Adaptogenic and nootropic activities of aqueous extracts of Carum carvi Linn (caraway) fruit: an experimental study in Wistar rats

Sushrutha Koppula*, Spandana Rajendra Kopalli, Satyanarayana Sreemanthalu
University College of Pharmaceutical Sciences, Andhra University
Visakhapatnam 530 003, Andhra Pradesh, India

*Corresponding author present address Department of Pharmacology, College of Medicine,
Seoul National University, Seoul 110 799, South Korea
Email sushrutak@gmail.com
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In the present investigation the aqueous extract of Carum carvi was evaluated for antistress activity in normal and stress induced rats. The extract was studied for nootropic activity in rats and in vitro antioxidant potential to correlate its antistress activity. For the evaluation of antistress activity groups of rats were subjected to forced swim stress one hour after daily treatment of Carum carvi extract. Urinary vanillylmandelic acid (VMA) and ascorbic acid were selected as non invasive biomarkers to assess the antistress activity. The 24 h urinary excretion of vanillylmandelic acid (VMA) and ascorbic acid was determined in all groups under normal and stressed conditions. The nootropic activity of the extract as determined from acquisition, retention and retrieval in rats was studied by conditioned avoidance response using Cook's pole climbing apparatus. The in vitro antioxidant activity was determined based on the ability of Carum carvi to inhibit lipid peroxidation in liver and brain homogenates. Daily administration of Carum carvi at doses of 100, 200 and 300 mg/kg body weight one hour prior to induction of stress inhibited the stress induced urinary biochemical changes in a dose dependent manner. However no change in the urinary excretion of VMA and ascorbic acid was observed in normal animals at all the doses studied. The cognition, as determined by the acquisition, retention and recovery in rats was observed to be dose dependent. The extract produced significant inhibition of lipid peroxide formation in comparison with ascorbic acid in a dose dependent manner in both liver and brain. The present study provides scientific support for the antistress (adaptogenic), antioxidant and nootropic activities of Carum carvi extract and substantiates its traditional use as a culinary spice in foods as beneficial and scientific in combating stress induced disorders.

Key words: Carum carvi, caraway, stress, nootropic, lipid peroxidation

Introduction

Stress can be described as the sum total of all the reactions of the body to a stimulus, which disturb the normal physiological condition and result in a state of threatened homeostasis and has been defined as a nonspecific response of the body to any demand imposed on it (Selye 1936). In its most simplified sense, stress is what one feels when life’s demands exceed one’s ability to meet those demands. Stress is an internationally recognised phenomenon fortifyed by advancement of industrialisation in a demanding civilisation. In fact every individual is likely to face stressful situations in day to day life from headaches to heart disease and immune deficiency to digestive problems. Thus stress is a factor in many illnesses (Selye 1998).

Increased production of stress hormones and subsequent decrease in immune function appear to contribute to the stress induced decline in health (Gregory 1999). Similarly increased physical and psychological stress leads to increased incidence of amnesia. There is increasing evidence that Alzheimer’s disease increases severe oxidative stress as a result of either beta amyloid mediated generation of free radicals or perturbed ionic calcium balance within neurons and their mitochondria (Gerard 2000). Supplementation with higher ascorbic acid and betacarotene was associated with better memory performance which indicates the role of potential antioxidants in brain aging and cognitive impairment (Kowalski 2000). Literature also indicates that the role of free radicals in the pathogenesis of cancer, aging, Alzheimer’s disease, diabetes and the compounds having capacity to scavenge these free radicals has great potential in mitigation of these disorders (Halliwell 1985).

Ayurveda is an ancient form of Indian medicine which deals with plants and plant products. This indigenous form of medicine uses the active ingredients present in plants for treating various diseases (Nair 1998). Since the introduction of adaptogens several plants have been investigated which were once used as tonics due to their adaptogenic and rejuvenating properties in Ayurvedic medicine (Rege 1999). The drugs of plant origin are gaining increasing popularity and are being investigated for remedies of a number of disorders including antistress (adaptogenic) activity (Wagner 1994, Katiyar 1997, Edzard 1998, Sreemanthalu 2005). Spices are dried herbs that have been effectively used in the indigenous systems of medicine in India and also in other countries (Nadkami 1976). Apart from their traditional use, arrays of beneficial pharmacological effects have been reported by extensive animal studies during the past three decades (Satyanarayana 2004, Sushruta 2006).

Carum carvi Linn, commonly known as caraway (Umbelliferae) is a globally distributed spice with a history...
as a medicinal plant since ancient times (Hartmans 1995). The dried ripe fruits of the plant are used in folk medicine especially in the treatment of digestive disorders in both adults and infants (Reynolds 1993, Thompson 2002). The main constituents of Carum carvi are the volatile oils including carvone (40–60%), limonene, carveol, dihydrocarveol and thymol in addition to glycosides and flavanoids (Zheng 1992, Matsumura 2002). Experimental studies have shown its antidiyspeptic (Holtmann 2003), antispasmodic (Eddouks 2004), antilucrogerenic (Khayyal 2001), antibacterial (Singh 2002), antitumor (Kamaleeswari 2006), antiproliferative (Nakano 1998), antioxidant (Kamaleeswari 2006), anidiuretic (Eddouks 2004), antithrombic (Lemhadri 2006) and diuretic (Lahlou 2007) activities.

In the present investigation the antistress (adaptogenic) activity of Carum carvi was evaluated in vivo in normal and stress induced rats following a non invasive biochemical approach. The antioxidant (lipid peroxidation inhibition) potential of the extract in both liver and brain homogenates was evaluated in vitro to support the antistress activity. The plant extract was further evaluated for nootropic activity using conditioned avoidance response in rats.

Materials and methods

Preparation of extract

The dried powdered fruit material of Carum carvi (1 kg) was obtained from Chemiloids, Vijayawada India and extracted with boiling water (5 L) for 30 minutes. The filtrate was evaporated under vacuum below 70°C in a vacuum drier to give a final yield of 66 g.

Chemicals used

Vanillylmandelic acid (VMA) and scopolamine butyl bromide (SBB) were purchased from Sigma-Aldrich, St. Louis USA, while ascorbic acid was purchased from Loba Chemie, Mumbai. All other reagents were analytical grade.

Animals

Wistar rats (200-250 g) of either sex obtained from Ghosh Enterprises, Kolkata were used in the study. They were housed six per cage at a temperature of 22 ± 2°C with 12 h light/dark cycle under controlled environment. Rats were fed with standard pellet diet (Rayan’s Biotechnologies Hyderabad) and water ad libitum. Animals were kept for seven days in laboratory for habituation. All animal experiments were performed in accordance with our Institutional Animal Ethics Committee (Reg 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.

Antistress activity

Rats of either sex were weight matched and divided into four groups (I, II, III, IV) each containing five animals. The 24 h urine samples from each group were collected into two different beakers, one containing 5 mL of 10% oxalic acid for determination of ascorbic acid (Roe 1943) and the other containing 0.5 mL of 6 N hydrochloric acid for determination of vanillylmandelic acid (VMA) (Pisano 1962). The experimental protocol was divided into four phases. In the first phase of the experiment 24 h urine samples were collected in all the four groups and subjected to analysis for both VMA and ascorbic acid and the normal values were recorded for five consecutive days. In this method rats were forced to swim until they were exhausted in a cylindrical vessel of height 60 cm and diameter 40 cm containing water at room temperature (28°C). Water depth was always maintained at 30 cm. After inducing stress in the animals 24 h urine samples were collected and the levels of both the parameters were determined based on the total volume of urine collected. The results were noted and the experiment was continued for five consecutive days.

In the second phase the animals in each group were subjected to fresh water swimming stress individually (Nagaraja 1999). In this method rats were forced to swim until they were exhausted (three to four minutes) in a cylindrical vessel of height 60 cm and diameter 45 cm containing water at room temperature (28°C). Water depth was maintained at 40 cm. The 24 h urinary levels of VMA and ascorbic acid under stressed conditions were determined as described above daily for five consecutive days. The third phase of the experiment consisted of administration of Carum carvi extract to the same groups of animals after having recovered completely to normal condition. Groups II, III and IV were administered orally with Carum carvi (dissolved in distilled water) at daily doses of 100, 200 and 300 mg/kg body weight respectively for five consecutive days while group I serving as control. The 24 h urine samples were collected and the levels of both VMA and ascorbic acid were determined.

The final phase of the experiment consisted of studying the influence of Carum carvi extract on stress induced changes in the same groups of animals after a recovery period of one week. Groups II, III and IV were administered with Carum carvi by oral gavage at daily doses of 100, 200 and 300 mg/kg body weight respectively, one hour prior to the daily induction of stress for five consecutive days with group I serving as control. The 24 h urine samples were collected and analysed for VMA and ascorbic acid for five consecutive days to study the influence of the extract on the stress induced biochemical changes.

Nootropic activity

The nootropic activity of Carum carvi was evaluated by using the conditioned avoidance response (CAR) in rats as described by Cook & Weidley (1957). Rats were divided into four groups each containing five animals. Groups II, III and IV were administered orally with 100, 200 and 300 mg/kg body weight respectively of Carum carvi (dissolved in distilled water) while animals in group I were served as control.

After 90 minutes all the groups of animals were subjected to a training schedule by placing them inside the perspex chamber of the apparatus. After an accustomed period of five minutes to 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 to 30 seconds given for each trial.

This procedure was repeated at 24 h intervals until all groups reached 95-99% avoidance. After attaining complete training of a particular group the animals were treated with a single dose of scopolamine butyl bromide (1 mg/kg body weight i.p.) to induce amnesia, thirty minutes before the next day dosing. 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.

Antioxidant activity: ex vivo inhibition of lipid peroxidation in the liver and brain of rats

Rats weighing 150-200 g were sacrificed by spinal traction and the whole brains and the livers were isolated. The pooled brains and livers were homogenised in four volumes of 40 mM Tris-HCl buffer (pH 7.0) using a tissue homogeniser. The antioxidant activity of *Carum carvi* was determined based on its ability to inhibit lipid peroxidation in homogenates of the liver and brain of rats (Okhawa 1979). The reaction mixture (0.5 mL) containing rat liver homogenate (0.1 mL), KCl (30 mM), ascorbic acid (0.06 mM), and ferrous iron (0.16 mM) and various concentrations of *Carum carvi* was incubated for 1 h at 37°C. At the end of the incubation period 0.4 mL of the
reaction mixture was treated with 0.2 mL of sodium dodecyl sulphate (8.1%), 1.5 mL of thiobarbituric acid (0.8 %), and 1.5 mL of acetic acid (20% pH 3.5). The total volume was then made up to 4 mL by adding distilled water and kept in an oil bath at 100°C for 1 hour. After the mixture had been cooled 1 mL of distilled water and 5 mL of butanol pyridine mixture (15:1 v/v) were added. Following vigorous shaking the tubes were centrifuged and the absorbance of the organic layer containing the chromophore was read at 532 nm. The percentage inhibition of lipid peroxidation by the extract was determined by comparing the absorbance values of the control and experimental tubes.

Data and statistical analysis

Results are expressed as means ± standard error of means. Statistical analysis used Student’s paired t test. In all the cases p<0.05 was considered statistically significant.

Results

Antistress activity

The urinary data of VMA and ascorbic acid observed in various phases of the experiment are shown in Fig 1 and Fig 2 respectively. Induction of forced swim stress to the animals produced a significant increase in VMA and decrease in ascorbic acid excretion compared with their respective basal excretion in normal condition. Both the parameters were found to return to their normal levels in three to four days after withdrawal of stress. Daily treatment of *Carum carvi* to the animals under normal condition produced no change in the excretion of VMA and ascorbic acid compared with normal basal levels indicating that *Carum carvi* did not alter excretion of VMA and ascorbic acid in normal condition. Daily administration of *Carum carvi* one hour prior to the induction of stress inhibited the increase in VMA and decrease in ascorbic acid excretion which was manifested during stress alone. The inhibition was found to be significant at all dose levels in a dose dependent manner.

Nootropic activity

The CAR of rats administered with the extract of *Carum carvi* or vehicle increased gradually to 95% over seven to eleven days. The acquisition (time to achieve 95% CAR) for rats administered with the extract of *Carum carvi* was found to be dose and time dependent compared with the vehicle treated control group which took 11 days for acquisition. The percent avoidance was always higher in the extract treated groups compared with the vehicle treated control group. Animals receiving 300 mg/kg body weight of the extract had taken seven days whereas groups treated with 200 and 100 mg/ kg doses of the extract required eight and ten days respectively to reach the point of acquisition (Fig 3).

Administration of scopolamine produced amnesia as seen from reduction in the observed CAR. The amnesia was found to be greater in controls compared with extract treated groups and was also found to be dose dependent. However continued treatment of *Carum carvi* produced better retention and recovery in a dose dependent manner than the vehicle treated animals.

Antioxidant activity

Lipid peroxides generated by the induction of ferrous/ascorbate on rat brain/liver homogenate was found to be inhibited by *Carum carvi*. The extract showed better activity in inhibiting lipid peroxides in brain homogenate compared with liver indicating that it is more effective in brain. The 50% inhibition values were calculated by plotting a graph between quantity (µg) vs optical density. The quantity of the *Carum carvi* extract needed for 50% inhibition of lipid peroxidation in rat liver homogenate was found to be 2350 µg (Fig 4A). A similar effect was produced by 5350 µg of ascorbic acid. The quantity of *Carum carvi* needed for 50% inhibition in brain lipid peroxidation was found to be 1684 μg. A similar effect was produced by 4690 µg of ascorbic acid.

Discussion

Stress is elicited by environmental, social or pathological conditions occurring during the life of living beings and determines changes in the nervous, endocrine and immune systems (Das 2002, Deepak 2003). Thus stress has been postulated to be involved in the
etopathogenesis of a diverse variety of diseases varying from psychiatric disorders such as depression and anxiety to immunosuppression, endocrine disorder including diabetes mellitus, male sexual dysfunction, cognitive dysfunction, peptic ulcers, hypertension and ulcerative colitis (Chrousos 1992). Considerable evidence published in the last decade has focused on alterations of neurochemical, biochemical and molecular effect caused by stress in these systems (Ben-Eltyahu 1991, Jiang 1990, Smith 1996, Ueyama 1997). Normally such stress induced changes are self limiting and adaptive in nature until and unless events that override threshold limits become irreversible and pathological (McCarty 1987).

Advancement in the understanding of processes leading to the reason for stress induced disorders cannot obscure the simple fact that the exhaustion of energy supply still forms the basis that triggers the disorders and collapse of energy metabolism following glucose deprivation in circulation (Aloe 2002). The desire to control the coping mechanism has led to the emergence of the science of adaptation, focusing on elucidating the mechanism that may help in modification so that insufficient, excessive and unnecessary responses can be prevented.

Literature reports indicate that noradrenaline is released during stressful conditions and metabolised to vanillyl mandelic acid (VMA) peripherally and 3-methoxy 4-hydroxyphenyl glycol (MOPEG) centrally (Ion 1969, Fukuda 1996). In the light of such reports VMA, the major metabolite of sympathetic amines, was taken as the indirect biochemical index to represent the increase in peripheral sympathetic activity during stress. In the present study the increase in the urinary VMA excretion during stress was used as a non invasive biochemical marker to study the antistress activity of Carum carvi.

L-ascorbic acid or vitamin C is synthesised biologically from D-glucose in rats (John 1998). 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. Several factors such as age, exposure to environmental situations, stress, dietary and biochemical changes produce alteration of L-ascorbic acid levels in body fluids (Cheng 1990, Kolb 1992).

Several studies indicated that the tissue levels of ascorbic acid decreased on application of stress (Rivero 1992, Kutlu 1993). Ascorbic acid, being a free radical scavenger, is more likely to be utilised in scavenging the free radicals involved in stress resulting in its decreased urinary concentration. Ascorbic acid also has a role in the biosynthesis of noradrenaline (Kalnner 1983) as a cofactor in the conversion of dopamine to noradrenaline (Goodman 2001).

Based on the above studies ascorbic acid excretion in urine was taken as an indirect biochemical index to indicate the influence of stress on catecholamine synthesis in rats and antistress (adaptogenic) activity of the Carum carvi extract on prior administration of stress induction.

Treatment with Carum carvi extract when associated with stress reversed the stress induced biochemical changes, i.e. increase in urinary VMA levels and decrease in urinary ascorbic acid levels, in a dose dependent manner. Previous studies have concluded that the antistress activity of some of the potential medicinal plants could be attributed to their antioxidant effect (Bhattacharya 2001, Satyanarayana 2004, Sreemantula 2005).

Based on these reports the antioxidant activity of Carum carvi extract was also done using ex vivo lipid peroxidation assay in brain and liver homogenates of rats. It was found that Carum carvi extract has significant antioxidant activity higher than that of ascorbic acid in both liver and brain.

It was reported that scopolamine impaired retrieval memory of rats and such amnesia was associated with elevated MDA and reduced GSH levels (El-Sherbiny 2003). Since oxidative stress was implicated in the pathophysiology of dementia and scopolamine was reported to elevate rat brain oxidative stress, scopolamine induced amnesia in rats could be used as a valid model to screen drugs with potential therapeutic benefit in dementia (El-Sherbiny 2003).

Earlier reports indicated that improvement in cognition through inhibition of central acetylcholine esterase activity and decrease in brain amyloid beta protein deposition has been, at least in part, mediated by antioxidant effect (Svensson 1998, Xiao 2000).

The antistress and antioxidant activities were correlated with the nootropic activity of the extract since the role of stress and free radicals have been implicated in loss of memory, concentration and in Alzheimer’s disease (Jodar 1995, Esch 2002). The process of nootropic activity involves acquisition, retention and retrieval and is measured using conditioned avoidance response. The acquisition was quicker in the extract treated rats (100, 200, 300 mg/kg body weight) in comparison with control, indicating significant antistress effect by the extract. When challenged with scopolamine bytylebromide (1 mg/kg body weight i.p.), the amnesia was less in the treated group showing better retention and recovery than the control group.

The Carum carvi extract showing a decrease in memory loss could be due to its central cholinomimetic activity as well as its free radical scavenging mechanism. The antioxidant activity of the seed extract provides a mechanistic basis for relieving stress by way of combating oxidative damage.

Conclusion

The present study provides scientific support for the antistress (adaptogenic), antioxidant and nootropic activities of Carum carvi fruit aqueous extract and substantiates the traditional claims for the usage of caraway fruits in stress induced disorders. Further investigations are required to characterise the active constituents responsible for the observed activities of the fruit extract.

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