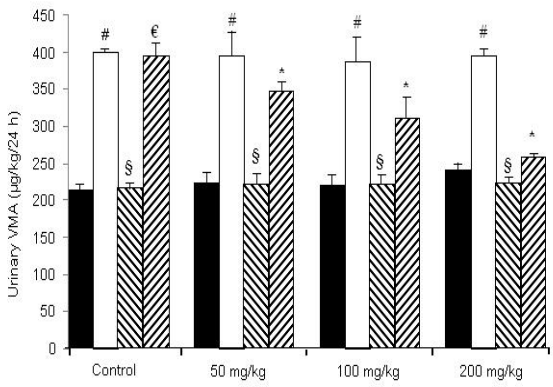

Fig 1: Influence of *F. vulgare* extract on the 24 h urinary levels of VMA in normal and stress-induced rats (mean ± S.E.M., n=6);
■ = *Normal,* □ = *Stress,* ▨ = *Normal + F. vulgare and* ▨ = *F. vulgare + Stress.*
$^{\#}p < 0.001$ *compared to normal condition of the corresponding groups.* $^{*}p < 0.05$, *compared with stressed condition of the corresponding groups.* §*No significant difference compared with normal condition of the corresponding groups.* $^{€}$*No significant difference from stressed condition*

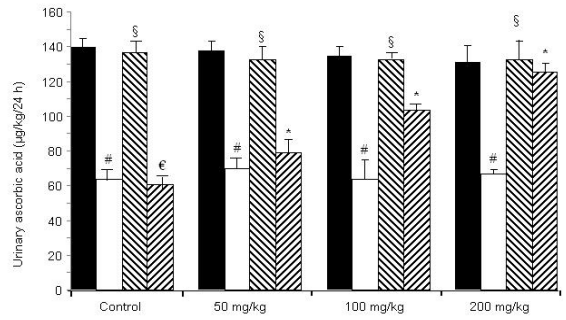

Fig 2: Effect of *F. vulgare* extract on the 24 h urinary levels of ascorbic acid in normal and stress-induced rats. Data are represented as mean ± SEM (n=6). #p< 0.001, compared to normal condition of the corresponding groups. *p<0.05, compared with stressed condition of the corresponding groups. §No significant difference compared with normal condition of the corresponding groups. €No significant difference compared to stressed condition.
■ *= Normal,* □ *Stress,* ▨ *Normal + F. vulgare and* ▨ *= F. vulgare + Stress.*

F. vulgare extract attenuates scopolamine-induced amnesia

The CAR of rats administered with the extract increased gradually to 95% over 7 to 12 days (Fig 3). The acquisition (time to achieve 95 % CAR) for rats administered with the extract was dose- and time-dependent compared to vehicle treated (control) group, which took 12 days for acquisition. The percent avoidance was always higher in the extract treated groups compared to vehicle treated (control) group. Animals receiving 200 mg/kg body weight of the extract took ten days while groups treated with 100 and 50 mg/kg doses of the extract required eleven and twelve days, respectively, to reach the point of acquisition. Administration of scopolamine produced amnesia as seen from reduction in the observed CAR. Amnesia was greater in the control group than in extract-treated groups. However, continued treatment with *F. vulgare* produced better retention and recovery in a dose-dependent manner than the vehicle-treated animals. Recovery from scopolamine-induced amnesia in the extract-treated groups took 3-5 days when compared to normal (control) group which took over 6 days.

F. vulgare extract inhibited the lipid peroxidation in rat brain and liver homogenate

As shown in Fig 4, generation of lipid peroxides by the induction of Fe^{2+}/ascorbate in rat liver and brain homogenates was inhibited by the extract in a dose-dependent fashion. The inhibition was higher in brain homogenate than in liver, indicating that it is more effective in brain. The quantity of the extract needed for 50 % inhibition of lipid peroxides in rat liver homogenate was 4480 µg (Fig 4A). A similar effect was produced by 5350 µg of ascorbic acid. The quantity of the extract needed for 50 % inhibition of brain lipid peroxidation was 3670 µg. A similar effect was produced by 4390 µg of ascorbic acid (Fig 4B).

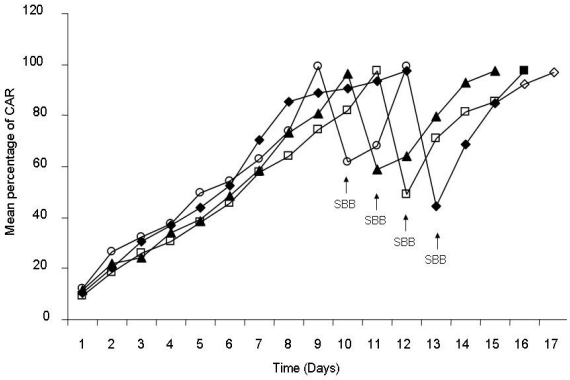

Fig 3: Effect of *F. vulgare* extract on the mean percent of conditioned avoidance response after oral administration in rats. Scopolamine butyl bromide (SBB) was administered 30 min prior to next day dosing with the extract after attaining complete acquisition. ♦ = Control, □ = 50 mg, ▲= 100 mg and o = 200 mg.

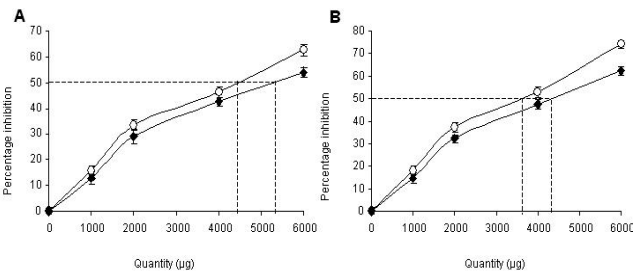

Fig 4: Effect of *F. vulgare* on the *in vitro* inhibition of lipid peroxidation in liver and brain of rat (n=6) showing the concentrations of *F. vulgare* and ascorbic acid required for 50 percent inhibition of lipid peroxidation in liver (A) and brain (B) homogenates. O = *F. vulgare* and ♦ = ascorbic acid.

DISCUSSION

In the present investigation *F. vulgare* extract showed significant inhibition of the stress-induced biochemical changes in VMA and ascorbic acid. The memory deficits observed in scopolamine-induced amnesic rats were also attenuated by *F. vulgare* extract. Further, the extract exhibited antioxidant action by inhibition of lipid peroxidation in both liver and brain homogenates of rats.

Mounting evidence suggests that stress induces considerable changes in the neurochemical,

biochemical and molecular processes [22, 23]. Noradrenaline and adrenaline, which are released normally or in response to stress are metabolized into 3-methoxy 4-hydroxyphenyl glycol (MOPEG) centrally and VMA (the metabolite of adrenaline) peripherally [24]. During increased stress, the blood level of VMA is observed in high concentration, and excreted in the urine [24]. L-ascorbic acid, which is synthesized from D-glucose in rats, is altered in body fluids due to several factors including stress. Ascorbic acid is present in adrenal glands as a metabolite of glucose in rats and glucaric acid is the corresponding metabolite in humans and primates. Studies indicate that the tissue levels of ascorbic acid are decreased on application of stress [25, 26]. Therefore, in the present study, the increase in the urinary VMA and decrease in the urinary ascorbic acid levels during stress were used as indirect biochemical indices to study the anti-stress activity of *F. vulgare* extract.

Earlier reports revealed that antioxidant herbs might be promising in reducing stress [6, 24, 27]. *F. vulgare* extract scavenged the lipid peroxide free radicals in a concentration dependent manner in both liver and brain homogenates of the rats. The concentration needed for 50% inhibition of lipid peroxides was lower in brain homogenates when compared to liver homogenates suggesting that *F. vulgare* has potential effects on central nervous system. In both liver and brain homogenates, *F. vulgare* showed higher lipid peroxide inhibition activity than that of a known antioxidant, ascorbic acid.

Stress and free radicals have been shown to affect memory function and processing [28, 29]. It is well documented that scopolamine induction impairs retrieval of memory in rats and such amnesia was associated with a significant increase in oxidative stress [30]. Therefore, scopolamine-induced amnesia in rats could be used as a valid model to screen several antioxidant agents, which may show potential therapeutic benefits in dementia.

In this study, the anti-stress and antioxidant activities of the extract are consistent with the memory-enhancing effects in scopolamine-induced rats. The antioxidant defense mechanism of the extract might be responsible for its anti-stress and memory enhancing activities.

CONCLUSION

This study provides scientific support for anti-stress, antioxidant and memory-enhancing activities of *F. vulgare* extract. Based on our present results and earlier traditional medicinal claims, *F. vulgare* extract can be developed as a therapeutic agent in the management of stress and stress-related diseases such as memory loss.

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