Research Article

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Reversal of memory deficits by *Coriandrum sativum* leaves in mice

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Abstract

BACKGROUND: *Coriandrum sativum* L., commonly known as coriander and belonging to the family Apiaceae (Umbelliferae), is cultivated throughout the world for its nutritional value. The present study was undertaken to investigate the effects of fresh *Coriandrum sativum* leaves (CSL) on cognitive functions, total serum cholesterol levels and brain cholinesterase activity in mice. In this study, CSL (5, 10 and 15% w/w of diet) was fed orally with a specially prepared diet for 45 days consecutively to experimental animals. Elevated plus-maze and passive avoidance apparatus served as the exteroceptive behavioral models for testing memory. Diazepam, scopolamine and ageing-induced amnesia served as the interoceptive behavioral models.

RESULTS: CSL (5, 10 and 15% w/w of diet) produced a dose-dependent improvement in memory scores of young as well as aged mice. CSL also reversed successfully the memory deficits induced by scopolamine (0.4 mg kg^{-1}, i.p.) and diazepam (1 mg kg^{-1}, i.p.). Interestingly, brain cholinesterase activity and serum total cholesterol levels were considerably reduced by CSL administration in daily diets concomitantly for 45 days.

CONCLUSION: CSL may be a useful remedy in the management of Alzheimer's disease on account of its multifarious effects such as, memory-improving property, cholesterol-lowering property and anticholinesterase activity.

Keywords: *Coriandrum sativum*; amnesia; memory; acetylcholinesterase; cholesterol

INTRODUCTION

Alzheimer's disease (AD) is a genetically heterogeneous, crippling neurodegenerative disorder, which is slow in onset but relentless in progress. The major symptoms of AD include dementia, aphasia, apraxia, agnosia, dyslexia and agraphia. Excessive deposits of extracellular β-amyloid (Aβ) plaques, intraneuronal fibrillary tangles and neuronal loss in certain brain areas constitute the three major hallmarks of AD.[1] Diminished cholinergic transmission in brain, elevated total cholesterol levels and vitamin B group deficiency all appear to be the causative factors responsible for development of AD.[2]

There has been a steady rise in the number of patients suffering from AD all over the world. There are around 35 million patients suffering from AD globally, of which the USA alone has around 4.5 million.[3] Despite the severity and high prevalence of this disease, an allopathic system of medicine is yet to provide a satisfactory remedy. Therefore, neurobiologists all over the world are looking for new directions and alternative strategies for managing this disease of senior citizens. In India AD patients are estimated to be fewer than 3.5 million,[4] a figure that is considerably smaller than that of the USA. There is the idea that certain nutrients in Indian dishes may be responsible for protection against AD.

Coriandrum sativum L., commonly known as coriander and belonging to the family Apiaceae (Umbelliferae), is cultivated throughout the world for its nutritional value. The fresh leaves of *C. sativum* are routinely added for their delicious taste and flavor which they impart to various dishes in Asian countries. The leaves contain volatile oil, proteins, flavonoid glycosides (quercetin, isoquercitrin and rutin), caffenic acid, traces of fats, minerals (like calcium, phosphorus, and iron), carotene, fiber and carbohydrates. Coriander leaves stimulate appetite and the fresh juice is often recommended for patients suffering from vitamin A, B and C deficiencies and also for the relief of anxiety and insomnia.[5,6] Coriander fruit is also reputed to be a refrigerant, tonic, diuretic and aphrodisiac, while the oil is considered useful in flatulent colic, rheumatism, neuralgia, etc.[7] Coriander is also used as antiedemic, anti-inflammatory, antiseptic, emmenagogue, antidiabetic, antihypertensive, lipolytic and myorelaxant, and possesses nerve-soothing properties.[8] Pharmacological studies have demonstrated the hypoglycemic,[9] hypolipidemic,[10] antimutagenic,[11] antihypertensive,[12] antioxidant,[13] antimicrobial,[14] hepatoprotective,[15] and post-coital

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antifertility[16] activity of *C. sativum*. It has also been used in heavy metal detoxification.[17] The present study was therefore undertaken to investigate the effects of *Coriandrum sativum* leaves (CSL) on memory, serum cholesterol and brain acetylcholinesterase activity (AChE) in mice.

MATERIALS AND METHODS

Plant material

The fresh leaves of *Coriandrum sativum* were obtained from a local market in Hisar, Haryana (India), and taxonomically identified by Dr HB Singh, Head, Raw Materials, Herbarium and Museum Division, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. A voucher specimen (GJU/PHARM/10) is preserved at the Pharmacology Division of the Department of Pharmaceutical Sciences, GJ University of Science and Technology, Hisar, India, for ready reference. These fresh leaves were cut into fine pieces with the help of a sharp knife and were mixed in varying concentrations (5%, 10% and 15% w/w) in normal animal diet. This special diet containing CSL was fed to mice for 45 days.

Animals

Either sex of Wistar rats (210–270 g) and male Swiss Albino mice, young (3–4 months old) and weighing around 20 g, as well as aged (12–15 months old) and weighing around 35 g, were used in the present study. All animals were procured from the disease-free small animal house of CCS Haryana Agricultural University, Hisar (Haryana), India. The animals were kept in propylene cages at 19–23 °C in a 12 h light : dark cycle, and had free access to food and water. The normal diet given to mice consisted of wheat flour kneaded with water and mixed with a small amount of refined vegetable oil. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the care of laboratory animals was taken as per the guidance of CPCSEA, Ministry of Forests and Environment, Government of India (registration number 0436).

Chemicals

The drugs used in this study were scopolamine hydrobromide (Sigma-Aldrich, St Louis, MO, USA), diazepam (Calmpose®, Ranbaxy, India), 5,5-dithiobis-2-nitrobenzoic acid (DTNB), acetylcholine iodide, eserine salicylate, sodium dihydrogen phosphate, disodium hydrogen phosphate (Hi Media, Mumbai, India), piracetam (Nootropil®, UCB India Ltd, Mumbai, India), metrifonate (Sigma-Aldrich), simvastatin (Krebs Biochemicals and Industries Ltd, Hyderabad, India) and cholesterol diagnostics kit (Erba Diagnostics, Mannheim, Germany).

Vehicle

Diazepam, scopolamine hydrobromide, piracetam and metrifonate were diluted separately in normal saline and injected intraperitoneally. Simvastatin was suspended in 0.5% (w/v) carboxymethylcellulose sodium (CMC) and administered orally to animals.

Toxicity study

Rats were divided into four groups of six animals each. CSL (5%, 10% and 15% w/w) was mixed in normal diet and was fed for 45 consecutive days to treated groups (II–IV) respectively. Individual body weight was measured and recorded weekly for 45 days. Detailed physical examination, including the nature, onset and duration of all gross or visible toxicological effects, were recorded daily for 45 days. On the 46th day, all animals were anesthetized with ether and blood samples were collected via cardiac puncture. Blood samples were used partly for the determination of hematological parameters with potassium ethylenediaminetetraacetic acid (EDTA) as anticoagulant. After centrifugation at 3000 rpm for 5 min, the collected serum was used to determine the biochemical parameters. Assessment of hematology parameters consisted of erythrocyte count, leukocyte count, and hemoglobin concentration. The collected blood serum was evaluated for alkaline phosphates (ALP), alanine aminotransferase (ALT), aspirate amino transferase (AST), total serum protein, albumin and creatinine. At the end of the experiment, selected organs (brain, liver and kidneys) were dissected for histopathological examination.[18]

Drug treatment

Mice were divided into different groups ($n = 6$) for investigations that employed various interoceptive as well as exteroceptive memory models and for biochemical estimations. CSL (5%, 10% and 15% w/w) was mixed in the normal diet and was fed for 45 consecutive days to young and aged mice of different groups. These mice were exposed to the training session using elevated plus-maze and passive avoidance apparatus on the 45th day 90 min after the last feed. Retention (memory) of the learned task was recorded after 24 h, i.e. on the 46th day. Amnesia was induced in separate groups (interoceptive model) of young mice by scopolamine (0.4 mg kg^{-1}, i.p.) or diazepam (1 mg kg^{-1}, i.p.) on the 45th day 90 min after the last feed. Piracetam (400 mg kg^{-1}, i.p.), an established nootropic agent, was injected for 7 days to the positive control group of animals. The blood samples and whole brain of the animal were collected after 45 days of administration of the diet containing various concentrations of CSL. Metrifonate (50 mg kg^{-1}, i.p.) served as the positive control to compare the extent of brain AChE inhibition. Simvastatin (5 mg kg^{-1}, p.o., for 7 days) served as the positive control to compare the extent of total cholesterol-lowering effect. The control group animals were fed with normal diet for 45 days.

Elevated plus-maze

Elevated plus-maze served as the exteroceptive behavioral model to evaluate memory in mice. The procedure, technique and endpoint for testing memory were followed as per the parameters described by the investigators working in the area of psychopharmacology.[19] The elevated plus-maze for mice consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 12 cm) extending from a central platform (5 cm × 5 cm) and the maze was elevated to a height of 25 cm from the floor. On the first day (i.e. 45th day of drug treatment), each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all its four legs and was recorded on the first day (training session) for each animal. The mouse was allowed to explore the maze for another 2 min and then returned to its home cage. Retention of this learned task (memory) was examined 24 h after the last dose, which was the 46th day, and corresponding to 24 h after the first day trial. Significant reduction in TL value of retention indicated improvement in memory.

Measurement of locomotor activity

Since the plus-maze experiment was affected by changes in locomotor activity, an additional experiment was carried out with the specific aim of monitoring the activity.[20] It was recorded using a digital actophotometer (INCO, Mumbai, India). Each mouse of young and aged elevated plus-maze groups (control, CSL 5%, CSL 10% and CSL 15%) was placed individually for 10 min in the activity cage on the 46th day immediately after finishing the experiment.

Passive avoidance paradigm

Passive avoidance behavior based on negative reinforcement was used to examine long-term memory.[21] The apparatus consisted of a box (27 cm × 27 cm × 27 cm) having three walls of wood and one wall of Plexiglas, featuring a grid floor (made up of 3 mm stainless steel rods set 8 mm apart), with a wooden platform (10 cm × 7 cm × 1.7 cm) in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock (20 V, AC) was delivered to the grid floor. Training (i.e. 30th day of drug treatment) was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the center of the grid floor. When the mouse stepped down, placing all its paws on the grid floor, shock was delivered for 15 s and the step-down latency (SDL), which was defined as the time (in seconds) taken by the mouse to step down from the wooden platform to the grid floor with all its paws on the grid floor, was recorded. Animals showing SDL in the range of 2–15 s during the first test were used for the second session and the retention test. The second session was carried out 90 min after the first test. During second session, if the animals stepped down before 60 s, electric shocks were delivered once again for 15 s; animals were removed from the shock-free zone if they did not step down for a period of 60 s and were subjected to retention test. Retention (memory) was tested after 24 h (i.e. 46th day, 24 h after last dose) in a similar manner, except that the electric shocks were not applied to the grid floor, observing an upper cut-off time of 300 s. Significant increase in SDL value indicates improvement in memory. SDL of the second day (46th day of drug treatment) reflects the long-term memory of animals.

Collection of blood and brain samples

The animals were sacrificed by cervical decapitation under light anesthesia on the 30th day, 90 min after administration of the last dose of CSL. Immediately after decapitation, the trunk blood was collected and the whole brain was carefully removed from the skull. The collected blood was centrifuged at 3000 rpm for 15 min to separate the serum. The serum was used for estimation of total cholesterol levels. For preparation of brain homogenate, the fresh whole brain was weighed and transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of normal saline solution. The homogenate was centrifuged at 3000 rpm for 10 min and the resultant cloudy supernatant liquid was used for estimation of brain AChE activity.

Estimation of brain cholinesterase

Brain cholinesterase activity was measured by the method of Ellman *et al.*[22,23] with a slight modification. A 0.5 mL portion of the cloudy supernatant liquid was pipetted out into a 25 mL volumetric flask and was made up to volume with freshly prepared 5,5-dithiobis-2-nitrobenzoic acid (DTNB) solution (10 mg DTNB in 100 mL of Sorensen's phosphate buffer, pH 8.0). From the volumetric flask, two 4 mL portions were pipetted out into two test tubes and 1 mL of substrate solution (75 mg acetylcholine iodide per 50 mL distilled water) was added into the tubes. One of the test tubes had additionally 2 drops of eserine solution. The contents of the tubes were incubated for 10 min at 30 °C. The resulting yellow color was due to reduction of DTNB by certain substances in the brain homogenate and non-enzymatic hydrolysis of substrate. Change in absorbance per minute of the sample was read at 420 nm.

Estimation of serum total cholesterol level

The CHOD-PAP method was used for the estimation of serum total cholesterol.[24] In this method, 20 µL of the blank, standard or test sample was pipetted into the respective reaction tubes containing 1000 µL of working reagent. These mixtures were incubated for 10 min at 37 °C. Absorbances were read at 510 nm and 630 nm against the blank sample by using an Autoanalyzer (Chem-5, Plus V_2, Erba Mannheim).

Statistical analysis

All the results were expressed as mean ± standard error (SEM). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test. *P*-values <0.05 were considered as statistically significant.

RESULTS

Toxicity study

The continuous 45-day administration of CSL with a normal diet did not cause any noticeable changes in the general behavior of the rats as compared to the control group. Moreover, no significant abnormal changes were noticed in body weight, hematology parameters, biochemistry estimations or histopathological examinations when compared with the control animals (results not shown).

Transfer latency using elevated plus-maze

In young animals (3–4 months), CSL at concentrations of 5% ($P < 0.01$), 10% ($P < 0.001$) and 15% ($P < 0.001$) w/w of diet produced a dose-dependent reduction in TL value when compared to the control group at the 46th day (Fig. 1). Aged animals (12–15 months old) receiving normal diet (without CSL) showed memory deficits (amnesia) as reflected by high ($P < 0.001$) TL value on the 46th day as compared to young animals. However, these memory deficits were successfully reversed by CSL when animals were fed for 45 days. Furthermore, CSL successfully reversed the amnesia induced by both scopolamine and diazepam in young mice (Fig. 2). Piracetam (used as a positive control) at a dose of 400 mg kg^{-1} i.p. improved memory ($P < 0.001$) of both young and aged mice and reversed the amnesia induced by scopolamine and diazepam.

Locomotor activity

There was no significant effect on the locomotor activity of young (223.17 ± 4.69, 220.50 ± 4.05, 227.17 ± 5.17, respectively) and aged (198.67 ± 6.15, 204.83 ± 7.39, 200.83 ± 6.44, respectively) mice treated with CSL (5%, 10% and 15% w/w of diet) for 45 successive days as compared to the respective control group (young 219.17 ± 6.22; aged 190.5 ± 6.15) when tested using actophotometer.

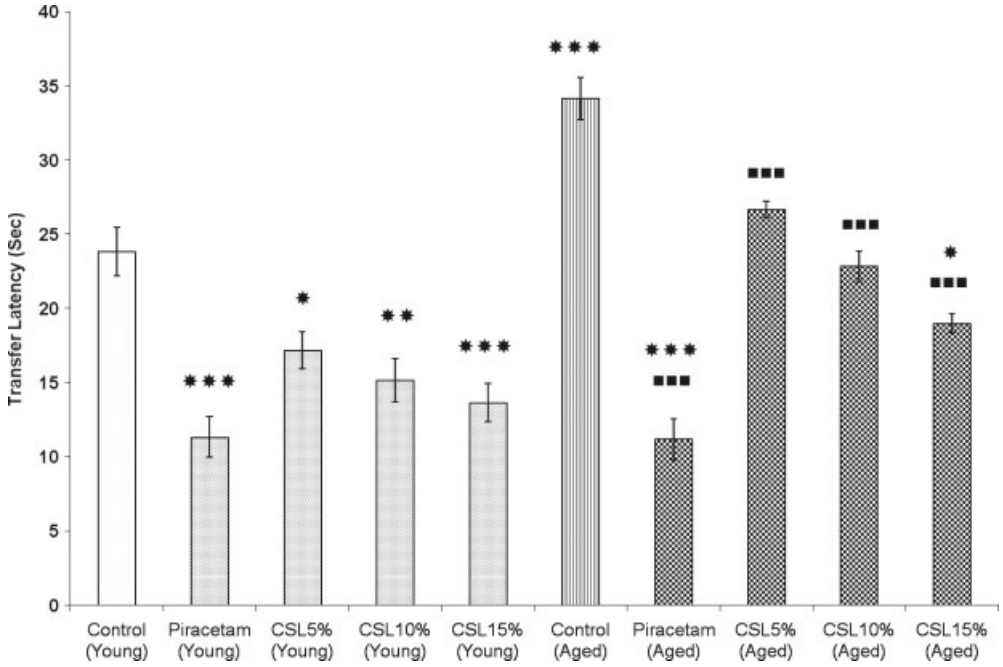

Figure 1. Effect of various concentrations of *C. sativum* leaves (5%, 10% and 15% w/w of diet) on transfer latency of young and aged mice using elevated plus-maze. Piracetam (400 mg kg^{-1}, i.p.) was used as a positive control. Values are in mean ± SEM ($n = 6$); $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ as compared to control group of young mice. $^{■■■}P < 0.001$ as compared to control group of aged mice (one-way ANOVA followed by Tukey–Kramer multiple comparisons test).

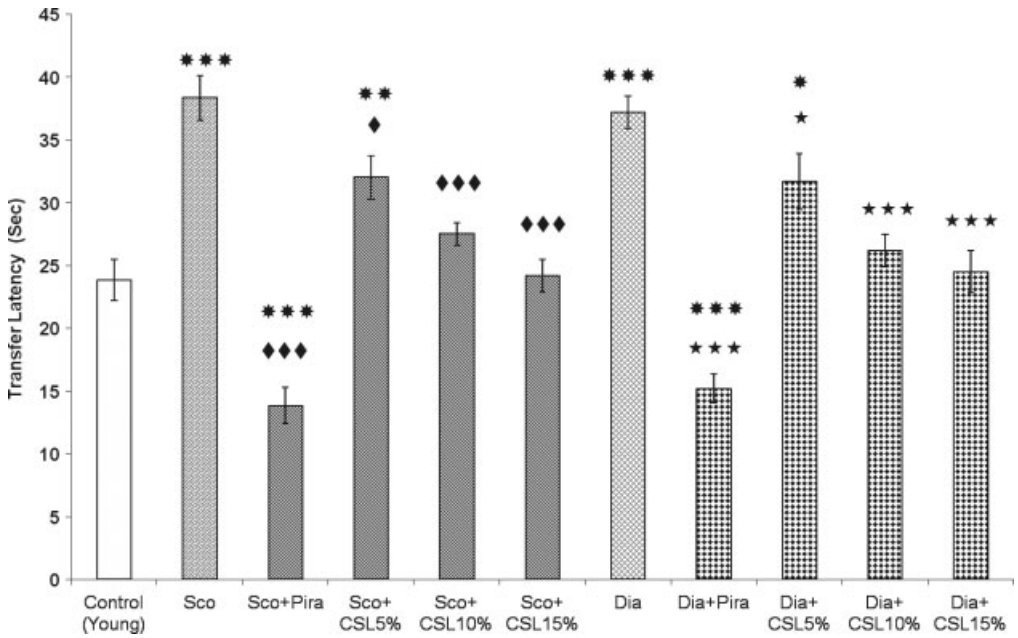

Figure 2. Reversal of scopolamine (0.4 mg kg^{-1}, i.p.) or diazepam (1 mg kg^{-1}, i.p.) induced amnesia by CSL (5%, 10% and 15% w/w of diet) in young mice using elevated plus-maze. Piracetam (Pira) 400 mg kg^{-1} i.p. was used as a positive control. Values are mean ± SEM ($n = 6$); $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ as compared to control group of young mice; $^{♦}P < 0.05$ and $^{♦♦♦}P < 0.001$ as compared to scopolamine (Sco) alone; $^{★}P < 0.05$ and $^{★★★}P < 0.001$ as compared to diazepam (Dia) alone (one-way ANOVA followed by Tukey–Kramer multiple comparisons test).

Step-down latency using passive avoidance paradigm

The concentrations of CSL (5%, 10% and 15% w/w of diet), fed to young and aged mice for 45 days, showed significant ($P < 0.05$) dose-dependent increases in SDL values when compared with their control groups (Fig. 3). Scopolamine and diazepam significantly ($P < 0.001$) decreased SDL values as compared to the control group of young mice, indicating impairment of memory (amnesia). CSL fed for 45 consecutive days caused a significant dose-dependent reversal of memory deficits induced by both scopolamine and diazepam (Fig. 4).

Brain cholinesterase activity

CSL showed a remarkable reduction in brain AChE activity in young and aged mice. In young mice, the percentage of decline in AChE

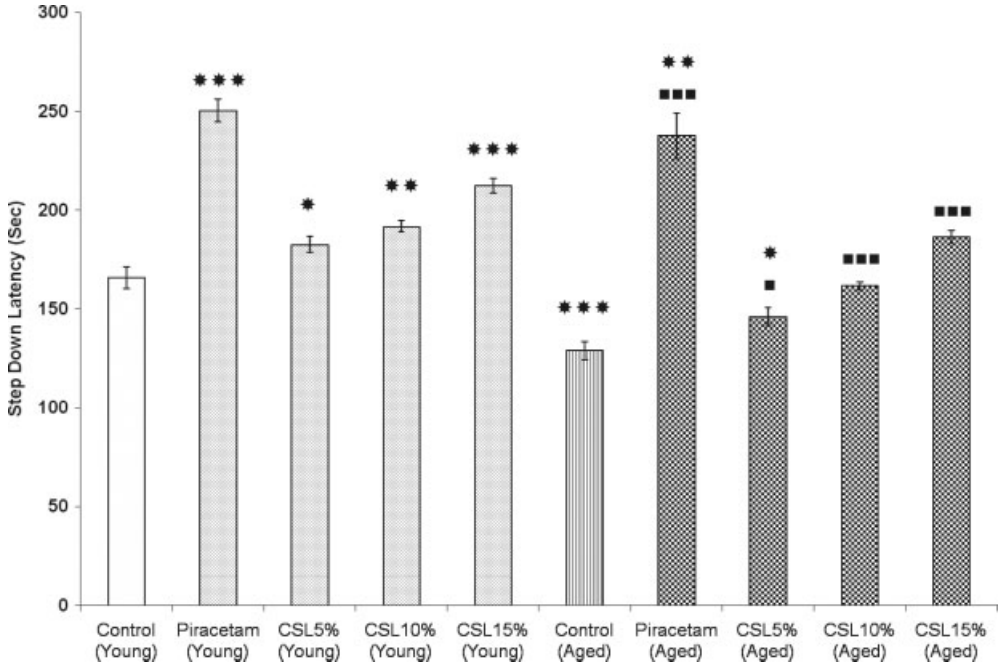

Figure 3. Effect of various concentrations of CSL (5%, 10% and 15% w/w of diet) on SDL of young and aged mice using passive avoidance paradigm. Piracetam (400 mg kg^{-1}, i.p.) was used as a positive control. Values are mean ± SEM ($n = 6$); $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ as compared to control group of young mice; $^{\blacksquare}P < 0.05$ and $^{\blacksquare\blacksquare\blacksquare}P < 0.001$ as compared to control group of aged mice (one-way ANOVA followed by Tukey–Kramer multiple comparisons test).

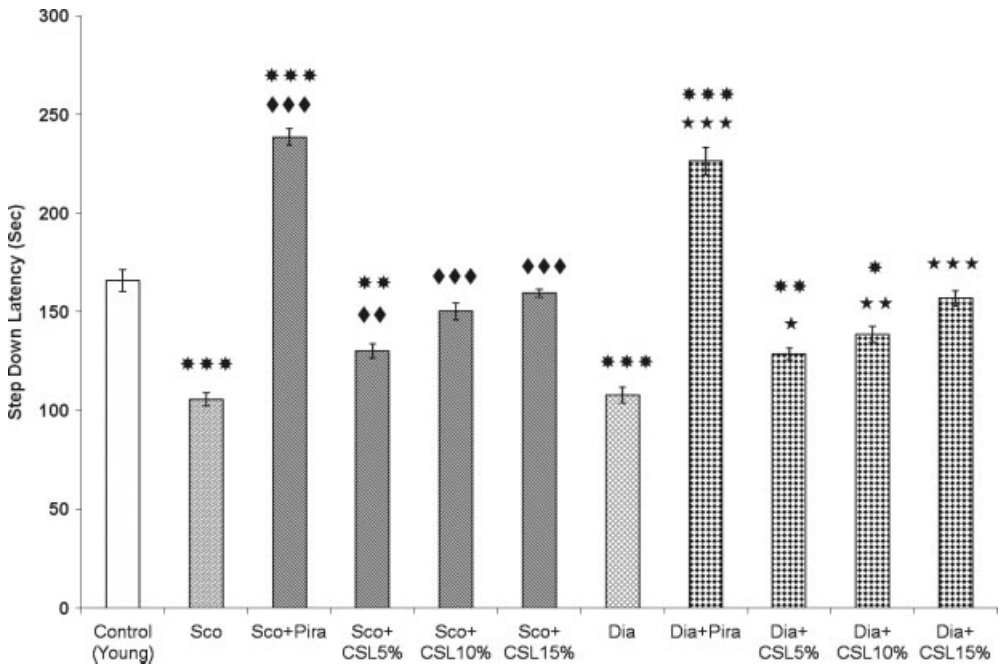

Figure 4. Reversal of scopolamine (0.4 mg kg^{-1}, i.p.) or diazepam (1 mg kg^{-1}, i.p.) induced amnesia by CSL (5%, 10% and 15% w/w of diet) in young mice using passive avoidance paradigm. Piracetam (Pira) 400 mg kg^{-1} i.p. was used as a positive control. Values are mean ± SEM ($n = 6$); $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ as compared to control group of young mice; $^{\blacklozenge\blacklozenge}P < 0.01$ and $^{\blacklozenge\blacklozenge\blacklozenge}P < 0.001$ as compared to scopolamine (Sco) alone; $^{\bigstar}P < 0.05$, $^{\bigstar\bigstar}P < 0.01$ and $^{\bigstar\bigstar\bigstar}P < 0.001$ as compared to diazepam (Dia) alone (one-way ANOVA followed by Tukey–Kramer multiple comparisons test).

activity was 10.3% when fed with CSL at concentrations of 5% w/w ($P < 0.05$), 17.2% at CSL concentrations of 10% w/w ($P < 0.001$) and 23.5% at CSL concentration of 15% w/w ($P < 0.001$) of diet. In the aged mice, they were 8.7% ($P < 0.05$), 12.9% ($P < 0.01$) and 17.3% ($P < 0.001$) at 5, 10 and 15% w/w diet, respectively (Fig. 5). Metrifonate (50 mg kg^{-1}, i.p.) used as a standard drug showed a significant ($P < 0.001$) 24.9% and 26.4% reduction of brain cholinesterase activity in young and aged mice, respectively.

Total serum cholesterol level

Animals receiving CSL showed significant reduction in total cholesterol levels in young ($P < 0.01$) and aged ($P < 0.001$)

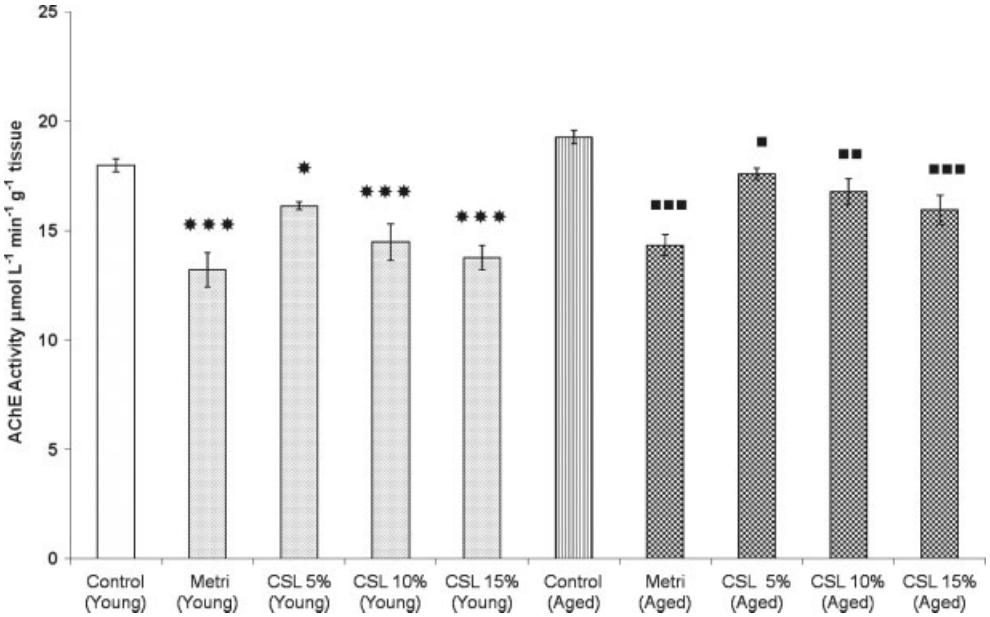

Figure 5. Effect of various concentrations of CSL (5%, 10% and 15% w/w of diet) on brain cholinesterase (AChE) activity of young and aged mice using Ellman's kinetic colorimetric method. Metrifonate (Metri) 50 mg kg^{-1} i.p. was used as a standard drug. Values are mean ± SEM ($n = 6$); $^{*}P < 0.05$ and $^{***}P < 0.001$ as compared to control group of young mice; $^{■}P < 0.05$, $^{■■}P < 0.01$ and $^{■■■}P < 0.001$ as compared to control group of aged mice (one-way ANOVA followed by Tukey–Kramer multiple comparisons test).

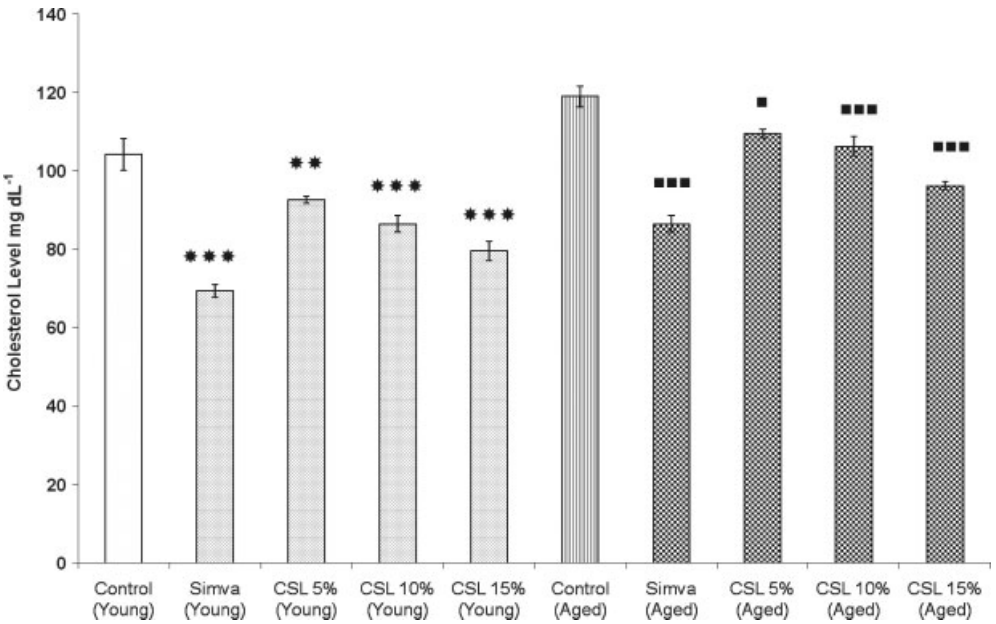

Figure 6. Effect of various concentrations of CSL (5%, 10% and 15% w/w of diet) on serum cholesterol level of young and aged mice using colorimetric method. Simvastatin (Simva) 5 mg kg^{-1} p.o. served as the positive control. Values are mean ± SEM ($n = 6$); $^{**}P < 0.01$ and $^{***}P < 0.001$ as compared to control group of young mice; $^{■}P < 0.05$ and $^{■■■}P < 0.001$ as compared to control group of aged mice (one-way ANOVA followed by Tukey–Kramer multiple comparisons test).

mice (Fig. 6). However, the extent of reduction ($P < 0.001$) in total cholesterol levels with simvastatin was high in both groups.

DISCUSSION

In the present study, CSL, when fed along with a normal diet for 45 consecutive days, improved the memory of mice as reflected by the diminished TL and enhanced SDL values when compared to control animals. Furthermore, chronic administration of CSL for 45 days protected the animals from the development of memory deficits observed after scopolamine/diazepam injection. These findings suggest the possible neuroprotective role of CSL leaves. Additionally, there is no toxicity effect or locomotor changes with CSL.

Acetylcholine is considered the most important neurotransmitter involved in the regulation of cognitive functions. Cognitive dysfunction has been shown to be associated with impaired cholinergic transmission and the facilitation of central cholinergic

transmission with improved memory. Moreover, selective loss of cholinergic neurons in certain brain parts appeared to be a characteristic feature of senile dementia.[25] AChE enzyme controls the concentrations of acetylcholine in brain by degrading acetylcholine. Therefore, AChE inhibitors like donepezil and rivastigmine elevated concentrations of acetylcholine in brain and are found to be useful in the treatment of Alzheimer patients.[26] On parallel lines, addition of coriander leaves to the daily diet may also prove to be beneficial for Alzheimer patients, owing to the AChE inhibitory property shown by CSL in the present study.

Several recent studies suggested a strong link between high cholesterol levels and high incidence of AD.[27] Therefore, a new therapeutic strategy aimed at reducing blood cholesterol levels is gathering momentum in the management of AD. The main histological features of AD include extracellular deposition of β-amyloid (Aβ) plaques, Aβ deposits in blood vessels and intraneuronal neurofibrillary tangles. Abnormal increase in total cholesterol levels increases Aβ in cellular and most animal models of AD; and drugs that inhibit cholesterol synthesis lower Aβ in these models.[28] Interestingly, the animals which received CSL chronically showed significant reduction in serum cholesterol levels.

Oxygen-free radicals and other byproducts of oxidative metabolism have been shown to be neurotoxic, in contrast to antioxidant-rich diets, which improve cerebellar physiology and motor learning in aged rats.[29] Antioxidant activity has been reported to be present in CSL as well.[13] The neuroprotective effect of CSL may be attributed to its antioxidant property, by virtue of which susceptible brain cells are exposed to less oxidative stress, resulting in reduced brain damage and improved neuronal function.

In the present study, we observed that CSL lowered serum cholesterol levels of mice and inhibited AChE enzyme, thereby elevating acetylcholine concentration in brain and ultimately improving memory in both young and aged mice. Furthermore, coriander reversed the memory deficits induced by the ageing process, diazepam or scopolamine. Therefore, the therapeutic potential of CSL in the management of Alzheimer patients may be of great interest.

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