Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: www.tandfonline.com/journals/iphb20

Cuminum cyminum extract attenuates scopolamine-induced memory loss and stress-induced urinary biochemical changes in rats: A noninvasive biochemical approach

Sushruta Koppula & Dong Kug Choi

To cite this article: Sushruta Koppula & Dong Kug Choi (2011) *Cuminum cyminum* extract attenuates scopolamine-induced memory loss and stress-induced urinary biochemical changes in rats: A noninvasive biochemical approach, Pharmaceutical Biology, 49:7, 702-708, DOI: 10.3109/13880209.2010.541923

To link to this article: https://doi.org/10.3109/13880209.2010.541923

Published online: 03 Jun 2011.

Pharmaceutical Biology, 2011; 49(7): 702–708

ISSN 1388-0209 print/ISSN 1744-5116 online
DOI: 10.3109/13880209.2010.541923

informa
healthcare

ORIGINAL ARTICLE

Cuminum cyminum extract attenuates scopolamine-induced memory loss and stress-induced urinary biochemical changes in rats: A noninvasive biochemical approach

Sushruta Koppula and Dong Kug Choi

Department of Biotechnology, College of Biomedical and Health Sciences, Konkuk University, Chungju, Republic of Korea

Abstract

Context: *Cuminum cyminum* Linn. (Apiaceae), cumin, is a popular spice with a long history of medicinal use to treat various symptoms such as diarrhea, flatulence, gynecological, and respiratory diseases.

Objective: To date, no scientific investigation was reported regarding memory-enhancing and antistress activity of cumin fruits. The present study deals with the memory-enhancing and antistress activities and further the antioxidant status via lipid peroxidation inhibition.

Materials and methods: Antistress activity was evaluated by inducing stress via forced swimming and the urinary vanillylmandelic acid (VMA) and ascorbic acid were estimated as biomarkers. Memory-enhancing activity was studied by conditioned avoidance response using Cook's pole climbing apparatus in normal and scopolamine-induced amnestic rats. Thiobarbituric acid reactive substances (TBARS) assay was used to evaluate the lipid peroxidation.

Results: Daily administration of cumin at doses of 100, 200, and 300 mg/kg body weight 1 h prior to induction of stress inhibited the stress-induced urinary biochemical changes in a dose-dependent manner without altering the levels in normal control groups. The cognition, as determined by the acquisition, retention, and recovery in rats, was observed to be dose-dependent. The extract also produced significant lipid peroxidation inhibition in comparison with known antioxidant ascorbic acid in both rat liver and brain.

Discussion and conclusion: This study provides scientific support for the antistress, antioxidant, and memory-enhancing activities of cumin extract and substantiates that its traditional use as a culinary spice in foods is beneficial and scientific in combating stress and related disorders.

Keywords: Cumin, stress, lipid peroxidation, antioxidant, forced swimming

Introduction

Stress is a broad ambiguous and poorly understood concept. In its most simplified sense, stress is what one feels when life demands exceed ones ability to meet those demands. In fact, every individual is likely to face stressful situations in day-to-day life from headache to heart disease and immune deficiency to digestive problems; stress is a factor in many illnesses (Selye, 1998). A substantial contribution to stress-induced decline in health appears to be increased production of stress hormones and subsequent decreased immune function (Kelly, 1999). Similarly increased physical and mental stress leads to increased incidence of amnesia.

Mounting evidence suggest that brain with Alzheimer's disease increases severe oxidative stress, as a result of either β-amyloid-mediated generation of oxyradicals or perturbed ionic calcium balance within neurons and their mitochondria (Perrig et al., 1997; Emilien et al., 2000). Supplementation with higher ascorbic acid and β-carotene was associated with better memory performance, which indicates the role of potential antioxidants in brain aging and cognitive impairment (Kowalski & Jawibowski, 2000). The therapeutic potential of several plant species and the necessity for scientific validation of the use of plants in popular medicine have prompted increased interest in the field, and a large number of

Address for Correspondence: Sushruta Koppula, Department of Biotechnology, College of Biomedical and Health Sciences, Konkuk University, Chungju, Republic of Korea. Tel.: +82-43-840-3609. Fax: +82-43852-3616. E-mail: koppula@kku.ac.kr

(Received 19 August 2010; revised 20 October 2010; accepted 08 November 2010)

plant species and their components have been shown to be potential immunomodulators acting as antistress and anticancer agents (Bin-Hafeez et al., 2003).

The drugs of plant origin are gaining increasing popularity and are being investigated as remedies for number of stress-related disorders including antistress activity (Edzard, 1998). It is well-documented that oxidative stress is associated with increased oxidant production and oxidative damage, and may enhance the risk of many diseases including physiological stress, aging, and neurodegenerative diseases (Kelly, 1999; Liu & Mori, 1999). Literature also indicates the role of free radicals in the pathogenesis of Alzheimer's disease, diabetes, cancer, aging, and the compounds having capacity to scavenge these free radicals have great potential in mitigation of these disorders/diseases (Halliwell & Gutteridge, 1985). Nutraceuticals such as ginseng, *Withania somnifera* Linn. (Solanaceae), *Ocimum sanctum* Linn. (Labiatae), and grape seed have been shown to produce antistress activity by way of their antioxidant/adaptogenic activity (Bhattacharya et al., 2001).

Spices widely recognized as food additives are used traditionally to prevent and treat diseases and are known to keep the immune system in a highly prepared state for any threat it may encounter. At present their pharmacologic activities, particularly stimulation of immune functions has been the focus of alternative medicine. Among the various spices, *Cuminum cyminum* Linn. (Apiaceae), cumin, is the second most popular spice in the world after black pepper. Native to east India and east Mediterranean, cumin has gained its place as a spice in Indian, African, Chinese, Cuban, and Mexican cuisines and is mainly used to increase the taste and flavor of food (Ishikawa et al., 2002).

Several therapeutic indications involving the disorders of gastrointestinal, gynecological, and respiratory systems (asthma and dysponea) have been described for the fruits of cumin in ancient medical books (Zargari, 1990). The main active constituents of cumin are cuminol, cymine, phellandrene, carvone, and cuminique alcohol (Ishikawa et al., 2002; Yan et al., 2002; Takayanagi et al., 2003). Cumin also contains cuminaldehyde, mixture of hydrocarbons, cymol, terpenes, small quantities of α-pinine, β-pinene, hydrated cuminaldehyde hydrocumin (Aruna & Sivaramakrishnan, 1992; Kokate et al., 2003), and glucosides (Gachkar et al., 2007).

Experimental studies have shown that cumin possesses antimicrobial (Agnihotri & Vaidya, 1996; Singh et al., 2002), anticarcinogenic (Aruna & Sivaramakrishnan, 1992; Gagandeep et al., 2003), antidiabetic (Dhandapani et al., 2002; Sushruta et al., 2006), anti-inflammatory (Srivastava, 1989; Shivakumar et al., 2010), antioxidant (Saito et al., 1976; Huang et al., 1981; Satyanarayana et al., 2004), antinociceptive (Sayyah et al., 2002), antitussive (Boskabady et al., 2006), antiepilepsy (Khatibi et al., 2008), hypocholesterolemic (Shirke & Jagtap, 2009), antifungal (Pai et al., 2010) properties, and improves chick production in poultry industry (Al-Kassi, 2010).

Recently published data revealed that *C. cyminum* is a potent immunomodulator. Cumin significantly increased T-cells (CD4 and CD8) count and Th1-predominant immune response in a dose-dependent manner, thereby suggesting immunomodulatory activity through modulation of T-lymphocytes expression. In restraint stress-induced immune-suppressed animal model, cumin and its active component countered the depleted T-lymphocytes, decreased the elevated corticosterone levels and size of adrenal glands, and increased the weight of thymus and spleen in mice. The authors suggest that cumin may be developed as a lead to recover the immunity of immunocompromised individuals (Chauhan et al., 2010). Another recent study revealed the protective effects of cumin against gentamicin-induced nephrotoxicity in rats (Mahesh et al., 2010). However, its memory-enhancing and stress-relieving activities have been reported.

Hence, the present study was planned to evaluate the aqueous extract of cumin for its antistress activity *in vivo* in normal and stress-induced rats following a noninvasive biochemical approach. Further, cumin was evaluated for its memory-enhancing activity using conditioned avoidance response (CAR) in normal and scopolamine-induced amnestic rats. The antioxidant (lipid peroxidation inhibition) potential of cumin in both liver and brain homogenates was also evaluated to support the memory-enhancing and antistress activities.

Materials and methods

Chemicals

Vanillylmandelic acid (VMA) and scopolamine butyl bromide (SBB) were purchased form Sigma-Aldrich, St. Louis, MO, whereas ascorbic acid was purchased from Loba Chemie, Mumbai, India. All other reagents used were of analytical grade.

Preparation of extract

The dried powdered fruit material of cumin (1 kg) was obtained from Chemiloids, Vijayawada, India and extracted with boiling water (5 L) for 30 min. The filtrate was evaporated under vacuum below 70°C in a vacuum-drier to give a final yield of 83.33 g. The extract was redissolved in distilled water as and when necessary.

Animals

Wistar rats of either sex obtained from Ghosh Enterprises, Kolkata, India were used in the study. They were housed five 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 Pvt. Ltd., Hyderabad, India) and water *ad libitum*. Animals were kept for 7 days in laboratory for habituation. All animal experiments were performed in accordance

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.

Evaluation of antistress activity

Rats of either sex weighing between 150 and 200 g were weight-matched and divided into four groups (I, II, III, and IV) each containing five animals. The 24 h urine samples from each group was collected into two different beakers, one containing 5 mL of 10% oxalic acid for spectrophotometric determination of ascorbic acid at 550 nm (Roe & Kuehter, 1943) and the other containing 0.5 mL of 6 N hydrochloric acid for determination of VMA spectrophotometrically at 360 nm (Pisano et al., 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 the second phase, the animals in each group were subjected to fresh water swimming stress individually (Nagaraja & Jaganathan, 1999). In this method, rats were forced to swim until they were exhausted (3–4 min) in a cylindrical vessel of height 60 cm and diameter 45 cm containing water at room temperature (28°C). Water depth was always maintained at 40 cm. The 24 h urinary levels of VMA and ascorbic acid under stressed conditions were determined again as described above daily for five consecutive days. The third phase of the experiment consists of administration of cumin extract to the same groups of animals after having recovered completely to normal condition. Groups II, III, and IV were administered orally with cumin (dissolved in distilled water) at daily doses of 100, 200, and 300 mg/kg body weight, respectively, for five consecutive days, whereas group I served 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 cumin extract on stress-induced changes in the same groups of animals after a recovery period of 1 week. Groups II, III, and IV were administered cumin by oral gavage at daily doses of 100, 200, and 300 mg/kg body weight, respectively, 1 h prior to the daily induction of stress for five consecutive days, whereas group I served as control. The 24 h urine samples were collected and analyzed for VMA and ascorbic acid for five consecutive days to study the influence of the extract on the stress-induced biochemical changes.

Evaluation of memory-enhancing activity

The memory-enhancing activity of cumin was evaluated by CAR technique in rats using Cook's pole climbing apparatus in rats (Cook & Weidley, 1957). Rats were divided into four groups each containing five animals. Groups II, III, and IV were treated orally with 100, 200, and 300 mg/kg body weight, respectively, of cumin (dissolved in distilled water), whereas animals in group I served as control. After 90 min, all the groups of animals were subjected to a training schedule individually by placing inside the Perspex chamber of the apparatus. After an accustomed period of 5 min 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 although 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 sec given for each trial. This procedure was repeated at 24 h intervals until all groups reach 95% to 99% avoidance. After attaining complete training of a particular group, the animals were treated with a single dose of SBB (1 mg/kg body weight, i.p.) to induce amnesia, 30 min 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.

Lipid peroxidation inhibition in liver and brain of rat using TBARS assay

Rats weighing between 150 and 200 g were sacrificed by spinal traction, and the whole brains and the livers were isolated. The pooled brains and livers were homogenized in four volumes of 40 mM Tris–HCl buffer (pH 7.0) using a tissue homogenizer. The antioxidant activity of cumin was determined based on its ability to inhibit lipid peroxidation in homogenates of liver and brain of rat (Ohkawa et al., 1979). In brief, 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 cumin were 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 sulfate (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 oil bath at 100°C for 1 h. 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

The results are expressed as means ± standard error of means. Statistical analysis was done using Student's paired *t*-test. In all the cases, $P < 0.05$ was considered statistically significant.

Results

Effect of cumin extract in inhibition of stress-induced urinary biochemical changes in rats

Induction of forced swim stress to the animals produced a significant increase in VMA levels from 226.90 ± 9.85 (basal levels) to 396.12 ± 15.68 μg/kg/24 h and decrease in ascorbic acid excretion levels from 141.13 ± 5.18 (basal levels) to 66.73 ± 3.20 μg/kg/24 h, respectively. Both the parameters were found to return to their normal basal levels in 3 to 4 days after withdrawal of stress. Daily treatment of cumin to the animals under normal condition produced no change in the excretion of VMA and ascorbic acid compared with normal basal levels indicating that cumin did not alter excretion of VMA and ascorbic acid in normal condition. Daily administration of cumin 1 h prior to the induction of stress inhibited the increase in urinary VMA levels to 291.71 ± 18.98, 263.78 ± 14.53, and 232.35 ± 13.04 μg/kg/24 h at 100, 200, and 300 mg dose of cumin, respectively. In contrast, daily administration of cumin 1 h prior to the induction of stress inhibited the decrease in ascorbic acid excretion to 92.50 ± 8.12, 111.16 ± 4.10, and 135.83 ± 8.10 μg/kg/24 h at 100, 200, and 300 mg doses of cumin, respectively. The inhibition increase in VMA levels and decrease in ascorbic acid levels was found to be significant at all dose levels in a dose-dependent manner. The urinary data of VMA and ascorbic acid observed in various phases of the experiment are shown in Figures 1 and 2, respectively.

Effect of cumin extract in attenuating scopolamine-induced memory loss in rats

The CAR of rats administered with the extract of cumin or vehicle increased gradually to 95% over 7 to 10 days. The acquisition (time to achieve 95% CAR) for rats administered with the extract of cumin was found to be dose- and time-dependent compared with vehicle-treated control group that took 11 days for acquisition. The percent avoidance was always higher in the extract-treated groups compared with vehicle-treated control group. Animals receiving 300 mg/kg body weight of the extract have taken 8 days, whereas groups treated with 200 and 100 mg/kg doses of the extract required 9 and 10 days, respectively, to reach the point of acquisition (Figure 3). Administration of scopolamine produced amnesia as seen from reduction in the observed CAR. The amnesia was found to be more in controls compared with extract-treated groups and was also found to be dose-dependent. However, continued treatment of cumin produced better retention and recovery in a dose-dependent manner than the vehicle-treated animals.

Effect of cumin extract in inhibition of lipid peroxidation in rat brain/liver homogenates

Lipid peroxides generated by the induction of ferrous/ascorbate on rat brain/liver homogenate were found to be inhibited by cumin. 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 cumin extract needed for 50% inhibition of lipid peroxidation in rat liver homogenate was found to be 4125 μg (Figure 4A). Similar effect was produced by 5350 μg of ascorbic acid. The quantity of cumin needed for 50% inhibition in brain lipid peroxidation was found to be 3220 μg, and similar effect was produced by 4950 μg of ascorbic acid (Figure 4B).

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

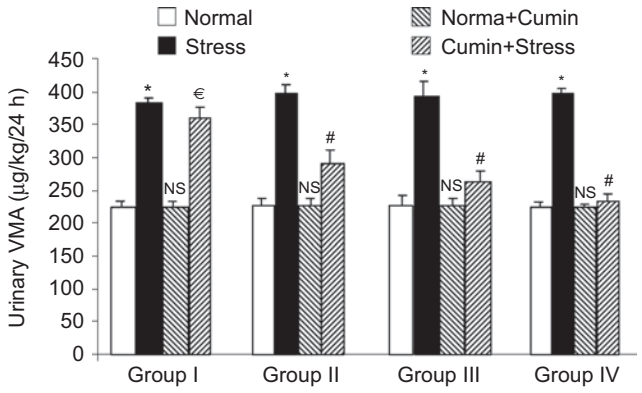

Figure 1. Influence of cumin extract on the 24 h urinary levels of VMA in normal and stress-induced rats. Each bar indicates the mean excretion of five animals. Data are presented as mean ± SEM ($n = 5$). *$P < 0.001$ compared with normal condition of the corresponding groups. #$P < 0.05$, compared with stressed condition of the corresponding groups. €No significant difference from stressed condition. Significance was determined using Student's t-test, $P < 0.05$ was considered statistically significant.

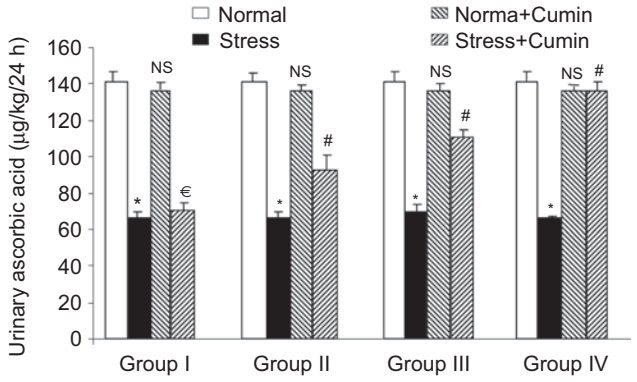

Figure 2. Effect of cumin extract on the 24 h urinary levels of ascorbic acid in normal and stress-induced rats. Each bar indicates the mean excretion of five animals. Data are presented as mean ± SEM ($n = 5$). *$P < 0.001$, compared with normal condition of the corresponding groups. #$P < 0.05$, compared with stressed condition of the corresponding groups. €No significant difference compared with stressed condition. Significance was determined using Student's t-test, $P < 0.05$ was considered statistically significant.

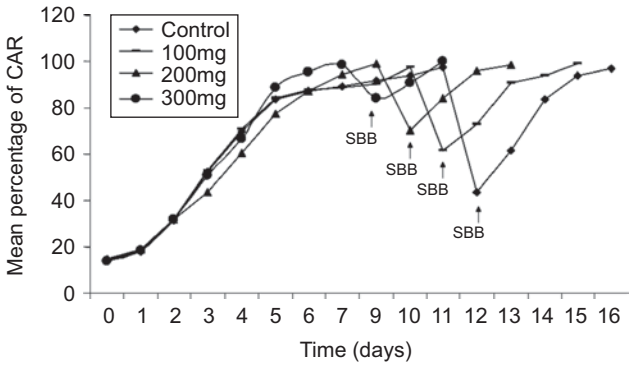

Figure 3. Effect of cumin extract on the mean percent of conditioned avoidance response after oral administration in rats. Scopolamine butyl bromide (SBB) was administered 30 min before the next day dosing with the extract after attaining complete acquisition.

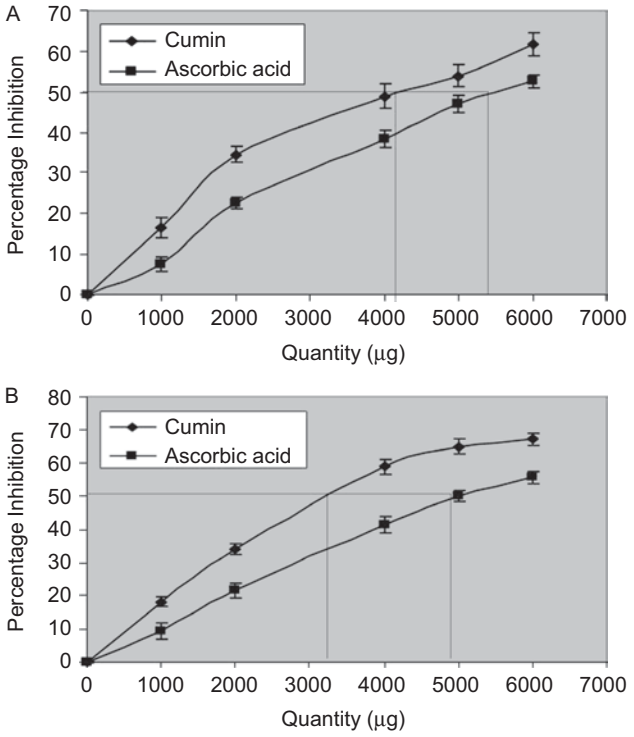

Figure 4. Effect of cumin on the inhibition of lipid peroxidation in liver and brain of rat. Graphical representations of the concentrations of cumin and ascorbic acid required to inhibit 50% of lipid peroxidation in liver (A) and brain (B) homogenates. Each point represents the mean percentage inhibition of six experiments.

immune systems (Das et al., 2002; Deepak et al., 2003). Stress has been postulated to be involved in the etiopathogenesis of a wide variety of diseases, including diabetes, sexual dysfunction, memory loss, ulcers, hypertension, and ulcerative colitis (Chrousos & Gold, 1992). Considerable evidence published in the last decade has focused on alterations of neurochemical, biochemical, and molecular effect caused by stress in these systems (Jiang et al., 1990; Ben-Eliyahu et al., 1991; Smith, 1996; Ueyama et al., 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).

Literature reports indicate that noradrenaline is released during stressful conditions and metabolized to VMA peripherally and 3-methoxy 4-hydroxyphenyl glycol (MOPEG) centrally (Ion, 1969; Fakuda et al., 1996). In the light of such reports, VMA, the major metabolite of sympathetic amines, was taken as 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 noninvasive biochemical marker to study the antistress activity of cumin.

L-Ascorbic acid or vitamin C is synthesized biologically from D-glucose in rat (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 like age, exposure to environmental situations, stress, dietary, biochemical changes, and so on produce alteration of L-ascorbic acid levels in body fluids (Cheng et al., 1990; Kolb, 1992).

Ascorbic acid, being a free radical scavenger (Jose & Kutan, 1995), is more likely utilized in scavenging the free radicals involved in stress resulting in its decreased urinary concentration and also has role in the biosynthesis of noradrenaline (Kallner, 1983; Goodman, 2001). Considering the above reports, 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 activity of the cumin extract on prior administration of stress induction.

Treatment with cumin extract along with stress reversed the stress-induced biochemical changes in a dose-dependent manner. Previously published reports have concluded that the antistress activity of some of the potential medicinal plants could be attributed to their antioxidant effects (Bhattacharya et al., 2001; Satyanarayana et al., 2004; Sreemantula et al., 2005). Based on these reports, the antioxidant activity of cumin extract was also done using *in vitro* lipid peroxidation assay in brain and liver homogenates of rat. It was found that cumin extract has significant antioxidant activity, which was 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 et al., 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 et al., 2003). Earlier reports also indicated that improvement in cognition through inhibition of central acetylcholine esterase activity and decrease in brain β-amyloid protein deposition have been at least, in part, mediated by antioxidant effect (Svensson & Nordberg, 1998; Xiao et al., 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 also in Alzheimer's disease (Jodar et al., 1995; Esch et al., 2002). The process of nootropic activity involves acquisition, retention, and retrieval and is measured using CAR. The acquisition was quicker in the extract-treated rats (100, 200, and 300 mg/ kg body weight) in comparison with control, indicating significant antistress effect by the extract. When challenged with SBB (1 mg/kg body weight, i.p.), the amnesia was less in treated group showing better retention and recovery than control group and the cumin extract was shown to attenuate memory loss that could be due to its central cholinomimetic activity apart from its free radical scavenging mechanisms. Furthermore, the antioxidant activity of the seed extract provides mechanistic basis in relieving stress by way of combating oxidative damage.

Conclusion

The present study provides scientific support for antistress, antioxidant, and memory-enhancing activities of cumin extract and substantiate the traditional claims for the usage in stress-induced disorders. The noninvasive biochemical approach used in this study to evaluate antistress activity helps researchers in reducing the unwanted sacrificing of experimental animals. Further investigations are required to characterize the active constituent(s) of cumin responsible for observed activities and conduct *in vitro* cytotoxicity studies in neuronal cell lines and *in vivo* behavioral changes to explore the exact molecular mechanisms.

Acknowledgement

This work was supported by Konkuk University in 2010.

Declaration of interest

The authors report no declaration of interest.

References

Agnihotri S, Vaidya AD. (1996). A novel approach to study antibacterial properties of volatile components of selected Indian medicinal herbs. *Indian J Exp Biol* 34:712–715.

Al-Kassi GAM. (2010). Effect of feeding cumin (*Cuminum cyminum*) on the performance and some blood traits of broiler chicks. *Pak J Nut* 9:72–75.

Aruna K, Sivaramakrishnan VM. (1992). Anticarcinogenic effects of some Indian plant products. *Food Chem Toxicol* 30:953–956.

Ben-Eliyahu S, Yirmiya R, Liebeskind JC, Taylor AN, Gale RP. (1991). Stress increases metastatic spread of a mammary tumor in rats: Evidence for mediation by the immune system. *Brain Behav Immun* 5:193–205.

Bhattacharya A, Ghosal S, Bhattacharya SK. (2001). Anti-oxidant effect of *Withania somnifera* glycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. *J Ethnopharmacol* 74:1–6.

Bin-Hafeez B, Haque R, Parvez S, Pandey S, Sayeed I, Raisuddin S. (2003). Immunomodulatory effects of fenugreek (*Trigonella foenum graecum* L.) extract in mice. *Int Immunopharmacol* 3:257–265.

Boskabady MH, Kiani S, Azizi H, Khatami T. (2006). Antitussive effect of *Cuminum cyminum* Linn. in guinea pig. *Nat Prod Radian* 5:266–269.

Chauhan PS, Satti NK, Suri KA, Amina M, Bani S. (2010). Stimulatory effects of *Cuminum cyminum* and flavonoid glycoside on cyclosporine-A and restraint stress induced immune-suppression in Swiss albino mice. *Chem Biol Interact* 185:66–72.

Cheng TK, Coon CN, Hamre ML. (1990). Effect of environmental stress on the ascorbic acid requirement of laying hens. *Poult Sci* 69:774–780.

Chrousos GP, Gold PW. (1992). The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA* 267:1244–1252.

Cook L, Weidley E. (1957). Behavioral effects of some psychopharmacological agents. *Ann N Y Acad Sci* 66:740–752.

Das A, Rai D, Dikshit M, Palit G, Nath C. (2002). Effect of unpredictable stress on cognition and brain acetyl choline saturation activity in adult and aged mice. *Ind J Pharmacol* 34:416–421.

Deepak R, Gitika B, Tuhinadri S, Gautam P. (2003). Comparative study of perturbations of peripheral markers in different stressors in rats. *Can J Physiol Pharmacol* 81:1139–1146.

Dhandapani S, Subramanian VR, Rajagopal S, Namasivayam N. (2002). Hypolipidemic effect of *Cuminum cyminum* L. on alloxan-induced diabetic rats. *Pharmacol Res* 46:251–255.

Edzard E. (1998). Harmless herbs? A review of recent literature. *Ann J Med* 104:170–178.

El-Sherbiny DA, Khalifa AE, Attia AS, Eldenshary Eel-D. (2003). *Hypericum perforatum* extract demonstrates antioxidant properties against elevated rat brain oxidative status induced by amnestic dose of scopolamine. *Pharmacol Biochem Behav* 76:525–533.

Emilien G, Beyreuther K, Masters CL, Maloteaux JM. (2000). Prospects for pharmacological intervention in Alzheimer disease. *Arch Neurol* 57:454–459.

Esch T, Stefano GB, Fricchione GL, Benson H. (2002). The role of stress in neurodegenerative diseases and mental disorders. *Neuro Endocrinol Lett* 23:199–208.

Fakuda M, Hata A, Niwa S, Hiramatsu K, Honda H, Nakagome K, Iwanami A. (1996). Plasma vanillylmandelic acid level as an index of psychological stress response in normal subjects. *Psychiatry Res* 63:7–16.

Gachkar L, Yadegari D, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I. (2007). Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. *Food Chem* 102:898–904.

Gagandeep, Dhanalakshmi S, Méndiz E, Rao AR, Kale RK. (2003). Chemopreventive effects of *Cuminum cyminum* in chemically induced forestomach and uterine cervix tumors in murine model systems. *Nutr Cancer* 47:171–180.

Goodman GA. (2001). *The Pharmacological Basis of Therapeutics,* 10th edition. McGraw-Hill: New York, pp. 1804–1805.

Halliwell B, Gutteridge JMC. (1985). *Free Radicals in Biology and Medicine*. Oxford, UK: Clarendon Press.

Huang JK, Wang GS, Chang WH. (1981). Studies on the antioxidative activities of spices grown in Taiwan. I. *Chung-kuo nung yeh hua hsueh hui chih* 19:200–207.

Ion M. (1969). Urinary excretion of catecholamines and vanillyl mandelic acid in rats exposed to cold. *Acta Physiol Scand* 76:393–395.

Ishikawa T, Takayanagi T, Kitajima J. (2002). Water-soluble constituents of cumin: monoterpenoid glucosides. *Chem Pharm Bull* 50:1471–1478.

Jiang CG, Morrow-Tesch JL, Beller DI, Levy EM, Black PH. (1990). Immunosuppression in mice induced by cold water stress. *Brain Behav Immunol* 4:278–291.

Jodar L, Takahashi M, Kaneto H. (1995). Effects of footshock-, psychological- and forced swimming-stress on the learning and

memory processes: involvement of opioidergic pathways. *Jpn J Pharmacol* 67:143–147.

John JC. (1998). The glucose/insulin system and vitamin C: implications in insulin dependent diabetes mellitus. *J Am Coll Nutr* 17:105–108.

Jose JK, Kutan R. (1995). Antioxidant activity of *Emblica officinalis*. *J Clin Biochem Nutr* 19:63–70.

Kallner A. (1983). Influence of vitamin C status on the urinary excretion of catecholamines in stress. *Hum Nutr Clin Nutr* 37:405–411.

Kelly GS. (1999). Nutritional and botanical interventions to assist with the adaptation to stress. *Altern Med Rev* 4:249–265.

Khatibi A, Haghparast A, Shams J, Dianati E, Komaki A, Kamalinejad M. (2008). Effects of the fruit essential oil of *Cuminum cyminum* L. on the acquisition and expression of morphine-induced conditioned place preference in mice. *Neurosci Lett* 448:94–98.

Kokate CK, Purohith AP, Gokhale SB. (2003). *Pharmacognosy*, 22nd edition. Nirali Prakashan, pp. 357–358.

Kolb E. (1992). Recent findings on the significance of ascorbic acid for domestic animals, its uses in veterinary science. *Tierztli Umschau* 47:163–175.

Kowalski A, Jawibowski K. (2000). Effect of different doses of vitamin C on selected hormonal, biochemical and immunological indices in rats subjected to acute stress. *Nat Sci* 4:273–281.

Liu J, Mori A. (1999). Stress, aging, and brain oxidative damage. *Neurochem Res* 24:1479–1497.

Mahesh CM, Shivalinge Gowda KP, Gupta AK. (2010). Protective action of *Cuminum cyminum* against gentamicin induced nephrotoxicity. *J Pharm Res* 3:753–757.

McCarty R. (1987). Stress—neurochemical and humoral mechanisms. In: *Stress Research: Principles, Problems, and Prospects*, Volume 1. Van Loon GR, McCarty RG (eds.). Breach Science: New York, pp. 3–13.

Nagaraja HA, Jaganathan PS. (1999). Influence of different types of stress on selected cardiovascular parameters in rats. *J Physiol Pharmacol* 43:296–304.

Ohkawa H, Ohishi N, Yagi K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95:351–358.

Pai MB, Prashant GM, Murlikrishna KS, Shivakumar KM, Chandu GN. (2010). Antifungal efficacy of *Punica granatum*, *Acacia nilotica*, *Cuminum cyminum* and *Foeniculum vulgare* on *Candida albicans*: an *in vitro* study. *Indian J Dent Res* 21:334–336.

Perrig WJ, Perrig P, Stähelin HB. (1997). The relation between antioxidants and memory performance in the old and very old. *J Am Geriatr Soc* 45:718–724.

Roe JH, Kuehter CA. (1943). The determination of ascorbic acid in bile blood and urine through 2,4 dinitrophenylhydrazine derivatives of dehydroascorbic acid. *J Biol Chem* 147:399–408.

Saito Y, Kimura Y, Sakamoto T. (1976). The antioxidant effects of petroleum ether soluble and insoluble fractions from spices. *Eiyo to shokuryo* 29:505–510.

Satyanarayana S, Sushruta K, Sarma GS, Srinivas N, Subba Raju GV. (2004). Antioxidant activity of the aqueous extracts of spicy food additives—evaluation and comparison with ascorbic acid in *in-vitro* systems. *J Herb Pharmacother* 4:1–10.

Sayyah M, Mahboubi A, Kamalinejad, M. (2002). Anticonvulsant effect of the fruit essential oil of *Cuminum cyminum* in mice. *Pharm Biol* 40:478–480.

Selye H. (1998). A syndrome produced by diverse nocuous agents. 1936. *J Neuropsychiatry Clin Neurosci* 10:230–231.

Shirke SS, Jagtap AG. (2009). Effects of methanolic extract of *Cuminum cyminum* on total serum cholesterol in ovariectomized rats. *Indian J Pharmacol* 41:92–93.

Shivakumar SI, Shahapurkar AA, Kalmath KV, Shivakumar B. (2010). Antiinflammatory activity of fruits of *Cuminum cyminum* Linn. *Der Pharma Lett* 2:22–24.

Singh G, Kapoor IP, Pandey SK, Singh UK, Singh RK. (2002). Studies on essential oils: part 10; antibacterial activity of volatile oils of some spices. *Phytother Res* 16:680–682.

Smith MA. (1996). Hippocampal vulnerability to stress and aging: possible role of neurotrophic factors. *Behav Brain Res* 78:25–36.

Sreemantula S, Nammi S, Kolanukonda R, Koppula S, Boini KM. (2005). Adaptogenic and nootropic activities of aqueous extract of *Vitis vinifera* (grape seed): an experimental study in rat model. *BMC Complement Altern Med* 5:1.

Srivastava KC. (1989). Extracts from two frequently consumed spices–cumin (*Cuminum cyminum*) and turmeric (*Curcuma longa*)—inhibit platelet aggregation and alter eicosanoid biosynthesis in human blood platelets. *Prostaglandins Leukot Essent Fatty Acids* 37:57–64.

Sushruta K, Satyanarayana S, Srinivas N, Raja Sekhar J. (2006). Evaluation of the blood-glucose reducing effects of aqueous extracts of the selected umbelliferous fruits used in culinary practices. *Trop J Pharm Res* 5:613–617.

Svensson AL, Nordberg A. (1998). Tacrine and donepezil attenuate the neurotoxic effect of A beta(25–35) in rat PC12 cells. *Neuroreport* 9:1519–1522.

Takayanagi T, Ishikawa T, Kitajima J. (2003). Sesquiterpene lactone glucosides and alkyl glycosides from the fruit of cumin. *Phytochemistry* 63:479–484.

Ueyama T, Kawai Y, Nemoto K, Sekimoto M, Toné S, Senba E. (1997). Immobilization stress reduced the expression of neurotrophins and their receptors in the rat brain. *Neurosci Res* 28:103–110.

Xiao XQ, Wang R, Tang XC. (2000). Huperzine A and tacrine attenuate beta-amyloid peptide-induced oxidative injury. *J Neurosci Res* 61:564–569.

Yan JH, Tang KW, Zhong M, Deng NH. (2002). [Determination of chemical components of volatile oil from *Cuminum cyminum* L. by gas chromatography–mass spectrometry]. *Se Pu* 20:569–572.

Zargari A. (1990). *Medicinal Plants*, 5th edition. Tehran, Iran: Tehran University Publications, pp. 312–314.