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# THE AMELIORATING AND ANTIOXIIDANT STUDY OF AQUEOUS EXTRACT OF *CITRULLUS LANATUS* (THUNB.) PULP USING SCOPOLAMINE INDUCED LEARNING AND MEMORY IMPAIRMENT IN SWISS ALBINO MICE

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**ABSTRACT**

Amnesia is a medical condition involving the loss of memory. While memory loss can be attributable to a number of illnesses or factors including Alzheimer's disease and dementia, amnesia is often caused by head injury, brain trauma, or brain surgery. Certain states of amnesia can also be precipitated by alcohol consumption, drug use, or the effects of a stroke. While the incidence of amnesia affects only a small percent of the world's population, it's study is becoming increasingly important with the rising numbers of people with Alzheimer's Disease. Alzheimer's is expected to strike 34 million people globally by 2025 and 14 million in the U.S. alone over the next 40 years. Half of all people who reach age 85 will exhibit symptoms of the disease. Diminished cholinergic transmission may be responsible for development of amnesia. Hence, the present study was undertaken to investigate the possible protective effect of aqueous extract of *Citrullus lanatus* (Thunb.)pulp (AECL) using scopolamine-induced amnesia in Swiss Albino mice. The mice were divided randomly into six groups each consisting of five mice (n = 5). Group IV, V and VI treated mice feed and AECL 25%, 50% and 100% instead of water for 21days. Groups I, II, III were treated with mice feed and water *ad libitum*. Then, they were subjected to single dose of scopolamine (1 mg/kg b.w., i.p) on 21st day 60 min after respective drug administration, except in group I. They were observed for the behavioural parameters for learning and memory using passive avoidance, Morris water Maze, Y-Maze and Elevated Plus Maze were as a tool for cognitive dysfunction study. In the brain homogenate estimation of Acetycholinesterase and antioxidant SOD, LPO, GPx, Catalase were done.

**Key words:** *Citrullus lanatus* (Thunb.), Watermelon, Antioxidant, Amnesia, Cognitive dysfunction.

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## INTRODUCTION

Decline in these cognitive abilities results in a neurodegenerative disorder called as amnesia which may be one of the symptoms of some neurodegenerative diseases such as Alzheimer's disease (Bowen DM *et al*., 1976).

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As per WHO guidelines, Amnesia is the main feature is loss of memory, usually of important recent events, that is not due to organic mental disorder, and is too great to be explained by ordinary forgetfulness or fatigue. The amnesia is usually centred on traumatic events, such as accidents or unexpected bereavements, and is usually partial and selective. Complete and generalized amnesia is rare, and is usually part of a fugue. The diagnosis should not be made in the presence of organic brain disorders, intoxication, or excessive fatigue. Scopolamine is a muscarinic receptor antagonist with profound amnesic effects in a variety of learning paradigms and a useful experimental pharmacological model to investigate the pathophysiology of the cognitive deficit in AD. It has been widely

implicated to cause amnesia and a viable model of dementia in humans and animals. The restoration of cholinergic function through prolongation of the availability of acetylcholine (ACh) released into the neuronal synaptic cleft by inhibiting acetylcholinesterase (AChE) activity, remains a rational target in the treatment of AD. Acetylcholinesterase inhibitors (piracetam,tacrine, donepezil, and rivastigmine) are the mainstay in the treatment of AD, though effective but not without adverse effects (Imam A *et al*., 2016; Bores GM *et al*., 1996; Allain H, Bentue´-Ferrer D *et al*., 2003).

According to the WHO more than 80% of the world's population relies on traditional herbal medicine for their primary healthcare. As we know that India, with its mega biodiversity and knowledge-rich ancient traditional system of medicine viz Ayurveda, Siddha, Unani and local health tradtions, Provides a strong base for the utilization of a large number of plants in general healthcare of the people (Bhavesh vaghela *et al*., 2013).

*Citrullus lanatus* citroides (CLC), commonly known as watermelon, belonging to the family *Cucurbitaceae* is native to India. It is found in forest lands, riversides, and wasteland, and also gets cultivated on a large scale. It is an excellent source of the arginine, Vitamin A, B and C, carotenoids, lycopene, carbohydrates, sodium, magnesium, potassium, and water *C. lanatus* sp. is a natural source of antioxidants such as beta carotene, vitamin C, citrulline (Yativ M, Harary I *et al*., 2010).

Its red flesh is also an excellent source of lycopene . The tissue protective effects of watermelon juice have been reported . Furthermore, the protective effects of watermelon as antimicrobial, antigiardial, hepatoprotective, anti-ulcerogenic, anti-diabetic, laxative, antiprostatic Hyperplasia, antioxidant, analgesic, anti-inflammator, Neuroprotective effects of watermelon were documented. It's also a brain tonic (Bhardwaj A *et al*., 2012; Sharma S *et al*., 2011; Olamide AA *et al*., 2011).

This study was undertaken to evaluate the potential benefits of *Citrullus lanatus* (Thunb.) pulp using mice as an animal model for the treatment of Amnesia.

## MATERIALS AND METHODS

### Collection, Authentication and Preparation of Juice

Watermelon fruits (green skin, red flesh) were procured identified and authenticated by Prof P.Jayaraman, Ph.D Reg.No PARC/2017/3433. A voucher specimen was submitted at C.L. Baid Metha College of Pharmacy, Chennai-97.

The mesocarp of the ripe watermelon fruit was chopped into thin slices, using a blender crushed to juice and filtered through a fine mesh muslin cloth to get the fresh watermelon fruit juice i.e 100% concentration. A 25% and 50% concentration was prepared by diluting a pure watermelon juice with filtered tap water in the ratio of (1 : 3 and 1 : 1) (v/v) (Oluwole FS *et al*., 2013).

### Animals and Experimental Design

A total of 30 Swiss Albino Mice (25–30 g) were procured acclimatised and maintained at 25 ± 2°C and kept in well ventilated animal house under with free access to food and water *ad libitum.* The experimental protocol described in the present study was approved by Institutional Animal Ethical Committee of C.L. Baid Metha College of Pharmacy. (Reg No:321/PO /Re/S/01/ CPCSEA).

Mice were divided into 6 groups of 5 animals each.
Group I – Normal Water ad libitum
Group II - Normal water ad libitum + Scopolamine was injected (1mg/kg,i.p.) on the 21st day
Group III- Normal water ad libitum + Piracetam (200mg/kg, i.p.) injected for 20 days + Scopolamine was injected (1mg/kg,i.p.) on the 21st day
Group IV V and VI- 25%, 50% and 100% AECL respectively ad libitum for 20days + Scopolamine was injected (1mg/kg,i.p.) on the 21st day

### Models Employed for Evaluation of Memory Enhancing Activity in Mice

### Passive-avoidance test[PA]

The apparatus consisted of a box (27x27x27 cm) having three walls of wood and one wall of Plexiglass,featuring a grid floor (3mm stainless steel rods set 8mm apart) with a wooden platform (10x7x1.7cm) in the centre of the grid floor. Electric shock (20V,A/C) was delivered to the grid floor. During training session ,each mouse was gently placed on the wooden platform set in the centre of the grid floor, when the mouse stepped down and placed all its paw on the grid floor, shocks were delivered for 15seconds and the Step Down Latency(SDL) was recorded. SDL was defined as time taken by the mouse to step down from the wooden platform to grid floor with its entire paw on the grid floor. Animals showing SDL in the range of 2 to 15 seconds during the first were used for the second session and the retention test. The second session was carried out 90minutes after the first test. During second session, if the animals stepped down before 60seconds, electric shocks were delivered once again for 15seconds. During the second test, animals were removed from shock free zone ,if they did not step down for a period of 60seconds and subjected to retention test. On the 20$^{th}$ day, after the treatment of last dose training was given and memory retention was examined after 24 hours (i.e., on 21$^{st}$ day) in a similar manner, except that the electric shocks were not applied to the grid floor observing an upper cut off time of 300 seconds (Mohammad MK *et al*., 2014).

### Elevated Plus Maze[EPM]

The elevated plus maze for mice consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 15 cm) extended from a central platform (5 cm × 5 cm) and the maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the

end of an open arm, facing away from the central platform. Transfer latency (TL) was defined as the time taken by the animal to move from the open arm into one of the covered arms with all its four legs. TL was recorded on the first day (i.e., 20th day of drug administration) for each animal. If the animal did not enter into one of the covered arm within 90 sec, it was gently pushed into one of the two covered arms and TL was assigned as 90 sec. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned task memory was examined 24h (21st day) (Kulkarni KS *et al*., 201).

**Morris Water Maze [MWM]**

Spatial learning and memory were evaluated by the Morris water maze. The procedure was to perform place navigation test from day 1 to 4, in which the escape latency (EL) (the time required to escape onto the hidden platform) was used to evaluate learning and memory function. Mice that found the platform were allowed to remain on the platform for 20 s and were then returned to the home cage. If mice did not reach the platform within 120 s, it was gently guided to the platform by the experimenter, where it remained for 20 s. The last trial was regarded as the probe test on day 5 after removal of the platform which was performed to test the ability of mice to find the removed platform by memory (Dhingra D *et al*., 2004).

**Y maze Test [YM]**

The behavioural test was conducted in a large quite room. A stop watch was used to score the behaviours and all events were observed manually. A Y-maze is made up of three equally spaced arms, labelled as A, B, and C which are 120° from each other, 41 cm long and 15 cm high. It was used to assess the spontaneous alternation in the mice. The floor of the apparatus is 5 cm wide and is levelled with saw shaves. Each mice was stationed in one of the arms and allowed to freely explore the apparatus. The sequence or consecutive entrance of the animals into the arms is termed an alternation. The total number of arms entered minus two is termed spontaneous alternations, and the percentage alternation was calculated as {(actual alternations/maximum alternations) × 100}. 5 min was assigned as the test time limit for each of the animals in the Y-maze apparatus. Recorded data is the total arm entries indicate the total number of a single arm entered (e.g. ABCBCABACBC, contain 11 entries), from which the correct and wrong alternations are recorded (Itoh J *et al*., 1990).

**BIOCHEMICAL ESTIMATION**

**Collection of Brain Sample**

After behavioral testing (retrieval) by screening models of memory, the animals were sacrificed by cervical dislocation. The whole brain was carefully removed from the skull and weighed. 10% w/v brain homogenate was then prepared by homogenizing it in ice-chilled phosphate buffer (pH 8, 0.1M). The homogenate was subsequently centrifuged using a refrigerated centrifuge at 3000 rpm for 10 min, and the supernatant was separated and used for the neurotransmitter and antioxidants estimation.

**Estimation of Neurotransmitter**

**Estimation of Acetylcholinesteras**

AChE present in the mice brain were estimated using the method described by Ellman *et al*.

**Estimation of Antioxidant**

**Estimation of lipid peroxidation (LPO):**

LPO was estimation of malondialdehyde (MDA) levels described by Ohkawa *et al.*

**Estimation of Superoxide dismutase (SOD):**

The activity of superoxide dismutase (SOD) was assayed by the method described by Kakkar, *et al***.**

**Estimation of glutathione peroxidase (GPX) activity**

Glutathione peroxidase (GPx) was measured by the method described by Rotruck *et al*.

**Estimation of Catalase (CAT)**

Catalase (CAT) activity was determined by the method described by Sinha *et al*.

**Histopathological studies**:

On the 21st day, after the behavioural study, Mice brains were collected after sacrifice and fixed in 10% neutral buffered formalin. Subsequently, brain tissues were further kept in 10% neutral buffered at 48°C. Then, the brains were routinely embedded in paraffin and stained with hematoxylin-eosin.

**Statistical analysis**

Data were analyzed using one-way ANOVA and expressed as mean ± standard deviation. Statistical analyses were performed using Graph Pad Prism version 7.04, for windows (Graph Pad software, San Diego, CA). Differences between mean values of different groups were considered statistically significant at *-$P$ <0.05, **-P<0.01,***- P<.0001, ns-Non significant

**RESULTS:**

**Effect of AECL on Passive Avoidance Test**

There was a significant difference between groups in the retention test.

When compared to Group I, Group II (P<0.001) significantly decreased, Group IV (P<0.001) and V(P<0.01) significant, Group III and VI non significant and increased latency in the retention test.

When compared to Group II, Group III, V and VI significant (P < 0.001), Group IV non significant and increased latency during retention test.

When compared to Group III, Group IV significant (P<0.01) Group V and VI non significant and decreased latency in retention test. Results were shown in figure 1, table 1

**3.2Effect of AECL on Elevated Plus maze**

Transfer Latency (TL) on retention of information or memory.

When compared to Group I, Group II, III and VI significantly increased (P < 0.001) Group V non significant and decreased, Group IV (P<0.01) significantly increased TL.

When compared to Group II, Group III, IV, V and VI significantly decreased TL (P < 0.001)

When compared to Group III, Group IV and VI (P<0.001), Group V (P<0.01) significantly increased TL. Results were shown in figure 2, table 1

**Effect of AECL on Morris Water Maze Test**

In the probe trial followed by last training session, Escape latency was calculated

When compared to Group I, Group II (P<0.01) significantly decreased, Group III and VI (P < 0.01), Group V(P<.0.05) significant and Group IV non significant and increased EL.

When compared to Group II, Group III, IV, V and VI significantly increased EL (P < 0.001)

When compared to Group III, Group IV (P<0.05) significant, Group V and VI non significant and decreased EL. Results were shown in figure 3, table 1

**Effect of AECL on Y maze**

In the probe trial followed by last training session,

When compared to Group I, Group II, IV and V significant (P < 0.001), Group III non significant, Group VI significant (P<0.05) and decreased Spontaneous alternation.

When compared to Group II, Group III significant (P<0.05), Group IV and V non significant, Group VI significant (P<0.05) and increased Spontaneous alternation.

When compared to Group III, Group IV significant (P<0.01), Group V (P<0.05) and Group VI non significant and decreased Spontaneous alternation. Results were shown in figure 4, table1

**Effect of AECL on Acetylcholinesterase**

When compared to Group I, Group II and IV (P<0.001), Group V (P<0.05) and Group VI (P<0.01) significant and Group III non significant with increased level of AChE.

When compared to Group II, Group III and V (P<0.001), Group VI (P<0.05) significant and Group IV non significant with decreased level of AChE.

When compared to Group III, Group IV (P<0.001), Group VI (P<0.05) significant and Group V non significant with increased level of AChE, Results were shown in figure 5 & Table 2.

**Effect of AECL on Lipid Peroxidation**

When compared to Group I, Group II (P<0.001) significant, Group III, IV, V and VI non significantly increased the level of LPO.

When compared to Group II, Group III and IV (P<0.01), Group V (P<0.001) and Group VI (P<0.05) significantly decreased the level of LPO.

When compared to Group III, Group IV and VI non significantly increased and Group V non significantly decreased the level of LPO. Results were shown in figure 6, table 3

**Effect of AECL on Superoxide Dismutase Level**

When compared to Group I, Group II (P < 0.001), Group V (P<0.01) significant, Group II, IV and VI non significant and decreased the level of SOD.

When compared to Group II, Group III and VI (P<0.05) and IV (P<0.001) significant, Group V non significant and increased the level of SOD.

When compared to Group III, Group IV non significant and increased, Group V and VI non significant and decreased the level of SOD. Results were shown in figure 7, table 3

**Effect of AECL on Glutathione Peroxidation**

When compared to Group I, Group II, IV, V and VI non significantly increased and Group III non significantly decreased the level of GPx.

When compared to Group II, Group III, IV, V and VI non significantly decreased the level of GPx .

When compared to Group III, Group IV, V and VI non significantly increased the level of GPx.

Results were shown in figure 8, table 3

**Effect of AECL on Catalase**

When compared to Group I, Group II (P<0.05) and Group III and VI (P<0.001), Group IV non significant and Group V (P<0.01) and increased the level of CAT.

When compared to Group II, Group III (P<0.05), Group V and VI non significantly increased and Group IV non significantly decreased the level of CAT.

When compared to Group III, Group IV (P<0.001) significant, Group V and VI non significant and decreased the level of CAT. Results were shown in figure 9, table 3.

**Table 1. Effect of AECL on scopolamine-induced alteration in behaviour parameter**

| Groups | PA-RL(sec) | EPM-TL(sec) | MWM-EL (sec) | Y M-%alterations |
|---|---|---|---|---|
| **Group I** | 43.83±1.24 | 31.21±1.04 | 10.22±0.96 | 47.85±1.45 |
| **Group II** | $26.50\pm0.53a^{***}$ | $65.53\pm1.12a^{***}$ | $6.08\pm1.20a^{**}$ | $31.97\pm2.54a^{***}$ |
| **Group III** | $39.50\pm0.74a^{ns}b^{***}$ | $18.01\pm1.56a^{***}b^{***}$ | $16.12\pm0.44a^{***}b^{***}$ | $42.95\pm1.32a^{ns}b^{**}$ |

| | | | | |
|---|---|---|---|---|
| **Group IV** | 32.03±1.31a$^{***}$b$^{ns}$c$^{**}$ | 38.53±0.60a$^{**}$b$^{***}$c$^{***}$ | 12.65±0.57a$^{ns}$b$^{***}$c$^{*}$ | 32.73±1.11a$^{***}$b$^{ns}$c$^{**}$ |
| **Group V** | 35.33±1.53a$^{**}$b$^{***}$c$^{ns}$ | 25.94±1.74a$^{ns}$b$^{***}$c$^{**}$ | 14.09±0.25a$^{*}$b$^{***}$c$^{ns}$ | 35.13±1.36a$^{***}$b$^{ns}$c$^{*}$ |
| **Group VI** | 38.59±1.85a$^{ns}$b$^{***}$c$^{ns}$ | 40.43±1.29a$^{***}$b$^{***}$c$^{***}$ | 15.74±0.54a$^{***}$b$^{***}$c$^{ns}$ | 39.56±1.78a$^{*}$b$^{*}$c$^{ns}$ |

All the values are expressed as mean±SEM. ***-P<0.001, **-P<0.01, *-P<0.05, ns-non significant; Group I Vs Group II, III, IV, V and VI is considered as a;Group II Vs Group III, IV, V and VI is considered as b; Group III Vs Group IV, V and VI is considered as c (one-way ANOVA). ANOVA – Analysis of variance; SEM – Standard error of the mean; AECL – Aqueous extract of Citrullus lanatus; % alterations- % of spontaneous alteration in the arms; EL—Escape Latency; TL-Transfer Latency; RL-Retention Latency.

**Table 2. Effect of AECL on scopolamine-induced alteration in Neurotransmitter**

| **Groups** | **AChE (**µmoles/min/mg tissue) |
|---|---|
| **Group I** | 17.53±1.45 |
| **Group II** | 32.40±1.26a$^{***}$ |
| **Group III** | 18.31±0.45a$^{ns}$b$^{***}$ |
| **Group IV** | 28.46±1.83a$^{***}$b$^{ns}$c$^{***}$ |
| **Group V** | 23.18±1.18a$^{*}$b$^{***}$c$^{ns}$ |
| **Group VI** | 24.46±0.68a$^{**}$b$^{**}$c$^{*}$ |

**Ache**

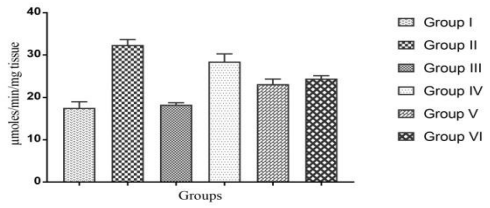



**Figure 5**

All the values are expressed as mean±SEM. ***-P<0.001, **-P<0.01, *-P<0.05, ns-non significant; Group I Vs Group II, III, IV, V and VI is considered as a;Group II Vs Group III, IV, V and VI is considered as b; Group III Vs Group IV, V and VI is considered as c (one-way ANOVA followed ). ANOVA – Analysis of variance; SEM – Standard error of the mean; AECL – Aqueous extract of Citrullus lanatus;AChE-Acetylcholinesterase

**Table 3. Effect of AECL on scopolamine-induced alteration in oxidative stress parameter**

| **Groups** | **LPO** (nmol MDA/mg protein) | **SOD** (Units/min/mg protein) | **Gpx** (units/mg protein) | **CAT** **(**µmols of H2O2 consumed/min/mg protein) |
|---|---|---|---|---|
| **GroupI** | 1.18±0.07 | 95.51±0.34 | 0.13±0.05 | 3.26±0.26 |
| **GroupII** | 3.12±0.26a$^{***}$ | 90.52±0.62a$^{***}$ | 0.21±0.03a$^{ns}$ | 4.15±0.21a$^{*}$ |
| **GroupIII** | 1.59±0.36a$^{ns}$b$^{**}$ | 93.36±0.45a$^{ns}$b$^{*}$ | 0.07±0.03a$^{ns}$b$^{ns}$ | 5.12±0.11a$^{***}$b$^{*}$ |
| **GroupIV** | 1.65±0.11a$^{ns}$b$^{**}$c$^{ns}$ | 94.42±0.83a$^{ns}$b$^{***}$c$^{ns}$ | 0.15±0.04a$^{ns}$b$^{ns}$c$^{ns}$ | 3.54±0.18a$^{ns}$b$^{ns}$c$^{***}$ |
| **GroupV** | 1.21±0.28a$^{ns}$b$^{***}$c$^{ns}$ | 92.20±0.58a$^{**}$b$^{ns}$c$^{ns}$ | 0.14±0.08 a$^{ns}$b$^{ns}$c$^{ns}$ | 4.54±0.20a$^{**}$b$^{ns}$c$^{ns}$ |
| **GroupVI** | 1.86±0.34a$^{ns}$b$^{*}$c$^{ns}$ | 93.18±0.43a$^{ns}$b$^{*}$c$^{ns}$ | 0.18±0.06 a$^{ns}$b$^{ns}$c$^{ns}$ | 4.76±0.19a$^{***}$b$^{ns}$c$^{ns}$ |

All the values are expressed as mean±SEM. ***-P<0.001, **-P<0.01, *-P<0.05; a-as compared to Group I Vs Group II, III, IV, V and VI is considered as a;Group II Vs Group III, IV, V and VI is considered as b; Group III Vs Group IV, V and VI is considered as c (one-way ANOVA). ANOVA – Analysis of variance; SEM – Standard error of the mean; AECL – Aqueous extract of Citrullus lanatus; SOD – Superoxide dismutase; LPO – Lipid peroxidation; GPx – Glutathione Peroidase;CAT-Catalse.

**Figure:1 PA**

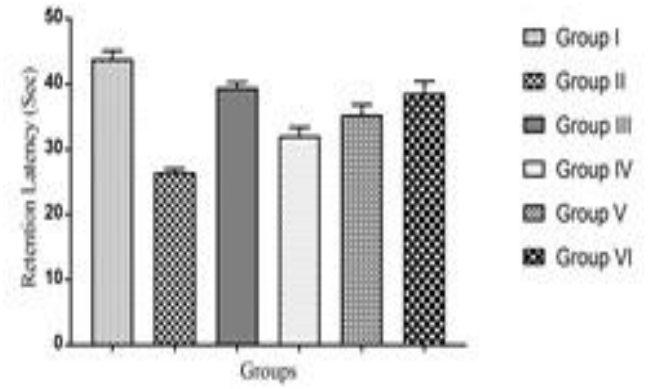



**Figure:2 EPM**

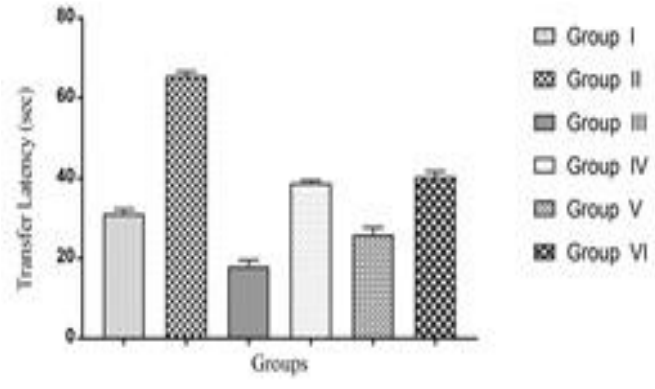


**Figure:3 MWM**

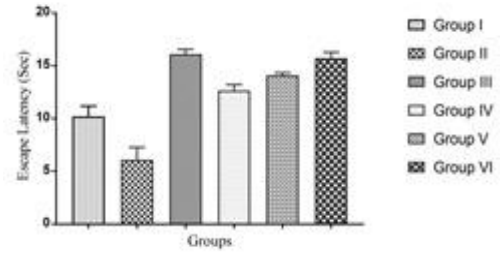



**Figure:4 YM**

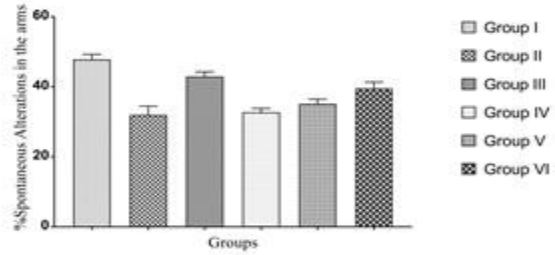



**Figure:6 LPO**

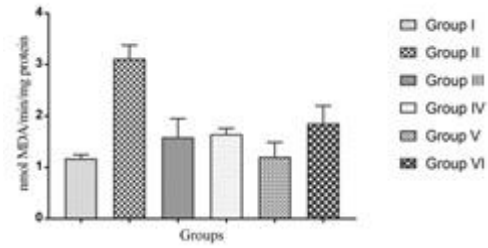



**Figure:7 SOD**

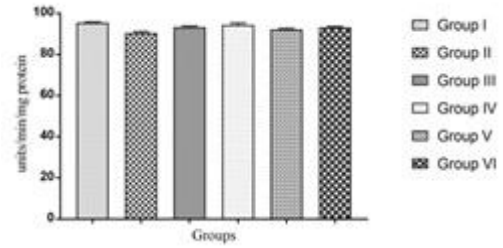



**Figure:8 GPx**

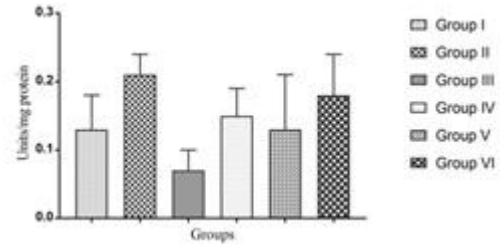
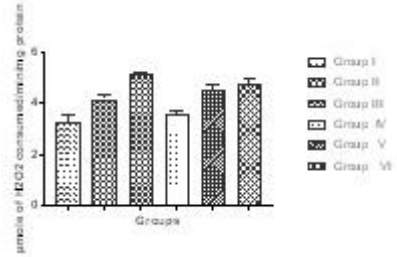
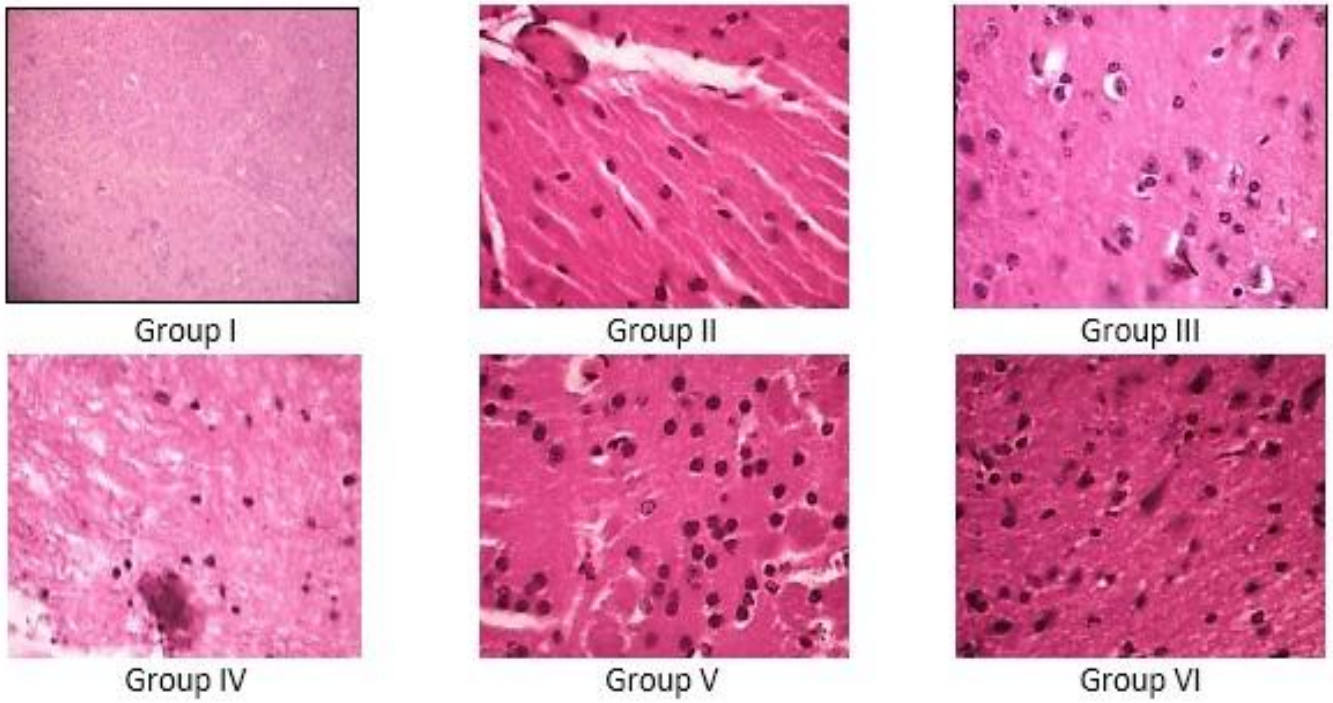



**Figure:9 CAT**




**Histopathology Assessment:**

Histopathology study of brain,



**DISCUSSION**

Alzheimer's disease is a neurodegenerative disorder associated with a decline in cognitive abilities (Cummings JL *et al*., 1998). which mostly affects the elderly population. It is a complex disease of multiple pathologies associated with degeneration of several neuronal populations, especially central cholinergic pathways (Praticò D *et al*., 2008) and the potential involvement of inflammatory and oxidative pathways Hence, the present study focuses on exploration of the memory enhancing activity of the aqueous extract of *Citrullus lanatus* (Thunb.) in scopolamine induced amnesia mice model.

Watermelon juice is a rich source of phenolics, α tocopherol, carotenoids such as beta carotene and lycopoene, and vitamin C. The beneficial effects of vitamin C are attributed mainly to its antioxidant properties. Watermelon juice is an excellent source of lycopene, having about 40% higher lycopene content than raw tomatoes Studies have attributed the antioxidant properties of water melon juice to its high lycopene content. There is a compelling evidence for the antioxidant role of lycopene in animal models of toxicant induced toxicities. Lycopene induces enzymes of the cellular antioxidant defense systems by activating the antioxidant response element transcription system

Piracetam a cyclic GABA derivative is the first representative of a new class if psychotropic drugs named as nootropic agents. These drugs were shown to improve learning acquisition and retention of learning (memory) both in normal animals and in conditions of impaired cognitive functions Scopolamine, an anti-muscarinic 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. Scopolamine diminishes cerebral blood flow due to cholinergic hypofunction. Scopolamine additionally triggers ROS, inducing free radical injury and deterioration in antioxidant status. Scopolamine induces neuro-inflammation by promoting high level of oxidative stress and pro inflammatory cytokines in the hippocampus. Scopolamine is proven to increase levels of APP and Tau. Administration of scopolamine led to marked histopathological alterations in the cerebral cortex, including neuronal degeneration (Perry G *et al*., 2008).

The present study evaluates, Scopolamine-induced cognitive dysfunction is extensively used to probe potential therapeutic agents attenuating cognitive deficits and the effect of AECL 25%, 50% and 100% in scopolamine-induced amnesia in mice. Screening methods such as the Morris water maze, elevated plus maze, passive avoidance paradigm and Y maze were performed to screen the effect of drugs. Furthermore, using mice brain homogenate acetylcholinesterase level, brain oxidative markers, histopathological studies were performed (Kang SY *et al*., 2005).

Passive avoidance task is fear-aggrevated test used to evaluate learning and memory in rodent models of CNS disorder. A method for evaluating passive avoidance- and escape-learning responses simultaneously has been developed for the study of learning and memory in mice. Therefore, the step-down latency may be good parameters of learning and memory performance and significant. Prolongation of the step-down latency in the retention test depended on the strength of the voltage of the electric shocks delivered during the training test. AECL increased SDL induced by i.p. treatment of scopolamine in the retention trail. This suggests that the animal has the retention of memory of the shock once entered in the shock-free zone (Charoensiri R *et al*., 2009).

An elevated plus-maze consisting of two open and two enclosed arms was employed for an evaluation of memory in mice. Mice in the plus-maze escaped from the open arm to the enclosed arm because mice apparently dislike open and high spaces. The time it took for the mice to move from the open arm to the enclosed arm (transfer latency) was recorded and the results suggested that transfer latency may be one of the parameters of learning and memory. In elevated plus maze, AECL decrease in transfer latency during probe trial indicated improvement of memory (Chaturvedi P *et al*., 2014).

Morris water maze were employed as behavioral models for evaluation of learning and memory. Morris water maze is generally accepted as an indicator of spatial learning and reference memory. It is a reliable and convenient method to assess hippocampal-dependent cognitive function in rodents. AECL reversed scopolamine-induced memory impairment in the MWM test by increasing the EL in the probe test. These results suggest that AECL attenuates long term and reference memory impairment induced by scopolamine through the rescue by antioxidant mechanisms and acetylcholine system.

Spontaneous alternation using a Y-maze is a test for habituation and spatial working memory. Spontaneous Alternation Tests are used to evaluate exploratory behavior in mice. Brain areas involved in this test include hippocampus, septum, basal forebrain, and prefrontal cortex. AECL treated mice showed increase in spontaneous alterations than the scopolamine treated mice (Bhattacharya SK *et al*., 1993).

Cholinergic neurotransmission is impaired by scopolamine; an increase of AChE activity, responsible for the hydrolysis of acetylcholine (Ach), and the subsequent reduction of Ach level is responsible primarily for impaired cognition. One of the most promising therapies to treat a cognitive deficit in AD is to increase the cholinergic activity and inhibition of AchE enzyme. In the brain, acetylcholine is produced in several locations including the basal forebrain. It may promote learning. Acetylcholine-producing cells in the basal forebrain are damaged in the early stages of Alzheimer's disease, which may contribute to the memory impairments which are an early symptom of

the disease. AECL decreased the AChE level in brain compared to the scopolamine treated mice (Izquierdo I, 1989).

Lipid peroxidation causes cell membrane destruction and cell damage. The abundance of polyunsaturated fatty acids (PUFAs) and redox active transition metal ions in the brain in addition to its high oxygen usage makes it highly susceptible to oxidative damage. Scopolamine significantly elevated the malondialdehyde (MDA) levels in the brain indicating enhanced peroxidation and breakdown of the antioxidant defense mechanisms. AECL treatment significantly reversed these alterations causing a significant decrease in MDA levels suggesting its protective effects against scopolamine-induced oxidative damage.

Superoxide Dismutase, an enzyme that alternately catalyzes the dismutation (or partitioning) of the superoxide radical into either ordinary molecular oxygen hydrogen peroxide. Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. *Citrullus lanatus* (Thunb.) contains Vitamin C which contribute to the antioxidant property. AECL treated significantly increased than scopolamine treated mice.

Glutathione preroxidase (GPX) is another enzymic anti-oxidant that acts as a defense against oxidative stress. There was no significant effect of GPx activity observed in our study after scopolamine treatment.

Catalase oxidation reaction occur in the presence of a hydrogen peroxide ($H_2O_2$) to form acetaldehyde. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). AECL treated mice showed decrease in catalase than the scopolamine treated animals

Histopathological study of mice brain proved damaging in scopolamine-treated group which showed neuronal death, while groups pretreated with AECL protected neurons by reversing the damage induced by scopolamine.

The protective effect of *Citrullus lanatus* (Thunb.) may be due to lycopene content. Lycopene has been shown as a neuroprotective agent against Ab-induced neurotoxicity in primary cultured rat and it was suggested as a promising candidate for Alzheimer Disease treatment and proven evidence for the potential of lycopene in the management of scopolamine induced amnesia (Kaur R *et al*., 2015; Kameyama T *et al*., 1986; Itoh J *et al*., 1990).

## CONCLUSION

The result of the study shows, neuroprotective and improved cognitive effect of AECL in scopolamine induced animal model could be due to the synergistic effect of the phytoconstituents, further can be explored on the phytoconstituent present for valued treatment of AD. Anti oxidant study give the significant result. *Citrullus lanatus* (Thunb.) has Vitamin C and could be the reason for the antioxidant property. Further, there was a significant decrease of acetylcholinesterase level in the brain and the histopathological study of brain shows future study is required to establish the mechanism of action of AECL

### Conflicts of Interest

There are no conflicts of interest.

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