Evaluation of anti-amnestic activity of few medicinal plants against scopolamine induced amnesia

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Dementia is a syndrome of progressive nature, characterized by impairment of memory and loss of intellectual ability. Subsequently, allopathic system of medicine is yet to provide a fundamental cure; it is meaningful to look for novel directions, which would curtail the memory loss seen in elderly patients. We have screened few plants for enhancement of memory and learning in our laboratory as pilot study and found two plants, viz. Acorus calamus L. and Gnetum gnemon L. to be more promising than others. Hence, the rationale for this study was to evaluate the prospective beneficial effect of Acorus calamus and Gnetum gnemon on learning and memory in rats using Radial-arm maze and Barnes maze and Morris water maze in mice using a repeated acquisition procedure. A suspension preparation of A. calamus (200mg/kg) and G. gnemon (200mg/kg) was administered orally after completion of the specific training period. Memory loss was induced by Scopolamine (0.4mg/kg i.p.), a muscarinic cholinergic antagonist and a classic amnestic drug. Tacrine (3mg/kg i.p) was used as the standard drug. The data obtained from behavioral and biochemical studies (Acetylcholinesterase, MDA and nitrite estimation) have shown that G. gnemon, and A. calamus possessed significant memory enhancing potency. However, further studies are necessitated to identify the exact mechanism of action.

Keywords: Dementia, Acorus calamus L., Gnetum gnemon L., Scopolamine, Acetylcholinesterase

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Dementia is a syndrome of progressive nature, characterized by impairment of memory and loss of intellectual ability. The dementing condition that has received the utmost attention in the past decade is Alzheimer’s disease (AD), which is the most common cause of dementia in the elderly, accounting for 60–70% of all cases. According to the World Health Organization (WHO), 5% of men and 6% of women aged above 60 yrs suffer from dementia of AD worldwide. Alzheimer’s disease (AD), the most common form of senile dementia, is characterized as memory loss accompanied by degeneration of basal forebrain cortical cholinergic neurons. The pathogenesis of AD is multifactorial and includes degeneration of cholinergic neurons, abnormal phosphorylation of the protein tau, oxidative stress, and altered protein processing resulting in abnormal β-amyloid peptide (Ab) accumulation. Brain areas associated with cognitive functions, particularly the neocortex and hippocampus, are the regions that mostly affected by the pathology which is characteristic of AD.

Acorus calamus (called Sweet Flag or Calamus) is a tall perennial wetland plant (Acoraceae family) in the genus Acorus. Sweet flag has a very long history of medicinal use in Chinese and Indian herbal traditions. Phytoconstituents of A. calamus include Isoeugenol methyl ether, λ-asarone, cis-asarone, trans-asarone, Acoramone, asarylaldehyde, Z-3-(2,4,5-trimethoxy phenyl)-2-propanal, 2,3-dihydro-4,5,7-trimethoxy-1-ethyl-2-methyl-3-(2,4,5-trimethoxy phenyl)indene, Shyobunone, Epishyobunone, 2,6-diepishyobunone, Isocalamendiol, Acoragermacrone, Preisocalamendiol, Calamusenone, Thujaone, Limonene and 5, 7-dihydroxyflavonol (Galangin).

Gnetum gnemon is an evergreen tree belonging to the Gnetaceae family. It has been used in folk...
medicines for the treatments of arthritis, bronchitis and asthma. Several bioactive compounds are found in *G. gnemon*, such as saponins, tannins and flavonoids. The seeds contain an abundance of resveratrol (stilbenoid) mainly in the form of dimers (gnetin C). Other resveratrol (stilbenoid) including 3, 4-dimethoxychlorogenic acid, 3-methoxyresveratrol, gnetin E, gnemonoside M, trans-resveratrol, gnetin L, gnemonoside A, gnemonoside C, and gnemonoside D.

Scopolamine, an anticholinergic drug, causes amnesia in human and also impairs learning in animals. Hence, it is widely utilized as a model simulating human dementia in general and AD, in particular. Although recently, several synthetic drugs have been introduced to treat learning and memory disorder, but their therapeutic effects are low and most of them have undesirable side effects. Today we can see the increasing tendency of people towards traditional medicine. In view of this, the present study is undertaken to investigate the beneficial effect of few medicinal plants in memory deficit rodents, employing scopolamine induced amnesia as an animal model and to compare such effects with tacrine, a commonly used agent in dementia and AD. In this study, medicinal plants that have shown the early promising signs of reversing memory and learning impairment were evaluated. One common feature of these plants is their ability to exert neuroprotective effects through inhibition of AChE or inhibition of oxidative stress.

**Methodology**

**Animals**

All experiments were performed in accordance to the guidelines on institutional animal ethical committee and care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (770/ac/CPCSEA/FVSc, AAU/IAEC/11-12/118). Swiss albino mice (male, age 3–5 months, weight 25–35 gm) and wistar rats (male, age 4-5 months, weighing 150–200 gm) were housed four animals per cage with access to food and water ad libitum under controlled laboratory conditions. Healthy mice and rats were screened on the basis of the swimming ability and normal behavior. Experiments were conducted between 9:00 and 18:00 hrs in a semi soundproof laboratory.

**Drugs and chemicals**

Scopolamine hydrobromide was purchased from Sigma–Aldrich (MO, USA). It was dissolved in saline and *i.p.* administered in a dose of 0.4 mg/kg. Tacrine hydrochloride (9-Amino-1, 2, 3, 4-tetrahydro-acridine hydrochloride hydrate) was purchased from Sigma–Aldrich (MO, USA). Acetylthiocholine iodide and 5, 5’-dithio- bis-nitro-benzoic acid (DTNB) were obtained from Sigma (St. Louis, USA).

**Preparation of extract**

The shade dried leaves of *Acorus calamus* and *Gnetum gnemon* were ground and finely powdered. For ethanol extraction 500 gm of air dried powder was extracted with 95% (v/v) ethanol (40-60 °C) in a Soxhlet extractor for 18-20 hrs. Then the solvent was evaporated using rotary evaporator (BUCHI, ROTAVAPOR R-210, Switzerland) to obtain the extract, which was kept in sealed bottles under refrigeration prior to use. The recovery percentage of *A. calamus* and *G. gnemon* was found to be 17.16% and 22.39%, respectively.

**Acute toxicity studies**

The acute toxicity studies of ethanolic extract of *Acorus calamus* and *Gnetum gnemon* was performed according to the Organization of Economic Corporation Development (OECD) Guidelines No. 425. The extracts were administered orally at 2000 mg/kg to a group of mice (n=3) and the percentage mortality, if any, was recorded for a period of 24 hrs. The animals were kept under observation for the next 14 days. No mortality or gross abnormality was observed with the given dose. Hence, based on the acute toxicity study, 200 mg/kg was selected as oral suspension for the present study.

**Preparation of formulation of a semi dry powder for oral suspension**

The ethanolic extracts were evaporated in order to attain a partially dry powder for the preparation of an oral drug dosage form, i.e., oral suspension (200mg/kg dose of extract). All the required constituents were added in appropriate amount and the formulation was tested for content uniformity, stability, density and viscosity. The results obtained for the pharmaceutical quality control of the suspension conform to Pharmacopoeias recommendations.
Experimental design

Animals were divided into 5 groups with 6 animals each. The suspension was administered at a dose 200 mg/kg body weight (b.w.) orally. The standard drug tacrine (3mg/kg b.w.)\(^{17}\), was administered intraperitoneally (i.p.).

Three different models universally used for learning and memory studies were done in the present study, i.e., Morris water maze in mice, Radial and Barnes maze in rats.

**Morris Water Maze\(^{18}\)**

The water maze contained a circular water pool with 150 cm diameter and 40 cm height. The water pool was divided into Northeast (NE), Southeast (SE), Southwest (SW), and Northwest (NW) equally spaced quadrants along the circumference of the pool. In the NW quadrant, an escape platform (10 cm diameter) was kept, 2 cm below the water surface. Throughout the acquisition trials the platform was maintained in a constant location in NW quadrant. The mouses were trained to locate this hidden platform. If the mice failed to find the platform within 60 s, it was gently guided to the platform and was allowed to stay there for 15 s. Animals had four acquisition trials per day for four consecutive days. To eliminate the quadrant effects, animal was positioned in each quadrant during each trial. Animals that failed to reach the platform in 20 s on the 4th trial day were excluded from the study. On probe day (day 5), 24 hrs after the last acquisition trial, escape platform was removed and retention trial was conducted. Animals were allowed to swim for 60 s before the end of session. Retention trials were repeated on day 65 on all groups to evaluate the memory consolidation. Data were obtained through a video camera attached to a computerized tracking system (ANY-maze™ - Stoelting Co. video tracking) fixed above the centre of the pool. Time to reach hidden platform (escape latency), were measured during retention trials.

**Radial Arm Maze\(^{19}\)**

In the present study, baited and unbaited arms were fixed throughout the tests. The 1\(^{st}\), 3\(^{rd}\), 6\(^{th}\), and 8\(^{th}\) arms were baited while the 2\(^{nd}\), 4\(^{th}\), 5\(^{th}\), and 7\(^{th}\) arms were unbaited. At the very beginning of each test session, each rat was placed in the central starting platform of the RAM in the position facing towards the 1\(^{st}\) arm. Food-deprived rats were expected to seek specific arms with rewards and subsequently register and retain the memory of each entered arm where food was present. Each rat was allowed to freely explore and consume food rewards for 3 min or until all food rewards of the four baited arms were eaten, whichever occurred first. An entry was recorded every time the rat placed all four paws into the initial part of the arm. The maze was then thoroughly cleaned with 70% alcohol prior to the next test session in order to minimize the effect of residual odours from the previous test.

**Scoring of Behaviour**

The first entry into never-baited arms was scored as a reference memory error (RME) and reentry into arms where the food reward had already been eaten was scored as a working memory error (WME).

**Barnes Maze\(^{20}\)**

The Barnes maze (BM) was created to evaluate spatial learning\(^{21,22}\). The Barnes maze consisted of PVC circular platform with 21 holes placed 6 cm from the edge and equally distributed around the surface. The platform was 122 cm in diameter and 92 cm high from the ground. The maze uses rodent’s natural aversion to open illuminated places, so the subjects were motivated by bright light (Lux level 300 – 500) to locate an escape hole which leads to a dark box (5.4 × 23 × 4.5 cm). Room design and equipment around the maze were used as fixed spatial cues (extra-maze cues) for navigational purposes. Barnes maze testing consisted of three phases, an adaptation period, an acquisition period and a probe trial. A pre-trial (adaptation period) was given prior the start of trial on day one and two. Each subject underwent four trials per day for four days (acquisition period) and then a probe trial was performed 24 hrs after the final acquisition trial. Thirty minutes prior to the first trial the test subjects received an injection of either scopolamine, saline vehicle, or scopolamine and tacrine. The test compounds were administered 30 min after scopolamine administration. Analysis of these recordings was performed using ANY-maze™ - Stoelting Co. video tracking system for the automatic tracking and analysis of animal movement, total latency, the latency for subject to complete the task. Total latency describes the time taken by the rat to enter the escape hole; on the maze are recorded.

**Biochemical estimation of markers of oxidative stress**

Biochemical tests were conducted 24 hrs after the last behavioral test. The animals were sacrificed by
anaesthesia. Brains were removed and rinsed with ice-cold isotonic saline, homogenized with ice-cold phosphate buffer (pH 8). The homogenates (10% w/v) were centrifuged at 10,000 rpm for 15 min and the supernatant was used for the biochemical estimations.

**Estimation of Acetylcholinesterase activity**

The AChE activity was measured by the method of Ellman *et al.* (1961)\(^{23}\) with slight modification. Change in absorbance per minute of the sample was read spectrophotometrically (MultiscanGo, Thermo Fisher) at 420 nm.

**Estimation of brain MDA level**

The whole brain MDA level was measured by the method of Colado *et al.* (1997)\(^{24}\) with slight modifications. The absorbance was measured spectrophotometrically (MultiscanGo, Thermo Fisher) at 532 nm.

**Estimation of brain nitric oxide content**

NO in brain homogenates was determined as total nitrate/nitrite (NOx) using Griess reagent\(^{25}\), after reduction of nitrate to nitrite by vanadium trichloride, and expressed as µmol/gm wet tissue. The absorbance was measured spectrophotometrically (MultiscanGo, Thermo Fisher) at 540 nm.

**Statistical Analysis**

All the results were expressed as mean ± SEM. Data were analyzed using one way analysis of variance followed by post hoc Tukey’s multiple range test. p < 0.05 was considered to be statistically significant.

**Results**

**Morris water maze**

**Effect of A. calamus and G. gnemon on Escape Latency (EL) of Mice**

There was significant reduction (p < 0.05) of EL in mice on 9\(^{th}\) day by *A. calamus* (12.94±2.044 s) *G. gnemon* (33.60±2.499 s) (NS) and Tacrine (11.48±0.9754 s) as compared to the control group (16.19±1.833 s). Scopolamine (0.4 mg/kg, i.p.) significantly increased EL in mice, indicating its amnesic effect (57.00±3.597). The results are presented in Fig. 1a.

**Effect of A. calamus and G. gnemon on brain Acetyl Cholinesterase (AChE) activity in mice**

AChE activity in the hippocampus and cortex of scopolamine treated group was 574.2±25.35 and 584.9±26.57 respectively. These values were significantly lower (p < 0.05) as compared to normal control. The AChE activity of the brain homogenates of *A. calamus* and *G. gnemon* treated group was 574.2±25.35 and 584.9±26.57 respectively. These values were significantly lower (p < 0.05) as compared to normal control.

**Effect of A. calamus and G. gnemon on brain MDA level of mice in Morris water maze.**

All the values are expressed as mean ± SEM (n = 6). ***p < 0.001, **p < 0.01, *p < 0.05, vs. normal control and ###p < 0.001, ##p < 0.01, #p < 0.05, vs. scopolamine treated group.**

**Effect of A. calamus and G. gnemon on brain Nitrite level of mice in Morris water maze.**

All the values are expressed as mean ± SEM (n = 6). ***p < 0.001, **p < 0.01, *p < 0.05, vs. normal control and ###p < 0.001, ##p < 0.01, #p < 0.05, vs. scopolamine treated group.**

Fig. 1a—Bar diagram representing the effect of *A. calamus* and *G. gnemon* on Escape Latency (EL) of Mice in Morris water maze. All the values are expressed as mean ± SEM (n = 6). ***p < 0.001, **p < 0.01, *p < 0.05, vs. normal control and ###p < 0.001, ##p < 0.01, #p < 0.05, vs. scopolamine treated group.**

Fig. 1b—Bar diagram representing the effect of *A. calamus* and *G. gnemon* on brain Acetyl Cholinesterase (AChE) Activity of mice in Morris water maze. All the values are expressed as mean ± SEM (n = 6). ***p < 0.001, **p < 0.01, *p < 0.05, vs. normal control and ###p < 0.001, ##p < 0.01, #p < 0.05, vs. scopolamine treated group.**

Fig. 1c—Bar diagram representing the effect of *A. calamus* and *G. gnemon* on brain MDA level of mice in Morris water maze. All the values are expressed as mean ± SEM (n = 6). ***p < 0.001, **p < 0.01, *p < 0.05, vs. normal control and ###p < 0.001, ##p < 0.01, #p < 0.05, vs. scopolamine treated group.**

Fig. 1d—Bar diagram representing the effect of *A. calamus* and *G. gnemon* on brain Nitrite level of mice in Morris water maze. All the values are expressed as mean ± SEM (n = 6). ***p < 0.001, **p < 0.01, *p < 0.05, vs. normal control and ###p < 0.001, ##p < 0.01, #p < 0.05, vs. scopolamine treated group.
560.2±31.05 nmol/min/mg tissue and was significantly (p< 0.05) higher than that of the control group (392.1±31.75 and 384.7±29.84 nmol/min/mg tissue). The results are presented in Fig. 1b. In scopolamine + A. calamus (200mg/kg, p.o.) and scopolamine + G. gnemon (200mg/kg, p.o.) treated groups, AChE activity in hippocampus and cortical tissues was 228.6±26.64, 207.1±17.05 and 352.3±24.62, 325.9±20.44 nmol/min/mg tissue (p< 0.05), respectively, which is lower than that of the scopolamine treated group.

Effect of A. calamus and G. gnemon on brain MDA level in mice

The MDA levels in hippocampus and cortex in the scopolamine treated group were higher (p< 0.05) than the control animals (0.2780±0.03596, 0.2520±0.02888 vs. 0.09856±0.01923, 0.0860±0.009524 nmol/mg tissue). Pretreatment with the extracts resulted in significant (p< 0.05) reduction of lipid peroxidation in the hippocampus and cortex MDA levels in scopolamine + A. calamus (200mg/kg, p.o.) and scopolamine + G. gnemon (200mg/kg, p.o.) treated groups to 0.1180±0.01612, 0.1080± 0.01582 and 0.1632±0.02066, 0.1470±0.0247 nmol/mg tissue, respectively in comparison with the scopolamine treated group (Fig. 1c).

Effect of A. calamus and G. gnemon on brain Nitrite level in mice

In scopolamine treated mice (4.130±0.4452 and 4.100±0.3435 nmol/mg protein), the level of nitrite in hippocampus and frontal cortex recorded a significant increase (p< 0.05) as compared with control group (1.910±31.2031 and 2.010 ± 0.3089 nmol/mg protein). Both A. calamus and G. gnemon significantly reversed the elevated nitrite levels. Interestingly, A. calamus showed better effect (2.18± 0.2061 and 2.320±0.2871 nmol/mg protein) on nitrite levels than G. gnemon (3.48±0.3061 in hippocampus and 3.630±0.3738 nmol/mg protein in cortex). The results are presented in Fig. 1d.

Radial Arm Maze

Effect of A. calamus and G. gnemon on working memory errors and reference memory errors in rat

In the radial arm maze, working memory errors were more in scopolamine treated group (2.670 ± 0.4350) when compared with the control group (0.784±0.1674) indicating memory impairment. Pretreatment with A. calamus, G. gnemon and Tacrine, showed significant (p< 0.05) reduction of working memory error by A. calamus (1.125±0.1823) and G. gnemon (1.875±0.3081) when compared to scopolamine treated group (0.66±0.0782).

The occurrence of reference memory errors were significantly (p< 0.05) less in Tacrine treated group (0.677±0.1275) and the groups treated with A. calamus (1.150±0.1487) and G. gnemon (1.555±0.2007) at 200 mg/kg, p.o. of each, when compared to scopolamine treated group (1.972±0.2454). Both the extracts showed protection of impaired memory by reducing reference memory errors. A. calamus (200 mg/kg, p.o.) showed better protection compared to G. gnemon (200 mg/kg, p.o.). The results are presented in Fig. 2a.

Effect of A. calamus and G. gnemon on brain Acetyl Cholinesterase (AChE) activity in rat

In Scopolamine treatment group (484.8±46.88 and 405.8±27.50 nmol/mg protein), increased Acetylcholinesterase activity in hippocampus and cortex region of brain was recorded as compared to control group (378.8±31.52 and 312.0±25.89 nmol/mg protein). However, the standard drug Tacrine (203.2±16.99 and 223.8±30.54 nmol/mg protein), A. calamus (230.1±19.83 and 210.4±26.01 nmol/mg protein) and G. gnemon (311.6±22.28 and 297.2±35.63 nmol/mg protein) treatment significantly (p< 0.05) decreased Acetylcholinesterase activity as compared to scopolamine treated group (Fig. 2b).

Effect of A. calamus and G. gnemon on brain MDA level in rat

Scopolamine treatment (0.2760±0.0304 and 0.2860±0.02556 nmol/mg protein) significantly (p< 0.05) increased MDA levels in hippocampus and cortex tissue as compared to control group (0.082±0.1106 and 0.812±0.0134 nmol/mg protein). A. calamus (0.1170±0.0152 and 0.1060±0.01143 nmol/mg protein) and G. gnemon (0.1380±0.0181 and 0.1410±0.01052 nmol/mg protein) significantly (p< 0.05) decreased hippocampal MDA levels compared to scopolamine treated group at the given dose. The results are presented in Fig. 2c. But in the cortex region there was better inhibition by A. calamus on the elevated MDA levels compared to scopolamine treated group.

Effect of A. calamus and G. gnemon on nitrite level in brain of rat

In the present study, the results show that in scopolamine treated group (3.82±0.3182 and 3.48±0.3061 in hippocampus and 3.630±0.3738 nmol/mg protein). The results are presented in Fig. 2d.
3.69±0.3358 nmol/mg protein) there was significant (p< 0.05) elevation of nitrite level in hippocampus and cortex. However, A. calamus (2.12±0.1767 and 2.050±0.2036 nmol/mg protein) and G. gnemon (3.096 ±0.2669 and 3.13±0.2390 nmol/mg protein) at a dose of 200 mg/kg each reversed the elevated nitrite level towards normal as compared to the scopolamine-treated group (Fig. 2d).

Barnes Maze

Effect of A. calamus and G. gnemon on escape latency (EL) in Scopolamine induced impairment of learning and memory using Barnes maze

There was significant (p< 0.05) attenuation of elevated EL indicating reversal of memory in A. calamus (34.18±3.612 s) and G. gnemon (26.14±2.796 s) (200 mg/kg, p.o.) treated animals compared to scopolamine treated (67.10±3.597 s) animals indicating reversal of scopolamine induced memory deficit (Fig. 3a).

Effect of A. calamus and G. gnemon on AchE activity of the rat brain in Barnes maze

Scopolamine (0.4 mg/kg, i.p.) significantly (p< 0.05) increased the AchE activity in hippocampus and cortex (551.6±21.74 and 484.5±46.88 nmol/mg protein) when compared to control rats (377.5±22.51 and 378.8±31.52 nmol/mg protein). Treatment with A. calamus (383.9±25.83 and 381.9±16 nmol/mg protein) and G. gnemon (350.3±25.07 and 311.6 ±22.83 nmol/mg protein) significantly attenuated the scopolamine induced increase in brain AchE activity (Fig. 3b).

Effect of A. calamus and G. gnemon on Scopolamine induced changes in the MDA levels of the rat brain

In this study also, scopolamine (0.4 mg/kg, i.p.) significantly (p< 0.05) increased hippocampal and cortical tissue MDA level (0.5246±0.0419 and 0.3320±0.065 nmol/mg protein) compared to the control group (0.3062±0.0445 and 0.2084 ±0.0239 nmol/mg protein) of animals, reflecting enhanced oxidative stress. However, treatment with A. calamus (0.3306±0.0211 and 0.2710±0.0183 nmol/mg protein) and G. gnemon (0.3392±0.0432 and 0.2490±0.0469 nmol/mg protein) (200 mg/kg, p.o.) significantly (p< 0.05) diminished scopolamine-induced raise in brain oxidative stress (Fig. 3c).
Effect of *A. calamus* and *G. gnemon* on Scopolamine - induced changes in the nitrite levels of the rat brain

A significant increase ($p<0.05$) in the nitrite level was observed in the hippocampus and cortex region of scopolamine treated rat ($5.13\pm0.3538$ and $4.7\pm0.3598$ nmol/mg protein), in comparison with the control group ($1.91\pm0.2031$ and $2.01\pm0.3089$ nmol/mg protein). Following treatment it was observed that *A. calamus* ($2.538\pm0.2654$ and $3.164\pm0.3346$ nmol/mg protein and *G. gnemon* ($3.48\pm0.3060$ and $3.63\pm0.3738$ nmol/mg protein) extract at a 200mg/kg dose significantly inhibited this effect ($p<0.05$). The results are presented in Fig. 3d.

Discussion

Memory is the process by which experiences are recorded and can be used to adapt their responses to the environment, and it is vital for survival. Central cholinergic system is considered to be the most important neurotransmitter involved in regulation of cognitive functions. The dementing condition that has received the most attention in the past decade is Alzheimer’s disease (AD), and impaired cognitive functions are the major features of AD.$^{26}$

The leaves of *G. gnemon* is regularly consumed by local tribes of Karbi Anglong district of Assam for its high nutritious value, but most of its medicinal properties are either not reported or yet to be studied. We have found very good adaptogenic activity of the plant, which has prompted us to study its role in enhancing memory and learning, as it is not yet reported. *A. calamus* has already been reported for its memory enhancing activity in rodents using radial arm maze models.$^{27}$ The leaves, stems, and roots are used widely in modern herbal medicine for its sedative, laxative, diuretic, and carminative properties.$^{28}$ It has also been reported to show neuroprotective effect against stroke and chemically induced neurodegeneration in rats.$^{29-30}$ In addition, the roots and leaves of *A. calamus* have potent antioxidant property.$^{31}$ The present study demonstrates the beneficial effect of ethanolic extract of *A. calamus* and *G. gnemon* on scopolamine-induced amnesia. Oral suspension (200 mg/kg) of the extracts was fed to the animals used in rodent models of memory and learning, viz. Morris water maze, Radial arm maze and Barnes maze. Behavioral study was further confirmed by estimation of the brain biochemical as well as *in vivo* oxidative stress markers. Acetylcholinesterase is the enzyme responsible for

![Bar diagram representing the effect of *A. calamus* and *G. gnemon* on Escape Latency (EL) in Scopolamine Induced Impairment of learning and memory of rat using Barnes maze. All the values are expressed as mean ± SEM (n = 6). ***p<0.001, **p<0.01,*p<0.05, vs. normal control and ###p<0.001, ##p<0.01, #p<0.05, vs. scopolamine treated group.](image)

![Bar diagram representing the effect of *A. calamus* and *G. gnemon* on AChE Activity of rat brain in Barnes maze. All the values are expressed as mean ± SEM (n = 6). ***p<0.001, **p<0.01,*p<0.05, vs. normal control and ###p<0.001, ##p<0.01, #p<0.05, vs. scopolamine treated group.](image)

![Bar diagram representing the effect of *A. calamus* and *G. gnemon* on scopolamine induced changes in the MDA levels of the rat brain using Barnes Maze. All the values are expressed as mean ± SEM (n = 6). ***p<0.001, **p<0.01,*p<0.05, vs. normal control and ###p<0.001, ##p<0.01, #p<0.05, vs. scopolamine treated group.](image)

![Bar diagram representing the effect of *A. calamus* and *G. gnemon* on scopolamine induced changes in the nitrite levels of the rat brain using Barnes maze. All the values are expressed as mean ± SEM (n = 6). ***p<0.001, **p<0.01,*p<0.05, vs. normal control and ###p<0.001, ##p<0.01, #p<0.05, vs. scopolamine treated group.](image)
acetylcholine hydrolysis which terminates the cholinergic transmission. Decrease in the cholinergic tone is associated with cognitive dysfunction and are reported in neurodegenerative diseases such as AD\textsuperscript{32-33}. In the present study, both the extracts were found to inhibit the Acetylcholinesterase enzyme and restoring the cholinergic functions. This property tends to allow more retention of acetylcholine in the brain, which is important for the cognitive function, learning and memory. It is well documented that scopolamine also leads to an increase in oxidative damage in the brain. In our study, scopolamine administration resulted significant increase in MDA, an important marker for lipid peroxidation and nitrite level in hippocampus and cortex in amnesic rats and mice. Pretreatment with \textit{A. calamus} (200 mg/ kg, \textit{p.o.}) and \textit{G. gnemon} (200 mg/ kg, \textit{p.o.}) as a suspension produced significant restoration of MDA and nitrite activities. These results evidently indicate towards the antioxidant potential of the plants.

Both the plants contain phytoconstituents mainly flavonoids, tannins and terpenoids which might be responsible for exhibiting anti-amnesic activity. The use of phytoconstituents as drug therapy to scavenge free radicals and to treat disorders leading to oxidative stress has proven to be clinically effective and relatively less toxic than the existing drugs. Our results suggest that the anti-amnesic effects of \textit{A. calamus} and \textit{G. gnemon} demonstrated in the present study might be due to anti-oxidant action. The root of \textit{Hemidesmus indicus} has antioxidant and nootropic (memory enhancer) potential in mice. Similarly, \textit{Withania sominifera} root extract was shown to exhibit nootropic effects in mice and caused inhibition of AchE, which suggested that indirect facilitation of cholinergic transmission may be of great value in neurodegeneration states associated with cholinergic deficiencies\textsuperscript{34-35}. Moreover, ethanolic extract of \textit{Ocimum sanctum} ameliorated scopolamine (0.4mg/kg) as well as aging induced memory deficit. This amelioration suggested possible cholinergic modulation as mechanism of its action\textsuperscript{16}.

In the present investigations, since \textit{A. calamus} and \textit{G. gnemon} has shown restoring effects on the MDA and nitrite levels, we propose that in addition to the Acetylcholinesterase inhibitory activity, an antioxidant effect might also play an important role in the memory enhancing effect of \textit{A. calamus} and \textit{G.gnemon}. Moreover, based on the results of behavioral and biochemical parameters, we hypothesize that \textit{A. calamus} and \textit{G. gnemon} could possibly act directly as a free radical scavenger or regulator to inhibit AChE, oxidative activity and ionic homeostasis imbalance in neurons induced by scopolamine.

**Conclusion**

The present study demonstrates the beneficial effect of \textit{A. calamus} and \textit{G. gnemon} in the form of oral suspension on scopolamine induced amnesia. The extract significantly ameliorated the cognitive deficit. It showed significant anti-amnesic activity as assessed by behavioral tests using Morris water, Radial arm maze and Barnes maze model. Isolation and identification of active molecule or pure compound are necessitated for confirming its precise mechanism of action.

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