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Precautionary Ellagic Acid Treatment Ameliorates Chronically Administered Scopolamine Induced Alzheimer's Type Memory and Cognitive Dysfunctions in Rats

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ABSTRACT

Background and Objective: The neuroprotective ability of Ellagic Acid (EA) as a constructive herbal drug to impede cholinergic dysfunctions and oxidative stress in Alzheimer's Disease (AD) in chronically administered scopolamine induced Alzheimer's type dementia in rats was evaluated. **Methodology:** Alzheimer's type dementia was induced by chronically administered intraperitoneal injection of scopolamine (0.7 mg kg^{-1}) to rats for period of 7 days. The EA (25 and 50 mg kg^{-1}) and Donepezil (0.5 mg kg^{-1}) were administrated to rats orally daily for a period of 13 days. Memory-related behavioral parameters were evaluated using the Elevated plus Maze (EPM) for 2 days and Morris Water Maze (MWM) for 5 days. At the end of protocol schedule i.e., day 14, biochemical parameters were estimated like AChE, MDA, GSH, catalase and SOD to evaluate the neuroprotective action of EA via AChE inhibition and antioxidant activity. **Result:** Chronically injected scopolamine treatment increased the transfer latency in EPM, escape latency time and shortened time spent in the target quadrant in MWM; these effects were reversed by EA. Scopolamine-mediated changes in malondialdehyde (MDA) and AChE activity were significantly attenuated by EA in rats. Recovery of antioxidant capacities, including reduced glutathione (GSH) content and the activities of SOD and catalase was also evident in EA treated rats. **Conclusion:** The present findings sufficiently encourage that EA has a major role in the neuroprotection in chronically injected Scopolamine induced Alzheimer type dementia. The EA can be used as an effectual herbal treatment to prevent cholinergic dysfunctions and oxidative stress associated with Alzheimer type dementia.

Key words: Neuroinflammation, oxidative stress, acetylcholinesterase, polyphenols

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INTRODUCTION

Alzheimer's Disease (AD) is a severe neurodegenerative disorder that gradually results in loss of memory and impairment of cognitive functions in the elderly[1-5]. In 2014, an estimated 5.2 million people of all ages have AD in U.S. This includes an estimated 5 million people of age 65 and older and approximately 200,000 individuals under age 65 who have younger-onset of Alzheimer's[6] disease. The pathological features of AD include extracellular amyloid deposition and intra-neuronal neurofibrillary tangles (NFTs) of hyperphosphorylated microtubule-associated tau protein[7-9]. The deposition of amyloid plaques is the primary event that leads to an inflammatory reaction, NFTs formation and ultimately cause neuronal death[10-12]. The mechanisms of neuronal cell loss in AD have not yet been fully elucidated but increased oxidative stress and inflammation are considered important mediators of neuronal damage in AD[13-17].

Many naturally occurring compounds have been proposed as potential therapies to slow or prevent the progression of AD, mostly by acting as antioxidants[18-24], but also with some direct anti-amyloid actions[18,23,25-30]. Recent studies have suggested the positive effects of dietary antioxidants as an aid in potentially reducing somatic cell and neuronal damage by free radicals[18-21,31-34]. The beneficial health effects of plant-derived products have been largely attributed to polyphenolic compounds, as well as vitamins, minerals and dietary fibers[18,19,35].

Ellagic acid (EA), a non flavonoid polyphenol, plays an essential role in explaining the sensory properties of fruits, food and beverages which exhibit this phyto-constituent[36-40]. The EA has been well proven to

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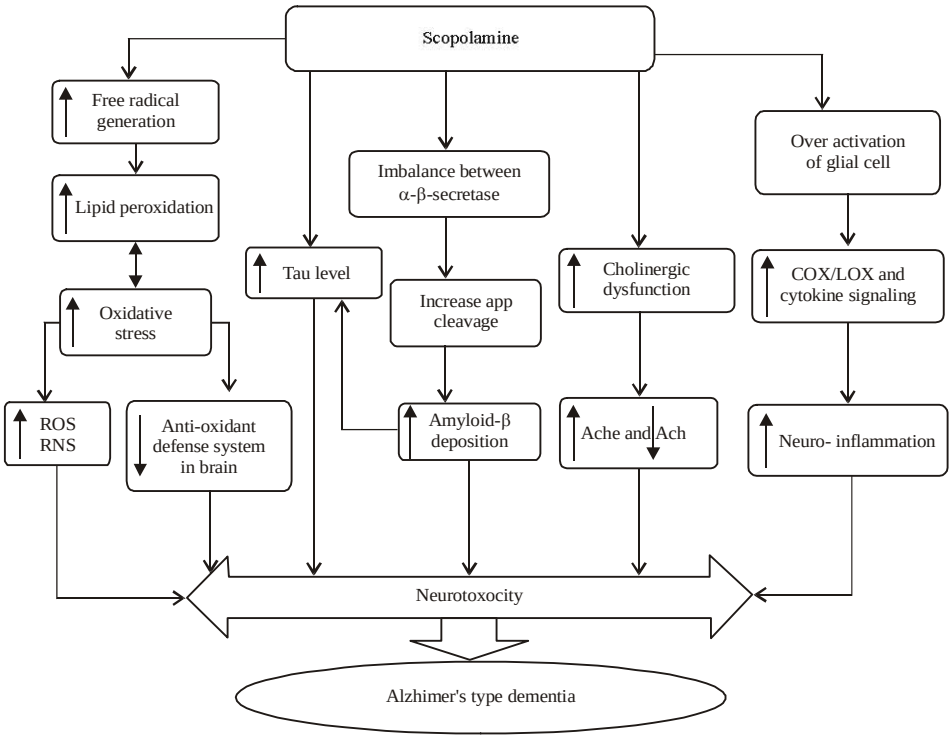

Fig. 1: Scopolamine induced experimental model of Alzheimer's type dementia

contain anti-oxidant[41-46], anti-inflammatory[47-51], anti-proliferative,[52-56] antidiabetic[57-59] and cardioprotective properties[60,61].

Neuroprotection can be a property of EA as it prevents both neuro-oxidation and neuroinflammation[62-68]. Moreover, by *in vitro* studies it was observed that EA inhibits β-secretase (BACE1), thus inhibiting Aβ-fibrillation and decrease AChE activity[4,69-71]. Recent studies suggested that glucose metabolism is affected during AD[72-75]. The EA stimulated GLUT4 translocation primary factor responsible for insulin induced glucose uptake and maintain glucose homeostasis[76,77]. The EA also shows modulation of monoaminergic system (serotonergic and noradrenergic systems) and GABAergic system[78-80]. Cognitive impairment in AD patients correlates with disturbance in various neurotransmitters, as the ratio of excitatory-inhibitory neurotransmitter levels disturb, cytotoxic damage to neurons and glia occurs and norepinephrine and serotonin levels declined[81-91]. Further, Gamma-Amino Butyric Acid (GABA) increases the formation of soluble receptor for advanced glycation end products (RAGE) and decreases the levels of full-length RAGE, lowering the Aβ uptake and inflammatory mediated reactions[92,93].

Scopolamine, an antimuscarinic agent, competitively antagonizes the effect of acetylcholine on the muscarinic receptors by occupying postsynaptic receptor sites with high affinity and increases AChE activity in the cortex and hippocampus[94-103]. Scopolamine abolishes cerebral blood flow due to cholinergic hypofunction[104-107]. Scopolamine additionally triggers ROS, inducing free radical injury and an increase in a scopolamine-treated group brain MDA levels and deterioration in antioxidant status[108-112]. Scopolamine induces neuro-inflammation by promoting high level of oxidative stress and pro inflammatory cytokines in the hippocampus[113-119]. Scopolamine is proven to increase levels of APP and Tau protein. Chronic administration of scopolamine led to marked histopathological alterations in the cerebral cortex, including neuronal degeneration[30,120-122]. Scopolamine administration has been used both in healthy human volunteers and in animals as a model of dementia to determine the effectiveness of potential new therapeutic agents for Alzheimer's disease[123-128] (Fig. 1).

Donepezil, a reversible inhibitor of AChE, is neuroprotective due to not only activation of cholinergic transmission but also by reducing the amount of the toxic form of amyloid β fibrils[129-136]. Donepezil

ameliorated the scopolamine induced memory impairment by reducing AChE activity and oxidative stress and restoring cerebral circulation[137-143]. With this background, EA might show neuroprotection via inhibiting neuronal dysfunctions. There is major requirement to determine therapeutic potential for EA in cases of AD with suitable behavioral and biochemical markers. This study was an attempt to investigate the neuroprotective effect of EA, potential of doses for the treatment of Alzheimer's disease.

MATERIAL AND METHOD

Chemicals: EA was purchased from Yucca Interprises, Mumbai, India and suspended in saline solution. Scopolamine hydrochloride was purchased from Sigma–Aldrich, St, Louis, MO, USA. Donepezil was obtained from Ranbaxy Pvt. Limited, Mumbai, India and both scopolamine and donepezil were dissolved in saline solution. All reagents used in this study were of analytical grade and high purity.

Animals: Male Wistar rats (weighing 220-250 g, aged 8-10 months) obtained from the Animal House of the Institute were employed in the studies. The animals were kept in polyacrylic cages with wire mesh top and soft bedding. They were kept under standard husbandry conditions of 12 h reverse light cycle with food and water *ad libitum*, maintained at 22±2°C. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (RITS/IAEC/2013/01/01). Animals were acclimatized to laboratory conditions prior to experimentation.

Drug administration: EA was administered by oral (p.o.) route in dose of 25 and 50 mg kg^{-1}. Scopolamine was administered by intraperitoneal (i.p.) route in dose of 0.7 mg kg^{-1}. Donepezil was administered by oral (p.o.) route in dose of 0.5 mg kg^{-1}.

Six groups (each group consist six rats) were employed in the present study. (1) Group 1: Normal Control (2) Group 2: Scopolamine Control (0.7 mg kg^{-1}, i.p.) (3) Group 3: EA perse (50 mg kg^{-1}, p.o.) 25 mg kg^{-1}, p.o.+Scopolamine (0.7 mg kg^{-1}, i.p.) (4) Group 6: EA 50 mg kg^{-1}, p.o.+Scopolamine (0.7 mg kg^{-1}, i.p.). After a 5 day habituation period, rats were given EA (25 and 50 mg kg^{-1}, p.o.) and Donepezil (0.5 mg kg^{-1}, p.o.) for total of 13 days. EA alone was treated for 6 days and then scopolamine (0.7 mg kg^{-1}, i.p.) was administered together with EA for another 7 days. Rats underwent locomotor activity (LMA) for 2 days i.e., 6th day and 13th day, MWM test for 5 days i.e., 7th day to 11th day. The day after completion of Morris Water Maze (MWM), the Elevated plus Maze (EPM) was conducted for 2 days i.e., 12th-13th day. The day after EPM, the rats were sacrificed and biochemical parameters were estimated (Fig. 2).

Elevated plus maze: Elevated plus Maze (EPM) served as the behavioral model (where in the stimulus existed outside the body) to evaluate learning and memory in rats. It consists of two opened arms (50×10 cm) and two covered arms (50×40×10 cm). The arms were extended from central platform (10×10 cm) and the maze was kept elevated to a height of 50 cm from the floor. The EPM was conducted for 2 days i.e., 12th-13th day of protocol schedule. Each animal was kept at the end of an open arm, facing away from the central platform on 12th day. Transfer Latency (TL) which was taken as the

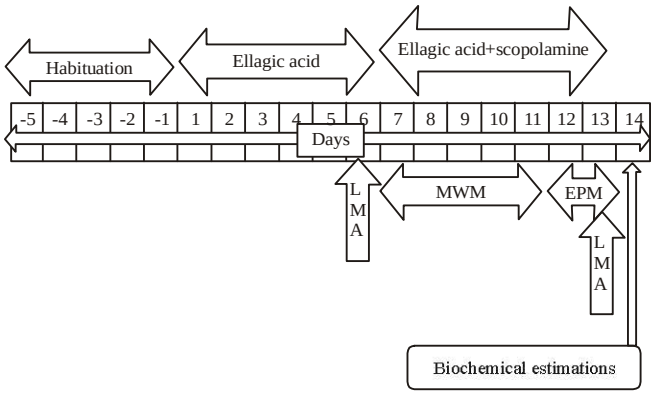

Fig. 2: Protocol schedule to determine the neuroprotective effect of ellagic acid in scopolamine induced Alzheimer' type memory and cognitive dysfunctions

time taken by the animal to move into any one of the covered arms with all its four legs, recorded on 12th day i.e., acquisition trial[144]. If the rat did not enter into one of the covered arms within 120 sec then it was gently pushed into one of the two covered arms and the transfer latency was assigned as 120 sec. The rats were allowed to explore the maze for 10 sec and then were returned to its home cage. TL was again examined 24 h after the first trial on 13th day of protocol schedule i.e., retention latency.

Spatial navigation task in morris water maze: Morris water maze employed in the present study was a model to evaluate spatial learning and memory. Escape from water itself acts as motivation and eliminates the use of other motivational stimuli such as food and water deprivation. Water provides uniform environment and eliminates interference due to olfactory clues[145]. Animals were trained to swim to a platform in a circular pool (180 cm diameter×60 cm) located in a sound attenuated dark test room. The pool was filled with water (28±2°C) to a depth of 40 cm. A movable circular platform, 9 cm in diameter and mounted on a column, was placed in the pool 2 cm below the water level for Escape Latency Time (ELT) while during Time Spent in the Target Quadrant (TSTQ), the platform was removed. Four equally spaced locations around the edge of the pool (N, S, E and W) were used to divide the pool into 4 quadrants and one of them is used as start point which was same during all trials. The pool was filled with opaque water to prevent visibility of the platform in the pool. The escape platform was placed in the middle of one of the random quadrants of the pool and kept in the same position throughout the experiments. Animals received a training session consisting of day 7-10 and ELT was recorded. ELT defined as the time taken by the animal to locate the hidden platform. ELT was noted as an index of learning. Each animal was subjected to single trial for four consecutive days (starting form 7th day of EA administration to 10th day), during which they were allowed to escape on the hidden platform and to remain there for 20 sec. If the rats failed to find the platform within 120 sec, it was guided gently onto the platform and allowed to remain there for 20 sec.

On fifth day (i.e., 11th day of EA administration) the platform was removed. Rats were placed in water maze and allowed to explore the maze for 120 sec. Time spent in three quadrants, that is, Q1, Q2 and Q3 was recorded and TSTQ in search of the missing platform provided as an index of retrieval. Care was taken not to disturb the relative location of water maze with respect to other objects in the laboratory.

Assessment of locomotor activity: Gross behavioral activity was assessed by digital actophotometer on 6th day and 13th day of protocol schedule to rule out any interference in locomotor activity by drugs which may affect the process of learning and memory, in before and after of MWM task. Each animal was observed over a period of 5 min in a square (30 cm) closed arena equipped with infrared light-sensitive photocells and values expressed as counts per 5 min[146]. The beams in the actophotometer, cut by the animal, were taken as measure of movements. The apparatus was placed in a darkened, sound-attenuated and ventilated testing room.

Preparation of brain homogenate: On 14th day of protocol schedule, Animals were sacrificed by decapitation, brains removed and rinsed with ice cold isotonic saline solution. Brain tissue samples were then homogenized with 10 times (w/v) ice cold 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000x g for 15 min, supernatant was separated and aliquots were used for biochemical estimations[146].

Protein estimation: The protein content was measured by using Agappe protein estimation kit (Biuret method).

Estimation of acetylcholinesterase levels: The quantitative measurement of AChE activity in brain was performed according to the method described by Ellman *et al*. (1961)[147]. The enzymatic activity in the supernatant was expressed as nmol per mg protein.

Estimation of malondialdehyde: The quantitative measurement of MDA-end product of lipid peroxidation-in brain homogenate was performed according to the method of Wills (1966)[148]. The concentration of MDA was expressed as nmol per mg protein.

Estimation of reduced glutathione: The GSH in brain was estimated according to the method described by Ellman *et al*. (1959)[149]. The concentration of glutathione in the supernatant expressed as μmol per mg protein.

Estimation of superoxide dismutase activity: The SOD activity was measured according to the method described by Misra and Frodvich (1972)[150]. The activity of SOD was expressed as % activity.

Estimation of catalase activity: Catalase activity was measured by the method of Aebi (1974)[151]. The activity of catalase was expressed as % activity.

Statistical analysis: All the results and data were expressed as Mean±Standard deviation. Data was analyzed using two way ANOVA followed by Post hoc test bonferroni and one way ANOVA followed by Post hoc test tukey's multi-comparison test. The $p<0.05$ was considered as statistically significant.

RESULTS

Effect of Ellagic acid on rats in elevated plus maze: On 12th day of protocol schedule, acquisition latency was recorded. Retention was observed as Transfer Latency (TL) on 13th day to evaluate learning and memory in rats using EPM. On 12th and 13th day Scopolamine administered rats showed remarkable increase (113±9.380 and 106.5±11.148 sec) in TL, when compared to normal (64±4.242 and 36.833±6.765 sec) and EA perse rats (63.333±10.385 and 32.833±3.311 sec). During experiment, EA perse administration did not reveal any change, when compared to normal rats in TL. Donepezil, a well established standard drug for AD considerably decrease (65.5±13.003 and 21.666±5.085 sec) TL, when compared to Scopolamine managed rats and reversed the memory impairment induced by Scopolamine. Administration of EA at the dose of 25 mg kg^{-1}, p.o. exhibit notable decrease (72.00±8.049 and 39.333±6.186 sec) in TL, when compared to Scopolamine treated rats. The EA (50 mg kg^{-1}, p.o.) administration also decreases (69.333±8.041 and 25.333±3.881 sec) TL, when differentiate to Scopolamine handled rats and there were expressively variation was found in between treatment doses of EA 25 and 50 mg kg^{-1}, p.o. indicating improved retention memory (Fig. 3).

Effect of ellagic acid on rats in spatial navigation task using Morris water maze: On 7th-10th day of 14 day protocol schedule, Escape Latency Time (ELT) was observed. On 7th day, there were no significant changes observed in Scopolamine (94.33±13.125 sec) treated rats, when compared to normal (89±9.859 sec) and EA perse governed (86.33±13.937 sec) rats. The EA perse administration did not show any significant change when compared to normal rats. Moreover, Donepezil treated rats did not show any considerable changes (88±9.033 sec), when compared to Scopolamine responded rats. In the treatment groups, administration of EA did not confirm notable changes (96.33±10.053;

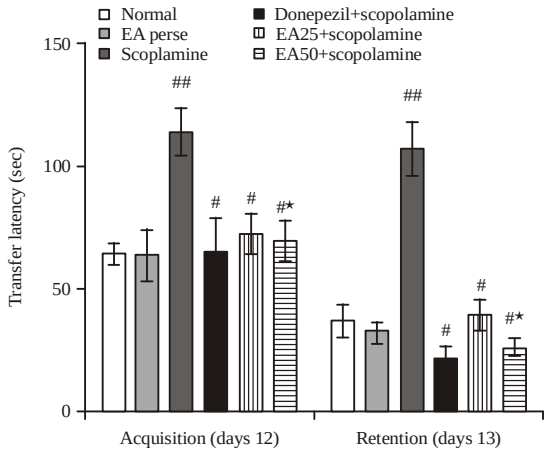

Fig. 3: Effect of ellagic acid on transfer latency of rats using elevated plus maze. Values were Mean±SD, $^{\#\#}p<0.05$ as compared to Normal and EA perse, $^{\#}p<0.05$ as compared to Scopolamine, $^{\#\star}p<0.05$ as compared to EA 25+Scopolamine

88.66±10.689 sec) in ELT at 25 and 50 mg kg^{-1}, p.o. when compared to Scopolamine treated rats. There were no changes found in ELT between treatment doses of EA 25 and 50 mg kg^{-1}, p.o.

Comparison data of 8th day, 9th day and 10th day ELT in MWM, showed that Scopolamine administered rats manifest remarkable increase (92±8.173, 85.33±12.75 and 83.33±8.664 sec) in ELT, when collate to normal (76.33±7.840, 29.16±7.808 and 15.33±3.723 sec) and EA perse (67.33±5.645, 29.33±8.710 and 15±2.898 sec) rats. EA perse administration did not show any significant difference, when compared to normal rats during ELT. Donepezil served rats outstandingly decreased (51±10.158, 26.16±6.40 and 10.83±4.622 sec) ELT when compared to Scopolamine dosed rats. EA at 25 mg kg^{-1}, p.o. proved remarkable decreased (79±10.807, 60.83±8.658 and 38.16±9.703 sec) in the ELT, when compared to Scopolamine employed rats. EA at the dose 50 mg kg^{-1}, p.o. significantly decreased (65.33±11.707, 43±9.838 and 24.5±8.312 sec) the ELT, when compared to Scopolamine and EA 25 mg kg^{-1}, p.o. treated rats, indicating remarkable improvement in learning (Fig. 4).

On 11th day of protocol schedule, TSTQ was performed. Time Spent in Target Quadrant (TSTQ) in search of missing platform provided as an index of retrieval. Scopolamine treated rats showed remarkable decrease (7.667±3.077 sec) in TSTQ when compared to normal (45.17±8.060 sec) and EA perse treated

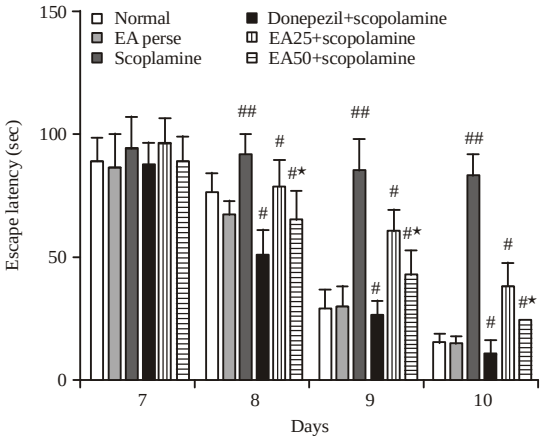

Fig. 4: Effect of ellagic acid on escape latency time of rats on 7th-10th day using morris water maze. Values were Mean±SD, ##p<0.05 as compared to Normal and EA perse, #p<0.05 as compared to Scopolamine, #⋆p<0.05 as compared to EA 25+Scopolamine

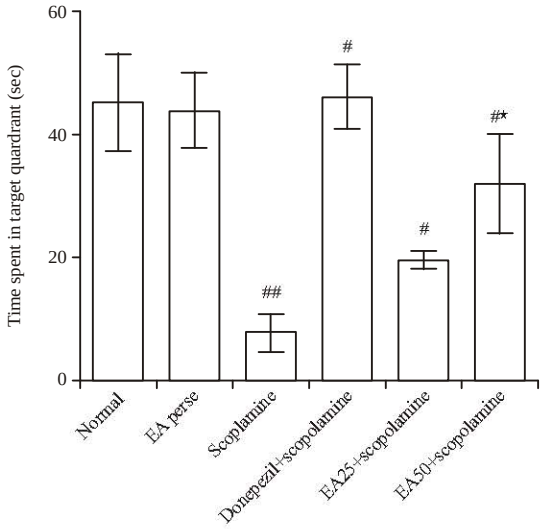

Fig. 5: Effect of ellagic acid on time spent in target quadrant of rats using morris water maze. Values were Mean±SD, ##p<0.05 as compared to Normal and EA perse, #p<0.05 as compared to Scopolamine, #⋆p<0.05 as compared to EA 25+Scopolamine

(43.83±6.242 sec) rats. In perse group of EA, there were no changes during TSTQ when compared to normal group. Further, Donepezil served rats improved (46.17±5.345 sec) memory when compared to Scopolamine treated rats. EA (25 mg kg^{-1}, p.o.) administration showed remarkable increase

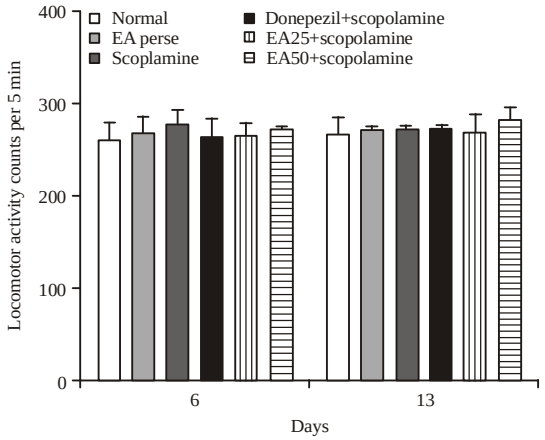

Fig. 6: Effect of ellagic acid on locomotor activity of rats using actophotometer. Values were Mean±SD

(19.50±1.517 sec) in TSTQ when compared to Scopolamine treated rats. EA (50 mg kg^{-1}, p.o.) administration indicated improvement (32.00±8.149 sec) in memory function when compared with Scopolamine governed rats. Moreover, markedly difference was also observed in between treatment doses of EA (Fig. 5).

Effect of ellagic acid on rats in locomotor activity: On 6th and 13th day of protocol schedule, locomotor activity was observed to rule out any interference in locomotor activity by treatment drugs. Scopolamine employed rats did not reveal any significant changes (281.333±15.318 and 274.833±5.344) in locomotor activity when compared to normal (263.833±17.474 and 274.5±21.314) and EA perse (270.666±18.250 and 274.5±4.764) rats. EA perse administration also did not show any considerable change in locomotor activity at 50 mg kg^{-1}, p.o. when compared to normal rats. Donepezil treated also showed trivially changes (267.5±21.314 and 274.833±5.344) when compared to Scopolamine treated rats. EA 25 mg kg^{-1}, p.o. (266.833±15.458 and 270.833±20.692) and 50 mg kg^{-1}, p.o. (274.5±4.764 and 283.5±16.208) administration did not showed any notable changes in locomotor activity of rats when differentiate to Scopolamine treated rats, indicating there were no effect on locomotor activity (Fig. 6).

Effect of ellagic acid on acetylcholinesterase levels: Prolongation of availability of acetylcholine has been used to enhancing cholinergic function. This prolongation may be achieved by inhibiting AChE. Scopolamine administered rats significantly increased

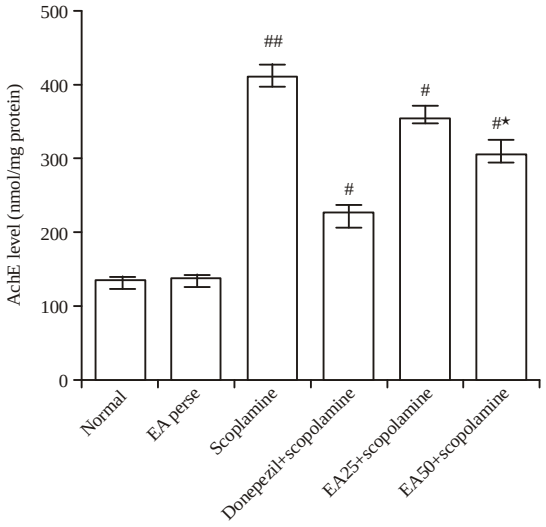

Fig. 7: Effect of ellagic acid on acetylcholinesterase levels. Values were Mean±SD, $^{\#\#}p<0.05$ as compared to Normal and EA perse, $^{\#}p<0.05$ as compared to Scopolamine, $^{\#\star}p<0.05$ as compared to EA 25+Scopolamine

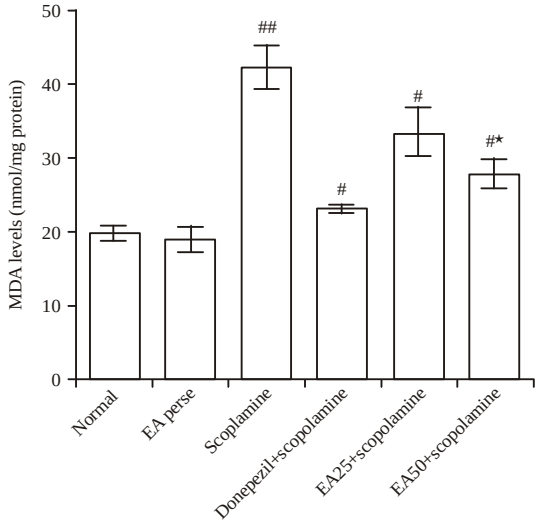

Fig. 8: Effect of ellagic acid on Malondialdehyde levels. Values were Mean±SD, $^{\#\#}p<0.05$ as compared to normal and EA perse, $^{\#}p<0.05$ as compared to Scopolamine, $^{\#\star}p<0.05$ as compared to EA 25+Scopolamine

(415.0±19.62) the AChE level when compared to normal (136.8±4.956) and EA perse (137.2±4.167) rats. EA perse administration did not show any appreciable changes in AChE level at the dose of 50 mg kg^{-1}, p.o. when compared to normal rats. Donepezil treated rats appreciably decreased (231.0±7.668) the AChE level in contrast to Scopolamine dosed rats. EA (25 mg kg^{-1}, p.o.) showed remarkably diminished the AChE level (360.8±15.96) when compared to Scopolamine rats. Administration of EA (50 mg kg^{-1}, p.o.) significantly reduced (311.7±17.63) the AChE level when compared to Scopolamine employed rats. Moreover, expressive distinction was present in between treatment doses of EA (Fig.7).

Effect of ellagic acid on malondialdehyde levels: MDA is an indicator of lipid peroxidation. Scopolamine administration increased (42.50±3.082) the MDA level when compared to normal (19.88±0.960) and EA perse (19.15±1.841) rats. Further, EA perse administration did not show any considerable changes in MDA levels when compared to normal rats. Donepezil appreciably decreased (23.12±0.511) the MDA level when compared to Scopolamine managed rats. EA (25 mg kg^{-1}, p.o.) administration showed remarkably decrease (33.57±3.347) in MDA level when compared to Scopolamine treated rats. EA administered rats at the dose of 50 mg kg^{-1}, p.o significantly decreased (27.97±2.089) in MDA level when compared to Scopolamine and EA 25 mg kg^{-1}, p.o. treated rats (Fig. 8).

Effect of ellagic acid on reduced glutathione levels: Reduced GSH is a marker of cellular antioxidant and provide protection against oxidative stress. Scopolamine governed rats remarkably decreased (2.067±0.417) the GSH level when compared to normal (9.833±0.776) and EA perse treated (9.733±0.799) rats. EA perse administration did not show any considerable changes in GSH levels in contrast to normal rats. Donepezil outstandingly increase (7.767±0.361) the GSH levels when compared to Scopolamine treated rats. The EA (25 mg kg^{-1}, p.o.) administration exhibited remarkable increase (5.250±0.575) in GSH level when compared to Scopolamine treated rats. The EA (50 mg kg^{-1}, p.o.) showed significantly increase (6.317±0.386) in GSH level when compared to Scopolamine treated rats. Moreover, in between treatment doses of EA, significance difference was present (Fig. 9).

Effect of Ellagic acid on superoxide dismutase activity: The SOD is an antioxidant enzyme which plays a key role in detoxifying superoxide anions. Scopolamine administered rats significantly decreased (27.33±3.386) the SOD levels in brain homogenate

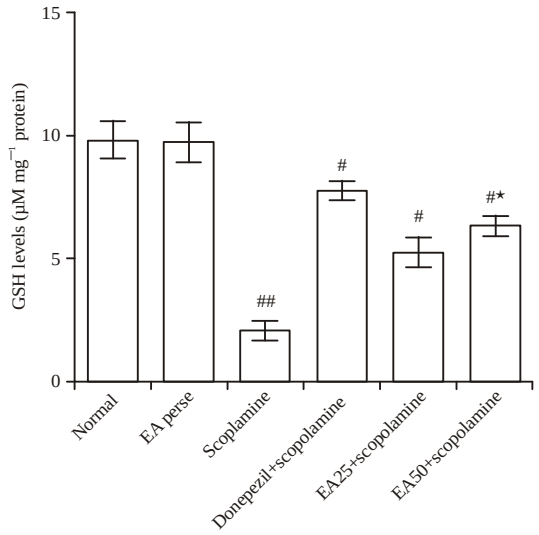

Fig. 9: Effect of ellagic acid on reduced glutathione levels. Values were Mean±SD, $^{\#\#}p<0.05$ as compared to Normal and EA perse, $^{\#}p<0.05$ as compared to Scopolamine, $^{\#\star}p<0.05$ as compared to EA 25+Scopolamine

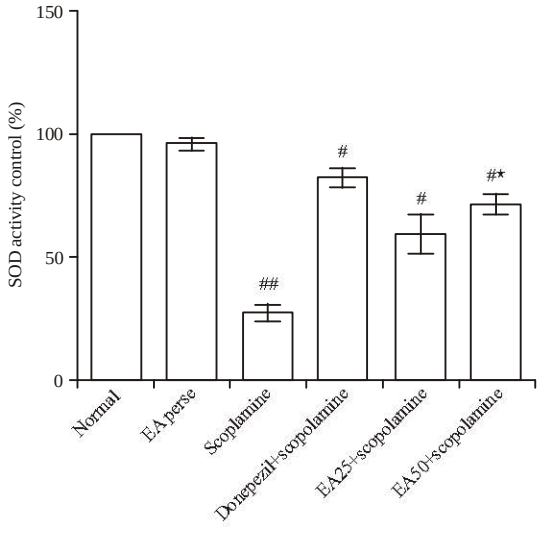

Fig. 10: Effect of ellagic acid on superoxide dismutase activity. Values were Mean±SD, $^{\#\#}p<0.05$ as compared to Normal and EA perse, $^{\#}p<0.05$ as compared to Scopolamine, $^{\#\star}p<0.05$ as compared to EA 25+Scopolamine

when compared to normal (100.0±0.0) and EA perse (95.83±2.639) rats. EA perse administration did not reveal any considerable change in SOD activity when collated to normal rats. Donepezil expressively increase (82.00±3.950) SOD activity when compared to

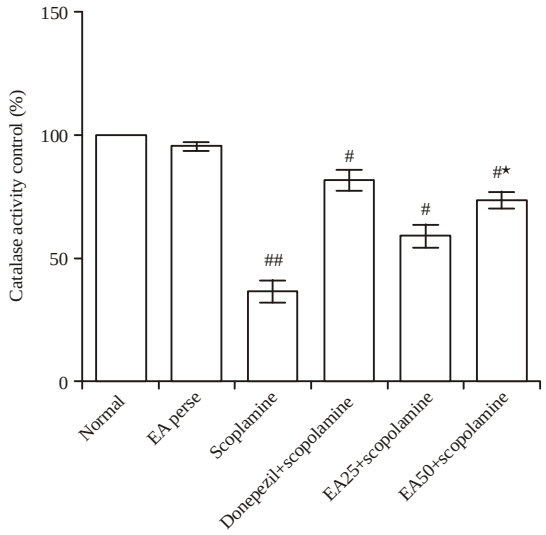

Fig. 11: Effect of ellagic acid on catalase activity. Values were Mean±SD, $^{\#\#}p<0.05$ as compared to normal and EA perse, $^{\#}p<0.05$ as compared to Scopolamine, $^{\#\star}p<0.05$ as compared to EA 25+Scopolamine

Scopolamine treated rats. In treatment group, EA (25 mg kg^{-1}, p.o.) administration showed remarkable increase (59.17±8.060) in SOD activity when compared to Scopolamine treated rats. EA (50 mg kg^{-1}, p.o.) administration showed significantly increase (71.33±4.033) in SOD activity when compared to Scopolamine treated rats and a remarkable disparity was found in between EA treated groups (Fig. 10).

Effect of ellagic acid on catalase activity: Catalase is also an antioxidant enzyme which has capability to detoxify oxidative free radicals. Scopolamine treated rats manifested remarkable decrease (36.50±4.461) in catalase activity in brain homogenate when differentiated to normal (100.0±0.0) and EA perse treated (95.50±1.871) rats. The EA perse administration did not show any considerable changes in catalase activity when compared to normal rats. Donepezil significantly increase (81.67±4.033) in catalase activity when compared to Scopolamine treated (36.50±4.461) rats. The EA (25 mg kg^{-1}, p.o.) remarkably increased (59.17±4.579) the catalase activity when compared to Scopolamine treated rats. The EA (50 mg kg^{-1}, p.o.) administration exhibited significantly increase (73.67±3.559) in catalase activity when compared to Scopolamine and EA 25 mg kg^{-1}, p.o. treated rats (Fig. 11).

DISCUSSION

Clinically AD is characterized by an insidious degradation of memory, associated with functional decline and neurobehavioral disturbances[152,153]. Despite the availability of various treatment strategies, the severity and prevalence of this disease are not yet under control. Therefore, alternative and complementary medicines including herbal supplements, phytochemicals and extracts are being utilized in the management of AD[154-160]. The current hypothesis about the mechanisms by which neurons come into necrotic or apoptotic processes has led to believe that the therapeutic use of natural antioxidants may be beneficial in aging and neurodegenerative disorders[161-163].

In the present study, the effect of improving memory deficit of EA was evaluated using chronically administered scopolamine induced Alzheimer's type dementia in rats.

It is well known that scopolamine as a cholinergic receptor antagonist has been shown to impair learning and memory processing[95,97,100,103]. Scopolamine produces deficits in acquisition, immediate retention and working memory[164-169].

The current study has revealed that long term administered scopolamine significantly increased the levels of lipid peroxidation products such as MDA and decreased the levels of antioxidants viz., GSH, SOD and catalase. The increase in oxidative stress was found to be associated with increase in AChE activity and spatial cognitive deficit. Present findings are in tune with previous reports[99,102,111,118].

Scopolamine induced Alzheimer's type dementia model has been widely used to provide a pharmacological model of memory dysfunction for screening potential cognition enhancing agents[99,110-112,170]. The cognitive-enhancing activity of EA on chronically administered scopolamine induced memory impairments in rats was investigated by using behavioral and biochemical parameters.

During elevated plus maze, decrease in retention latency indicated improvement of memory and vice versa[142,171-173]. In EPM, it was shown that long term injected scopolamine also drastically increase in TL, demonstrating that the central cholinergic neuronal system plays an important role in learning acquisition. EA dose-dependently decreased TL prolongation induced by scopolamine. These results suggested that the neuroprotective effect of EA on scopolamine-induced memory impairment may be related to mediation of the cholinergic nervous system. In order to confirm the effects of EA, MWM was used to test spatial learning in rats, where scopolamine treated rats were taking more time to reach at the hidden platform which shows memory impairments in this spatial task. EA treated rats impressively reduced the escape latency prolonged by scopolamine. Moreover, EA exhibited appreciable improvement of cognitive performance as indicated by significant decrease in ELT. It is important to notice that MWM test investigating spatial learning and memory has been used in detecting changes of the central cholinergic system[174-178]. If the animals spent more time in target quadrant where the platform had previously been placed during the training session, this would indicate that the animals learned from prior experience with the MWM test, showing the spatial memory improvement. Scopolamine treated rats decreased TSTQ, on the other side EA treated rats expressively increased the TSTQ. Both the test doses viz., 25 and 50 mg kg^{-1}, p.o. significantly attenuated these behavioral changes in rats with chronically administered scopolamine induced memory and cognitive impairment.

Along with EPM and MWM, Locomotor activity also was investigated using actophotometer to determine any modulation in locomotor activity by treatment drugs which may affect locomotion in EPM and MWM. However, no significant difference in locomotor activity was observed in any of the animal groups. These results suggest that there was not any sedative effect or interference in EPM and MWM locomotion. Therefore, transfer latency in EPM, escape latency and TSTQ in MWM were purely result of improved memory. Therefore, EA can repair the long-term memory in chronically injected scopolamine-induced memory impairments.

To investigate the effect of EA on cholinergic function, that governs vital aspects of memory and other cognitive functions, brain acetylcholinesterase activity was measured in the present study. The hippocampus, amygdala and cortical regions of the brain are mainly involved in cholinergic transmission to monitor learning and memory processing and seem to be more prone to oxidative damage[9,179-181]. Moreover, oxidative damage to the rat synapse in these regions of brain has been reported to contribute to cognitive deficits[182,183]. The AD is characterized by alterations at the level of various neurotransmitters. The most severely affected is the cholinergic system which is responsible for the storage and retrieval of items in memory and its degradation correlates well with the severity of cognitive and memory impairment[10,184].

In this study, scopolamine was found to significantly elevate AChE activity, an enzyme responsible for degradation of ACh which is in tune with earlier reports[102,118]. This increase in AChE activity was significantly restored dose dependently by EA. These observations suggest the modulation of cholinergic neurotransmission and/or prevention of cholinergic neuronal loss.

Recently, many studies have reported that memory impairments is associated to oxidative damage in the scopolamine-induced dementia in rats[110-112]. Moreover, many clinical studies have reported that oxidative stress is closely involved in the pathogenesis of AD[13,185-188].

Lipid peroxidation is an important indicator of neurodegenration of brain. Unlike other body membranes, neuronal membranes contain a very high percentage of long chain polyunsaturated fatty acids because they are used to construct complex structures needed for high rates of signal transfer. The ROS are generated continuously in nervous tissues during normal metabolism and neuronal activity. The brain is subjected to free radical induced lipid peroxidation because it uses one-third of the inspired oxygen[189,190]. Lipids and proteins, the major structural and functional components of the cell membrane are the target of oxidative modification by free radicals in neurodegenerative disorders[191]. Extensive evidence exists on lipid peroxidation and protein oxidation leading to loss of membrane integrity, an important factor in acceleration of aging and age-related neurodegenerative disorders. Oxidative stress has been implicated in the pathogenesis of AD in humans[192-194].

In the present study, scopolamine-injection in rats significantly induced peroxidation of lipids and proteins and reduced antioxidant defense indicating increased oxidative stress. MDA is an end product of lipid peroxidation and is a measure of free radical generation and scopolamine injected rats showed extensive lipid peroxidation as evidenced by increase in MDA levels. In order to evaluate the effect of EA on lipid peroxidation in brain, MDA level was assessed. MDA level was remarkably increased by scopolamine and EA dose-dependently reduced MDA level, indicating the reduced peroxidation of lipids.

Lipid peroxidation may enhance due to depletion of GSH content in the brain which is often considered as the first line of defense of the cell by this endogenous antioxidant against oxidative stress[191,195-197]. Evidence has been presented that the neuronal defense against H_2O_2 which is the most toxic molecule to the brain, is mediated primarily by the glutathione system[198-200]. The GSH is a tri-peptide, an endogenous antioxidant found in all animal cells in variable amounts and is a very accurate indicator of oxidative stress[197]. Consistent with previous studies, in present study, scopolamine treatment significantly decreased the GSH levels. Further, co-administration of EA markedly improved GSH levels.

The most important antioxidant enzymes are SOD and catalase. The SOD plays a key role in detoxifying superoxide anions which otherwise damages the cell membranes and macromolecules. Scopolamine administration showed a significant reduction in enzymatic activity of SOD and catalase. On the other side, Catalase has the capability to detoxify H_2O_2 radicals. Release of H_2O_2 promotes the formation of numerous other oxidant species that greatly contributes for oxidative stress leading to the pathogenesis of AD[189,201]. Scopolamine treatment was found to be decreased SOD and catalase activities. Treatment of rats with EA significantly preserved the activities of SOD and catalase.

It has been well documented that persistent administration of scopolamine in response to degradation of ACh and increase the level of AChE enzyme, further responsible for the production of oxidative stress and pro-inflammatory mediators viz., cytokines and further activation of these cells[99,110-112]. A strong and long lasting administration of scopolamine has been demonstrated to cause cholinergic dysfunction while inhibition of this scopolamine mediated abnormalities has shown to reverse cholinergic dysfunction as well as inhibit the release of oxidative and inflammatory markers[99,103,112]. The results of the present study suggest that chronic administration of EA perse did not have any significant effect on cognitive performance in normal animals. But, EA treatment groups at the dose of 25 and 50 mg kg^{-1}, p.o. showed marked improvement in cognitive tasks when compared to scopolamine treated rats suggesting the significant role of ACh in long lasting administrated scopolamine mediated cognitive dysfunction. Reports also support that ACh is involved in memory acquisition and retention[10,155,202,203]. Moreover, scopolamine injection drastically impaired memory retention, resembling Alzheimer's dementia[103,112]. The same has been reported to be attenuated by pretreatment with herbal supplements and extracts and phytochemicals[156-158,160].

The presented data in this study also suggests that EA possesses potent antioxidant activity by scavenging ROS and exerting a neuro-protective effect against oxidative damage induced by long term administration

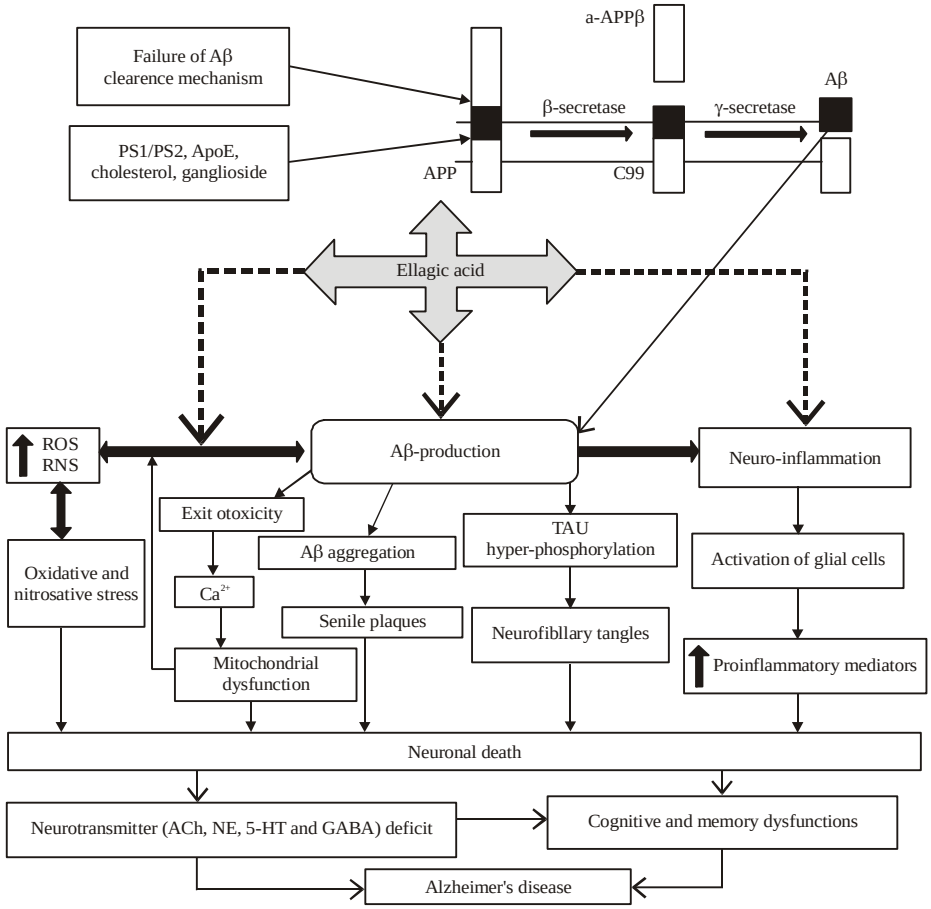

Fig. 12: Neuroprotective action of ellagic acid via modulating various signaling pathways involved in the progression of Alzheimer's disease

of scopolamine (Fig. 12). Predominant role of AChE inhibition, antioxidant activity reveal an important contributory factor to the beneficial effects of EA against dementia. Higher dose of Ellagic acid i.e., 50 mg kg^{-1}, p.o. was found more neuroprotective in all behavioral and biochemical evaluations. At lastly, the neuroprotective effects of EA might result from the regulation of AChE and the anti-oxidative defense system. These results suggest that EA can be used as a constructive herbal drug to impede cholinergic dysfunctions and oxidative stress in AD.

CONCLUSION

It was concluded that long term injected scopolamine could persuade Alzheimer's type dementia via increase AChE levels and oxidative stress like bio-markers. Scopolamine mediated Alzheimer's type dementia is mainly associated with cognitive and memory impairments in behavioral models like elevated plus maze and morris water maze. Ellagic acid diminished the acetylcholinesterase level and improves the anti-oxidant defense system. Further, Ellagic acid down turned the cognitive impairments induced by scopolamine. Like Donepezil, Ellagic acid reversed the scopolamine induced Alzheimer's type dementia in rats. Therefore, Ellagic Acid can be used as an effectual herbal treatment to prevent cholinergic dysfunctions and oxidative stress associated with Alzheimer's type dementia.

On the basis of this study, the major bio-markers of Alzheimer's disease like amyloid beta, inflammatory cytokines and histopathological changes can be further evaluated according to current protocol schedule to confirm and justify the strong evidence of Ellagic acid in long term injected scopolamine mediated dementia.

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