

## Pharmacological Evidence for the Potential of *Daucus carota* in the Management of Cognitive Dysfunctions

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The present study was aimed at investigating the effects of *Daucus carota* seeds on cognitive functions, total serum cholesterol levels and brain cholinesterase activity in mice. The ethanolic extract of *Daucus carota* seeds (DCE) was administered orally in three doses (100, 200, 400 mg/kg) for seven successive days to different groups of young and aged mice. 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. DCE (200, 400 mg/kg, *p.o.*) showed significant improvement in memory scores of young and aged mice. The extent of memory improvement evoked by DCE was 23% at the dose of 200 mg/kg and 35% at the dose of 400 mg/kg in young mice using elevated plus maze. Similarly, significant improvements in memory scores were observed using passive avoidance apparatus and aged mice. Furthermore, DCE reversed the amnesia induced by scopolamine (0.4 mg/kg, *i.p.*) and diazepam (1 mg/kg, *i.p.*). *Daucus carota* extract (200, 400 mg/kg, *p.o.*) reduced significantly the brain acetylcholinesterase activity and cholesterol levels in young and aged mice. The extent of inhibition of brain cholinesterase activity evoked by DCE at the dose of 400 mg/kg was 22% in young and 19% in aged mice. There was a remarkable reduction in total cholesterol level as well, to the extent of 23% in young and 21% in aged animals with this dose of DCE. Therefore, DCE may prove to be a useful remedy for the management of cognitive dysfunctions on account of its multifarious beneficial effects such as, memory improving property, cholesterol lowering property and anticholinesterase activity.

**Key words** *Daucus carota*; amnesia; memory; anti-cholinesterase; cholesterol

Cognition is that operation of mind by means of which, we become aware of our surroundings, objects and thoughts. Alzheimer's disease (AD) represents the most common cause of dementia, affecting more than 15 million individuals worldwide.<sup>1)</sup> It is characterized by progressive memory loss, cognitive impairments, and personality defects accompanied by diffuse structural abnormalities in the brain.<sup>2,3)</sup> Memory forms one of the most complex functions of the brain and ultimately involves multiple neuronal pathways and neurotransmitter systems. One of the most consistent and profound change associated with AD is diminished central cholinergic neurotransmission.<sup>4)</sup> Therefore, facilitation of cholinergic transmission by inhibition of acetyl cholinesterase enzyme can be looked upon as an important strategy in improving cognitive and behavioral functions.<sup>2)</sup> The main histological hallmarks of AD are extraneuronal deposits of  $\beta$ -amyloid (A $\beta$ ) plaques and intra neuronal fibrillary tangles.<sup>5)</sup> Recently, substantial evidence is accumulating in the literature showing a strong link between high cholesterol and high incidence of Alzheimer's disease.<sup>5–7)</sup> Both the generation and clearance of A $\beta$  appear to be regulated by cholesterol. Elevated cholesterol levels increased A $\beta$  in cellular and most animal models of AD whereas, drugs that inhibited cholesterol synthesis lowered A $\beta$ .<sup>6)</sup> Clinical studies indicated that patients with elevated serum cholesterol levels have increased susceptibility to AD,<sup>8–11)</sup> and that the prevalence of AD is higher in countries with high-fat intake.<sup>12)</sup> Therefore, a new approach aimed at reducing blood cholesterol level may prove worthwhile in the management of Alzheimer's disease. Since the Allopathic system of medicine is yet to provide a radical cure for Alzheimer's disease, it is worthwhile to look for new directions, which would minimize the memory loss of patients with neuropsychiatric disorders. The utility of non-toxic plants may be explored for treating patients with dementia.

*Daucus carota* LINN. commonly known as "Carrot" belongs to the family Apiaceae (Umbelliferae) and is cultivated almost all over the world as a useful vegetable. Different parts of this plant are used in Indian systems of medicine for the treatment of a broad spectrum of ailments including kidney dysfunction, asthma, dropsy, inflammation, leprosy and worm troubles.<sup>13,14)</sup> The plant has undergone extensive phytochemical studies and a large number of active ingredients have been isolated. These include volatile oils, steroids, triterpenes, carbohydrates, glycerides, tannins, flavonoids, amino acid, carotene and hydrocarotene.<sup>15–17)</sup> Pharmacological studies showed that *Daucus carota* seeds exhibit antifertility properties.<sup>18,19)</sup> The crude extract of root of the plant has been found to be hypoglycemic<sup>20)</sup> and hepatoprotective.<sup>21)</sup> Indian Materia Medica makes a mention of *Daucus carota* seeds as a brain tonic.<sup>14)</sup> Cholinergic activity was also found in water-soluble fraction of the alcoholic extract of seeds.<sup>22,23)</sup> Since, *Daucus carota* seeds have been reported to contain choline,<sup>22,23)</sup> there is a possibility to elevate the brain acetylcholine levels *via* increased synthesis of Ach, which would in turn prove beneficial in cognitive dysfunctions. Therefore, we were motivated to study the potential of DCE in the management of cognitive dysfunctions.

### MATERIALS AND METHODS

**Plant Material** The seeds of *Daucus carota* were obtained from local market of Hisar, Haryana (India) in the month of January 2005, which were taxonomically identified and authenticated by The Head, Department of Plant Breeding, Chaudhary Charan Singh Agricultural University, Hisar, Haryana (India). A voucher specimen (GJU/PHARM/09/2005) has been preserved at Pharmacology Division of Department of Pharmaceutical Sciences, G. J. University, Hisar,

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India for ready reference.

**Preparation of the Extract** Collected seeds were powdered in hand grinder and defatted with petroleum ether (bp 60–80 °C). The defatted seeds (2 kg) were extracted with 95% ethanol using a soxhlet extractor, at room temperature. After exhaustive extraction, the ethanolic extract was filtered and concentrated by distillation process. A brownish-green colored residue was obtained (yield 8.4% w/w), which was kept in a desiccator. This ethanolic extract of *Daucus carota* seeds (DCE) was used for further experiments.

**Animals** All the experiments were carried out using male, Swiss Albino mice procured from the disease-free small animal house of CCS Haryana Agricultural University, Hisar (Haryana), India. Memory capacities of young and aged animals differ.<sup>24,25</sup> There are several studies showing memory enhancement by estrogen, therefore we focused our project on male young and aged mice.<sup>26,27</sup> Young (3–4 months old) mice weighing around 20 g and aged (12–15 months old) mice weighing around 35 g were used in the present study. The animals had free access to food and water, and they were housed in a natural (12 h each) light–dark cycle. Food given to mice consisted of wheat flour kneaded with water and mixed with a small amount of refined vegetable oil. The animals were acclimatized for at least 5 days (d) to the laboratory conditions before behavioral experiments. Experiments were carried out between 0900 h and 1800 h. 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).

**Drugs** The drugs used in this study were obtained from following drug houses. Scopolamine hydrobromide (Sigma-Aldrich, U.S.A.), diazepam injection (Calmpose, Ranbaxy, India), 5,5-dithiobis-2-nitrobenzoic acid (DTNB), acetylcholine iodide, eserine salicylate, sodium dihydrogen phosphate, disodium hydrogen phosphate (Hi Media, India), piracetam (UCB India Ltd., India), metrifonate (Sigma-Aldrich, U.S.A.), simvastatin (Krebs Biochemicals and Industries Limited, India) and cholesterol diagnostics kit (Erba Diagnostics, Germany).

**Vehicle** Scopolamine hydrobromide, diazepam injection, piracetam and metrifonate were dissolved separately in normal saline and injected intra peritoneally. Plant extract (DCE) and simvastatin were suspended separately with 0.5% w/v carboxymethylcellulose sodium (CMC) and given orally. Volume of oral administrations and i.p. injections were 1 ml/100 g of mouse.

**Acute Toxicity Studies** Acute toxicity studies were performed according to OECD/OCDE guidelines (revised document, October 2000).<sup>28,29</sup> Male Swiss mice selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. DCE was administered orally at a dose of 5 mg/kg initially and mortality if any was observed for 3 d. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then only higher (50, 300, 2000 mg/kg) doses of DCE were employed for further toxic-

ity studies.

**Drug Treatment** In the present investigation, the mice were divided into 52 different groups for employing various interoceptive and exteroceptive memory models and for biochemical estimations. Each group comprised of a minimum of six animals. DCE (100, 200, 400 mg/kg) was administered orally for seven successive days to young and aged mice. After 90 min of the administration of the last dose (on seventh day), mice were exposed to the training session using elevated plus maze and passive avoidance apparatus. Retention (memory) was recorded after 24 h (on eighth day). Amnesia was induced in separate groups (interoceptive models) of young mice by scopolamine (0.4 mg/kg, i.p.) or diazepam (1 mg/kg, i.p.) after 90 min of the last dose of extract (100, 200, 400 mg/kg, *p.o.*) administration on seventh day. The animals were exposed to the training session (on seventh day) after 45 min of scopolamine or diazepam injection. The retention (memory) was measured after 24 h (on eighth day). Piracetam (400 mg/kg, i.p.) was used as an established nootropic agent and was injected for 7 d to positive control groups. DCE (100, 200, 400 mg/kg) was administered orally for seven successive days to separate groups of young and aged mice for biochemical studies. Metrifonate (50 mg/kg, i.p., 60 min before dissecting brain) served as the positive control for comparison of brain cholinesterase activities. Simvastatin (5 mg/kg, *p.o.*, for 7 d) served as the positive control for comparing total serum cholesterol levels. All control group animals received vehicle (0.5% w/v CMC) for seven consecutive days.

**Elevated Plus-Maze** Elevated plus-maze served as the exteroceptive behavioral model to evaluate memory in mice. The procedure, technique and end point for testing memory was followed as per the parameters described by the investigators working in the area of psychopharmacology.<sup>24,25</sup> The elevated plus maze for mice consisted of two open arms (16×5 cm<sup>2</sup>) and two covered arms (16×5×12 cm<sup>3</sup>) extended from a central platform (5×5 cm<sup>2</sup>), and the maze was elevated to a height of 25 cm from the floor.<sup>30</sup> On the first day (*i.e.* seventh 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. TL 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 first day trial (*i.e.* eighth day, 24 h after last dose). Significant reduction in TL value of retention indicated improvement in memory.

**Passive Avoidance Paradigm** Passive Avoidance Behavior based on negative reinforcement was used to examine the long-term memory.<sup>31</sup> The apparatus consisted of a box (27×27×27 cm<sup>3</sup>) having three walls of wood and one wall of Plexiglass, featuring a grid floor (made up of 3 mm stainless steel rods set 8 mm apart), with a wooden platform (10×7×1.7 cm<sup>3</sup>) in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock (20 V, A.C.) was delivered to the grid floor.<sup>32</sup> Training (*i.e.* seventh 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, shocks were delivered for 15 s and the step-down latency (SDL) was recorded. SDL was defined as the time (in seconds) taken by the mouse to step down from the wooden platform to grid floor with all its paws on the grid floor. 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. During the second test, animals were removed from 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.* eighth 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.<sup>33)</sup>

**Collection of Blood and Brain Samples** The animals were sacrificed by cervical decapitation under light anesthesia on the seventh day 90 min after administration of the last doses of DCE or standard drugs or vehicle. Immediately after decapitation, the trunk blood was collected. Then whole brain was carefully removed from the skull. The collected blood was centrifuged at 3000 rpm for 15 min so as to separate serum. The serum was used for estimation of cholesterol levels. The fresh whole brain was weighed and transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of sterile normal saline injection. The homogenate was centrifuged at 3000 rpm for 10 min and the resultant cloudy supernatant liquid was used for estimation of cholinesterase activities.

**Estimation of Brain Cholinesterase** Cholinesterase activity was measured by the method of Ellman *et al.* with a slight modification.<sup>34,35)</sup> Half milliliter of the cloudy supernatant liquid was pipetted out into 25 ml volumetric flask and dilution was made with a freshly prepared DTNB (5,5-dithio-bis-2-nitrobenzoic acid) solution (10 mg DTNB in 100 ml of Sorenson phosphate buffer, pH 8.0). From the volumetric flask, two 4-ml portions were pipetted out into two test tubes. Into one of the test tubes, 2 drops of eserine solution was added. One milliliter of substrate solution (75 mg of acetylcholine iodide per 50 ml of distilled water) was pipetted out into both tubes and incubated for 10 min at 30 °C. The solution in the tube containing eserine was used for zeroing the colorimeter. The resulting yellow color is due to reduction of DTNB by certain substances in the brain homogenate and due to non-enzymatic hydrolysis of substrate. After having calibrated the instrument, change in absorbance per min of the sample was read at 420 nm.<sup>36)</sup>

**Estimation of Serum Total Cholesterol** CHOD-PAP method was used for the estimation of serum total cholesterol.<sup>37,38)</sup> In this method, the blank sample, standard sample and test sample were pipetted into the respective reaction vessels using a micro pipette. For the blank sample, 20  $\mu$ l distilled water and 1000  $\mu$ l working reagent were mixed. For the standard sample, 20  $\mu$ l standard cholesterol and 1000  $\mu$ l working reagent, while for the test sample, 20  $\mu$ l serum and 1000  $\mu$ l working reagent were mixed. These mixtures were incubated for 10 min at 37 °C. The absorbance was read at 510 and 630 nm (Filter 1, Filter 2) against the blank sample by using Autoanalyzer (Erba Mannheim, Chem-5, Plus V<sub>2</sub>).

**Statistical Analysis** All the results were expressed as mean  $\pm$  standard error (S.E.M.). Data was analyzed using one-way ANOVA followed by Dunnett's *t*-test and Student's unpaired *t*-test. *p*-Values < 0.05 were considered as statistically significant.

## RESULTS

**Acute Toxicity Studies** All the doses (5, 50, 300, 2000 mg/kg, *p.o.*) of DCE employed for acute oral toxicity studies were found to be non-toxic. *Daucus carota* extract did not produce any mortality even at the highest dose (2000 mg/kg, *p.o.*) employed. Three submaximal doses (100, 200, 400 mg/kg, *p.o.*), which were found to be safe in mice were employed for further psychopharmacological and biochemical investigations.

**Effect on Transfer Latency (Using Elevated Plus-Maze)** DCE (100 mg/kg) administered for 7 d orally did not have any significant effect on TL of eighth day in elevated plus maze test. DCE (200, 400 mg/kg, *p.o.*) showed dose-dependent reduction in TL of eighth day in young ( $p < 0.05$ ) and aged ( $p < 0.001$ ) animals, when compared to respective control groups indicating significant improvement in memory (Fig. 1). The extent of memory improvement in young mice was 23% at the dose of 200 mg/kg and 35% at the dose of 400 mg/kg of DCE using elevated plus maze. Whereas, the improvement in memory was 31% at the dose of 200 mg/kg and 44% at the dose of 400 mg/kg of DCE in aged mice. Scopolamine (0.4 mg/kg, *i.p.*) and diazepam (1 mg/kg, *i.p.*) injected before training significantly increased ( $p < 0.001$ ) TL of eighth day indicating impairment in memory. The DCE (200, 400 mg/kg, *p.o.* for 7 d) successfully reversed memory deficits induced by scopolamine and diazepam (Fig. 2). Piracetam (used as the positive control) at the dose of 400 mg/kg, *i.p.* improved memory ( $p < 0.001$ ) of both young and aged mice and reversed the amnesia induced by scopolamine and diazepam as expected.

**Effect on Step-Down Latency (Using Passive Avoidance Paradigm)** Step Down Latency (SDL) of eighth day (24 h after last dose) reflected the long-term memory of animals. Significant increase in SDL value indicated improvement in memory. DCE (100 mg/kg, *p.o.*) did not show any significant effect on SDL as compared to the control group of young mice. Ageing process remarkably ( $p < 0.001$ ) reduced SDL of aged mice (Fig. 3). DCE (100 mg/kg, *p.o.*) did not have any effect on ageing induced amnesia as indicated by non-significant changes in SDL values. On the other hand, the higher doses of 200 and 400 mg/kg of DCE administered orally in young ( $p < 0.001$ ) and aged ( $p < 0.05$ ) mice for 7 d markedly increased SDL as compared to the respective control groups (Fig. 3). The extent of improvement in memory scores were 16% in young mice at the dose of 200 mg/kg and 27% at the dose of 400 mg/kg of DCE. Similarly, the improvement in memory scores were 19% at the dose of 200 mg/kg and 33% at the dose of 400 mg/kg in aged mice. Scopolamine (0.4 mg/kg, *i.p.*) and diazepam (1 mg/kg, *i.p.*) significantly ( $p < 0.001$ ) decreased SDL as compared to control group of young mice, indicating impairment of memory (amnesia). DCE administered for 7 d reversed the amnesia induced by both scopolamine and diazepam (Fig. 4). The groups of mice, which were treated with piracetam (400 mg/kg, *i.p.*) for

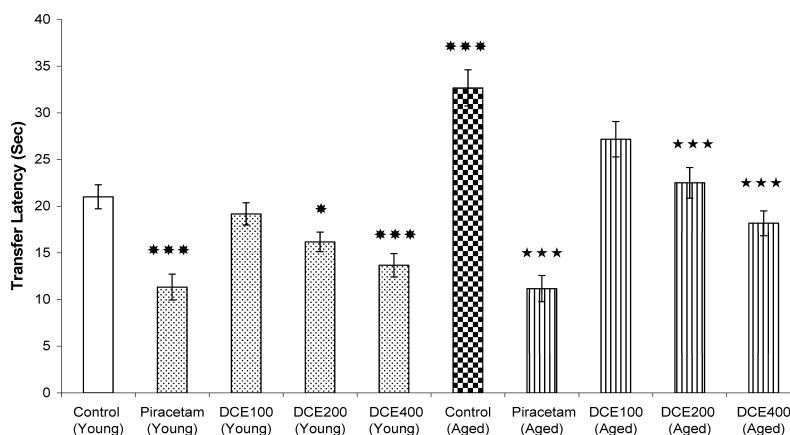


Fig. 1. Effect of Various Concentration of *Daucus carota* Seed Extract (DCE 100, 200, 400 mg/kg) Administered Orally for Seven Successive Days on Transfer Latency of Young (3—4 Months) and Aged (12—15 Months) Mice Using Elevated Plus Maze

Piracetam (400 mg/kg, i.p.) was used as a standard drug. Values are in mean  $\pm$  S.E.M. ( $n=6$ ). \* Denotes  $p<0.05$  as compared to control group of young mice. \*\*\* Denotes  $p<0.001$  as compared to control group of young mice. \*\*\* Denotes  $p<0.001$  as compared to control group of aged mice. One-way ANOVA followed by Dunnett's  $t$ -test and Student's unpaired  $t$ -test.

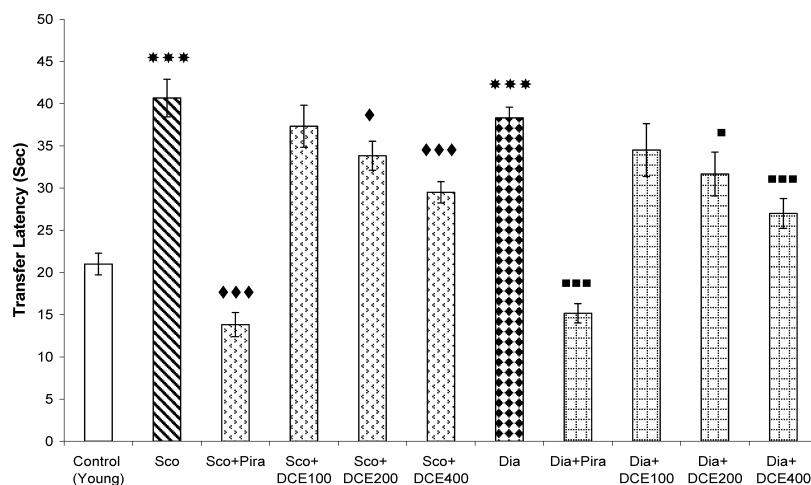


Fig. 2. Reversal of Scopolamine (0.4 mg/kg, i.p.) or Diazepam (1 mg/kg, i.p.) Induced Amnesia by *Daucus carota* Seed Extract (DCE 100, 200, 400 mg/kg) in Young Mice Using Elevated Plus Maze

Piracetam (Pira) 400 mg/kg, i.p. was used as a standard drug. Values are in mean  $\pm$  S.E.M. ( $n=6$ ). \*\*\* Denotes  $p<0.001$  as compared to control group of young mice. ♦ Denotes  $p<0.05$  as compared to scopolamine (Sco) alone. ♦♦♦ Denotes  $p<0.001$  as compared to scopolamine (Sco) alone. ■ Denotes  $p<0.05$  as compared to diazepam (Dia) alone. ■■■ Denotes  $p<0.001$  as compared to diazepam (Dia) alone. One-way ANOVA followed by Dunnett's  $t$ -test and Student's unpaired  $t$ -test.

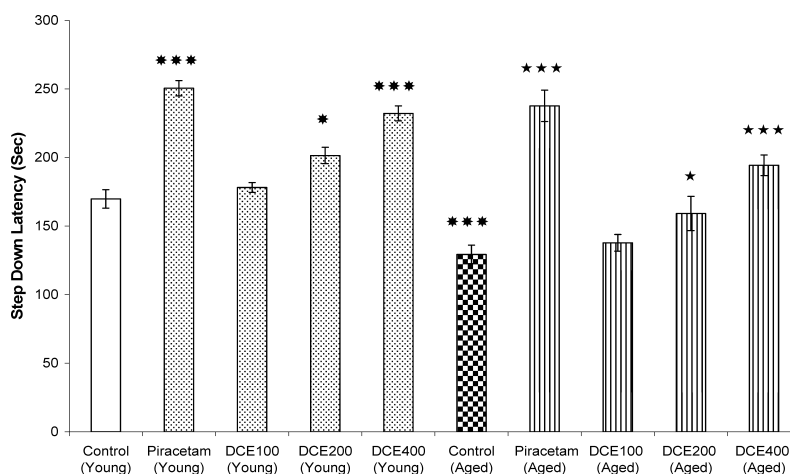


Fig. 3. Effect of Various Concentration of *Daucus carota* Seed Extract (DCE 100, 200, 400 mg/kg) Administered Orally for Seven Successive Days on Step-Down Latency of Young (3—4 Months) and Aged (12—15 Months) Mice Using Passive Avoidance Paradigm

Piracetam (400 mg/kg, i.p.) was used as a standard drug. Values are in mean  $\pm$  S.E.M. ( $n=6$ ). \* Denotes  $p<0.05$  as compared to control group of young mice. \*\*\* Denotes  $p<0.001$  as compared to control group of young mice. \* Denotes  $p<0.05$  as compared to control group of aged mice. \*\*\* Denotes  $p<0.001$  as compared to control group of aged mice. One-way ANOVA followed by Dunnett's  $t$ -test and Student's unpaired  $t$ -test.

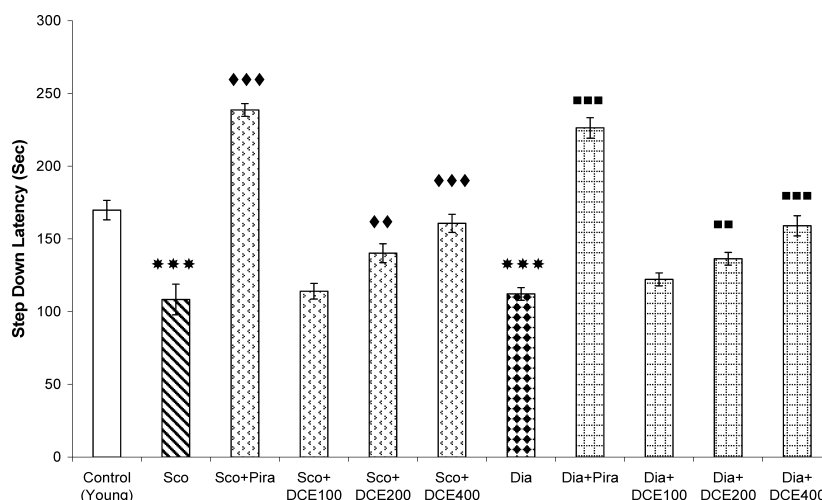


Fig. 4. Reversal of Scopolamine (0.4 mg/kg, i.p.) or Diazepam (1 mg/kg, i.p.) Induced Amnesia by *Daucus carota* Seed Extract (DCE 100, 200, 400 mg/kg) in Young Mice Using Passive Avoidance Paradigm

Piracetam (Pira) 400 mg/kg, i.p. was used as a standard drug. Values are in mean  $\pm$  S.E.M. ( $n=6$ ). \*\*\* Denotes  $p<0.001$  as compared to control group of young mice. ♦♦ Denotes  $p<0.01$  as compared to scopolamine (Sco) alone. ♦♦♦ Denotes  $p<0.001$  as compared to scopolamine (Sco) alone. ■ Denotes  $p<0.01$  as compared to diazepam (Dia) alone. ■■■ Denotes  $p<0.001$  as compared to diazepam (Dia) alone. One-way ANOVA followed by Dunnett's  $t$ -test and Student's unpaired  $t$ -test.

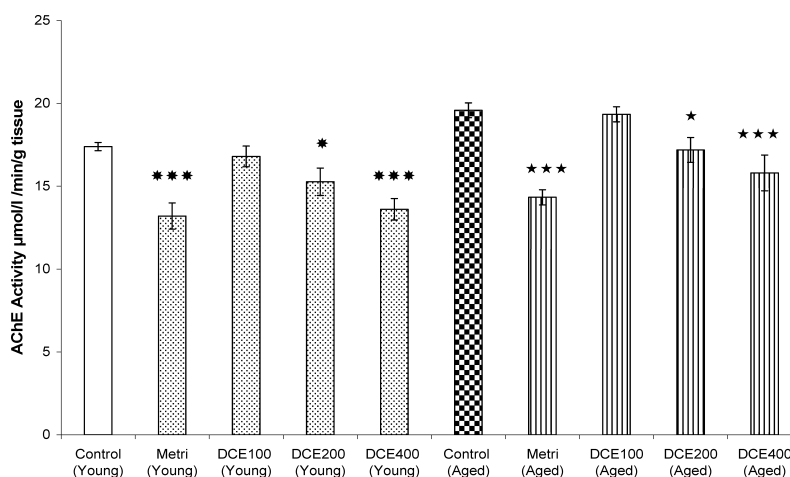


Fig. 5. Effect of Various Concentrations of *Daucus carota* Seed Extract (DCE 100, 200, 400 mg/kg) Administered Orally for Seven Successive Days on Brain Cholinesterase (AChE) Activity of Young (3–4 Months) and Aged (12–15 Months) Mice Using Ellman's Kinetic Colorimetric Method

Metrifonate (Metri) 50 mg/kg, i.p. was used as a standard drug. Values are in mean  $\pm$  S.E.M. ( $n=6$ ). \* Denotes  $p<0.05$  as compared to control group of young mice. \*\*\* Denotes  $p<0.001$  as compared to control group of young mice. \* Denotes  $p<0.05$  as compared to control group of aged mice. \*\*\* Denotes  $p<0.001$  as compared to control group of aged mice. One-way ANOVA followed by Dunnett's  $t$ -test.

seven successive days showed improvement ( $p<0.001$ ) in memory of young as well as aged mice. Piracetam also reversed amnesia induced by scopolamine and diazepam.

**Effect on Brain Cholinesterase Activity** The lowest dose of DCE (100 mg/kg, *p.o.*) employed in the present study, did not produce any effect on cholinesterase activity in young and aged mice. However, in higher doses (200, 400 mg/kg, *p.o.*) DCE showed a remarkable reduction ( $p<0.05$ ) in brain cholinesterase activity in young and aged mice, as compared to respective control groups by using Ellman's kinetic colorimetric method (Fig. 5). The percentage reductions in cholinesterase activity were 12% at the dose of 200 mg/kg and 22% at the dose of 400 mg/kg in young mice. Whereas, the inhibition of cholinesterase activity was 12% at the dose of 200 mg/kg and 19% at the dose of 400 mg/kg in aged mice. Metrifonate (50 mg/kg, i.p.) used as a standard drug showed significant ( $p<0.001$ ) reduction of brain

cholinesterase activity in young (24%) and aged (27%) mice as expected (Fig. 5).

**Effect on Total Cholesterol Level** DCE (100 mg/kg, *p.o.*) did not show any significant change in total serum cholesterol levels of young and aged mice. The animals receiving DCE (200, 400 mg/kg, *p.o.*) for 7 d consecutively showed significant reduction in total serum cholesterol levels of young ( $p<0.05$ ) as well as aged ( $p<0.001$ ) mice, when tested using Autoanalyzer following colorimetric method (Fig. 6). The extent of reduction in total cholesterol levels of young mice were 10% and 23% at doses of 200 and 400 mg/kg of DCE respectively. Similarly, the reductions were 14% at the dose of 200 mg/kg and 21% at the dose of 400 mg/kg in aged mice. The extent of reductions ( $p<0.001$ ) in total cholesterol levels with simvastatin (a standard cholesterol lowering agent) were 32% in young animals and 28% in aged animals (Fig. 6).

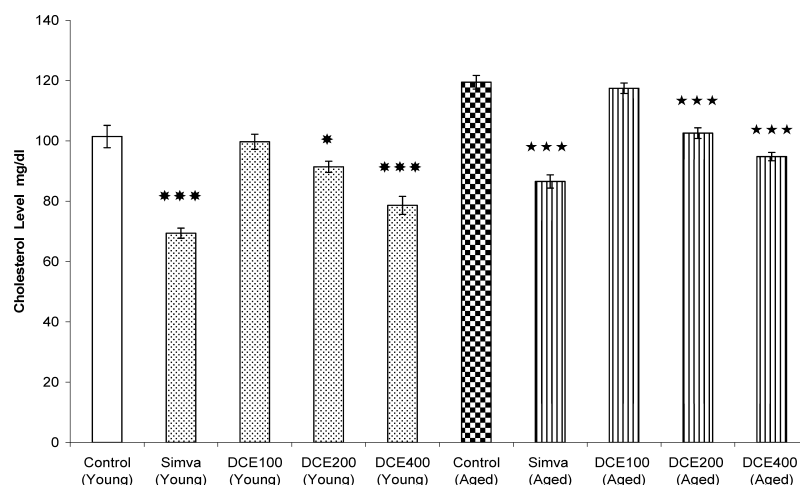


Fig. 6. Effect of Various Concentrations of *Daucus carota* Seed Extract (DCE 100, 200, 400 mg/kg) Administered Orally for Seven Successive Days on Serum Cholesterol Level of Young (3—4 Months) and Aged (12—15 Months) Mice Using Colorimetric Method

Simvastatin (Simva) 5 mg/kg, *p.o.* served as the positive control. Values are in mean  $\pm$  S.E.M. ( $n=6$ ). \* Denotes  $p<0.05$  as compared to control group of young mice. \*\*\* Denotes  $p<0.001$  as compared to control group of young mice. \*\*\* Denotes  $p<0.001$  as compared to control group of aged mice. One-way ANOVA followed by Dunnett's *t*-test.

## DISCUSSION

Cognition includes all aspects of perceiving, learning, thinking and remembering. The cognitive dysfunctions include delirium, behavioral disorders and dementia. Dementia (memory loss) is a common disorder of elderly individuals.<sup>39</sup> Presently, there are no satisfactory diagnostic procedures and therapeutic regimens available for the management of cognitive dysfunctions. In the present study, we have focused upon exploring the potential of DCE in reversing the memory deficits. Amnesia was induced in mice by intraperitoneal injection of scopolamine or diazepam, in addition to ageing induced amnesia (a natural process). DCE successfully reversed scopolamine, diazepam or ageing-induced amnesia, when administered for 7 d. Piracetam, the established nootropic agent was used in the present study for comparison because, it improves memory as a net result of several protective actions such as increased resistance to adverse conditions, brain protection against physical and chemical injuries and enhancement of reserve energy stores.<sup>40</sup> Piracetam also increases choline uptake in cholinergic nerve endings, thereby facilitating cholinergic transmission in brain.<sup>41,42</sup> There is a serious decline in the density of acetylcholine receptors in elderly individuals. Piracetam elevates the density of frontal cortex acetylcholine receptors by 30—40% restoring the levels of acetylcholine in the brain.<sup>43</sup>

There are extensive evidences linking the central cholinergic system to memory.<sup>44—46</sup> Cognitive dysfunction has been shown to be associated with impaired cholinergic function and the facilitation of central cholinergic activity with improved memory.<sup>47</sup> Selective loss of cholinergic neurons and decrease in cholinacetyltransferase activity was reported to be a characteristic feature of senile dementia of the Alzheimer's type.<sup>48</sup> Anticholinesterases such as Metrifonate,<sup>49</sup> Physostigmine, Tacarine, Donepezil,<sup>50</sup> Huperzine-A,<sup>51</sup> Rivastigmine,<sup>52</sup> Galanthamine<sup>53</sup> and Eptastigmine<sup>54</sup> have all been shown to reverse amnesia produced by disruption of cholinergic system. Enzyme choline acetyltransferase is involved in the synthesis of acetylcholine and acetylcholinesterase is involved in the degradation of acetyl-

choline. Acetylcholine is synthesized from choline and acetyl Co. enzyme A in the presence of choline acetyltransferase.<sup>41,42</sup> The quaternary base chlorides separated from the seeds of *Daucus carota* were rich in choline content and exhibited procholinergic activity.<sup>22,23</sup> Thus, it is possible that enhanced cholinergic transmission resulting from increased acetylcholine synthesis in brain due to abundant availability of choline and reduction of brain cholinesterase activity in young and aged mice may explain the memory improving effect exhibited by DCE.

Cholesterol turnover appears to play a crucial role in the deposition and clearance of amyloid peptides in brain. Clinical studies suggested that the net brain cholesterol concentration is regulated by serum cholesterol level and that there is a cross-talk between the CNS and peripheral cholesterol pools.<sup>55—58</sup> Therefore, it is plausible that peripheral cholesterol levels modulate CNS cholesterol levels and *vice versa*. Epidemiological studies revealed that individuals with high peripheral cholesterol levels show more susceptibility to AD,<sup>8—11</sup> and that the incidence of AD is higher in countries with high-fat and high-calorie diets.<sup>12</sup> In addition, lovastatin and pravastatin, which reduce serum cholesterol levels provided protection against AD.<sup>59,60</sup> Furthermore, serum cholesterol, atherosclerosis, apolipoprotein E (ApoE), and AD all appear to be interconnected.<sup>8—10,61</sup> ApoE is a cholesterol transporting protein that is associated with amyloid deposits.<sup>61—63</sup> Elevated serum cholesterol levels not only lead to atherosclerosis but also carry a high risk of developing AD.<sup>64</sup> Thus, the cholesterol lowering activity exhibited by DCE in the present study may be preventing the accumulation of  $\beta$ -amyloid plaques and intraneuronal neurofibrillary tangles.

It has been observed that elderly patients suffering from Alzheimer's disease showed reduction in symptoms of Alzheimer's disease upon chronic use of anti-inflammatory drugs.<sup>65,66</sup> Epidemiological studies have almost confirmed that non steroidal anti-inflammatory drugs reduced the incidence of AD.<sup>65—67</sup> Compounds such as Geraniol, 2,4,5-trimethoxy benzaldehyde (TMB), oleic acid and transasarone isolated from *Daucus carota* seeds have been shown

to possess anti-inflammatory action in rodents.<sup>68)</sup> The compound TMB showed higher selectivity towards cyclooxygenase (COX)-II enzyme and the COX-II/COX-I ratio was 17.68, which was much higher than ibuprofen and naproxen.<sup>68)</sup> Interestingly, indomethacin (*Indocin*, *Indomethagan*) and ibuprofen (*Profen*, *Ibuprohm*), which are marketed for their non steroidal anti-inflammatory activity have been found to be beneficial in atherosclerosis as well as AD by virtue of their cholesterol lowering property.<sup>69–72)</sup> Thus, the memory enhancing effect showed by DCE in the present study may be dependent upon its cholinesterase inhibiting activity, cholesterol lowering effect and the anti-inflammatory action.

## CONCLUSION

In the present study, *Daucus carota* seeds extract reversed the memory deficits induced by scopolamine or diazepam in young mice. Furthermore, DCE improved the retention capacity of aged mice, when administrated orally for 7 d. These behavioral effects of DCE might prove helpful in the management of cognitive dysfunctions seen in elderly patients.

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