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## Screening of the Ethanolic Extract of Rhizomes of *Alpinia Galanga* (Linn.) On Nootropic and its Influence on Brain Cholinergic System in Rats

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### ABSTRACT

Loss of memory is the major disorder in the modern world. In ayurvedic system of medicine rhizomes of *Alpinia galanga* linn. family Zingiberaceae is used as a brain tonic and nervine tonic. But since its effect on learning and memory is not scientifically documented. The present study was carried out to assess the effects of ethanolic extract of Rhizomes of *Alpinia galanga* Linn. on memory deficits caused by MES and Scopolamine in rats. In Albino rats of either sex, amnesia was induced by subjecting to MES (150mA for 0.2s) through corneal electrodes or administration of Scopolamine (0.3mg/kg i.p) for seven days. Ethanolic extract of Rhizomes of *Alpinia galanga* Linn. (500mg/kg, p.o) was evaluated for its nootropic activity in terms of Transfer Latency (TL) by using elevated plus maze and step down passive avoidance test. Rats were sacrificed at the end of study and Acetyl Cholinesterase (AChE) enzyme activity was estimated. The extract showed significant improvement in 'learning' and 'memory' as compared to 'control' group in all the models and also showed significant reduction in 'acetylcholine-esterase activity'. The present study indicate that treatment with Rhizomes of *Alpinia galanga* Linn. extract enhances the memory function and this could be mediated through brain cholinergic system.

**Keywords:** Amnesia, Transfer Latency, *Alpinia galanga* Linn., Acetylcholine esterase, learning and memory.

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## INTRODUCTION

An enhanced life expectancy in developed countries has been accompanied by an increased number of people suffering from age-associated dementia. Dementia is a syndrome 'due to disease of the brain, usually of chronic or progressive nature in which there is disturbance of multiple higher cortical functions, including memory, thinking, orientation, calculation, learning capacity, language and judgment, without clouding of consciousness'. Cognition in a broad sense means information processing. It denotes a relatively high level of processing of specific information including thinking, memory, perception, motivation, skilled movements and language. The hippocampus contains the neural circuitry crucial for cognitive functions such as learning and memory. It refers to the perceptual and intellectual aspects of mental functioning. Among the specific function that may be assessed in determining the intactness or adequacy of cognition are orientation, the ability to learn necessary skills, solve problems, think abstractly, reason and make judgments, the ability to retain and recall events, mathematical ability and other forms of symbol manipulation, control over primitive reaction and behavior, language use and comprehension, attention, perception and praxis.<sup>2</sup>

## MATERIALS AND METHOD

### **Drugs:**

#### **Mentat:**

A poly herbal preparation containing around 25 different herbs, and is a proven memory enhancing drug available in the market. It was procured from Himalaya Herbal Healthcare, Bangalore.

#### **Scopolamine:**

An antimuscarinic agent for induction of loss of memory. It was purchased from Sigma Chemicals, USA.

#### **Rhizomes for Extraction:**

The fruits of *Alpinia galanga* Linn. were purchased from the local market of Belgaum, and authenticated at Department of Botany, Regional Medical Research Centre, Belgaum Branch by Dr. Harsha Hegde, Research Supervisor.

The rhizomes were later powdered and used for the extraction process. The ethanolic extract of rhizomes of *Alpinia galangal* Linn. obtained was used as the test drugs for the evaluation of memory enhancing activity.

### **Reagents:**

The reagents required for acetylcholine esterase enzyme estimation were:

- Acetylthiocholine iodide – Sigma Chemicals, USA.
- Ellman's Reagent [5,5'-Dithiobis(2-Nitrobenzoic acid)] – Sigma Chemicals, USA.

**Chemicals:**

- Sodium dihydrogen orthophosphate – Poona Chemicals, Pune.
- Disodium hydrogen phosphate – Poona Chemicals, Pune.
- Tween 60 – Himedia

**Instruments used:**

- Electronic Digital Balance (Afcoset®)
- Digital pH meter (Systronics®)
- Electroconvulsimeter (INCO)
- High Speed Tissue Homogenizer (Remi Motors, Mumbai)
- UV Visible Spectrophotometer [UV-1201] (Shimadzu)
- Cooke's Pole Climbing response apparatus (ROLEX)
- Elevated Plus Maze.

**Animals:**

The Albino Wistar strain rats of both sexes weighing 150-250g were procured from Venkateshwara Enterprises, Bangalore. They were housed in a group of five per cage and were maintained under natural day and night cycle at  $25\pm 2^\circ\text{C}$  ambient temperature, 45-55% relative humidity. They were allowed to acclimatize one week before the experiment. The rats were allowed with free access to standard pellet and water *ad libitum*.

The experimental protocol was cleared from the Institutional Animal Ethical Committee, K. L. E. S's College of Pharmacy, Belgaum.

**METHOD****Extraction of Rhizomes:**

The rhizomes of *Alpinia galanga* Linn. were purchased from the local market. The rhizomes were dried, powdered and then used for extraction. The extraction was carried out by maceration process taking absolute ethanol (99.5%) as the solvent. Later, the extract was condensed on water bath to remove excess of solvent and then dried using flash evaporator to maximum dryness. The extracts obtained were in the form of thick paste due to the presence of resinous matter.

**Preparation of Drugs:****Mentat:**

The tablets were crushed and used for preparing the drug suspension. Specified quantity of Mentat powder was weighed and mixed with Tween 60, triturated well and suspended in distilled water quantity sufficient to produce a suspension of 10mg/ml and was administered orally at a dose of 100mg/kg b.w.<sup>7</sup>

**Extract:**

The rhizomes extract was weighed and triturated with tween 60 (0.5%) and then was suspended in distilled water quantity sufficient to produce a suspension of the strength: 100mg/ml. Dose: 500mg/kg b.w.<sup>6</sup> by oral route.

#### **Scopolamine:**

The scopolamine was administered intraperitoneally. The ampoule containing a solution of 20mg/ml was diluted with distilled water in a volumetric flask to get the final volume of 100ml with a final concentration of 0.2mg/ml and was administered at a dose of 0.3mg/kg b.w.<sup>7</sup>

#### **Solutions used in Ellman's Method for Estimation of Acetyl Cholinesterase enzyme activity:**

**Phosphate buffer:** 0.05M Phosphate Buffer (pH – 7.2)

**Solution A:** 6.85g Sodium dihydrogen orthophosphate dissolved in 100ml distilled water.

**Solution B:** 13.40g Disodium hydrogen phosphate dissolved in 100ml distilled water.

*Solution A* was mixed with *Solution B* until pH reached 7.2 and then was diluted in a ration of 1:10 with distilled water. This diluted solution was used for estimation.

**Substrate:** Acetyl thiocholine iodide (0.075M Solution)

21.68mg/ml solution was prepared in 0.05M Phosphate buffer pH 7.2. The solution was used successfully for 10 – 15 days by keeping it in the refrigerator.

**Ellman's Reagent:** 5, 5'-Dithio bis (2-Nitro benzoic acid) [DTNB] (0.01M Solution)

19.8mg/5ml (3.96mg/ml) solution was prepared in 0.05M Phosphate buffer pH 7.2. At this pH, the reagent was more stable and was used successfully for 2 – 3 days by keeping it in the refrigerator.

#### **Grouping of Animals:**

The animals were divided into 9 groups, each consisting of 5 rats, viz;

Group 1 – Normal Control: Treated with vehicle.

Group 2 – Positive Control: Treated with Extract of *Alpinia galanga* Linn.

Group 3 – Positive Control: Treated with Mentat (Standard Drug).

Group 4 – Negative Control: MES Induced.

Group 5 – Negative Control: Scopolamine Induced.

Group 6 – Treatment Group: MES + Treated with Extract of *Alpinia galanga* Linn.

Group 7 – Treatment Group: MES + Treated with Mentat (Standard Drug).

Group 8 – Treatment Group: Scopolamine + Treated with Extract of *Alpinia galanga* Linn.

Group 9 – Treatment Group: Scopolamine + Treated with Mentat (Standard Drug).

#### **Experimental Schedule:**

All the animals were dosed once in a day with respective drugs for seven consecutive days. Group-1 animals received only the vehicle (0.5% tween 60), Group-2 received test drug only, Group-3 received standard drug only, Group-4 received only electric shock (150mA for 0.2s), Group-5 received only scopolamine (0.3mg/kg b.w.), Group-6 and

Group-7 received both electric shock and respective drug treatment, and Group-8 and Group-9 received both scopolamine and respective drug treatment.

The animals were trained on the 0 (zero) day and the acquisition of memory was tested on the day 1, later the animals were subjected to induction followed by drug treatment, that was continued for up to day 7. Then, the animals were subjected for the retention test on the day 7. Soon after the passive avoidance task (step-down latency) elevated plus maze (transfer latency) and open field behavioral test the animals were sacrificed for Acetyl Cholinesterase enzyme estimation.

#### **. *In-vivo* methods:**

#### **Models used for induction of Amnesia:**

#### **Electroshock induced amnesia:**<sup>7,9</sup>

Amnesia can be induced in experimental animals by electroshock stimulation. According to standard procedure, presentation of electroshock by silver corneal electrodes induced clonic-tonic seizures and impaired memory. The electroshock was applied immediately after the training trials in the task being tested. A sham electroshock was given to the control animals. Alternatively, electroconvulsive shock (ECS)-induced convulsions in animals may produce a more severe form of amnesia. In this technique, an electroshock (150mA for 0.2sec) was applied through commercially available electro stimulators. Electric shock gives very unpleasant feeling to the animal. The model offers evaluations of various nootropic agents.

#### **Drug induced (Scopolamine) amnesia:**<sup>10</sup>

Scopolamine is a powerful muscarinic antagonist capable of crossing blood brain barrier, acts both peripherally by blocking the receptors for ACh at the synapse. It impairs memory storage of new information (short term memory) and learning acquisition. The dose of 0.3mg/Kg is approved to produce cognitive and memory changes without causing debilitating peripheral anticholinergic effect. Though the several models for amnesia are available, but the scopolamine induced memory deficits has been proposed to have symptomatological similarities with AD and related disorders.

#### **Models used for Screening of memory:**<sup>10-11</sup>

#### **Elevated Plus Maze (Transfer Latency):**

An elevated plus maze consists of two open arms (50 x 10cm) and two closed arms (50 x 10 x 40cm) with an open roof. The maze was elevated to a height of 50cm. The animals were

individually placed at the end of either of the open arms and the time taken for the animal to move from open to closed arm (Transfer latency, TL) was taken as the criterion of task. If the animal didn't move into the closed arms it was later pushed into one of the closed arm. The animals were allowed to explore the apparatus for 30s.

After 24h of the first exposure; TL was again noted on the day-1 of the study for determining the acquisition. The criterion was reached when the animal moved into the closed arms in very short period keeping the cut-off time of 60s (as maximum time taken for moving from open arms to closed one). Five minutes later the animals of Group – 4, 6 and 7 received electroshock of 150mA for 0.2s through a pair of ear electrode from an Electroconvulsimeter and then the animals were dosed with respective drug and kept in their home cage. Similarly, animals of Group – 5, 8 and 9 received scopolamine i.p. (0.3mg/kg b.w.) and then were dosed with respective drug and returned to their home cage. The electroshock/scopolamine and dosing with drug continued for up to 7 days and on 7<sup>th</sup> day, the animals were subjected to the retention test 30min. after the last dose, for evaluating the step-down latency keeping the time period of 60s as cut-off criterion.

#### ***In-vitro* method:**

#### **Estimation of Acetyl Cholinesterase Enzyme Activity of Discrete Parts of Brain:**

##### **Dissection:**

Exactly 60min. after the electroshock and scopolamine treatment the rats were decapitated by Gillette, and the whole brain were taken out quickly. The cerebral cortex, cerebellum, medulla oblongata and midbrain were dissected out as described by Glowinsky and Iverson 1966 suspended in phosphate buffer and weighed accurately.

##### **Preparation of Brain Homogenate:**

###### **Procedure:**

The different regions of the brain viz. cortex, cerebellum, medulla oblongata and midbrain were homogenized in a tissue homogenizer. [Approximately 20mg of tissue per ml of phosphate buffer pH 7.2

A 0.4ml aliquot of this homogenate was added to a cuvette containing 2.6ml phosphate buffer (pH 7.2, 0.05M). To this, 100µl of Ellman's reagent was added and then taken into the photocell. The absorbance was set at 412nm when the fluctuations stopped.

Of the substrate (Acetyl thiocholine iodide) 20µl was added. A change in the absorbance per minute was noted. The rate of moles of substrate hydrolyzed per minute per gram of tissue was later calculated as per the following equation:

$$R = \frac{\Delta A}{1.36 (10^4)} \times \frac{1}{(400/3120) C_0} = 5.74(10^{-4}) \frac{\Delta A}{C_0}$$

Where,

$\Delta A$  = Change in absorbance per minute (mean change in absorbance from the 1<sup>st</sup> to 7<sup>th</sup> min. was taken)

$C_0$  = Original concentration of the tissue.

R = Rate in moles substrate hydrolyzed per minute per gram of tissue.

### Statistical Analysis:

The transfer latency was analyzed using the Student's paired 't' test (two tailed). A probability level of  $P < 0.01$  was considered as significant. The AChE activity of different groups were analyzed using One Way Analysis of Variance (ANOVA), followed by Dunnett's test for individual comparison of groups, viz.; A probability level of  $P < 0.0001$  for One way ANOVA was considered as significant, and for post test (Dunnett's test), a probability level of  $P < 0.01$  was considered as significant.

## RESULTS AND DISCUSSION

Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs. Traditionally, *Alpinia galanga* Linn. Known to be very effective in enhancing the memory, promoting intellect and is used in treating cognitive decline and weakness of brain. Despite its long-time use, there are no scientific studies to explain the influence of *Alpinia galanga* Linn. on learning and memory. Therefore, in this study, the hypotheses that this drug can enhance cognitive function in different regions of the brain. To delineate the mechanism by which *Alpinia galanga* Linn. exerts nootropic action, its effect on brain ACh levels was also determined. The findings of the present investigation indicate that *Alpinia galanga* Linn. can be regarded as nootropic agent since it improved learning and memory of rats significantly in both the exteroceptive behavioral models (i.e. Elevated Plus maze and Passive avoidance paradigm). In our present study, chronic exposure to MES for 7 days produced a significant decrease in latency to expose to electroshock grid in SDL and increased the time of TL in elevated plus maze. The same effect was also seen in Scopolamine exposed animals. This suggested that application of MES and Scopolamine disrupts the acquisition, retention and consolidation of a learned task. It is also been proved that AChE enzyme activity decreased significantly after electroshock and Scopolamine treatment.<sup>11,12</sup> Furthermore, pretreatment with extract protected the animals from learning and memory impairment produced by the exteroceptive (MES) and interoceptive (Scopolamine) stimuli which resulted in significant decrease in TL and increase in retrieval of memory in PAT, suggesting an underlying cholinergic mechanism. These findings suggest the possible neuroprotective role for *Alpinia galanga* Linn. *Alpinia galanga* Linn. also

produced a similar effect (in the absence of both stimuli), which may indicate drug-induced increase in cholinergic function. Thus, it meets a major criterion for nootropic activity, namely improvement of memory in the absence of cognitive deficit. Its influence on cholinergic activity of the rat brain was studied because there are many reports suggesting loss of memory is associated with decreased cholinergic activity.<sup>11</sup> A consistent finding is, “highly diminished or deficient central cholinergic system in AD, characterized by decreased pre-synaptic cholinergic markers such as choline acetyl transferase, and degeneration of cholinergic neurons in the nucleus basalis of Meynert.” Hence, in the present study it was observed that there was significant decrease in AChE activity in all parts of the brain where mainly the memory centres are located. This shows more availability of ACh in brain. The relationship between memory retention, ACh activity and AChE activity is controversial. Madepalli *et al.* 1994, reported that ACh content and AChE activity have an inverse relationship on memory retention. Whereas, Agnolli *et al.* reported that ACh content and AChE enzyme activity were decreased in senile dementia suggesting a direct relationship between them.<sup>11,12</sup> This intensifies the result showing that sandalwood oil has an effect on brain cholinergic system. Reports suggest that drugs like carbamazepine, certain cholinergic and anticholinergic drugs also enhance memory by decreasing AChE activity<sup>11</sup>. The results from the present study suggest that extract of *Alpinia galanga* Linn. possesses anti-amnesic activity as it reversed the memory impairment produced by MES and Scopolamine. The results suggest that these extracts improve cognition by decreasing the level of AChE Enzyme activity. Immuno-histochemical studies suggested the existence of chronic inflammation in certain regions of the brain in AD patients. Since inflammation can be damaging to host tissue, it was hypothesized that anti-inflammatory drugs might be inhibiting both the onset and the progression of AD. Indomethacin, a non-steroidal anti-inflammatory drug exhibited a memory protective effect against electroconvulsive shock-induced retrograde amnesia and also against amyloid deposits in the brain<sup>4</sup>. Anti-inflammatory action of *Alpinia galanga* Linn. might also be contributing to the observed memory-enhancing activity.<sup>1</sup> Oxygen free-radicals are implicated in the process of ageing and may be responsible for the development of Alzheimer’s disease in elderly persons. Oxygen-free radicals and other products of oxidative metabolism have been shown to be neurotoxic and antioxidant-rich diets improved cerebellar physiology and motor learning in aged-rats.<sup>5</sup> The protective effect of *Alpinia galanga* Linn. may be attributed to its antioxidant property by virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improved neuronal function, thereby enhancing the memory. Thus, a combination of anti-inflammatory, antioxidant and neuroprotective role could all be leading

to the net memory-enhancing effect. The present study indicates that *Alpinia galanga* linn has nootropic activity. However, further studies are needed to better evaluate the potential effectiveness of ethanolic extract of *Alpinia galanga* linn. as a nootropic agent.



**Figure 1: Isolated Brains Ready for Estimation of Acetyl cholinesterase Enzyme**



**First Row: (A) Cerebral Cortex (B) Cerebellum**

**Second Row: (C) Medulla Oblongata (D) Midbrain**

**Figure 2: Parts of Rat Brain used for AchE Activity Determination**

**Effect on Transfer Latency (using Elevated Plus maze):**

The animals were subjected to transfer latency (TL) to evaluate the retrieval of memory in behavioral paradigm after a period of 7 days of acquisition trial, to know the effect of extract

on the long term memory. TL of day 1 reflects learning behavior of the animals whereas; TL of day 7 reflects the retention of the information or memory.

The Normal Control animals have showed highly-significant retrieval of the memory in this behavioral paradigm (Table No. 3). In the Positive Control group, the animals treated with both extract of *Alpinia galanga Linn.* produced highly significant ( $P<0.0001$ ) activity whereas, Standard(Mentat), reduced the time taken in TL, did not produced significant activity (Table No. 4 and 5)

In the Negative Control group, the animals exposed to MES and Scopolamine produced significant ( $P<0.01$ ) loss of memory in behavioral paradigm (Table No. 6&7), which resulted in increase in TL on day 7 when compared to day 1.

In the Treatment group, the animals exposed to MES and treated with Mentat produced significant ( $P<0.01$ ) (table No. 8) activity and animals treated with extract of *Alpinia galanga Linn.* did not produced moderately significant ( $P<0.05$ ) (Table No. 9) retrieval of memory in behavioral paradigm, but the animals showed reduction of time taken to perform the task in Elevated plus maze.

In the treatment Group, the animals exposed to Scopolamine and treated with Mentat and extract of *Alpinia galanga Linn.*; although reduced the time taken to perform the task in Elevated plus maze it did not produced moderately significant retrieval of memory in behavioral paradigm.(Table No. 10 and 11).

#### **Acetyl Cholinesterase (AChE) Enzyme Activity:**

The animals were sacrificed at the end of the study period of 7 days after last dosing and evaluating SDL and TL, to dissect and isolate the brain. Then different parts of brain were separated and subjected for the estimation of AChE enzyme activity and the results were expressed as Mean $\pm$ SEM (moles  $\times 10^{-6}$ /minute/gram of tissue).

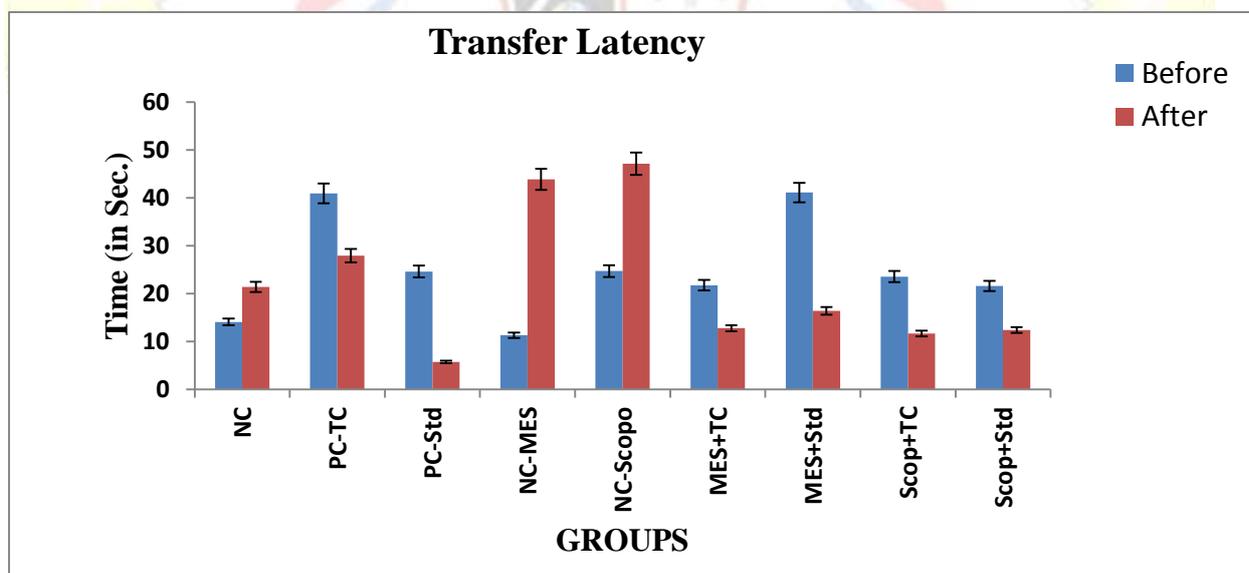
The animals of Positive Control group, Negative Control (MES Exposed) and Negative Control (Scopolamine Exposed) groups were compared with Normal Control group, whereas the MES induced + Treatment Group was compared to Negative Control (MES induced group) and Scopolamine induced + Treatment group was compared to Negative Control Scopolamine induced group.

The animals of Positive Control group treated with extract of *Alpinia galangal Linn.* (4.447 $\pm$ 0.3174, 4.547 $\pm$ 0.4872, 3.443 $\pm$ 0.4831 and 2.110 $\pm$ 0.2173) and Standard (Mentat) (3.110 $\pm$ 0.08327, 3.200 $\pm$ 0.1400, 3.437 $\pm$ 1.032 and 2.400 $\pm$ 0.1531) produced significant ( $P<0.01$ ) (Table No. 4 and 5) reduction of AChE enzyme activity in comparison with Normal Control (7.387 $\pm$ 0.3078, 9.223 $\pm$ 0.2955, 9.783 $\pm$ 0.5349 and 3.930 $\pm$ 0.4251) in different parts of brain viz. cortex, medulla, midbrain and cerebellum respectively (Table No. 3).

The animals of Negative control group exposed to MES ( $9.307\pm 0.2143$ ,  $11.31\pm 0.2857$ ,  $12.53\pm 0.1904$  and  $7.420\pm 0.3205$ ) and animals exposed to Scopolamine ( $8.927\pm 0.2392$ ,  $11.20\pm 0.1444$ ,  $13.58\pm 0.1910$  and  $6.367\pm 0.2834$ ) showed significant ( $P<0.01$ ) increase in ACh Enzyme activity compared to the Normal Control group (Table No. 6, 7 and 3 respectively).

In the Treatment group, the animals exposed to MES and treated with Standard (Mentat) ( $4.887\pm 0.1178$ ,  $4.213\pm 0.644$ ,  $4.130\pm 0.6245$  and  $1.680\pm 0.2117$ ) and extracts of *Alpinia galanga* Linn ( $5.390\pm 0.4763$ ,  $4.203\pm 0.1683$ ,  $4.217\pm 0.4199$  and  $2.167\pm 0.2817$ ) significantly ( $P<0.01$ ) decreased the Acetyl Cholinesterase Enzyme activity in comparison with negative control MES exposed group in different parts of brain viz. cortex, medulla, midbrain and cerebellum respectively (Table No.8 & 9).

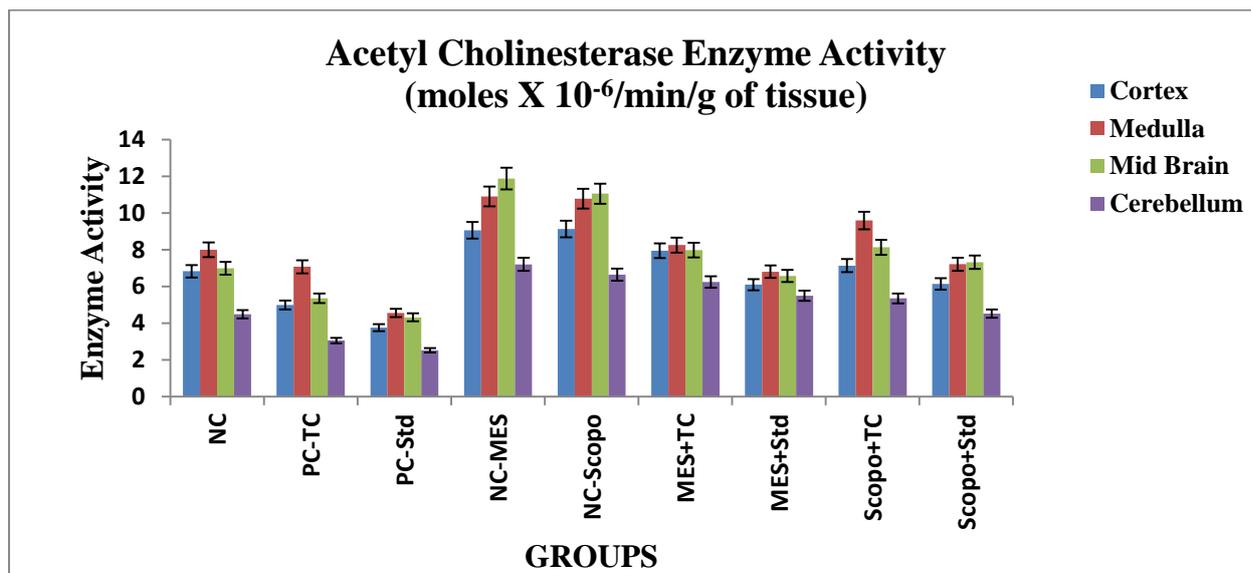
In the Treatment group, the animals exposed to Scopolamine and treated with Standard (Mentat) ( $4.717\pm 0.3060$ ,  $4.743\pm 0.4879$ ,  $4.537\pm 0.3606$  and  $2.530\pm 0.06557$ ) and extracts of *Alpinia galanga* Linn. ( $2.793\pm 0.5124$ ,  $5.923\pm 0.4642$ ,  $6.143\pm 0.576$  and  $2.717\pm 0.2599$ ) produced significant ( $P<0.01$ ) reduction in Acetyl Cholinesterase Enzyme activity in comparison with negative control Scopolamine exposed group. (Table No.11, 10 & 7 respectively).



**Graph 1: Transfer Latency Before and After (Induction and Treatment) in different groups**

NC= Normal Control, PC-TC= Positive Control treated with Terminalia chebula Retz., PC-Std= Positive Control treated with Standard (Mentat), NC-MES= Negative Control (MES induced), NC-Scopo= Negative Control (Scopolamine induced), MES+TC= Treatment group: MES induced + Treated with Terminalia chebula Retz. extract, MES + Std= Treatment group: MES induced + Treated with Standard (Mentat), Scopo + TC= Treatment

group: Scopolamine induced + Treated with *Terminalia chebula* Retz. extract, Scopo + Std= Treatment group: Scopolamine induced + Treated with Standard (Mentat).



**Graph 2: Mean Acetyl Cholinesterase Enzyme activity in different parts of Rat brain**

NC= Normal Control, PC-TC= Positive Control treated with *Terminalia chebula* Retz., PC-Std= Positive Control treated with Standard (Mentat), NC-MES= Negative Control (MES induced), NC-Scopo= Negative Control (Scopolamine induced), MES+TC= Treatment group: MES induced + Treated with *Terminalia chebula* Retz. extract, MES + Std= Treatment group: MES induced + Treated with Standard (Mentat), Scopo + TC= Treatment group: Scopolamine induced + Treated with *Terminalia chebula* Retz. extract, Scopo + Std= Treatment group: Scopolamine induced + Treated with Standard (Mentat).

**Table 1: Summary Of Transfer Latency And Step-Down Latency**

Group	Sub Group	Transfer Latency (in Sec.) in Elevated Plus Maze	
		Before Day 1	After Day 7
Normal Control Positive Control	Normal control	22.36±1.634	6.492±0.5852***
	Treated with <i>Alpinia galanga</i> Linn.. extract	55.11±1.420	27.96±2.891***
	Treated with Standard (Mentat)	28.89±6.007	19.04±4.447*
Negative Control	MES Induced	6.074±1.094	22.18±1.277**
	Scopolamine Induced	24.71±4.325	47.12±5.485**
Treatment Group MES + Drug Treated	Treated with <i>Alpinia galanga</i> Linn extract	41.29±4.518	29.12±2.5218*
	Treated with Standard (Mentat)	26.96±3.601	13.03±1.872**
Treatment Group	Treated with <i>Alpinia galanga</i> Linn extract	33.79±7.701	22.50±4.80*

Scopolamine + Drug Treated	Treated with Standard (Mentat)	21.30±9.572	15.48±7.693*
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N = 5

Values are expressed as Mean±SEM.

Student's 't' Test – Paired, two tailed

\*\*\*P&lt;0.0001, \*\*P&lt; 0.01, \*P&lt; 0.05

**Table 2: Summary Of AChE Enzyme Activity In Different Parts Of Rat Brain**

Group	Sub-Group	Acetylcholinesterase Enzyme activity (Mean± SEM) (in moles x 10 <sup>-6</sup> /min/g of tissue)			
		Cortex	Medulla	Midbrain	Cerebellum
Normal Control	Normal control	7.387±0.3078	9.223±0.2955	9.783±0.5349	3.93±0.425
Positive Control	Treated with <i>Alpinia galanga</i> Linn extract	4.447±0.3174**	4.547±0.4874**	3.443±0.4831**	2.11±0.2173**
	Treated with Standard (Mentat)	3.11±0.08327**	3.2±0.14**	3.437±1.032**	2.4±0.1531**
Negative Control	MES Induced	9.307±0.2143**	11.310.2857**	12.53±0.1904**	7.42±0.3205**
	Scopolamine Induced	8.927±0.2392**	11.2±0.1444**	13.58±0.1910**	6.637±0.2834**
Treatment Group MES + Drug Treated	Treated with <i>Alpinia galanga</i> Linn extract	5.390±0.476 <sup>#</sup>	4.203±0.1683 <sup>#</sup>	4.21±0.4199 <sup>#</sup>	2.167±0.2817 <sup>#</sup>
	Treated with Standard (Mentat)	4.887±1.178 <sup>#</sup>	4.213±0.6444 <sup>#</sup>	4.130±0.6245 <sup>#</sup>	1.68±0.2117 <sup>#</sup>
Treatment Group	Treated with <i>Alpinia galanga</i> Linn extract	5.203±0.4068 <sup>††</sup>	2.750±0.28229 <sup>††</sup>	3.6±0.7842 <sup>††</sup>	1.773±0.1977 <sup>††</sup>
Scopolamine + Drug Treated	Treated with Standard (Mentat)	4.717±0.3060 <sup>††</sup>	4.743±0.4879 <sup>††</sup>	4.537±0.3606 <sup>††</sup>	2.53±0.06557 <sup>††</sup>

N = 3

Values are expressed as Mean± SEM.

One Way Analysis of Variance (ANOVA) followed by Dunnett's 't' Test

\*\*P&lt;0.01 – Compared to Normal Control

<sup>#</sup>P<0.01 – Compared to Negative Control – MES Induced<sup>††</sup> P<0.01– Compared to Negative Control – Scopolamine Induced.

## CONCLUSION

In the present study, alcoholic extract of *Alpinia galanga* linn. Rhizomes possess cognitive enhancement activity. The cognitive enhancement activity is further supported by the decrease in AChE enzyme activity in different regions of the brain. Further studies to evaluate the efficacy of the extract of *Alpinia galanga* linn., in neuro- protection and to establish the therapeutic values in the treatment of dementia, where combination of this extract along with other drugs like Cholinesterase inhibitors might also represent future alternative to the present monotherapy. Extensive preclinical and clinical work is desirable.

We have designed the study to understand the effects of ethanolic extract of *Alpinia galanga* Linn. on cognitive dysfunction, for that we produced amnesia using Electroshock induced memory disruption model and Scopolamine induced amnesia model. After 7 days of chronic treatment, the animals were dissected to estimate AChE level in different parts of the rat brain, and that is considered as an additional parameter as a cholinergic marker of learning and memory. Data obtained from the study showed significant neuro-protection and memory enhancement by extract of *Alpinia galanga* Linn. at a dose of 500mg/kg b.w. which might also be useful as supportive adjuvant in treatment of elderly memory loss, but to establish its therapeutic value in treatment of dementia, extensive work is needed.

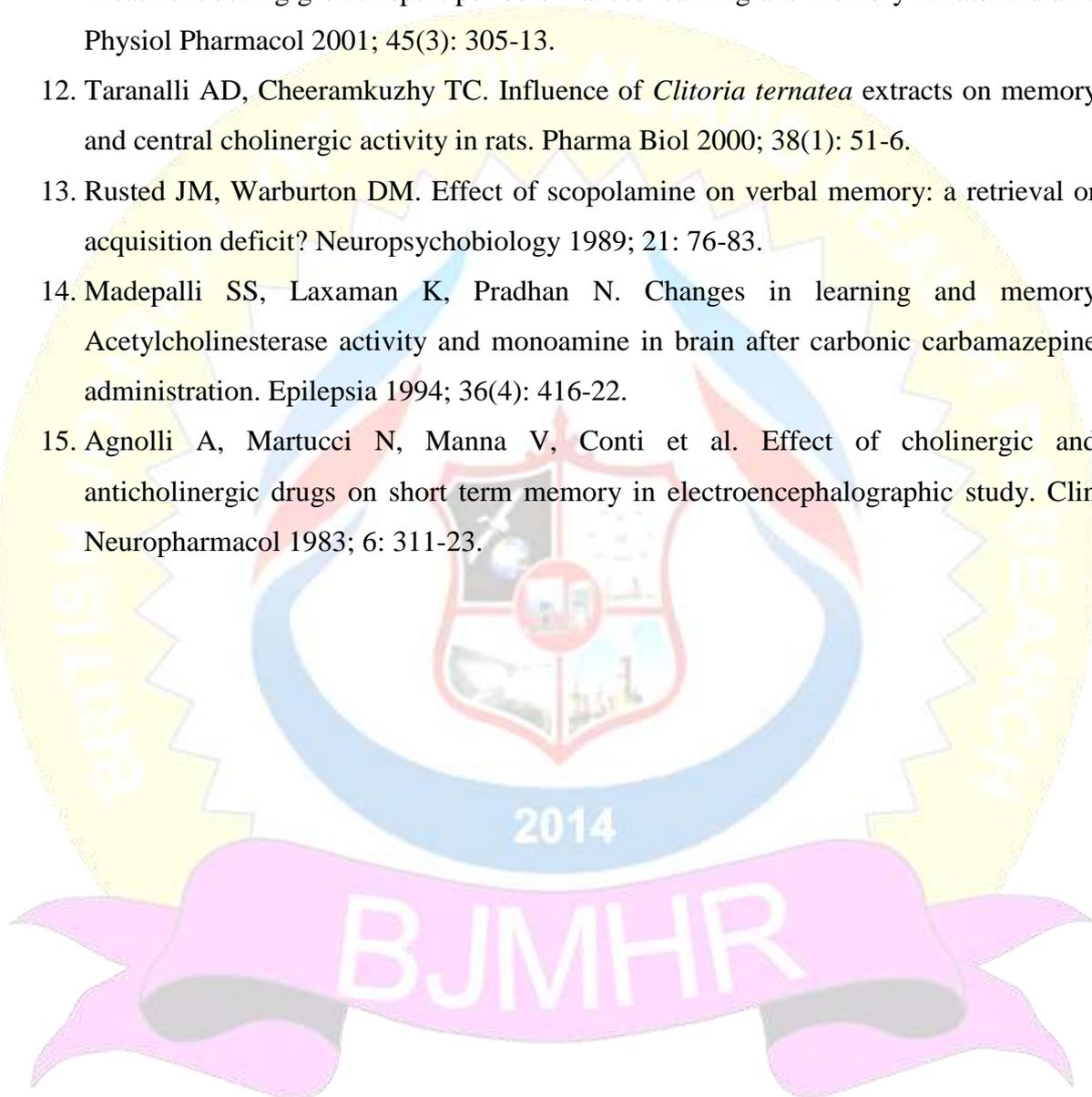
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