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Antiamnesic Effects of *Desmodium gangeticum* in Mice

Hanumanthachar JOSHI,*,[a] and Milind PARLE[b]

[a]*Department of Pharmacognosy, SET'S College of Pharmacy, S.R. Nagar, Dharwad–580 002 Karnataka, India and* [b]*Division of Pharmacology, Department of Pharmaceutical Sciences, Guru Jambheshwar University, Hisar, Haryana, India*

(Received May 12, 2006; Accepted June 21, 2006)

Dementia is a mental disorder characterized by loss of intellectual ability sufficiently severe enough to interfere with one's occupational or social activities. *Desmodium gangeticum* commonly known as Salparni, is widely used in ayurveda for the treatment of neurological disorders. The present work was designed to assess the potential of aqueous extract of *D. gangeticum* (DG) as a nootropic agent in mice. DG (50, 100 and 200 mg/kg, *p.o.*) was administered for 7 successive days to both young and older mice. Exteroceptive behavioral models such as elevated plus maze and passive avoidance paradigm were employed to evaluate learning and memory. Scopolamine (0.4 mg/kg, *i.p.*) induced amnesia and ageing induced amnesia were the interoceptive behavioral models. To delineate the mechanism by which DG exerts nootropic activity, the effect of DG on whole brain AChE activity was also assessed. Piracetam (200 mg/kg, *i.p.*) was used as a standard nootropic agent. Pretreatment with DG (50, 100 and 200 mg/kg *p.o.*) for seven successive days significantly improved learning and memory in mice and reversed the amnesia induced by both, scopolamine (0.4 mg/kg, *i.p.*) and natural ageing. DG also decreased whole brain acetyl cholinesterase activity. Hence, *D. gangeticum* 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 dementia and Alzheimer disease.

Key words——acetylcholine; nootropic; *D. gangeticum*; memory

INTRODUCTION

The most common cause of dementia in the elderly is probably Alzheimer's disease (AD), a chronic, progressive disabling organic brain disorder characterized by disturbance of multiple cortical functions, including memory, judgment, orientation, comprehension, learning capacity and language.[1] The National Institute of Health predicts, if the current trend continues, there will be more than 8.5 million AD patients by the year 2030 in USA alone.[2] Amnesic mild cognitive impairment represents a transitional state between the cognitive changes of normal ageing and the earliest critical features of Alzheimer's disease.[3] Although there is no cure for dementia of AD type at present, alternative pharmacologic treatment modalities can reduce the symptoms of cognitive impairment and slow disease progression.[4] Nootropic agents like, piracetam and cholinesterase inhibitors like, Donepezil® are commonly used for improving memory, mood and behavior. However, the resulting adverse effects of these drugs such as diarrhea, insomnia, nausea, bronchitis, loose stools, muscular cramps and other known side effects,[5,6] has made their use limited and it is worthwhile to explore the utility of traditional medicines in the treatment of various cognitive disorders.

Desmodium gangeticum DC. (Leguminosae) is commonly known as Salpan, Salpani in Hindi and Shalparni in Sanskrit. It is abundantly found throughout India and is one of the important plants used in indigenous system of medicine as bitter tonic, febrifuge, digestive, anticatarrhal, antiemitic,[7] in inflammatory conditions of chest and in various other inflammatory conditions which are due to *vata* disorder.[8] It is used in ayurvedic preparations like 'Dashmoolarishta' and 'Dashmoolakwaath' for the post-natal care to avoid secondary complications and also in nervous debility.[9] The sterols, *N*,*N*-dimethyltryptamine,5-methoxy-*N*,*N*-dimethyltryptamine, their oxides and other derivatives have been isolated from aerial parts, three pterocarpenoids, gangetin, gangetinin and desmodin, are the major chemical constituents of the roots.[10] Alkaloid isolated from aerial part comprises indol-3-alkyl-aminesand β-carbolines and has anticholinesterase, smooth muscle stimulant, CNS stimulant response.[11] Gangetin, a pterocarpan, shows anti-fertility activity by affecting

*e-mail: amanjoshi17@yahoo.com

alkaline phosphatase activity in uterine fluids.[12] It is reported to possess antiulcer,[13] antioxidant,[14] cardiotonic,[15] anti-inflammatory, anti-nociceptive[16] activities and useful in neurological disorders.[17] The present study was undertaken to assess the anti-amnesic potential of *D. gangeticum* in mice.

MATERIALS AND METHODS

Plant Materials The aerial and root parts of *D. gangeticum* were collected from Gopeshwar, Tehri Garhwal district, Uttaranchal, India, during October 2003. The plant parts were identified and authenticated taxonomically at Department of Systemic Botany, Forest Research Institute, Dehradun, Uttaranchal, India. Voucher specimens of the collected samples were deposited in the Department of Pharm. Sciences, Guru Jambheshwar University, Hisar, Haryana, India.

Preparation of Extract The shade-dried roots and aerial parts were powdered and passed through 10-mesh sieve. The coarsely powdered materials (1000 g) were soaked in distilled water in the ratio of 1 : 16 (w/v) and boiled for 20 min. The combined extract was filtered, first concentrated on rotavapour and then freeze dried with high vacuum (yield of the dried extract: 16.7% w/w). The chemical constituents of the decoction was identified by qualitative analysis and confirmed by thin layer chromatography.[18] This indicates the presence of alkaloids and flavonoids. A suspension was prepared using distilled water containing 1% (w/v) carboxymethyl cellulose (CMC).

Drugs and Chemicals Scopolamine hydrobromide (Sigma Aldrich, USA) and piracetam (Nootropil®, UCB India Pvt. Ltd., Vapi, Gujarat) were diluted in normal saline and injected intraperitoneally. Phenytoin (Dilantin® suspension, Parke Davis) was administered orally. Volume of administration was 1 ml/100 g. All the drugs were administered in the morning session i.e. 8 AM—9 AM on each day.

Acute Toxicity Studies *D. gangeticum aqueous extract* (DG) at different doses (50—2000 mg/kg) was administered orally to normal mice. During the first four hrs after the drug administration, the animals were observed for gross behavioral changes if any for 7 days. The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia, mortality were observed and doses selected were 50, 100 and 200 mg/kg/day.

Animals Swiss mice of either sex weighing around 18 g (younger ones, aged 8 weeks) and 25 g (older ones, aged 28 weeks) were used in present study. Animals were procured from disease free animal house of CCS Haryana Agriculture University, Hisar (Haryana, India). They were acclimatized to the laboratory conditions for 5 days before behavioral studies. The animals had free access to food and water and were maintained under 12 : 12 h light and dark cycles. All the readings were taken during same time of the day i.e. between 8 AM—11 AM. Institutional Animals Ethics Committee (IAEC) had approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Dept. of Animal Welfare, Govt. of India.

Elevated Plus-maze Elevated plus-maze served as the exteroceptive behavioral model to evaluate learning and memory in mice. The procedure, technique and end point for testing learning and memory was followed as per the parameters described by the investigators working in the area of psychopharmacology.[18—20] 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) extended 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, 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 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 for each animal. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned-task was examined 24 h after the first day trial.

Group I: Control group for young mice. Vehicle of the extract was administered orally for seven successive days. TL was recorded after 90 minutes of vehicle administration on day seven and retention was examined after 24 h (i.e. on eighth day).

Group II: Positive control for young mice. Piracetam (200 mg/kg *i.p.*) was injected to young mice for seven successive days. TL was recorded after 60 minutes of *i.p.* injection on day seven and retention was examined after 24 h (i.e. on eighth day).

Groups III, IV and V: DG (50, 100 and 200 mg/kg, respectively) were administered orally for seven successive days to young mice. TL was noted after 90

minutes of the extract administration on day seven and after 24 h (i.e. on eighth day).

Group VI: Scopolamine (0.4 mg/kg) was injected intraperitoneally into young mice and TL was recorded 45 minutes after injection. Retention was examined after 24 h (i.e. on eighth day).

Group VII: Piracetam (200 mg/kg, *i.p*) was injected to young mice for seven successive days. At 60 minutes after the injection of Piracetam on the seventh day, scopolamine (0.4 mg/kg, *i.p.*) was injected. TL was noted after 45 minutes of injection of scopolamine and retention was examined after 24 h (i.e. on eighth day).

Groups VIII, IX and X: DG (50, 100 and 200 mg/kg, respectively) were administered for seven successive days orally. Scopolamine (0.4 mg/kg) was injected intraperitoneally to young mice at 90 minutes after administration of extract on day seven. TL was recorded 45 minutes after injection and after 24 h (i.e. on eighth day).

Group XI: Control group for older mice. The vehicle was administered orally for seven successive days. TL was recorded after 90 minutes of vehicle administration on day seven and retention was examined after 24 h (i.e. on eighth day).

Group XII: Positive control for older mice. Piracetam (200 mg/kg *i.p.*) was injected to older mice for seven successive days. TL was recorded after 60 minutes of *i.p.* injection on day seven and retention was examined after 24 h (i.e. on eighth day).

Groups XIII, XIV and XV: DG (50, 100 and 200 mg/kg, respectively) were administered orally for seven successive days to older mice. TL was noted after 90 minutes of the extract administration on day seven and after 24 h (i.e. on eighth day).

Passive Shock Avoidance Paradigm Passive avoidance behavior based on negative reinforcement was recorded to examine the long-term memory. The apparatus consisted of a box (27×27×27 cm) having three walls of wood and one wall of Plexiglas, featuring a grid floor (3 mm stainless steel rods set 8 mm apart), with a wooden platform (10×7×1.7 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 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 and placed all its paws on the grid floor, shocks were delivered for 15 sec and the step-down latency (SDL) was recorded.[21,22] SDL was defined as the time taken by the mouse to step down from wooden platform to grid floor with its entire paw on the grid floor. Animals showing SDL in the range (2—15 sec) 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. When the animals stepped down before 60 sec, electric shocks were delivered for 15 sec. During the second test, animals were removed from shock free zone if they did not step down for a period of 60 sec. Retention was tested after 24 h in a similar manner, except that the electric shocks were not applied to the grid floor. Each mouse was again placed on the platform, and the SDL was recorded, with an upper cut-off time of 300 sec.

Group I: Control group for young mice. The vehicle was administered orally for seven successive days. Shock was delivered for 15 seconds after 90 minutes of vehicle administration on day seven, and SDL was recorded after 24 h (i.e. on eighth day).

Group II: Positive control for young mice. Piracetam (200 mg/kg *i.p.*) was injected to young mice for seven successive days. Shock was delivered for 15 seconds after 60 minutes of *i.p.* injection on day seven and SDL was examined after 24 h (i.e. on eighth day).

Groups III, IV and V: DG (50, 100 and 200 mg/kg, respectively) were administered orally for seven successive days to young mice. Shock was delivered for 15 seconds after 90 minutes of the extract administration on day seven and SDL was noted after 24 h (i.e. on eighth day).

Group VI: Scopolamine (0.4 mg/kg) was injected intraperitoneally into young mice and shock was delivered for 15 seconds after 45 minutes of injection and SDL was noted after 24 h (i.e. on eighth day).

Group VII: Piracetam (200 mg/kg, *i.p*) was injected to young mice for seven successive days. At 60 minutes after the injection of Piracetam on the seventh day, scopolamine (0.4 mg/kg, *i.p.*) was injected. Shock was delivered for 15 seconds after 45 minutes of injection of scopolamine and SDL was examined after 24 h (i.e. on eighth day).

Groups VIII, IX and X: DG (50, 100 and 200 mg/kg, respectively) were administered orally for seven successive days to young mice. Scopolamine (0.4 mg/kg) was injected intraperitoneally to young mice at 90

minutes after administration of extract on day seven. Shock was delivered for 15 seconds after 45 minutes of injection and SDL was noted after 24 h (i.e. on eighth day).

Group XI: Control group for older mice. The vehicle was administered orally for seven successive days. Shock was delivered for 15 seconds after 90 minutes of vehicle administration on day seven, and SDL was examined after 24 h (i.e. on eighth day).

Group XII: Positive control for older mice. Piracetam (200 mg/kg *i.p.*) was injected to older mice for seven successive days. Shock was delivered for 15 seconds after 60 minutes of *i.p.* injection on day seven and SDL was examined after 24 h (i.e. on eighth day).

Groups XIII, XVI and XV: DG (50, 100 and 200 mg/kg, respectively) were administered orally for seven successive days to older mice. Shock was delivered for 15 seconds after 90 minutes of extract administration on day seven and SDL was noted after 24 h (i.e. on eighth day).

Estimation of Brain Acetyl Cholinesterase (AChE) Levels The time frame of cholinesterase activity estimation was similar to behavioral tests i.e. 8 AM—11 AM on each day. On the 9th day the animals were euthanized by cervical dislocation carefully to avoid any injuries to the tissue. The whole brain AChE levels was measured using the Ellman method.[23] This was measured on the basis of the formation of yellow color due to the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The sample was first treated with 5, 5′-dithionitrobenzoic acid (DTNB) and the optical density (OD) of the yellow color compound formed during the reaction at 412 nm every minute for a period of three minutes was measured. Protein estimation was done using Folin's method. AChE activity was calculated using the following formula:

$$R=\frac{\delta \text{O.D.}\times \text{Volume of Assay (3 ml)}}{\text{E}\times \text{mg of protein}}$$

Where R=rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed/min/mg protein, δO.D.=Change in absorbance/min, E=Extinction coefficient=13600/M/cm

Group I: Served as control and treated with saline water, Group II: was treated with phenytoin (12 mg/kg, *p.o.*) and Group III: was treated with piracetam (200 mg/kg, *p.o.*). Groups IV, V and VI: were treated with DG (50, 100 and 200 mg/kg, *p.o.*) respectively for 7 days and acetyl cholinesterase levels were determined.

Locomotor Function Locomotor activity of control and drug-treated animals was measured with the help of a photoactometer (INCO, Ambala, India).

Statistical Analysis All the results were expressed as mean±Standard error. The data from passive avoidance tasks was analyzed using ANOVA followed by Student's (Unpaired) '*t*' test. Kruskal Wallis one-way ANOVA followed by multiple range tests was used for the analysis of non-normally distributed data of whole brain AChE activity. $P<0.001$ and $p<0.05$ considered significant.

RESULTS

Acute Toxicity Study No mortality was observed following oral administration of DG even with the highest dose (2000 mg/kg). Both the doses of DG had no toxic effect on the normal behavior of the rats.

Effect on Locomotor Activity In the present study, DG (50, 100 and 200 mg/kg) did not show any significant change in the locomotor function of animals (score: 222.6±8, 218±2 and 211±15) when tested on photoactometer as compared to control group (score 216.4±12).

Effect on Transfer Latency (By Elevated Plus Maze) Transfer Latency (TL) of first day (on seventh day of drug treatment) reflected acquisition or learning behavior of animals. Whereas, TL of next day reflected retention of information or memory. DG (50 mg/kg) administered for 7 days orally did not have any significant effect on TL of seventh day and eighth day in elevated plus maze test. The young and older animals treated orally with 100 mg/kg and 200 mg/kg showed remarkable reduction ($p<0.001$) in TL of seventh day as well as eighth day, indicating significant improvement in learning and memory (Figs. 1 and 2). Scopolamine hydrobromide (0.4 mg/kg, *i.p.*) injected before training significantly increased ($p<0.001$) TL on days seven and eight indicating impairment in learning and memory (Fig. 3). The DG at higher dose levels (50 and 100 mg/kg, *p.o.* for 7 successive days) successfully reversed memory deficits induced by scopolamine ($p<0.001$). Piracetam (used as the positive control) at a dose of 200 mg/kg, *i.p.* also improved learning and memory

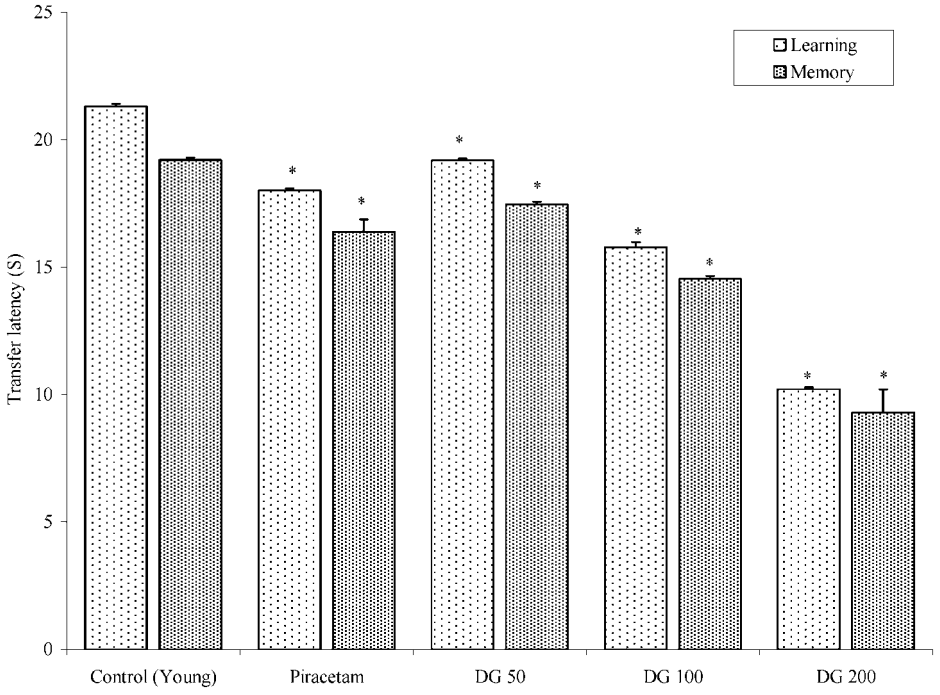

Fig. 1. Effects of *D. gangeticum* (DG) on Transfer Latencies of Young Mice in Elevated Plus Maze

Values are mean±S.E.M. (n=6). *indicates $p<0.001$ compared to control (young mice). ANOVA followed by Tukey-kramer multiple comparison test ($F=152.62$).

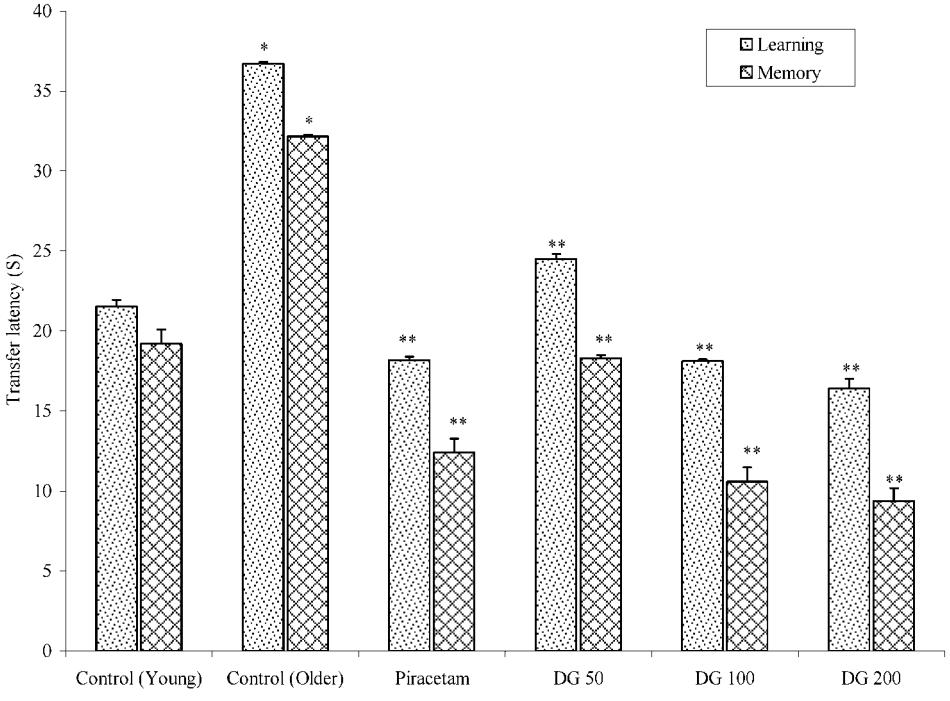

Fig. 2. Effects of *D. gangeticum* (DG) on Transfer Latencies of Older Mice in Elevated Plus Maze

Values are mean±S.E.M. (n=6). *indicates $p<0.001$ compared to control (young mice) alone. **indicated $p<0.001$ compared to control (older mice) alone. ANOVA followed by Tukey-kramer multiple comparison test (Day 1, $F=7235.0$; Day 2, $F=7747.6$).

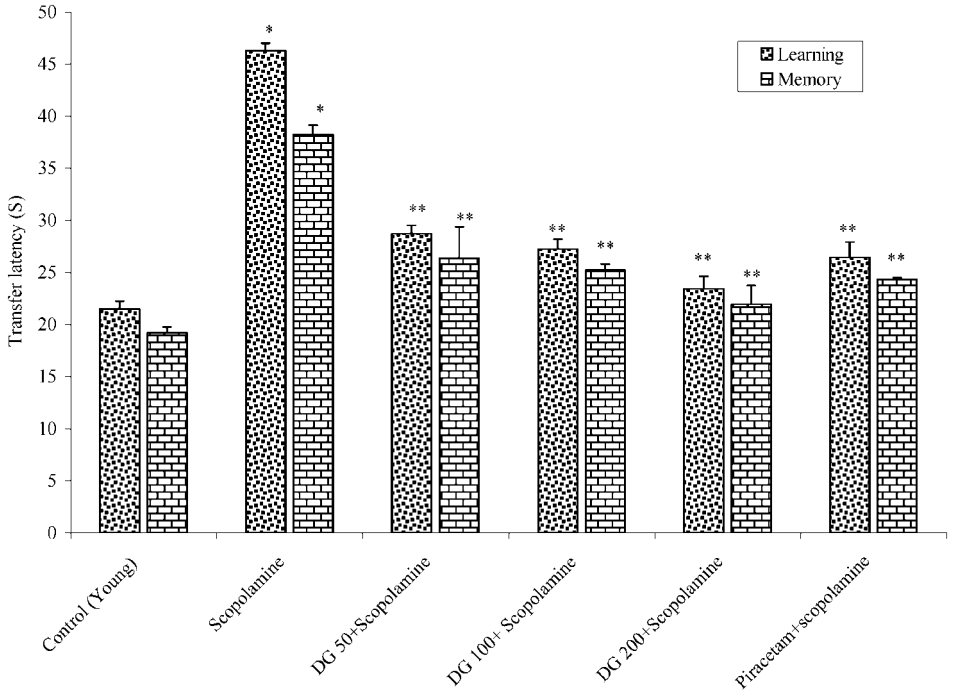

Fig. 3. Effects of *D. gangeticum* (DG) on Transfer Latencies of Scopolamine Induced Amnesic Mice

Values are mean±S.E.M. ($n=6$). *indicates $p<0.001$ compared to control (young mice) alone. ** indicated $p<0.001$ compared to control (scopolamine) alone. ANOVA followed by Tukey-kramer multiple comparison test (Day 1, F=7203; Day 2, F=3634.1).

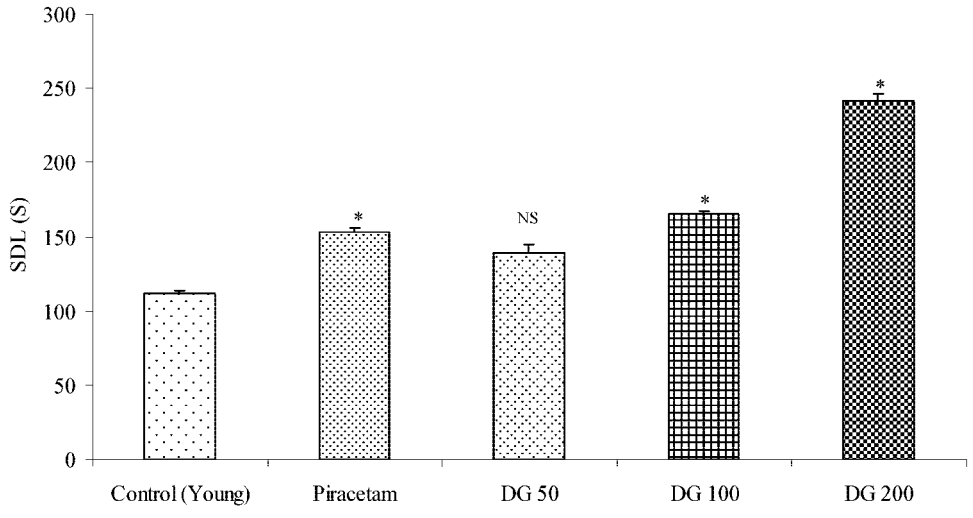

Fig. 4. Effects of *D. gangeticum* (DG) on Passive Avoidance Behavior of Young Mice

Values are mean±S.E.M. ($n=6$). *indicates $p<0.001$ compared to control (young mice) alone. ANOVA followed by Tukey-kramer multiple comparison test (SDL, F=3512.0).

in both young and older mice and reversed the amnesia induced by scopolamine as expected.

Effect on Step-down Latency (Using Passive Avoidance Paradigm) Step down latency (SDL) of second day/eighth day of drug treatment reflected the long-term memory of animals. DG (50 mg/kg, *p.o.*) did not exert any significant effect on SDL of young mice as compared to control group (Fig. 4). On the other hand, the higher doses of 100 and 200 mg/kg of the extract administered orally in young mice for 7 days markedly ($p<0.001$) increased SDL as compared to the control group. Scopolamine (0.4 mg/kg, *i.p*) significantly ($p<0.001$) decreased SDL as compared to the control group of young mice, in-

dicating impairment of memory (amnesia). DG (100 and 200 mg/kg, *p.o.*) administered for 7 days significantly reversed amnesia induced by both scopolamine (Fig. 5). The groups of mice, which were treated with Piracetam (200 mg/kg, *i.p.*) for seven successive days showed improvement ($p<0.001$) in memory of young as well as older mice and reversed amnesia induced by scopolamine. Older mice showed significantly ($p<0.001$) low SDL thereby indicating that ageing had produced amnesia in these animals. DG (100 and 200 mg/kg, *p.o.*) successfully reversed ($p<0.001$) aging induced amnesia (Fig. 6).

Effect on Whole Brain Acetylcholinesterase Levels The whole brain AChE activity with phenytoin (12 mg/kg, *p.o.*) demonstrated significant rise in AChE activity as compared to control and piracetam (200 mg/kg, *p.o.*). DG (50, 100 and 200 mg/kg, *p.o.*) significantly ($p<0.001$) decreased AChE activity in older mice (Fig. 7).

DISCUSSION

Alzheimer's disease is a genetically heterogeneous neurodegenerative disorder, which is slow in onset but relentless in progress. It is characterized by aphasia, apraxia and agnosia with loss of memory as the main symptom.[24,25] Despite the severity and high prevalence of this disease, allopathic system of medicine is yet to provide a satisfactory antidote. Therefore, we were motivated to explore the potential of medicinal plants from Himalayan flora to manage this deadly disease (AD). In the present study, *D. gangeticum* extract administered orally for 7 days improved the memory of mice as reflected by diminished TL and enhanced SDL values as compared to control animals. Additionally, DG reduced central cholinesterase activity. Furthermore, pretreatment with DG for 7 days protected the animals from memory deficits produced by scopolamine. These findings suggest the possible neuroprotective role for *D. gangeticum*.

Nootropics represent a new class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system, particularly on intellectual performance, learning capability and memory.[26] Piracetam, the first representation of a class of nootropic agents, has been shown to improve memory deficits in geriatric individuals. Repeated injections of piracetam had improved learning abilities and memory capacities of laboratory animals. Passive avoidance behavior is based on negative reinforcement and is used to examine long-term memory. Both piracetam and *D. gangeticum* meet major criteria for nootropic activity, namely improvement of memory

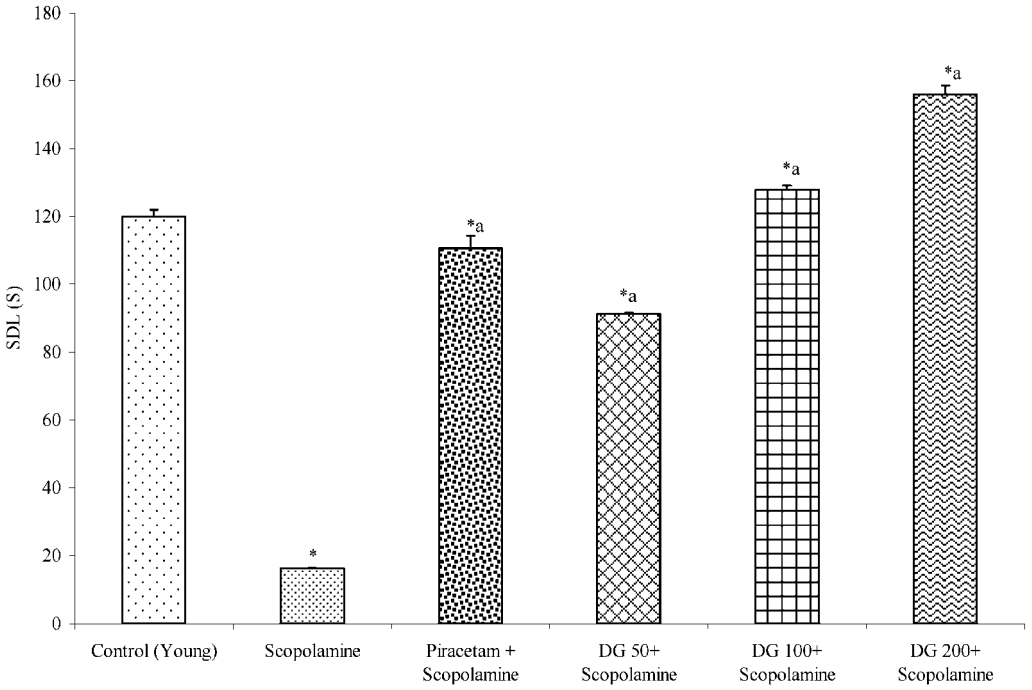

Fig. 5. Effects of *D. gangeticum* (DG) on Scopolamine Induced Amnesic Young Mice

Values are mean±S.E.M. ($n=6$). *indicates $p<0.001$ compared to control (young mice) alone. a indicated $p<0.001$ compared to control (scopolamine) alone. ANOVA followed by Tukey-kramer multiple comparison test (SDL, $F=7216.9$).

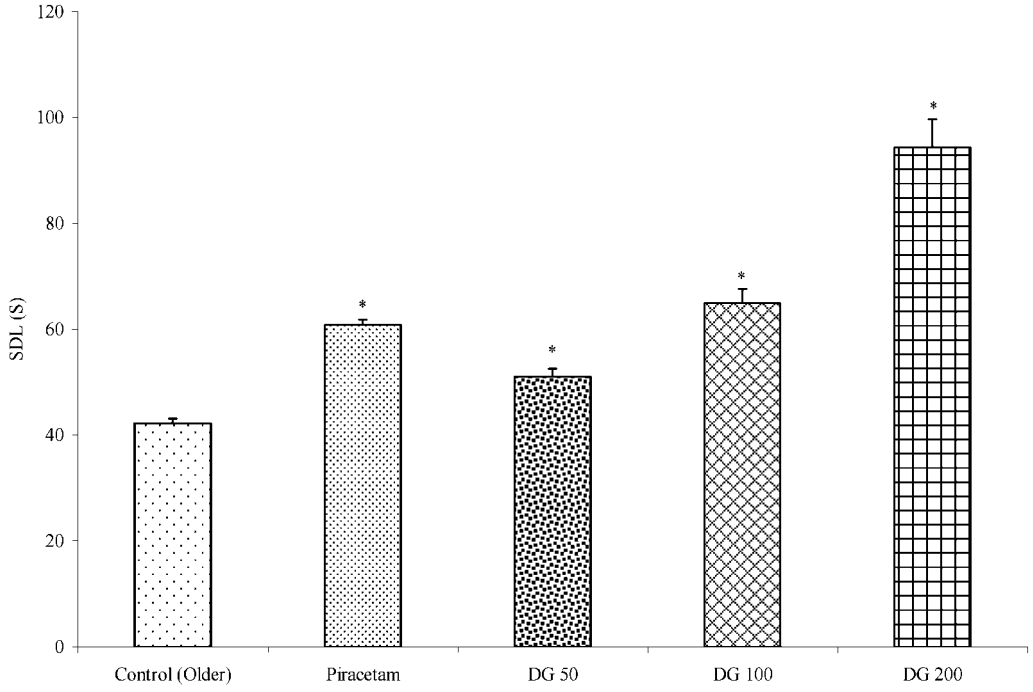

Fig. 6. Effects of *D. gangeticum* (DG) on Passive Avoidance Behavior of Older Mice

Values are mean ± S.E.M. ($n=6$). *indicates $p<0.001$ compared to control (older mice) alone. ANOVA followed by Tukey-kramer multiple comparison test (SDL, F=3783.0).

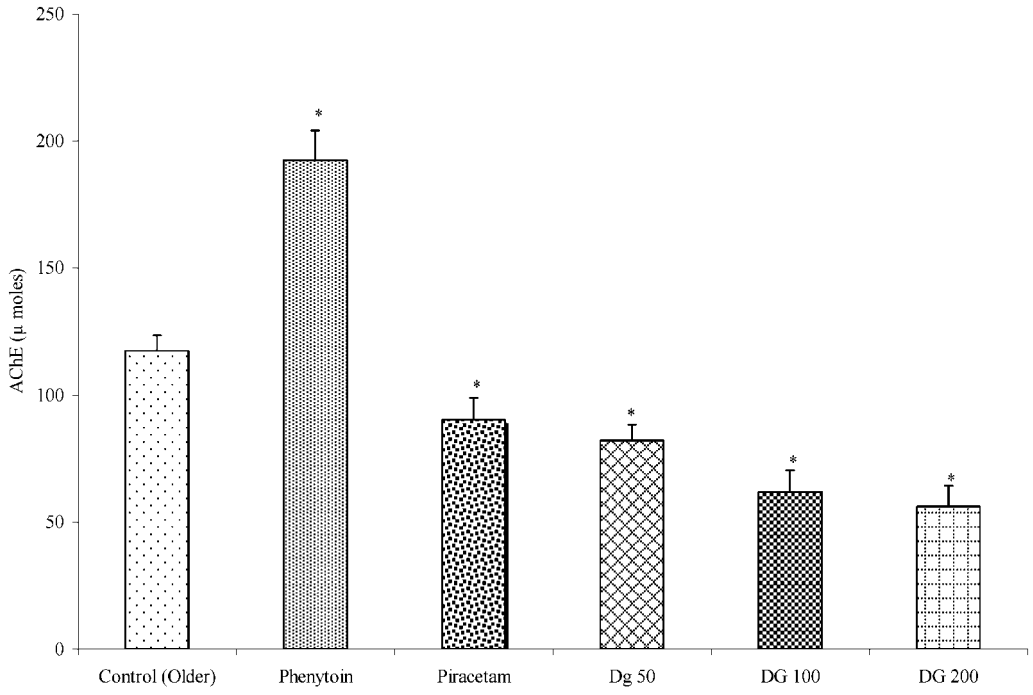

Fig. 7. Effects of *D. gangeticum* (DG) on AChE Activity in Older Mice

Values are mean ± S.E.M. ($n=6$). *indicates $p<0.001$ compared to control (older mice) alone. ANOVA followed by Tukey-kramer multiple comparison test (SDL, F=5305.7).

in absence of cognitive deficit.[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.[28,29] Epidemiological studies have almost confirmed that non steroidal anti-inflam-

matory drugs reduce the incidence of AD.[30] *D. gangeticum* has been shown to produce anti-inflammatory action in rodents.[15] This anti-inflammatory effect of *D. gangeticum* would certainly help Alzheimer patients by taking care of the inflammatory component of the Alzheimer's disease. Oxygen free-radicals are implicated in the process of age-related decline in cognitive performance and may be responsible for the development of Alzheimer's disease in elderly persons.[31—38] *D. gangeticum* has been reported to possess antioxidant property as well.[13] The neuroprotective effect of DG may be attributed to its antioxidant property by virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improved neuronal function.

The symptoms of dementia are presumed to be related to impaired neurotransmission and degeneration of neuronal circuits in the affected brain areas.[39] Cognitive deterioration occurring in patients with probably AD is associated with progressive loss of cholinergic neurons and consequent decline in levels of acetylcholine (Ach) in brain.[40] Cholinergic deficits occur in the brain of patients with AD and vascular dementia.[41,42] The indol-3-alkyl-amines and β-carbolines isolated from *D. gangeticum* are reported to have anticholinesterase activity. Phenytoin is known to reduce hippocampal ACh concentration and causes cognitive impairment.[43] Our research findings using *Zingiber officinale*, *Brahmi rasayana* and *Hibiscus sabdariffa* have displayed a link between memory improving effect and cholinesterase activity.[44—46] In the present study, the aqueous extract of salparni significantly decreased the AChE levels in the mice whole brain homogenate, indicating its potential in the attenuation of severity of Alzheimer's disease.

Previous pharmacodynamic studies with *D. gangeticum* showed that this plant possessed useful anti-inflammatory and antioxidant properties. In the present study, we observed that *D. gangeticum* extract (i) decreased acetylcholinesterase enzyme levels and (ii) ultimately improved memory of both young and older mice in both exteroceptive and interoceptive behavioral models. Thus, a combination of anticholinesterase, anti-inflammatory, antioxidant and neuroprotective effects exhibited by *D. gangeticum* may all be eventually responsible for the memory improving effect observed in the present study. However, investigations using more experimental paradigms may be warranted for further confirmation of nootropic potential of *D. gangeticum* in the treatment of dementia and Alzheimer's disease.

Acknowledgements Authors are deeply grateful to Dr. R. P. Bajpai, Honorable Vice-Chancellor, Guru Jambheshwar University, Hisar, for research facilities and encouragement. We are thankful to UCB India Pvt. LTD., (Gujarat), for supply of piracetam.

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