

Anti-Dementia Potential of *Daucus carota* Seed Extract in Rats

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Summary

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. Carrot is widely consumed as an aphrodisiac and nervine tonic and its scraped root is used as a local stimulant for indolent ulcers. In light of the above, the present study was undertaken to investigate the effects of *D. carota* seeds on memory in rats. The ethanolic extract of *Daucus carota* (DCE) was administered orally in three doses (100, 200 and 400 mg/kg) for seven successive days to different groups of young and aged rats. Elevated plus-maze, Hebb-Williams maze and hexagonal swimming pool served as the exteroceptive behavioral models for testing memory. Diazepam-, scopolamine- and ageing-induced amnesia served as the interoceptive behavioral models. DCE (200 and 400 mg/kg, p.o.) showed significant improvement in memory of young and aged rats by using elevated plus maze, Hebb Williams maze and hexagonal swimming pool. DCE also reversed the amnesia induced by scopolamine (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.). These finding suggest that *D. carota* seeds appear to be a promising candidate for improving memory and it would be worthwhile to explore the potential of this plant in the management of Alzheimer patients.

Key words: *Daucus carota*, amnesia, memory, Elevated plus maze, Hebb Williams maze, Hexagonal swimming pool.

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Introduction

Dementia is related to progressive loss of memory, and one other cognitive disturbance, like speech disorder and loss of space orientation. It is a common disorder in older persons as approximately 10 percent of adults 65 years and older, and 50 percent of adults older than 90 years, have dementia. According to the latest estimate, 35 million will suffer from dementia by 2010 [1]. At present, there is no effective treatment available, causing research on reducing or preventing dementia particularly important. In fact, there is growing evidence in the recent years on nutritional interventions especially fruits and vegetables in reducing the risk of developing dementia [2,3].

Daucus carota Linn. commonly known as "carrot" belongs to the family Apiaceae (Umbelliferae), is widely consumed as an aphrodisiac and nervine tonic and its scraped root is used as a local stimulant for indolent ulcers [4]. Phytochemical analysis of the plant showed that it contains volatile oils, steroids, triterpenes, carbohydrates, glycerides, tannins, flavonoids, amino acid, carotene and hydrocarotene [5-7]. Pharmacological studies found the crude extract of *D. carota* roots to be hypoglycemic and hepatoprotective while the seeds to exhibit antifertility properties [8-11]. *D. carota* seeds have been noted as a brain tonic in Indian Materia Medica [12]. These seeds which contain choline, have been reported to inhibit brain cholinesterase activity, there is a possibility to elevate the brain acetylcholine levels via increased synthesis of acetylcholine, which would in turn prove beneficial in cognitive dysfunctions [13, 14]. In the present study the potential of DCE in the management of cognitive dysfunctions is evaluated.

Materials and Methods

Plant Material

The seeds of *D. carota* were obtained from a local market of Hisar, Haryana (India), which were taxonomically identified and authenticated by Chaudhary Charan Singh of Plant Breeding Department, Agricultural University, Hisar, Haryana, India. A voucher specimen (GJU/PHARM/09) was deposited at Pharmacology Division of Department of Pharmaceutical Sciences, G.J. University of Science and Technology, Hisar, India for ready reference.

Preparation of the Extract

Collected seeds were powdered in hand grinder and defatted with petroleum ether (b.p. 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 *D. carota* seeds (DCE) was used for further experiments.

Animals

The experiments were carried out using male, Wistar rats procured from the disease-free small animal house of CCS Haryana Agricultural University, Hisar (Haryana), India. Young (3-4 months old) rats weighing around 150 g and aged (12-15 months old) rats weighing around 250 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 animals 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 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 and Vehicle

Scopolamine hydrobromide (Sigma-Aldrich, USA), diazepam injection (Calmpose®, Ranbaxy, India) and piracetam (UCB India Ltd., India) were used. Plant extract (DCE) was suspended in 2 % w/v gum acacia and administered orally in rats. Scopolamine hydrobromide, diazepam and piracetam were dissolved separately in normal saline and injected i.p. Volume of oral administration and i.p. injection was 1 ml/ kg of rat.

Drug Treatment

In the present investigation, the rats were divided into different groups (six animals per group) for the various interoceptive and exteroceptive memory models. The DCE (100, 200 and 400 mg/kg) was administered orally for seven successive days to young and aged rats. After 90 min of the administration of the last dose (on seventh day), rats were exposed to the training session using elevated plus maze, Hebb-Williams maze and hexagonal swimming pool. Retention (memory) was recorded after 24 h (on eighth day). Amnesia was induced in separate groups (interoceptive models) of young rats 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 and 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 seven days to positive control groups. All control group animals received vehicle (2 % w/v gum acacia) for seven consecutive days.

Elevated Plus-maze

Elevated plus-maze served as the exteroceptive behavioral model to evaluate memory in rats. 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 [15-17]. The elevated plus maze apparatus for rats consisted of a central platform (10 cm²) connected to two open arms (50 cm × 10 cm) and two covered (enclosed) arms (50 cm × 40 cm × 10 cm) and the maze was elevated to a height of 50 cm from the floor [18]. On the first day (i.e. seventh day of drug treatment), each rat 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 any one of the covered arms with all its four legs. TL was recorded on the first day (training session) for each animal. The rat 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.

Hebb-Williams Maze

Hebb-Williams maze is an incentive based exteroceptive behavioural model useful for measuring spatial working memory of rats [18]. It consists of mainly three components. Animal Chamber (or start box), which is attached to the middle chamber (or exploratory area) and a reward chamber at the other end of the maze in which the reward (food) is kept. All the three components are provided with guillotine removable doors. On the first day (i.e. seventh day of drug treatment), each rat was placed in the animal chamber or start box and the door was opened to facilitate the entry of the animal into the next chamber. The door of start box was closed immediately after the animal moved into the next chamber so as to prevent back-entry. Time taken by the animal to reach reward chamber (TRC) from start box was recorded on first day (training session) for each animal. Each animal was allowed to explore the maze for 3 minutes with all the doors opened before returning to its home cage. Retention of this learned task (memory) was examined 24 h after the first day trial (i.e. eighth day, 24h after last dose) [17].

Hexagonal Swimming Pool

A specially designed hexagonal swimming pool (with each side of hexagon 75 cm, diagonal length of 150 cm and depth around 60 cm) was employed for swimming task. A hidden platform was provided to the animals as the only means of escape from water. The rigid square (11 X 11 cm), and 29 cm long platform was placed 1 cm below the water surface. The pool was filled with water up to a height of 30 cm, which was made opaque by adding non-toxic white colour to it so that there is no visible cue to animals regarding the spatial location of the platform. On the first day (i.e. seventh day of drug treatment) each rat was placed in the swimming pool just opposite to the hidden platform. The time taken in seconds by the animal to swim from the starting point to the hidden platform was taken as escape latency time (ELT). ELT recorded on the first day was considered as training session. Animals were allowed to explore the platform for additional 20 seconds. Retention of this learned-task (memory) was examined 24 h after the first day trial (i.e. eighth day, 24h after last dose). Significant reduction in ELT value of retention indicated improvement in memory [19-21].

Statistical Analysis

All the results were expressed as mean \pm standard error (SEM). 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

Effect on Transfer Latency (Using Elevated Plus-Maze)

Transfer latency of second day reflected retention of information or memory. DCE (100 mg/kg) administered for seven days orally did not have any significant effect on TL of eighth day in elevated plus maze test. The young ($P <0.01$; $P <0.001$, respectively) and aged ($P <0.05$; $P <0.001$, respectively) animals treated orally with 200 mg/kg and 400 mg/kg showed remarkable reduction in TL of eighth day, indicating significant improvement in memory (Fig.1). Scopolamine hydrobromide (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) injected before training significantly increased ($P <0.001$) TL on day eighth indicating impairment in memory (Fig. 2). The DCE at higher dose levels (200 and 400 mg/kg, p.o.) for 7 successive days successfully reversed memory deficits induced by scopolamine and diazepam. Piracetam (used as the positive control) at a dose of 400 mg/kg, i.p. also improved memory ($P <0.001$) in both

young and aged rats and reversed the amnesia induced by scopolamine and diazepam as expected.

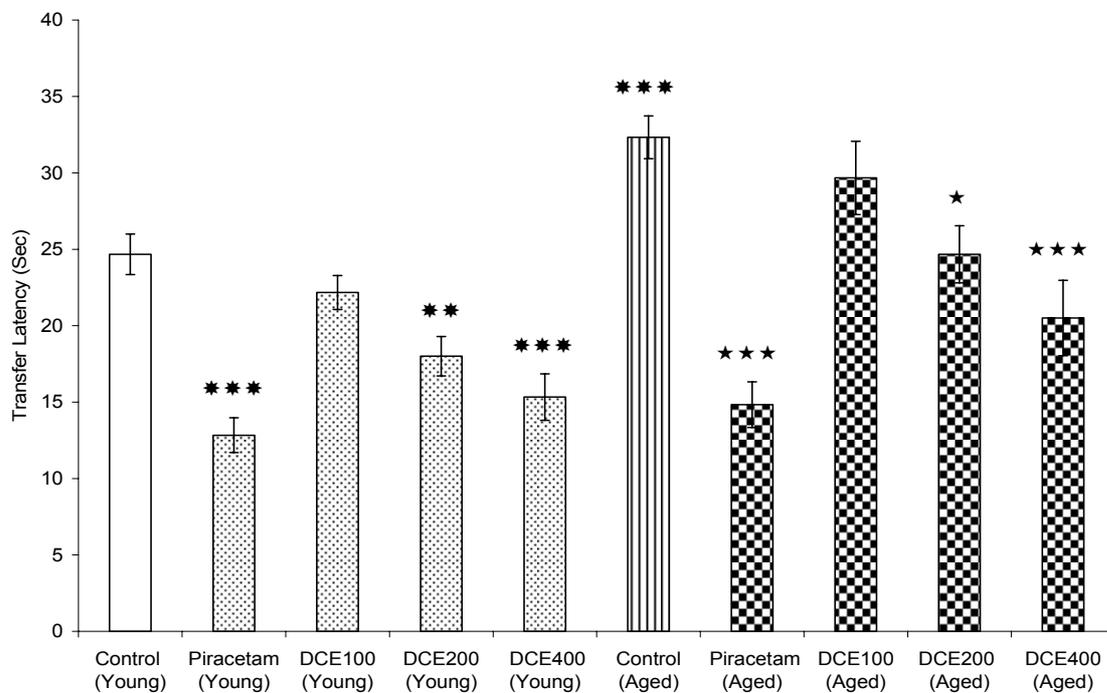


Fig 1

Effect of various concentration of *Daucus carota* seeds extract (DCE 100, 200 and 400mg/kg) administered orally for seven successive days on transfer latency of young (3-4 months) and aged (12-15 months) rats using elevated plus maze. Piracetam (400 mg/kg, i.p.) was used as a standard drug.

Values are in mean ± SEM. (n=6)

- *** $P < 0.01$ as compared to control group of young rats.
- *** $P < 0.001$ as compared to control group of young rats.
- * $P < 0.05$ as compared to control group of aged rats.
- *** $P < 0.001$ as compared to control group of aged rats.

(One-way ANOVA followed by Dunnett's t-test and student's unpaired t-test)

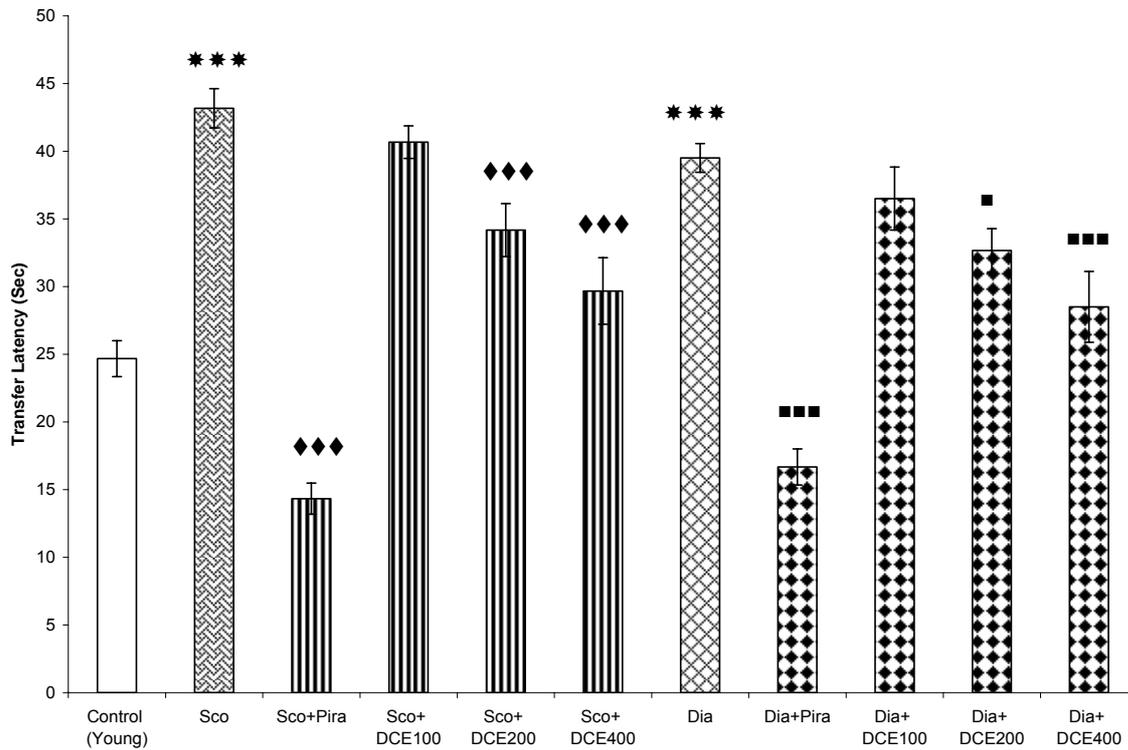


Fig 2
Reversal of scopolamine (0.4 mg/kg, i.p.) or diazepam (1 mg/kg, i.p.) induced amnesia by *Daucus carota* seeds extract (DCE 100, 200 and 400mg/kg) in young rats using elevated plus maze. Piracetam (Pira) 400 mg/kg, i.p. was used as a standard drug.

Values are in mean ± SEM. (n=6)

*** $P < 0.001$ as compared to control group of young rats.

◆◆◆ $P < 0.001$ as compared to scopolamine (Sco) alone.

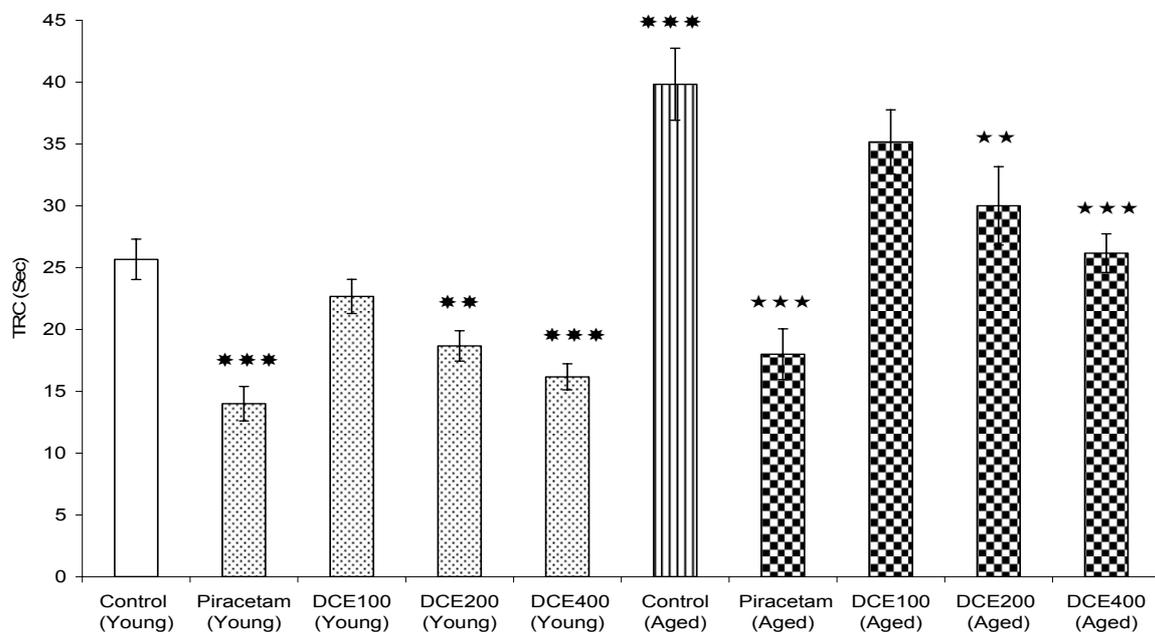
■ $P < 0.05$ as compared to diazepam (Dia) alone.

■■■ $P < 0.001$ as compared to diazepam (Dia) alone.

(One-way ANOVA followed by Dunnett's t-test and student's unpaired t-test)

Effect on Time Taken to Reach Reward Chamber (Using Hebb-Williams Maze)

Time taken to reach reward chamber (TRC) of eighth day (24h after last dose) reflected the memory of animals. Significant reduction in TRC value indicated improvement in memory. DCE (100 mg/kg, p.o.) did not show any significant effect on TRC in young or aged rats when compared to the control group but ageing process remarkably ($P < 0.001$) increased TRC (Fig. 3). On the other hand, at higher doses of 200 and 400 mg/kg of DCE administered orally in young and aged rats for seven days markedly reduced ($P < 0.01$) TRC as compared to the respective control groups (Fig. 3). Scopolamine (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) significantly increased ($P < 0.001$) TRC as compared to control group of young rats, indicating impairment of memory (amnesia). DCE administered for seven days reversed the amnesia induced by both scopolamine and diazepam (Fig. 4). The groups of rats, which were treated with piracetam (400 mg/kg, i.p.) for seven successive days showed improvement ($P < 0.001$) in memory of young as well as aged rats. Piracetam also reversed amnesia induced by scopolamine and diazepam.

**Fig 3**

Effect of various concentration of *Daucus carota* seeds extract (DCE 100, 200 and 400mg/kg) administered orally for seven successive days on TRC of young (3-4 months) and aged (12-15 months) rats using Hebb -Williams maze. Piracetam (400 mg/kg, i.p.) was used as a standard drug.

Values are in mean ± SEM. (n=6)

- *** $P < 0.01$ as compared to control group of young rats.
- *** $P < 0.001$ as compared to control group of young rats.
- ** $P < 0.01$ as compared to control group of aged rats.
- *** $P < 0.001$ as compared to control group of aged rats.

(One-way ANOVA followed by Dunnett's t-test and student's unpaired t-test)

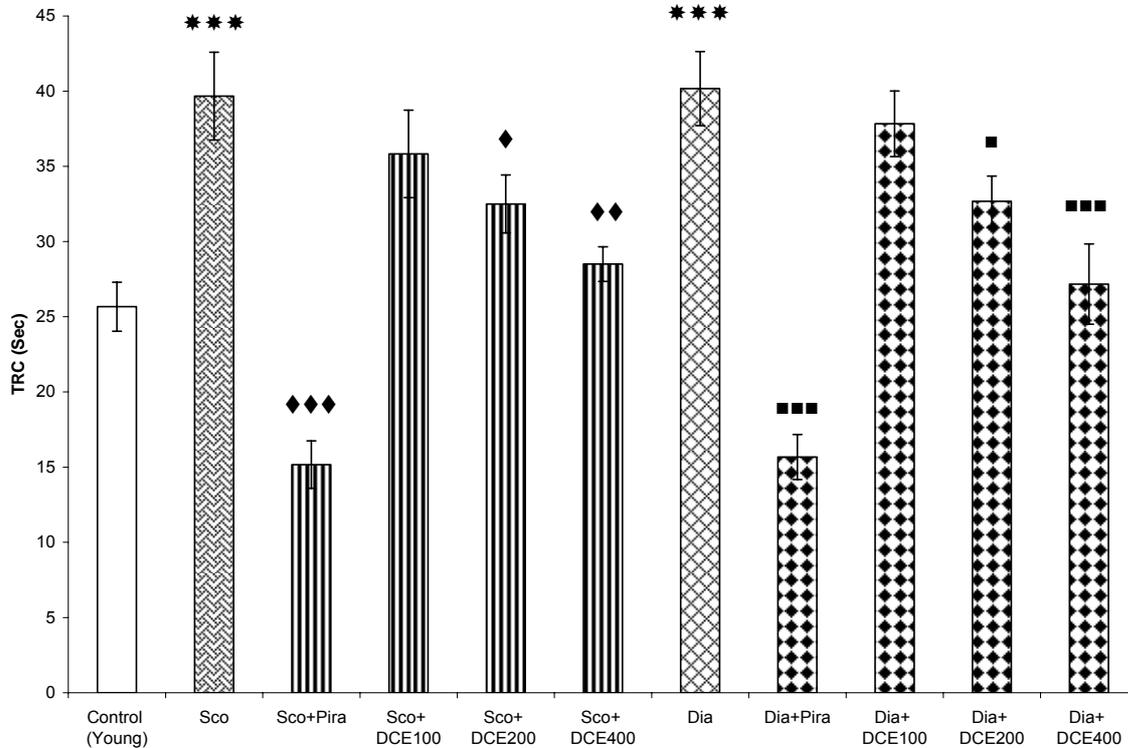


Fig 4
Reversal of scopolamine (0.4 mg/kg, i.p.) or diazepam (1 mg/kg, i.p.) induced amnesia by *Daucus carota* seeds extract (DCE 100, 200 and 400mg/kg) in young rats using Hebb-Williams maze. Piracetam (Pira) 400 mg/kg, i.p. was used as a standard drug.

Values are in mean ± SEM. (n=6)

***P<0.001 as compared to control group of young rats.

◆P<0.05 as compared to scopolamine (Sco) alone.

◆◆P<0.01 as compared to scopolamine (Sco) alone.

◆◆◆P<0.001 as compared to scopolamine (Sco) alone.

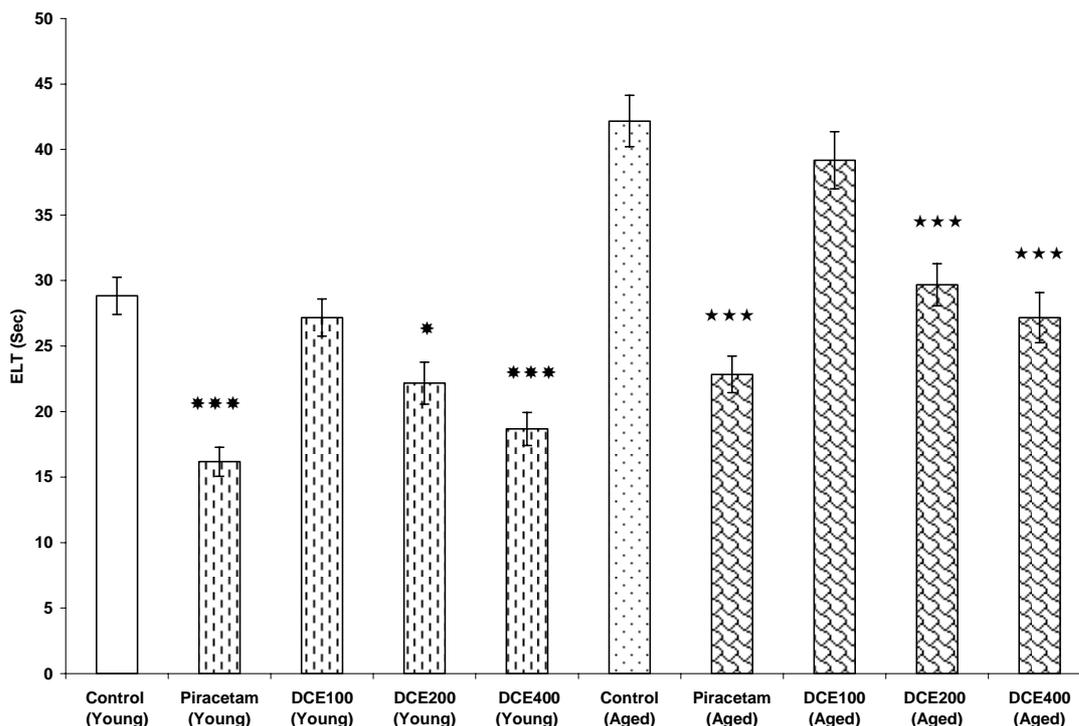
■P<0.05 as compared to diazepam (Dia) alone.

■◆◆P<0.001 as compared to diazepam (Dia) alone.

(One-way ANOVA followed by Dunnett's t-test and student's unpaired t-test)

Effect on Escape Latency Time (ELT) of Rats Using Hexagonal Swimming Pool

DCE (200 and 400 mg/kg) administered orally in young ($P < 0.05$) and aged ($P < 0.001$) rats for 7 days markedly decreased ELT as compared to the respective control groups (Fig.5). Scopolamine (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) significantly ($P < 0.001$) increased ELT as compared to control group of young mice, indicating impairment of memory (amnesia). DCE administered for 7 days reversed the amnesia induced by both scopolamine and diazepam (Fig.6). The group of rats, which were treated with piracetam (400 mg/kg, i.p.) showed improvement ($P < 0.001$) in memory of young as well as aged rats. Piracetam also reversed amnesia induced by scopolamine and diazepam.

**Fig 5**

Effect of various concentration of *Daucus carota* seeds extract (DCE 100, 200 and 400mg/kg) administered orally for seven successive days on ELT of young (3-4 months) and aged (12-15 months) rats using hexagonal swimming pool. Piracetam (400 mg/kg, i.p.) was used as a standard drug.

Values are in mean \pm SEM. (n=6)

* $P < 0.05$ as compared to control group of young rats.

*** $P < 0.001$ as compared to control group of young rats.

*** $P < 0.001$ as compared to control group of aged rats.

(One-way ANOVA followed by Dunnett's t-test and student's unpaired t-test)

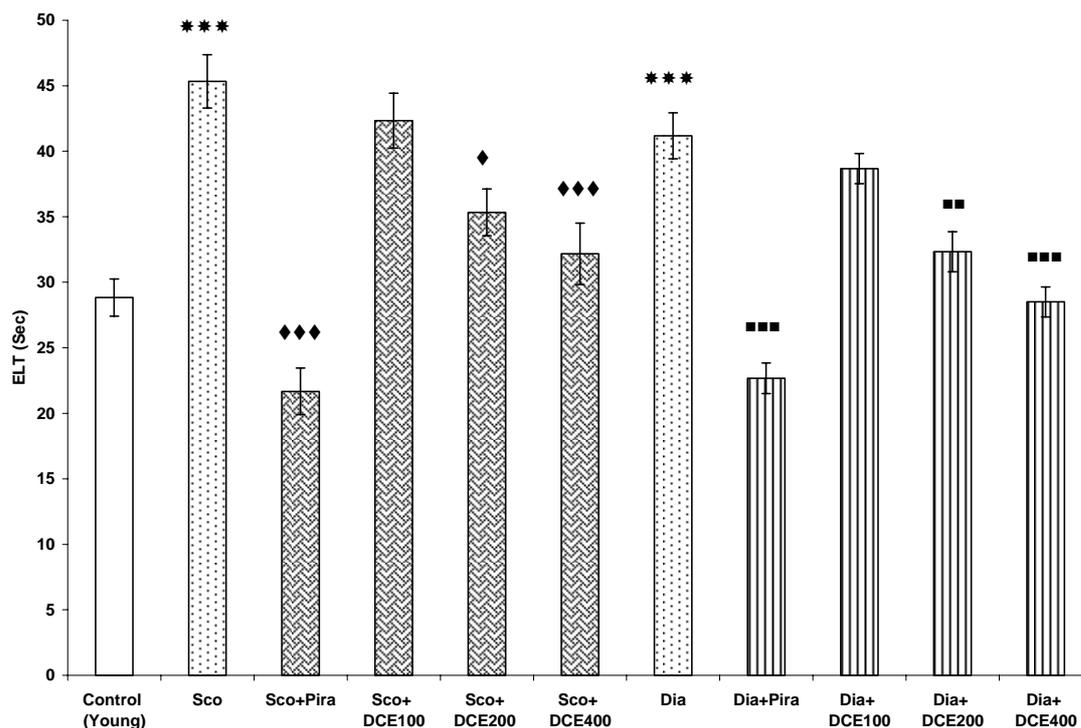


Fig 6

Reversal of scopolamine (0.4 mg/kg, i.p.) or diazepam (1 mg/kg, i.p.) induced amnesia by *Daucus carota* seeds extract (DCE 100, 200 and 400mg/kg) in young rats using hexagonal swimming pool. Piracetam (Pira) 400 mg/kg, i.p. was used as a standard drug.

Values are in mean \pm SEM. (n=6)

*** P <0.001 as compared to control group of young rats.

◆ P <0.05 as compared to scopolamine (Sco) alone.

◆◆◆ P <0.001 as compared to scopolamine (Sco) alone.

■■ P <0.01 as compared to diazepam (Dia) alone.

■■■ P <0.001 as compared to diazepam (Dia) alone.

(One-way ANOVA followed by Dunnett's t-test and student's unpaired t-test)

Discussion

Cognition includes all aspects of perceiving, learning, thinking and remembering. The cognitive dysfunctions include delirium, behavioural disorders and dementia. Dementia (memory loss) is a common disorder of elderly individuals [22]. Currently; 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 (200 and 400 mg/kg) in reversing the memory deficits. Amnesia was induced in rats 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 seven days. Piracetam, the established nootropic agent was used in the present study for comparison.

The main histological features of AD include extracellular protein deposits termed as β -amyloid ($A\beta$) plaques, $A\beta$ deposits in blood vessels and intraneuronal neurofibrillary tangles [23, 24]. Abnormal accumulation of cholesterol levels increase $A\beta$ in cellular and most animal models of AD; and drugs that inhibit cholesterol synthesis lower $A\beta$ in these models [25, 26]. Our previous finding indicated that the animals which were treated with DCE showed significant reduction in cholesterol levels in young and aged mice as compared to control group [27]. Therefore, it seems likely that *Daucus carota* may prove to be a useful anti-Alzheimer agent, in view of its memory enhancing property observed in the present study.

Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions. There is extensive evidence linking the central cholinergic system to memory [28-31]. Cognitive dysfunction has been shown to be associated with impaired cholinergic function and the facilitation of central cholinergic activity with improved memory [32]. Selective loss of cholinergic neurons and decrease in cholinesterase activity was reported to be a characteristic feature of senile dementia of the Alzheimer's type [33]. Anticholinesterases such as metrifonate, physostigmine, tacarine, donepezil, huperzine-A, rivastigmine, galanthamine and eptastigmine have all been shown to reverse amnesia produced by disruption of cholinergic system [34-39]. Enzyme choline acetyltransferase is involved in the synthesis of acetylcholine and acetylcholinesterase is involved in the degradation of acetylcholine. Acetylcholine is synthesized from choline and acetyl Co. enzyme A in the presence of choline acetyltransferase [40,41]. The quaternary base chlorides separated from the seeds of *Daucus carota* were rich in choline content and exhibited procholinergic activity [13, 14]. 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 [27].

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 pulsed controlled-release system for potential [42,43]. Epidemiological studies have almost confirmed that non steroidal anti-inflammatory drugs reduce the incidence of AD [42-44]. Compounds such as Geraniol, 2,4,5-trimethoxy benzaldehyde, oleic acid and trans-asarone isolated from *D. carota* seeds have been shown to possess anti-inflammatory action in rodents.⁴⁵ Interestingly, indomethacin and ibuprofen, 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 [46-49]. Thus, the memory enhancing effect exhibited by DCE in the present study may be dependent upon its cholinesterase inhibiting activity, cholesterol lowering effect and the anti-inflammatory action. Therefore, *D. carota* bark appears to be a promising candidate for improving memory and it would be worthwhile to explore the potential of this plant in the management of Alzheimer patients.

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