EFFEY OF ECLIPTA ALBA ON SCOPOLAMINE INDUCED AMNESIA IN MICE

Chahal Harmel Singh¹*, Sharma Shailendra²

¹ Jodhpur Institute of Pharmacy, Jodhpur National University, Jodhpur 342003, Rajasthan, India
² Department of Pharmacy, Jai Narain Vyas University, Jodhpur 342011, Rajasthan, India

ABSTRACT

The present study deals with the evaluation of potential effects of Eclipta alba (EA) in memory impairment of mice. Memory impairment was induced by scopolamine (3 mg/kg, i.p) in animals. To assess learning and memory in mice Morris water maze test was employed. The acetylcholinesterase enzyme (AChE) activity in brain was measured to evaluate the central cholinergic activity. The levels of thiobarbituric acid reactive species (TBARS) and reduced glutathione (GSH) in brain were estimated to assess the degree of oxidative stress. Scopolamine treatment produces significant impairment of learning and memory in mice, as reflected by a significant decrease in MWM performance. Scopolamine also produced a significant enhancement of brain AChE activity and brain oxidative stress (increase in TBARS and decrease in GSH) levels. EA (300 and 600 mg/kg, oral) significantly prevented scopolamine-induced learning and memory deficits along with decrease of scopolamine-induced rise in brain AChE activity and brain oxidative stress levels. It may be concluded that Eclipta alba has significant protective action against scopolamine induced memory deficits in mice that can be attributed to its anti AChE and anti oxidant actions.

Keywords: Alzheimer disease, Oxidative stress, Morris water Maze, Scopolamine

INTRODUCTION

Alzheimer’s disease (AD) a neurodegenerative disorder of the elderly is a leading cause of dementia in developed countries. AD is a progressive, degenerative disease characterized by memory loss, language deterioration, poor judgment, impaired visuospatial skills, etc. Dysfunction of cholinergic neurotransmission in the brain contributes to the salient cognitive decline in AD. Loss of cholinergic cells, particularly in the basal forebrain, is accompanied by loss of the neurotransmitter acetylcholine.¹ Pathologically, dementia in AD patients is caused by at least two distinct characteristic events. Deposition of the amyloid beta (Ab) peptide in the extracellular space and formation of intra-neuronal tangle owing to hyperphosphorylation of axonal Tau protein. Although deposition of Ab peptide and associated reactive oxygen species mediated neuronal damage is one of the major hallmarks of AD, the exact sequence of neuronal loss, synaptic dysfunction, and biochemical cascade of events therein are still unknown.² The common symptoms of AD, such as loss of memory and tremor, have been recognized as a feature of increasing age for a long time and are acknowledged in many traditional medical systems.³ AD has affected more than 37 million worldwide and the economic burden in US alone is estimated to be around $100 billion.⁴

Since no cure for patients with AD is currently available, symptomatic treatment for AD focuses on the restoration of cholinergic function. One of the most accepted strategies in AD treatment is the use of cholinesterase inhibitors. Their clinical efficacy is thought to result from prolonging the half-life of...
acetylcholine (Ach) through inhibition of acetylcholinesterase enzyme (AChE). Rivastigmine, galantamine and donepezil are some of the AChE inhibitors which are main stay of treatment of cognitive deficit in AD. Memantine a NMDA receptor antagonist is another drug being used clinically. However these limited therapeutic modalities provide only symptomatic relief and are associated with number of undesirable side effects. So there is a need for the agent that not only will provide symptomatic relief but also halt the progression of the disease. Recently scientists have directed their focus towards herbal products as a source of treatment. Several herbal drugs with antioxidant properties have been reported to have beneficial effects in AD. Clinical studies in AD patients have already shown that Ginkgo biloba is as effective as donepezil in improving dementia. Although the mechanisms of the ant amnesic effects of most herbal extracts and constituents are not yet fully understood, one or more of the components could be responsible for the activation of the central ACh function through the inhibition of AChE and activation of ACh synthesis. Recent studies have pointed out that AD is associated with inflammatory processes. Reactive oxidative species (ROS) are able to damage cellular constituents and act as secondary messenger in inflammation. The use of antioxidants may be useful in the treatment of AD.

**Eclipta alba** (L) Hassk. Family-Asteraceae has been mentioned in ancient texts to be a nervine tonic. In addition to possessing hepato protective, hair growth promoting and anti-aging properties. A Small much-branched annual herb with white flower heads, which is found in most situations throughout India ascending up to 600 feet on the hills. Commonly, it is known as ‘white bhringraj’ when in flower and as ‘black bhringraj’ when in fruit. The plant is reported to contain the phytoconstituents eclalatin, alphanamyrin, ursolic acid, oleanolic acid, eclipta saponin, daucosterol, stigmasterol-3-O-glucoside and coumestans (wedelolactone and demethylwedelolactone) as main active principles. A number of herbal preparations comprising of *Eclipta alba* are available for treatment of jaundice and viral hepatitis.

In view of the above observations, we have selected the *Eclipta Alba* to explore its role in the treatment of AD. Exploring ameliorative potential and possible mechanism of *Eclipta Alba* in AD will open up new avenues in the drug therapy of this neurodegenerative disorder.

Scopolamine has been used to induce experimental models of AD. Scopolamine significantly increases AChE and malondialdehyde (MDA) levels in the cortex and hippocampus and has been used to screen anti-amnesic drugs for the age-related CNS dysfunction. The elevation of brain oxidative status after administration of amnesic doses of scopolamine further substantiates the value of scopolamine-induced amnesia as an animal model to test for drugs with potential therapeutic benefits in dementia. To assess the anti-amnesic effects of EA in mice, we evaluated the effect of EA on scopolamine-induced learning and memory deficits in the Morris water maze test. This study also evaluated the effect of EA on the biochemical parameters like AChE, TBARS and GSH activity in the brains of mice with scopolamine-induced dementia.

**MATERIALS AND METHODS**

**Animals**

Swiss albino mice of either sex weighing 25±2g were employed in present study. The animals were housed in the departmental animal house and were exposed to 12hr light and dark cycle. Before experiments the animals were acclimatize to the laboratory conditions. The experimental protocol was duly approved by the institutional animal ethical committee and care of the animals was carried out as per the guidelines of CPCSEA.

**Drugs and Chemicals**

Memantine (Almenta®, 5mg/tablet, Sun Pharmaceutical Ltd, Mumbai, India), suspended in tween 80, were given orally and used as reference standard drugs in this study. Scopolamine was purchased from sigma-Aldrich (MO, USA). The dose of drugs and chemicals were selected based on previous literature report. All chemicals of analytical grade were used in the study.

**Preparation of Aqueous extract of Eclipta alba**

The plant drug was collected from the local market and authenticated by department of Pharmacognosy. The drug was coarsely powdered. The powdered plant material was then subjected to extraction with water by decoction for 18 h in Soxhlet’s extractor, to obtain the aqueous extracts. The yield of aqueous extract (Aq. Ext.) was found to be 31.44% (w/w). Preliminary photochemical investigations of the extract revealed presence of saponins, tannins, carbohydrates and phenolic compounds.

**Experimental design**

Mice were randomly allotted to groups of 6 animals each and treatment was carried out as outlined below:

- **Group I (Vehicle control):** Vehicle control group were treated with 1% tween 80 (p.o) 30 min before acquisition trials for four consecutive days and 30 min before retrieval trial (on day 5).
- **Group II (Amnesic control):** Amnesic control group were treated with 3mg/kg Scopolamine intra-peritoneal 30 min before acquisition trial conducted from day 1 to day 4 and then vehicle only 30 min prior to retrieval trial conducted on day 5.
- **Group III (Standard Treated Group):** This group was treated with 3mg/kg Memantine and 3mg/kg/day 30 min before acquisition trial conducted from day 1 to day 4 and then vehicle only 30 min prior to retrieval trial conducted on day 5.
- **Group IV (Test dose 1 treated group):** This group was treated with 3mg/kg scopolamine and extract of EA 300mg/kg/day 30 min before acquisition trial conducted from day 1 to day 4 and then vehicle only 30 min prior to retrieval trial conducted on day 5.
Evaluation of Memory using Morris water Maze:
Morris water maze was used to assess learning and memory in experimental mice. A circular pool (140 cm in diameter, 50 cm high) was filled to depth of 30 cm with water at a temperature of 22±18°C. The pool was divided into four quadrants of equal area, Q1, Q2, Q3, and Q4. A glass platform (20 cm in diameter) was placed 1 cm below the surface, midway between the center and rim of the pool in the quadrant (Q4). The mice were introduced into the pool in the Q4 quadrant, and an uninformed observer measured the time taken for it to find the escape platform. In the event the animal was unable to locate the hidden platform within 180 s it was gently guided to it, kept for 20 s and then removed from the pool. The point of entry of the mice into the pool and the location of the escape platform remained unchanged throughout training session. Each animal was subjected to a daily session of three trials per day (with a rest period of 30–45 min inter trial periods) for five consecutive days. Escape latency time (ELT) in seconds was recorded and day 4 ELT was taken as an index of acquisition whereas day 5 time spent in target quadrant (TSTQ) served as an index of retrieval or memory.

Brain Tissue Sampling and Preparation
After completion of behavior tests, the animals were kept fasting for 12 hours. Blood samples were collected using the orbital sinus technique. Each animal was sacrificed and the whole brain of each animal was rapidly dissected, thoroughly washed with isotonic saline, dried and then weighed. The brain was homogenized with ice-cold 0.1M phosphate buffer (pH 7.4, 10% w/v). The homogenate was centrifuged at 3000 rpm for 10 min at 4°C. The supernatant (10%) was separated for biochemical analysis.

Estimation of brain AChE activity
The whole brain acetyl cholinesterase activity (AChE) was measured by the method Ellman et al. and Voss and Sachsse. The change in absorbance per min. of the sample was read spectrophotometrically at 420nm.

Estimation of thiobarbituric acid reactive substances
The quantitative measurement of Thiobarbituric acid reactive substance (TBARS) as an index of lipid per oxidation in brain was performed according to the method Ohkawa et al. The absorbance of developed pink color was measured spectrophotometrically at 532nm. TBARS value was expressed as nano moles per mg of protein.

Estimation of reduced glutathione
The reduced glutathione content (GSH) in tissue was estimated using Beutler et al. Absorbance was noted spectrophotometrically at 412nm. All the values were expressed as micromoles of reduced glutathione per mg of protein.

Statistical Analysis
The results were expressed as mean ± standard deviation (S.D). The data obtained from various groups were statistically analysed using one-way ANOVA followed by Turkey’s multiple range test. A value of p<0.05 was considered to be statistically significant.

RESULT
Effect on ELT and time spent in target quadrant (TSTQ) using Morris water maze:
Control untreated mice exhibit a significant fall in day4 ELT as compared to its value on day1, reflecting acquisition (learning). Further significantly more time was spent in the target quadrant (Q4) in search of the missing platform as compare to the total time in the other quadrant (Q1, Q2, and Q3) during retrieval trial on 5th day, signifying memory or retrieval. Administration of scopolamine (3mg/kg) significantly prevented the diminish in day4 ELT as compared to the vehicle control group and markedly diminished TSTQ (Q4) in search of missing platform during the retrieval trial, reflecting impairment of both learning as well as memory. Administration of Memantine10mg/kg/day to scopolamine-treated animals significantly attenuated day 4 rise in the ELT as well as day 5th decrease in TSTQ (P<0.05). Treatment with EA (300 and 600mg/kg) significantly decreased day4 ELT and increased day 5th TSTQ (P<0.05) in dose dependent manner, reflecting reversal of scopolamine-induced memory deficits [Figure 1].

Effect on Brain AChE Activity
Scopolamine administration significantly, increase the brain AChE activity (µmol/min/mg protein) on 15th day as compared to the control group (p<0.05). Treatment of EA (300 and 600 mg/kg) significantly decreased AChE activity when compared to the scopolamine (amnesic control) group (p<0.05). Memantine also significantly decreased AChE activity when compare to the scopolamine (amnesic control) group (p<0.05).Figure 2.
Effect on Brain TBARS Activity

Scopolamine treated group significantly increase the TBARS activity on 15th day as compare to control group, reflecting enhanced oxidative stress (p<0.05). EA (300 and 600mg/kg) treated group significantly decreased the TBARS level (p<0.05) and abolished the scopolamine induced rise in brain oxidative stress level. Memantine also significantly decreased TBARS activity when compare to the scopolamine (amnesic control) group (p<0.05). Figure 3.
Figure 3: Effect of EA 300mg/kg, 600mg/kg on TBARS level in mice brain. a = p<0.01 brain TBARS level of scopolamine treated group. b = p<0.05 Vs brain TBARS level of scopolamine treated group. c = p<0.05 Vs brain TBARS level of scopolamine treated group.

Effect on Brain GSH Activity
Scopolamine treated group significantly reduce the GSH level on 15th day as compare to control group (p<0.05) and reflecting enhanced oxidative stress. Treatment with EA (300 and 600mg/kg) significantly abolished the scopolamine induced rise in brain oxidative stress level (p<0.05) and significantly increase the level of GSH. The standard drug Memantine also reversed the effect of scopolamine on the GSH level. Figure 4.

Figure 4: Effect of EA 300mg/kg, 600mg/kg on GSH level in mice brain. a = p<0.01 brain GSH level of scopolamine treated group. b = p<0.05 Vs brain GSH level of scopolamine treated group. c = p<0.05 Vs brain GSH level of scopolamine treated group.
DISCUSSION

MWM test is one of the most widely accepted models to evaluate learning and memory of the animals. A significant decrease in day 4 ELT of control animals during the ongoing acquisition trials indicate normal acquisition of memory and an increase in TSTQ in search of missing platform during retrieval trial indicate retrieval of memory. In the present study, scopolamine produced impairment of acquisition and retrieval of memory, as reflected by significant increase in day 4 ELT and decrease in day 5 TSTQ, respectively. These results are consistent with earlier findings. In the present investigation, pretreatment of EA (300 and 600mg/kg) abolished scopolamine-induced memory deficits.

The central cholinergic system plays a major role in the process of learning and memory and has been carefully expressed over the last several years. The cholinergic cell bodies are destroyed by the disease process in AD patients, leading to a deficiency of the neurotransmitter Ach. Several studies have revealed that blockade of Ach effects by muscarinic receptor antagonist scopolamine caused memory deficits in both normal and elderly groups. In our study, EA treatment significantly inhibited AchE activity in dose related manner. Therefore, it can be revealed that the anti-amnesic effect of EA on scopolamine-induced impairment of learning and memory may be related to modification of cholinergic neuronal systems. Increased TBARS activity has been shown to be an important marker for in vivo lipid peroxidation in the learning and memory deficient mouse brains. In addition, it has been reported that scopolamine significantly increases TBARS activity in the hippocampus and frontal cortex. Levels of TBARS were elevated in all AD brain regions except the middle frontal gyrus. Elevated levels reached statistical significance in the hippocampus and pyriform cortex and marginal significance in the amygdala of AD subjects compared with age-matched controls. because thiobarbituric acid is highly reactive with non-lipid moieties as well as lipid per oxidation products; TBARS provide a non-specific marker of lipid peroxidation in AD brain. In our study, EA treatment significantly inhibited TBARS activity in dose related manner. These findings are in line with earlier studies. This study further evaluated whether such impaired cognition by scopolamine is associated with altered oxidative stress indices. Scopolamine-treated mice had with reduced GSH activity. Oxidative stress results from a marked imbalance between free radical production and elimination by antioxidant systems. Many studies have reported the strong positive correlation that memory impairments in the scopolamine-induced amnesic mice show with patterns of oxidative damage in patients with amnesic mild cognitive impairment. A clinical study have reported that oxidative stress is closely related in the pathogenesis of AD. GSH is the principal intracellular nonprotein thiol and plays a major role in the maintenance of the intracellular redox state. The level of GSH diminishes with an increase in the generation of free radicals. In the present study, GSH was estimated on the 15th day after the first injection of scopolamine. Scopolamine-treated mice showed a significant decrease in the GSH in the brain compared to control values, indicating elevated oxidative stress. But EA treatment produced significant increase in the activities of GSH in mice brain. These observations suggest that EA produced significant antioxidant activity against scopolamine-induced oxidative stress. The effective anti-amnesic effect of EA might result, in the reduction in oxidative stress and Inhibition of AchE activity which is the basis of AD management. Our study showed that EA can be effective neuroprotective agents which could be linked to the inhibition of both AchE and TBARS activity and increase in GSH activity in the brain of mice with scopolamine-induced amnesia. The anti-AChE, antioxidant and anti-amnesic effects of EA on scopolamine-induced cognitive impairments suggest that this may be the possible herbal drug for the treatment of AD.

REFERENCES
