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Research Article

Anti-Stress and Anti-Amnesic Effects of *Coriandrum sativum* Linn (Umbelliferae) Extract – an Experimental Study in Rats

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Abstract

Purpose: *Coriandrum sativum Linn. (Umbelliferae, C. sativum) is cultivated throughout the world for its use as spice and as a folk medicine. This study deals with the anti-stress and anti-amnestic properties of C. sativum extract in rats.*
Methods: *Urinary levels of vanillylmandelic acid (VMA) and ascorbic acid were used to evaluate anti-stress activity in rats, while conditioned avoidance response test in normal and scopolamine-induced amnesic rats was used to evaluate anti-amnesic effects. C. sativum extract was also evaluated for its antioxidant activities by inhibition of lipid peroxidation in brain and liver homogenates of the rats.*
Results: *Daily administration of C. sativum extract (100, 200 and 300 mg/kg body weight) 1 h prior to induction of stress significantly decreased the stress-induced urinary levels of VMA from 382.79 ± 10.70 to 350.66 ± 15.15, 291.21 ± 16.53 and 248.86 ± 13.56 µg/kg/24 h and increased the ascorbic acid excretion levels from 66.73 ± 9.25 to 69.99 ± 7.37, 105.28 ± 13.74 and 135.32 ± 12.54 µg/kg/24 h at 100, 200 and 300 mg/kg, respectively, in a dose-dependent fashion without affecting the normal levels in control groups. The amnesic deficits (acquisition, retention and recovery) induced by scopolamine (1mg/kg, i.p.) in rats was reversed by C. sativum dose dependently. The extract also inhibited lipid peroxidation in both rat liver and brain to a greater extent than the standard antioxidant, ascorbic acid.*
Conclusion: *C. sativum may be useful remedy in the management of stress and stress related disorders on account of its multiple actions such as anti-stress, anti-amnestic and antioxidant effects.*

Keywords: *C. sativum, Stress, Lipid peroxidation, Vanillylmandelic acid, Memory.*

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INTRODUCTION

Stress is experienced by every individual and is a reaction of mind and body resulting in threatened homeostasis. Extreme stress may lead to the pathogenesis of a stream of disease conditions including hypertension, peptic ulcer, diabetes, immunosuppression, reproductive dysfunctions, metabolic and endocrine disorders [1]. Research has revealed that stress impairs brain functioning, which leads to memory loss and can negatively affect cognitive skills leading to the pathogenesis of various neurodegenerative disorders [2,3]. Further, overload of stress build up the free radicals and induce potential damage to neuronal receptors and damage a variety of other organ tissues [4]. Thus agents that scavenge free radicals may have great potential in mitigation of these disorders/diseases.

Spices are used not only to add flavor, color and nutritional values to food, but also to treat various diseases [5]. *Coriandrum sativum* Linn. (Umbelliferae, *C. sativum*), commonly known as coriander, is a widely cultivated spice all over the world. Traditionally, *C. sativum* is used in the disorders of digestive, respiratory, urinary, convulsions, insomnia and anxiety. Pharmacologically, *C. sativum* was reported to possess antidiabetic, hepatoprotective, antimutagenic, antihypertensive, antioxidant, anxiolytic, antimicrobial activity and heavy metal detoxification [6-8].

The major chemical components identified in *C. sativum* are flavonoids, polyphenols and carotenoids [9,10]. Despite the potential medicinal benefits of *C. sativum*, the stress relieving and memory enhancing effects have not been fully investigated. The aim of the present study was to assess the anti-stress and anti-amnesic effects of aqueous extract of *C. sativum in vivo,* and to correlate the anti-stress and anti-amnesic effects with *in vitro* inhibition of lipid peroxidation.

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. 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 *C. sativum* (1 kg) was obtained from a local market in Vijayawada, India and the authenticity of the fruit material was confirmed by taxonomist Dr. Hemadri K, Regional Research Institute, India. A voucher specimen (CS-2003) 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 108.69 g. *C. sativum* 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. 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 [11].

Evaluation of anti-stress activity

Induction of stress was carried out using the forced swimming test in rats [12]. 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 [13]. 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) spectrophotometrically at 360 nm [14].

The experimental protocol was divided into four phases. In the first phase of the experiment, 24 h urine samples were collected in all the 4 groups and subjected to analysis for both VMA and ascorbic acid separately and the values were recorded for 5 consecutive days. In the second phase, the animals in each group were individually subjected to fresh water swimming stress [12]. In this method, the rats were forced to swim until they were exhausted (usually after 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 these stressed conditions were determined daily as outlined above for 5 consecutive days. The third phase of the experiment consisted of administration of *C. sativum* extract to the animals following recovery to normal condition (usually in 3-4 days). Groups II, III and IV were administered orally with *C. sativum* (dissolved in distilled water) at daily doses of 100, 200 and 300 mg/kg body weight respectively for 5 consecutive days, while group I served as control and received only distilled water. 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 evaluating the influence of *C. sativum* extract on stress-induced changes in the animals after a recovery period of one week. Groups II, III and IV were administered *C. sativum* by oral gavage at daily doses of 100, 200 and 300 mg/kg, respectively, one hour to the daily induction of stress for 5 consecutive days. Group I served as control received only distilled water. Urine samples (24 h) were collected and analyzed for VMA and ascorbic acid for 5 consecutive days to assess the influence of the extract on stress-induced biochemical changes.

Evaluation of anti-amnesic activity

This experiment was evaluated by conditioned avoidance response (CAR) technique in rats using Cook's pole climbing apparatus, Techno, India [15]. The rats were divided into 4 groups of 5 animals each. Groups II, III and IV were administered orally with 100, 200 and 300 mg/kg, respectively of *C.sativum* (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.

Inhibition of lipid peroxidation in rat liver and brain (TBARS assay)

Rats weighing between 150-200 g were sacrificed by spinal traction and liver was excised and washed in ice-cold Tris-HCl buffer (0.1M, pH 7.4), and cytosolic samples of liver homogenate were prepared by centrifuging at 10,000 rpm for 30 min at 4 °C. 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 *C. sativum* extract. The lipid peroxide formed was measured by TBARS formation [16]. 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 ^{o}C for 1 h. On cooling, 1ml of distilled water and 5ml 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 optical density.

Statistical analysis

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

RESULTS

As shown in Figs 1 and 2, a significant increase (p<0.05) in urinary levels of VMA and decrease in ascorbic acid excretion levels was observed in animals subjected to forced swim stress when compared to the normal basal levels in control animals. The altered levels returned to normal basal levels in 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 normal basal levels. Treatment with extract (100, 200 and 300 mg/kg) one hour prior to the induction of stress significantly inhibited increase in urinary VMA levels dose-dependently (Fig 1). In contrast, administration of the extract one hour prior to the induction of stress significantly inhibited the decrease in urinary levels of ascorbic acid (Fig 2). The inhibition of the increase in VMA levels and decrease in ascorbic acid levels were dose-dependent ($p < 0.05$).

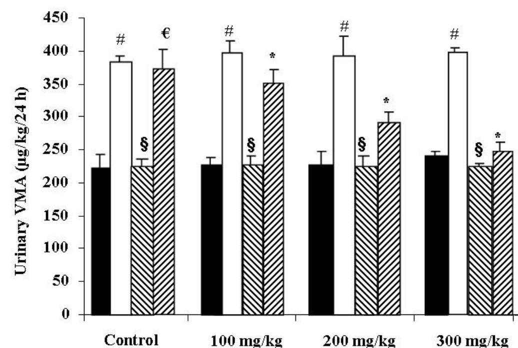

Fig 1: Influence of *C. sativum* extract on the 24 h urinary levels of VMA in normal and stress-induced rats (mean ± SEM, n=6). $^{\#}$p< 0.001 compared to normal condition of the corresponding groups. *p<0.05, compared with stressed condition of the corresponding groups. §No significant difference from normal 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.
■ = Group I (Untreated), □ = Group II (Stress), ▧ = Group III (Extract alone), ▨ = Group IV (Extract + Stress).

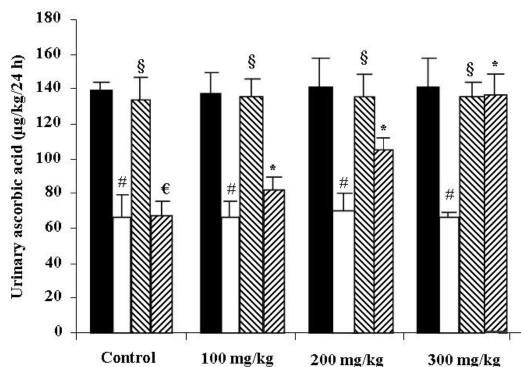

Fig 2: Effect of *C. sativum* extract on the 24 h urinary levels of ascorbic acid in normal and stress induced rats. Data are represented as mean ± SEM (n=6). $^{\#}$p< 0.001, compared to normal condition of the corresponding groups. *p<0.05, compared with stressed condition of the corresponding groups. §No significant difference from normal condition of the corresponding groups. $^{€}$No significant difference compared to stressed condition. Significance was determined using Student's *t*-test, p<0.05 was considered statistically significant.
■ *= Group I (Untreated),* □ *Group II (Stress),* ▨ *= Group III (Extract alone),* ▧ *= Group IV (Extract + Stress).*

The CAR of rats administered with the extract increased gradually to 95% over seven to eleven 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 11 days for acquisition. The percent avoidance was always higher in the extract treated groups compared to vehicle treated (control) group. Animals receiving 300 mg/kg body weight of the extract took eight days while groups treated with 200 and 100 mg/kg doses of the extract required nine to ten 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 *C. sativum* 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 3-4 days when compared to normal (control) group which took over 5 days.

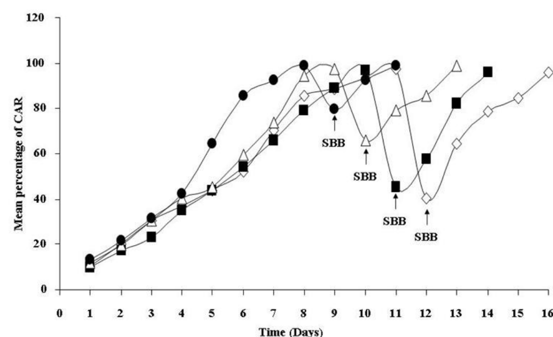

Fig 3: Effect of *C. sativum* extract on the mean percent of conditioned avoidance response after oral administration in rats. Scopolamine butyl bromide (SBB) was administered thirty minutes before the next day dosing with the extract after attaining complete acquisition. ◊ = Control, ■ = 100 mg, Δ= 200 mg and ● = 300 mg.

The extract inhibited the producvtiuon of peroxides by the induction of fe^{2+}/ascorbate in rat liver and brain homogenates in a dose-dependent fashion (Fig. 4). 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 4660 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 3780 µg. A similar effect was produced by 4690 µg of ascorbic acid (Fig 4B).

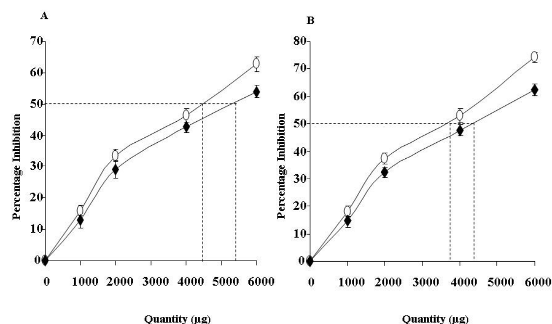

Fig 4: Effect of *C. sativum* on the *in vitro* inhibition of lipid peroxidation in liver and brain of rat (n=6). Graphical representations of the concentrations of *C. sativum* and ascorbic acid required to inhibit 50 percent of lipid peroxidation in liver (A) and brain (B) homogenates. O = *C. sativum* and ♦ = ascorbic acid.

DISCUSSION

Functional foods and food additives for improving quality of life have received a lot of attention in recent times. Spices and condiments widely recognized as food additives have been used traditionally to prevent and treat diseases.

The results from this study show that administration of *C. sativum* aqueous extract had a profound influence on the urinary levels of VMA and ascorbic acid in rats subjected to forced swim stress. The extract also improved memory deficits induced by scopolamine injection and exhibited higher inhibition of lipid peroxidation when compared with ascorbic acid in both rat liver and brain homogenates.

It is well known that noradrenaline and adrenaline, which are released normally or in response to stress are metabolized into 3-methoxy 4-hydroxyphenyl glycol (MOPEG) centrally and vanillyl mandelic acid (VMA) peripherally. During increased stress, blood levels of VMA increase, leading to the excretion of the metabolite in the urine [17].

L-ascorbic acid which is synthesized from D-glucose in rats is altered in body fluids due to several factors like age, exposure to environmental situations, stress and diet etc. 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 studies indicated that the tissue levels of ascorbic acid decrease on application of stress [18, 19]. Furthermore ascorbic acid being a free radical scavenger, it is more likely utilized in scavenging the free radicals involved in stress resulting in its decreased urinary concentration and also it has role in the biosynthesis of noradrenaline as a cofactor in the conversion of dopamine to noradrenaline [20]. These findings justify the use of urinary VMA ascorbic acid levels during stress as indirect biochemical indices for studying the anti-stress activity of *C. sativum*.

Several reports suggested that medicinal plants with immense antioxidant properties might be beneficial in combating stress [21, 22]. Stress and free radicals have been implicated in loss of memory and cognitive deficits [2, 3, 23]. Earlier reports indicated that scopolamine impaired retrieval memory of rats [24, 25]. The amnesia was associated with a significant increase in oxidative stress as indicated by rise in brain thiobarbituric acid reactive species (TBARS) with elevated MDA and reduced glutathione (GSH) levels. Ideally, scopolamine induced amnesia in rats could be used as a valid model to screen agents with potential therapeutic benefit in dementia [26].

The mechanism by which *C.sativum* extract acts in attenuating the memory deficits induced by scopolamine could be due to its free radical scavenging activity. Furthermore, the antioxidant activity of the *C. sativum* extract provide mechanistic basis in relieving stress by way of combating oxidative damage.

CONCLUSION

The present study provides scientific support for anti-stress, antioxidant and anti-amnestic activities of *C.sativum* extract and usage in the management of stress and stress-related diseases like memory loss may be of great interest.

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