

Anti-amnesic effect of aqueous extract of *Crataeva nurvala* stem bark in scopolamine induced amnesia

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ARTICLE INFO

Article history:

Received on: 08/05/2014

Revised on: 05/06/2014

Accepted on: 17/07/2014

Available online: 27/09/2014

Key words:

Alzheimer; Morris water maze; acetylcholinesterase; lipid peroxidation.

ABSTRACT

Aim of the study was to evaluate the anti-amnesic effects of *Crataeva nurvala* stem bark in scopolamine induced amnesia in rats. The standardized aqueous extract of *Crataeva nurvala* (AECN) was freshly dissolved in distilled water and administered orally to rats at the dose of 500 mg/kg for evaluation of its effects against scopolamine (3 mg/kg, i.p.) induced retrograde and anterograde amnesia. The effects of the extract on escape latency during the acquisition trial (Days 1-4) and the time spent in target quadrant (TSTQ) during the retrieval trial (Day 5) were determined using the Morris water maze test. In order to indicate the probable mechanism of *C. nurvala*, its effects on acetylcholinesterase (AChE) and TBARS levels in the brain were determined. In scopolamine induced retrograde amnesia treatment with AECN showed a significant ($p < 0.05$) increase in TSTQ and reduction ($p < 0.001$) in levels of both AChE and TBARS in brain. In the anterograde amnesia model AECN significantly ($p < 0.001$) reduced the escape latency and increased ($p < 0.01$) the TSTQ. The extract also lowered the levels of AChE ($p < 0.01$) and TBARS ($p < 0.001$) in the brain of scopolamine induced anterograde amnesic rats. The study proved that AECN possesses positive effects on memory and learning, which may be due to decrease in AChE levels and inhibition of lipid peroxidation in the brain.

INTRODUCTION

Neurodegeneration is an umbrella term for the progressive loss of structure or function of neurons including their death. The examples of neurodegenerative disorders include Parkinson's, Alzheimer's and Huntington's disease. Aging is the greatest risk factor for neurodegenerative diseases. Mitochondrial DNA as well as oxidative stress both contributes to aging. Alzheimer's disease (AD) is a progressive and neurodegenerative disorder that mainly affects the elderly population (Singhal *et al.*, 2012). It is a brain disease that slowly destroys memory and thinking skills and eventually the ability to carry out the simplest tasks. AD is the commonest form of dementia and affects approximately 60 million people worldwide and 5.2 millions in US. Dementia is the loss of cognitive functioning like thinking, remembering, and reasoning and behavioral abilities, to such an extent that it interferes with a person's daily life and activities. It is estimated that by 2050, more than 115 million people worldwide will be affected by dementia (Dhingra and Kumar, 2012).

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The main causes of AD appear to be: (1) decreased cholinergic activity (2) deposition of beta amyloid peptides in brain, (3) formation of neurofibrillary tangles from tau proteins inside nerve cells and (4) oxidative stress.

There is no permanent cure for AD but the available drug treatments can improve the symptoms or temporarily slow down its progression. The therapeutic strategies include inhibition of acetylcholinesterase enzyme and blockade of NMDA glutamate receptors. Acetylcholine (ACh) is the neurotransmitter in cholinergic neurons and acetylcholinesterase (AChE) inhibitors such as donepezil, galantamine and rivastigmine prevent enzyme AChE from breaking down ACh in the brain. Thus increased concentration of ACh in the neurons increase cholinergic activity and thus temporarily improve the symptoms of AD. NMDA receptor antagonists (like Memantine) block glutamate release and protect brain cells from damaging effects of excess glutamate. Though these conventional medicines are successfully used for the treatment of memory loss, they suffer from various side effects. AChE inhibitors cause loss of appetite, nausea, vomiting and diarrhea while memantine causes headache, insomnia, dizziness, mental confusion and hallucinations (MacShane, 2012).

Herbal medicines are natural and relatively safe with fewer side effects and thus can be used with less caution. These are also cheaper than the conventional medicines and easily available. Some important plants known to possess memory enhancing effects are *Bacopa moniera* Linn. (Plantaginaceae; Saraf *et al.*, 2011), *Ginkgo biloba* Linn. (Ginkgoaceae; Shi *et al.*, 2010), *Myricaria elegans* Royle (Tamariscineae; Mukherjee *et al.*, 2007), *Acorus calamus* Linn. (Acoraceae; Oh *et al.*, 2004), *Rhododendron ponticum* Linn. (Ericaceae; Orhan *et al.*, 2004), *Vicia faba* Linn. (Fabaceae; Orhan *et al.*, 2004), *Tribulus terrestris* Linn. (Zygophyllaceae; Orhan *et al.*, 2004), and many more.

Crataeva nurvala Buch-Ham (Capparidaceae) is a common small to medium sized soft wooded tree that grows on the banks of canals, rivers, lakes etc. This evergreen plant grows widely in all parts of Bangladesh, Pakistan, India and China. Its common names are 'Varuna' and 'Three leaved caper' (Parvin *et al.*, 2011).

The plant contains various chemical constituents including alkaloids (cadabicine), tannins, saponins (diosgenin), flavonoids (rutin and quercetin) and triterpenes (lupeol and its acetates). *Crataeva nurvala* is traditionally used in treating blood flow, waste elimination, fever, metabolic disorders, wounds, weak immune system and memory loss (Bhattacharjee *et al.*, 2013). Despite its traditional claims for treating memory loss there is no scientific data to prove its anti-amnesic property; thus the present work was undertaken to substantiate or validate the traditional claim in a scientific manner.

MATERIALS AND METHODS

Drugs and Chemicals

Authentic dried aqueous extract of *Crataeva nurvala* (AECN) stem bark was provided by Amsar Pvt. Ltd. (Indore, M.P.) as a gift sample. Piracetam (Nootropil[®]) and scopolamine (Buscopan[®]) injections (ampoules) which contain scopolamine butylbromide were purchased locally from the market. Chemicals like DTNB, acetylthiocholine iodide, trichloroacetic acid, thiobarbituric acid, were procured from Himedia Pvt. Ltd. (Mumbai) and S.D. Fine Chemicals Ltd. (Mumbai).

Experimental Animals

Adult albino rats of Wistar strain, weighing 120-150 g and belonging to either sex were housed in polypropylene cages in groups of 5-6 animals per cage under laboratory conditions with alternating light and dark cycle of 12 h each. Animals had free access to food and water. Animals were acclimatized for a week before the commencement of the experiment in order to avoid any stress due to handling. The experimental protocols were approved (Approval No.: RIP/IAEC/2012-13/04) by the Institutional Animal Ethics Committee (IAEC) and conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Morris Water Maze Test

Morris water maze test is used to test learning including acquisition of spatial memory. The method described by Saraf *et al.* (2011) and Dhingra and Kumar (2012) was adopted for the present study. Morris water maze (142 × 60 cm) consisted of a circular pool (28 × 13 cm) filled upto a depth of 14 cm with water maintained at 25°C. Water was made opaque by adding titanium dioxide. The tank was divided into four equal quadrants by using two threads fixed at right angles to each other on the rim of the pool.

A submerged platform was placed inside the tank at the centre of the pool 1 cm below water level. The position of the platform was unaltered throughout the training session. Each animal was subjected to four consecutive trials each day with different points with 5 min gap between each trial for four consecutive days, during which they are allowed to escape on the hidden platform and to remain there for 20 sec. During the training session the rat was gently placed in the water from different locations facing the wall of the pool and allowed 120 sec to locate the submerged platform. If the rat failed to locate the platform within 120 sec, it was guided gently on to the platform and allowed to remain there for 20 sec. Each animal was subjected to training trials for four consecutive days and the starting position was changed with each exposure as mentioned below:

Day 1: (A), (B), (C), (D)

Day 2: (B), (D), (A), (C)

Day 3: (C), (B), (A), (D)

Day 4: (D), (C), (A), (B)

On the last day (retrieval day) of training session (i.e. Day 5), the platform was removed and the rat was placed in the pool from any of the point and allowed to explore the target quadrant (centre of the pool where the platform was placed) for 300 sec. The mean time spend in the centre of the pool in search of the missing platform (i.e. time spend in target quadrant, TSTQ), which is an index of retrieval or memory, was recorded.

Experimental design

Animals were divided into four groups consisting of five rats per group. Group I animals served as vehicle control and were administered distilled water (4 ml/kg, p.o.) followed by saline (1 ml/kg, i.p.) after 45 min. Group II animals served as the negative control and were administered the vehicle, distilled water (4 ml/kg, p.o.) followed by scopolamine (3 mg/kg, i.p.; dissolved in normal saline) after 45 min of vehicle administration. Group III animals represented the drug treated group and were administered 500 mg/kg (p.o.) of aqueous extract of *C.nurvala* (AECN), followed by scopolamine after 45 min of drug administration. AECN was dissolved in distilled water just before use. Group IV animals served as standard group and received piracetam (120 mg/kg, i.p.), followed by scopolamine after 30 min of piracetam administration. After 30 min of scopolamine administration the animals were subjected to the Morris water maze test. Actophotometer and rota rod tests were performed to screen the locomotor activity and

muscle coordination activity of rats, respectively before subjecting them to water maze test. Rats showing abnormal swimming pattern in water maze coupled with altered locomotor or muscle coordination activities were excluded from the study.

Effect of AECN on Scopolamine-Induced Retrograde Amnesia

To study the effect of the drug extract on retrieval or retention, the animals received the vehicle during the acquisition trial (days 1-4) and were subjected to four consecutive trials per day for four consecutive days to locate the platform in the centre of the water tank. On the fifth day, the animals received the drug treatment followed by scopolamine administration. The time spent in the target quadrant (TSTQ) by the animal in the centre of the tank in search of the missing platform was noted as an index of retrieval.

Effect of AECN on Scopolamine-Induced Anterograde Amnesia

To study the effect of drug on acquisition, the drug extract (AECN) or scopolamine was administered prior to the acquisition trial for 4 days. The animals were subjected to four consecutive trials per day for four consecutive days to locate the platform in the centre of the water tank. On fifth day, the vehicle distilled water (4 ml/kg, p.o.) was administered 45 min before the retrieval trial to all the groups. The platform was then removed and time spent in the target quadrant (TSTQ) was noted as an index of retrieval.

Biochemical estimations

Collection of Brain Sample

Immediately after behavioral testing (retrieval) in Morris water maze, the animals were sacrificed by cervical dislocation. The whole brain was carefully removed from the skull and weighed. A 10% w/v brain homogenate was then prepared by homogenizing it in ice chilled phosphate buffer (pH 8, 0.1 M). The homogenate was then centrifuged using a refrigerated centrifuge at 3000 rpm for 10 min and the resultant supernatant was used for the biochemical estimations.

Estimation of Brain Acetylcholinesterase (AChE) Activity

Brain acetylcholinesterase was estimated using the method of Ellman *et al.* (1961). Briefly, 0.4 ml of brain homogenate was added to a test tube containing 2.6 ml of phosphate buffer (0.1 M, pH 8). 100 μ l of DTNB reagent was added to the above mixture followed by the addition of 20 μ l of acetylthiocholine iodide solution. The absorbance was then noted at 412 nm for 5 min thereafter and the change in absorbance per min was calculated. The rate of moles of substrate hydrolyzed per min per gram of tissue was calculated by the following equation:

$$R = 5.74 \times 10^{-4} (\Delta A / C_0)$$

Where;

R= Rate, in moles of substrate hydrolysed per min per gram of tissue.

ΔA = Change in absorbance per min.

C_0 = Original concentration of tissue (mg/ml)

Estimation of brain thiobarbituric acid reactive substrate (TBARS) level

The TBARS level in the brain homogenate of animals was determined by the method of Slater & Sawyer (1971). Briefly, 2.0 ml of the tissue homogenate (supernatant) was added to 2 ml of freshly prepared 10% w/v trichloroacetic acid (TCA) and the mixture was allowed to stand in an ice bath for 15 min. After 15 min the precipitate was separated by centrifugation and 2 ml of clear supernatant solution was mixed with 2 ml of freshly prepared thiobarbituric acid (TBA).

The resulting solution was then heated in a boiling water bath for 10 min. It was then immediately cooled in an ice bath for 5 min. The colour developed was measured at 532 nm against reagent blank. Different concentrations (0-230nM) of standard malondialdehyde (1,1,3,3-tetraethoxypropane) were taken and processed as above for standard graph. The values were then expressed as nM/mg of tissue weight.

Statistical evaluation

The data was expressed as Mean \pm SEM and analyzed by one-way and two way analysis of variance (ANOVA) followed by Turkey's multiple comparison test and, with the level of significance set at $p < 0.05$.

RESULTS

Effects of AECN on scopolamine induced retrograde amnesia

Effect of AECN on retrieval of memory

In the animals of all groups (Groups I-IV) a gradual reduction in escape latency (ELT) was observed during the on-going acquisition trail period (Day 1 to Day 4), where the ELT on day 4 was found to be similar in all the groups (Table 1).

Table 1: Effect of AECN on Scopolamine induced retrograde amnesia.

Groups	ESCAPE LATENCY TIME (ELT) (in sec)				TSTQ (in sec)
	Day 1	Day 2	Day 3	Day 4	Day 5
I	72.8 \pm 8.83	45.2 \pm 11.24	29.1 \pm 8.32	21.9 \pm 6.33	66.6 \pm 3.66
	II	57.8 \pm 9.16	34.5 \pm 5.47	24.7 \pm 4.27	13.7 \pm 2.11
III		73.4 \pm 8.36	54.6 \pm 8.58	25.6 \pm 4.67	14.4 \pm 3.25
	IV	56.1 \pm 11.30	36.4 \pm 7.90	24.4 \pm 5.14	17.9 \pm 3.05

Values are expressed as Mean \pm SEM; ^{*} $p < 0.05$, ^{**} $p < 0.01$, ^{***} $p < 0.001$; Negative control group was compared with the vehicle control group, Standard and test groups were compared with the negative control group. Group I: Vehicle treated control group, Group II: Scopolamine treated negative control group, Group III: Standard drug (Piracetam) treated group, Group IV: Test drug (AECN) treated group.

On the retrieval day (i.e. day 5) when the platform was removed, the control group animals (Group I) were found to spend 66.6 \pm 3.67 sec in the target quadrant (TSTQ). In Group II where the animals on the retrieval day (day 5) received scopolamine 30 min before the trial, a significant ($p < 0.001$) reduction in TSTQ as compared to the control group (Group I) was observed (Table 1). Animals of the standard group (Group III) which were

administered the drug, piracetam (120 mg/kg, i.p.) followed by scopolamine on the 5th day showed a significant ($p < 0.01$) increase in TSTQ as compared to the scopolamine treated negative control group (Group II). Similarly the animals of the test group (Group IV) which on the 5th day received AECN (500 mg/kg, p.o.) followed by scopolamine showed a significant ($p < 0.05$) increase in the time spent in the target quadrant (TSTQ) as compared to the negative control group animals (Table 1).

Estimation of AECN on brain AchE level

In the vehicle treated control group (Group I) animals the levels of AchE in the brain was found to be 5.68 ± 1.24 mmol/l/min $\times 10^{-6}$ /g of tissue. scopolamine administration was found to significantly ($p < 0.001$) increase the levels of AchE in the brain of negative control group (Group II) animals as compared to control group (Group I) animals. Treatment with AECN (500 mg/kg, p.o.) significantly ($p < 0.001$) reduced the levels of AchE in the brain of test group (Group III) animals as compared to the scopolamine treated (negative control) group. Similarly treatment with the standard drug, piracetam (120 mg/kg, i.p.) in Group IV animals was also found to significantly ($p < 0.001$) reduce the level of AchE in the brain (Fig 1).

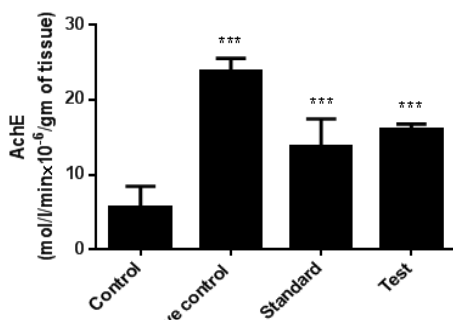


Fig. 1: Values are expressed as Mean \pm SEM; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Negative control group was compared with the vehicle control group, Standard and test groups were compared with the negative control group. Group I: Vehicle treated control group, Group II: Scopolamine treated negative control group, Group III: Standard drug (Piracetam) treated group, Group IV: Test drug (AECN) treated group.

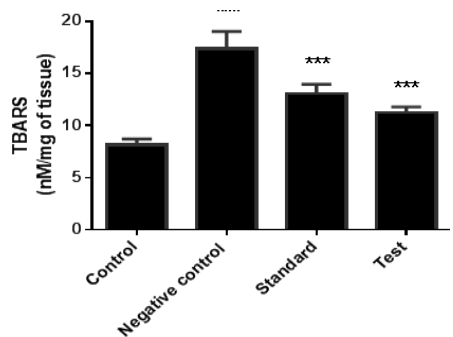


Fig. 2: Values are expressed as Mean \pm SEM; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; Negative control group was compared with the vehicle control group, Standard and test groups were compared with the negative control group. Group I: Vehicle treated control group, Group II: Scopolamine treated negative control group, Group III: Standard drug (Piracetam) treated group, Group IV: Test drug (AECN) treated group.

Effect of AECN on brain TBARS level

In vehicle treated control group (Group I), the MDA content was found to be 8.16 ± 0.24 nM/mg of tissue. Administration of scopolamine to Group II animals significantly ($p < 0.001$) increased the level of MDA in the brain as compared to the control group (Fig 2). Treatment with standard drug (piracetam) at the dose of 120 mg/kg (i.p) and test drug (AECN) at the dose of 500 mg/kg (p.o.) to Groups III and IV animals, respectively, showed significant ($p < 0.001$) decrease in the level of MDA in the brain as compared to the scopolamine treated negative control group (Fig.2).

Effects of AECN on scopolamine induced anterograde amnesia

Effect of AECN on acquisition of memory

The vehicle treated control group (Group I) animals showed a gradual and significant reduction in the escape latency time (ELT) from day 1-4 as compared to day 1 (Table 2). On the last day (day 5) when the platform was removed these animals were found to spend 66.6 ± 3.67 sec in the target quadrant (TSTQ). Scopolamine treated negative control group (Group II) animals showed a progressive and significant increase in ELT from day 1-4 as compared to day 1. On the 5th day, after removal of the platform scopolamine was found to significantly ($p < 0.001$) reduce the time spent in the target quadrant (TSTQ) as compared to the vehicle treated control group (Table 2). The animals treated with the standard drug, piracetam (120 mg/kg, i.p) in Group III or with AECN (500 mg/kg, p.o.) in Group IV showed a gradual and significant reduction in ELT from day 1-4 when compared to day 1. On the retrieval day (Day 5) the animals of both the groups, Group III ($p < 0.05$) and Group IV ($p < 0.01$) showed a significant increase in the TSTQ as compared to the negative control group (Group II).

Table 2: Effect of AECN on acquisition in scopolamine induced anterograde amnesia.

Groups	ESCAPE LATENCY TIME (ELT) (in sec)				TSTQ (in sec)
	Day 1	Day 2	Day 3	Day 4	Day 5
I	72.8 \pm 8.83	45.2 \pm 11.24**	29.1 \pm 8.32***	21.9 \pm 6.33***	66.6 \pm 3.66
	62.9 \pm 11.60	87.6 \pm 8.14*	88.3 \pm 4.70**	109.3 \pm 3.60***	21.3 \pm 3.70***
III	73.2 \pm 10.99	36.7 \pm 3.51**	24.7 \pm 3.81***	22.6 \pm 2.64***	44.0 \pm 2.08*
	81.8 \pm 10.01	60.4 \pm 10.44*	46.4 \pm 9.88**	28.9 \pm 3.70***	50.3 \pm 3.18**

Values are expressed as Mean \pm SEM; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ELT on days 2, 3 and 4 were compared with ELT on day 1, within the group. TSTQ of Group II was compared with that of Group I, while TSTQ of groups III and IV were compared with group II. Group I: Vehicle treated control group; Group II: Scopolamine treated negative control group; Group III: Standard drug (Piracetam) treated group; Group IV: Test (AECN) treated group.

Effect of AECN on brain AchE levels:

In the vehicle treated control group (Group I) animals the levels of AchE in the brain was found to be 6.83 ± 1.83 mol/l/min $\times 10^{-6}$ /g of tissue. Scopolamine administration was found to significantly ($p < 0.001$) increase the levels of AchE in the brain

of negative control group (Group II) animals as compared to control group (Group I) animals (Fig. 3). Treatment with piracetam (120 mg/kg, i.p.) and AECN (500 mg/kg, i.p) to Groups III ($p<0.05$) and IV ($p<0.01$) significantly reduced the level of AchE in the brain of animals as compared to scopolamine treated negative control (Group II) animals (Fig. 3).

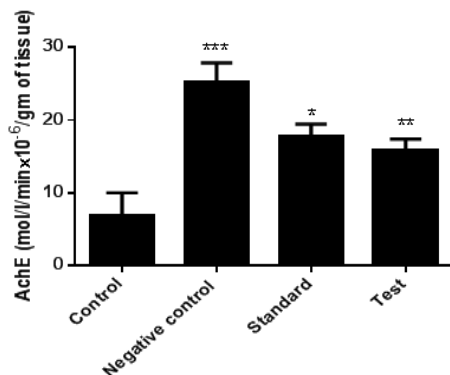


Fig. 3: Values are expressed as Mean \pm SEM; * $p<0.05$, ** $p<0.01$, *** $p<0.001$; Negative control group was compared with the vehicle control group, Standard and test groups were compared with the negative control group. Group I: Vehicle treated control group, Group II: Scopolamine treated negative control group, Group III: Standard drug (Piracetam) treated group, Group IV: Test drug (AECN) treated group.

Effect of AECN on the brain levels of TBARS:

In vehicle treated control group (Group I), the brain MDA content was found to be 8.13 ± 0.26 nM/mg of tissue. Administration of scopolamine significantly ($p<0.001$) increased the level of MDA in the brain of scopolamine treated negative control group (Group II) animals as compared to the control group (Group I) animals (Fig. 4).

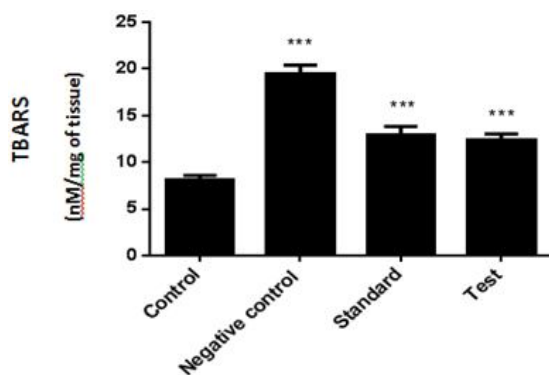


Fig. 4: Values are expressed as Mean \pm SEM; * $p<0.05$, ** $p<0.01$, *** $p<0.001$; Negative control group was compared with the vehicle control group, Standard and test groups were compared with the negative control group. Group I: Vehicle treated control group, Group II: Scopolamine treated negative control group, Group III: Standard drug (Piracetam) treated group, Group IV: Test drug (AECN) treated group.

Treatment with standard drug (piracetam) at the dose of 120 mg/kg (i.p.) and test drug (AECN) at the dose of 500 mg/kg (p.o.) to the animals of Groups III and IV, respectively, showed significant ($p<0.001$) decrease in the level of MDA in the brain as compared to the scopolamine treated negative control group (Group II).

DISCUSSION

Cognition is the psychological process of knowing including awareness, perception reasoning and judgement. Central cholinergic system plays an important and major role in regulation of cognitive function. Scopolamine, a muscarinic receptor antagonist, has been widely adopted to study cognitive deficits in experimental animals. Scopolamine is reported to impair cognitive performances especially spatial learning and acquisition (Saraf *et al.*, 2011) and also reduces the cholinergic function and produces amnesia in laboratory animals (Attrey *et al.*, 2012). The scopolamine amnesia test is widely used as primary screening test for so-called anti-Alzheimer drugs.

There are two main types of amnesia, retrograde amnesia and anterograde amnesia. Retrograde amnesia is the inability to retrieve information that was acquired before a particular event whereas anterograde amnesia is the inability to acquire new information. Earlier studies proved that scopolamine significantly impaired the acquisition and retrieval of memory producing both anterograde and retrograde amnesia (Saraf *et al.*, 2011). Recently many studies showed that scopolamine induced animal model is associated with increased oxidative stress and the increased level of acetylcholinesterase enzyme (AchE) within the brain (Goverdhan *et al.*, 2012).

Morris water maze is used to assess learning and memory in experimental mice. There are several advantages of Morris water maze over other models of learning and memory including absence of motivational stimuli such as food and water deprivation, electrical stimulations, and buzzer sounds (Morris, 1984). In morris water maze, memory is developed in animals progressively with repetitive trials that resemble the human interactions. During the acquisition trials the rat learns to locate a hidden platform and subsequently develops spatial memory. This model is very helpful to analyze the reversal of amnesic effect with investigational drugs because repetitive trials confirm the progress of reversal of amnesia. Scopolamine is reported to exert amnesic effects in various behavioral models including the Morris water maze test (Saraf *et al.*, 2011).

In Morris water maze test the animals are trained to locate a platform for 4 days (acquisition period) and then to test the retrieval of memory of animals the platform is removed on the 5th day and the time spent in the target quadrant (TSTQ) is taken as an index of memory. Normal animals have been reported to show a gradual decrease in escape latency (ELT) in Morris water maze during the acquisition period, which is an indication of learning (Saraf *et al.*, 2011). In previous studies it has been proved that scopolamine impairs both acquisition and retrieval of memory.

In earlier studies, after the normal acquisition period administration of scopolamine on the retrieval day (day after acquisition) before the removal of the platform was found to reduce the TSTQ as compared to the vehicle control animals. This effect proved that scopolamine impairs the retrieval of memory or produces retrograde amnesia (Saraf *et al.*, 2011). Similarly in the present study, administration of scopolamine (3 mg/kg, i.p) on the retrieval day (day 5) significantly reduced the TSTQ, confirming

the induction of retrograde amnesia. Piracetam at the dose of 400 mg/kg (i.p.) was reported to reverse the scopolamine (0.4 mg/kg) induced retrograde amnesia in mice. Administration of scopolamine after 45 min of piracetam administration during the retrieval (Day 5) trial showed a marked increase in TSTQ in scopolamine induced retrograde amnesic mice (Ashwlayan & Singh, 2011). In the present study, treatment with piracetam (120 mg/kg, i.p) and AECN (500 mg/kg, p.o.) on the 5th day significantly reversed the reduction in TSTQ during the retrieval trial (Day 5) as compared to the scopolamine treated negative control group. In the present study AECN was used at the dose of 500 mg/kg (p.o.) based on previous studies where the similar extract of *C.nurvala* stem bark was proved for its anti-diarrheal (Inayathulla *et al.*, 2010) and anti-hyperlipidemic activities (Sikarwar & Patil, 2012). The results thus showed that piracetam and AECN reverses the scopolamine induced retrograde amnesia.

Previous reports indicated that administration of scopolamine during the acquisition trial (Day 1 to Day 4) rather than on the retrieval trial day, showed a gradual increase in ELT which suggests that scopolamine impairs the process of acquisition and thus produces anterograde amnesia (Saraf *et al.*, 2011). The administration of scopolamine during the acquisition trial not only affected the learning but also reduced the TSTQ on the 5th day which reflected effects on retrieval of memory also. Similarly, in the present study, administration of scopolamine (3 mg/kg, i.p) during the acquisition trial (Day1 to Day 4) gradually increased the ELT and reduced the TSTQ of negative control animals as compared to vehicle treated control group. This indicated that scopolamine induced anterograde amnesia in the animals by impairing the learning process and also affected retrieval.

Piracetam at the dose of 400 mg/kg (i.p.) was reported to reverse the scopolamine (0.4 mg/kg) induced anterograde amnesic effects by decreasing ELT during acquisition trial and increasing TSTQ on the retrieval day (Ashwlayan & Singh, 2011). In the present study also, both piracetam (120 mg/kg, i.p) and AECN (500 mg/kg, p.o.) significantly affected both ELT and TSTQ. They gradually and significantly decreased the ELT during acquisition trial (Day 1 to Day 4) and increased the TSTQ during retrieval trial (Day 5) as compared to scopolamine treated negative control group. This effect showed that piracetam and AECN reversed the scopolamine induced anterograde amnesia by both increasing the learning and retrieval of memory in animals.

Acetylcholine (Ach) is the major neurotransmitter of cholinergic system, while acetylcholinesterase (AChE) is a serine protease that hydrolyses the neurotransmitter Ach. Alzheimer's disease (AD) progresses to mainly affect the cholinergic system. A decline in cortical cholinergic activity in the brain correlates with the severity of the AD symptoms and with the observed intellectual deterioration in the patient. As the disease worsens, cholinergic neurons are progressively lost and the level of acetylcholine (Ach) and number of Ach receptors declines. Enhanced AChE activity in the brain leads to a decline in the level of Ach in the brain of AD patients (Zhang *et al.*, 2004). To improve the cholinergic transmission, different strategies are

adopted including the stimulation of cholinergic post synaptic muscarinic and nicotinic receptors and inhibition of Ach synaptic degradation by employing acetyl cholinesterase inhibitors (Parle & Bansal, 2011).

It was earlier reported that scopolamine significantly increases the levels of AChE in the brain of the animal as compared to the vehicle treated control animals. Scopolamine, a non-selective anti-muscarinic agent causes impairment of memory and its administration decreases Ach concentration and increases the level of AChE in the brain of animals (Attrey *et al.*, 2012; Otari *et al.*, 2012). A study revealed that administration of scopolamine on the retrieval day (day 5) at the dose of 1.4 mg/kg (i.p.) significantly increased the activity of AChE in the brain of scopolamine treated negative control animals as compared to vehicle treated control group (Goverdhan *et al.*, 2012). This suggests that scopolamine increases the levels of AChE in retrograde amnesic animals. In another study, animals administered with scopolamine at the dose of 0.5 mg/kg (i.p.) 30 min before acquisition trials for four consecutive days (Day 1 to Day 4) and the vehicle (Distilled water) on the retrieval day (5th day), showed a significant increase in AChE levels in the brain of scopolamine treated negative control group animals as compared to the animals of vehicle treated control group. This suggested that administration of scopolamine increases the levels of AChE in the brain of anterograde amnesic animals (Sharma *et al.*, 2010). In the present study, administration of scopolamine (3 mg/kg, i.p) to the negative control animals in both retrograde and anterograde amnesia models showed an increase in AChE levels in the brain as compared to the vehicle treated control groups.

Piracetam is reported to reduce the levels of AChE in the brain of both scopolamine induced retrograde (Joshi & Parle, 2006) and anterograde (Parle & Bansal, 2011) amnesic animals at the doses of 200 and 400 mg/kg, respectively. Similarly piracetam (120 mg/kg, i.p) treatment in the present study also significantly reduced the levels of AChE in retrograde and anterograde amnesic rats. Administration of AECN at the dose of 500 mg/kg (p.o.) significantly reduced the levels of AChE in test group animals in both retrograde and anterograde amnesia as compared to scopolamine treated negative control group, indicating a direct or indirect anticholinesterase effect of AECN.

The brain is especially vulnerable to oxidative damage as a result of high oxygen consumption rate and abundant content of easily peroxidizable fatty acids. The generation of ROS and oxidative stress is reported to be involved in the pathogenesis of neurodegenerative diseases (Melo *et al.*, 2011). Oxidative stress is linked to neuronal protein misfolding, membrane dysfunction, cell death and glial cell activation that are associated with normal ageing or certain neurodegenerative diseases (Lamari *et al.*, 2009). Scopolamine administration is reported to increase the brain levels of malondialdehyde (MDA) which is a measure of lipid peroxidation and free radical generation (Goverdhan *et al.*, 2012). Piracetam at the dose of 150 mg/kg has been reported to significantly reduce the levels of brain TBARS as compared to the scopolamine (1 mg/kg, i.p) treated negative control group during

both acquisition and retrieval trials (Kshirsagar *et al.*, 2012). It has also been proposed that piracetam reduces oxidative stress by improving mitochondrial dysfunction (Keil *et al.*, 2006). In the present study also scopolamine (3 mg/kg, i.p) significantly increased the levels of brain TBARS, while administration of piracetam (120 mg/kg, i.p) and AECN (500 mg/kg, p.o.) significantly reduced the level of TBARS, indicating inhibitory effects on lipid peroxidation.

CONCLUSION

Thus the present study revealed that aqueous extract of *Crataeva nurvala* stem bark (AECN) possesses positive effects in improving memory in amnesic animals, which either may be due to the inhibition of lipid peroxidation or due to its anti-AchE activity. The results thus not only confirmed the traditional claims but also indicated a therapeutic utility of stem bark of *Crataeva nurvala* in memory related disorders like Alzheimer's disease, provided clinical studies indicate the same.

ACKNOWLEDGEMENT

The authors would like to thank The Director, Rayat Institute of Pharmacy (Punjab) for providing the necessary facilities for the research work. The authors also thank Amsar Pvt Ltd. (Indore, M.P., India) for providing the aqueous extract of the plant as a gift sample.

Declaration of interest

The authors declare no conflict of interest.

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How to cite this article:

Anshul Tanwar, Pallavi A Bafna, Anand R Bafna. Anti-amnesic effect of aqueous extract of *Crataeva nurvala* stem bark in scopolamine induced amnesia. *J App Pharm Sci*, 2014; 4 (09): 066-072.