RESEARCH ARTICLE

Anti-amnesic activity of *Mangifera indica* L. Leaves extract against Scopalamine Induced Memory Impairment in Rats

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ABSTRACT:

BACKGROUND: Alzheimer's disease is a degenerative brain disease and is the most common cause of dementia. Recognized factors in Alzheimer'sdisease include acetylcholine deficiency, increased free radicals and inflammation of the brain tissue. Thus, we intended to evaluate anti-amnesic activity of ethanolic extract of *Mangifera indica* L. leaves against scopolamine-induced memory impairments in experimental rat model.

METHODS: The investigation lasted for a total of 28 days. On the 29th day, a behavioral assessment was conducted using the rota rod, actophotometer, elevated plus maze, open field model, and biochemical parameters like lipid peroxidation, catalase, acetyl cholinesterase activity, and protein estimation. Rats (30) were divided into five groups of six animals each, including a negative control, positive control (scopolamine1mg/kg i.p), standard (donepezil 2.5mg/kg p.o) and two different doses of EEMI (250 & 500mg/kg) along with scopolamine. One-way Anova was used to analyze all the data, and it was followed by a tukey Kramer multiple comparison test.

RESULTS*:* The current study's findings showed that, in comparison to the positive control group, EEMI treatment (250mg/kg and 500 mg/kg) increased motor activity, improved muscle grip strength, decreased amnesic-like behavior, and increased exploratory activity. When compared to the positive control group, brain homogenate showed decreased MDA level, increased cathalase level, decreased acetyl cholinesterase level, and increased protein level in EEMI in a dose-dependent manner.

Conclusion: The study concludes that ethanolic extract of *Mangifera indica* L possess significant Anti-amnesic activity.

Keywords: *Mangifera indica L.*, Antiamnesic activity, Donepezil, Scopolamine,

Ethanolic extract.

Introduction:

Amnesia is usually self-limiting, transient, and situation-specific. However, a small minority of persons experience debilitating persistent fatigue. When amnesia cannot be explained by a medical condition like hypothyroidism or anemia, Alzheimer's disease (AD) may be the cause. It is one of the most common neurodegenerative disorders. Alzheimer's disease is the cause of more than 80% of dementia cases in older adults worldwide. Learning, mental, behavioral, and functional capacities gradually decline as a result of it. Scopolamine is an anti-muscarinic drug that acts by highly binding to postsynaptic receptor sites and enhancing acetyl cholinesterase (AChe) activity in the cortex and hippocampus.[1] By encouraging a high level of oxidative stress and pro-inflammatory cytokines in the hippocampal regions, scopolamine causes neuro-inflammation. Scopolamine is proven to increase levels of amyloid precursor protein (APP) and tubulin associated unit (Tau).[2]

Mangifera indica L. is a large climber that has branches that are delicately grey-downy and prickly, with strong yellow prickles that are hooked and straight. Typically growing like a vine, the stem can also produce flowers and fruits like a shrub. On the stems, there may be a few or many spines. Blaze is a huge, white, pith-white bean that has an odor similar to fresh green beans.[3] This plant has extensive medical applications and has been shown to contain anti-inflammatory, anti-helminthic, and anti-malarial medications. In the treatment of jaundice and other liver problems, it has also shown promise as a stomachic, digestive, and liver tonic.[4]

Hence, the present study aimed to evaluate the anti-amnesic activity of the ethanolic extract of *Mangifera indica* L .leaves against scopolamine induced memory impairment in rats.

MATERIALS AND METHODS:

Plant materials:

Leaves of *Mangifera indica* L. were collected from medicinal garden of SJM college of pharmacy, Chitradurga, Karnataka and they were washed, powdered, and dried under shade. The leaves was identified and authenticated by botanist, Chitradurga, Karnataka.

Extraction Procedure: [5]

The leaves were dried and powdered with the help of electric grinder and extracted with ethanol as a solvent in a Soxhlet extractor, further, the extract was filtered and concentrate on Rota evaporator to get extract that is Ethanolic extract of leaves of *Mangifera indica L*. (EEMI).

Preliminary phytochemical screening : [6, 7]

Preliminary phytochemical investigations were carried out on the ethanolic extract of *Mangifera indica L*.leaves for the detection of various phytochemicals by using standard methods prescribed in practical pharmacognosy by C K Kokate and R K Khandelwal.

Experimental animals:

Animal ethical clearance was obtained from Institution Animal Ethics Committee (IAEC) for the research purpose **(Ref no: Ref no: 06B/SJMCP/IAEC/Aug 2022/2021- 22).** Healthy Adult Wistar Albino rats weighing about 150-200g of either sex was used for the study. The animals were obtained from Biogen Laboratory Animal Facility, Bangalore – 562107. Before the initiation of the experiment, the animals were acclimatized for 10 days and randomized under standard environmental conditions such as temperature (26±2ºC), relative humidity (45-55%), and 12hrs light/dark cycle maintained as per Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) guidelines. All the animals were allowed free access to standard laboratory pellets and drinking water ad libitum under strict hygiene conditions.

Selection of screening dose: [8]

In accordance with OECD-423 recommendations, an acute oral toxicity test was conducted. In this investigation, healthy adult rats were employed that had been acclimated to the lab environment for one week prior to dosing. Each animal was given a unique cage number at random and had its fur marked with picric acid. The test ingredient was administered to the rats three hours after they had not eaten for the previous night. During this time, water was not denied. Rats were given the test drug, which was dissolved in demineralized water, by gavage for a period of 14 days. A syringe with an appropriate-sized intubation needle was used for this purpose. Based on the literature of acute toxicity tests (The Lorke technique) of ethanolic extract of *Mangifera indica* L. leaves, screening of antiamnesic activity dose was taken into consideration.

Screening of Anti-amnesic Activity

Experimental Design :[9]

The Wistar rats weighing 150-200g were randomly divided into 5 groups each containing 6 animals. (n=6).

1. Group A: Negative Control group, Standard diet and water *ad libitum.*
2. Group B: Positive control group, Scopolamine 1 mg/kg (i.p.) for 28 days.
3. Group C: Standard, Donepezil (2.5mg/kg .p.o) + (scopolamine 1 mg/kg i.p.) for 28 days.
4. Group D: Test group I, Low dose of *Mangifera indica* L**.** 250 mg/kg (p.o.) + (scopolamine 1 mg/kg i.p.) for 28 days.
5. Group E: Test group II, High dose of *Mangifera indica* L**.** 500 mg/kg (p.o.)+ (scopolamine 1 mg/kg. i.p.) for 28 days.

Doses of the extract were calculated according to the body weight of the animals. All drugs were freshly prepared before each experiment.

Behavioral parameters assessment:

Elevated plus maze[10]

The Elevated Plus Maze test, a widely utilized behavioral assay, serves not only to assess anxiolytic activity but also to evaluate potential anti-amnesic effects. Following the

methodology outlined by Handley and Mithani, the Elevated Plus Maze consists of four arms (two open and two closed) joined at a central platform, elevated 50cm above ground level. Rats all 5 groups were positioned at the junction of the four arms, facing an open arm, and their entries, time spent in each arm, and central area were recorded for 5 minutes. An increase in open-arm activity indicates antiamnesic behavior. To ensure accuracy, the apparatus was cleaned with alcohol between trials, and measures were taken to maintain a noise-free environment.

Rota rod model[11]

Effect of motor coordination was assessed using rota rod model. Rota rod consists of a base platform and a non-slippery surface rotating rod of 6cm diameter and divided into five equal sections. The animals were pre-selected in a training session based on ability to remain on rod (at 12 rpm) for 2 minutes. Animals were placed on rod at a speed of 12 rpm. Falling off time was automatically recorded. Time spent in the apparatus was observed for 5min duration (300s). The apparatus was cleaned thoroughly with alcohol in between trials.

Actophotometer model[12]

To assess rat locomotor activity, a Digital Actophotometer was utilized. This device comprises a 35×35 cm square field enclosed by walls equipped with photocells on all sides, rigorously tested before the experiment. Over a 5-minute period, the Actophotometer automatically tallied the total counts as each animal crossed the light beam. Subsequently, locomotor activity was assessed in all rat groups.

Open field[13]

The open field test (OFT) is used to assess how medications are affecting rodents' overall performance and locomotor behaviours, which may have an impact on their ability to learn and remember. The automated open field consists of a 40 cm× 40 cm× 35 cm measurements, which are divided into equal squares. After treatment, each animal will be placed individually at the centre of the open field, and locomotion will be recorded for 20 min. During this period, the number of rearing, assisted rearing, number of entries to central zone, and time spent in central zone will be recorded.

Catalase assay[14]

The rat brain was homogenized, centrifuged, and then phosphate buffer (2.5ml, pH 7.8) was added to the supernatant and incubated at 25°C for 30 minutes. Subsequently, the absorbance was measured at 240 nm using a spectrophotometer after transferring it to a cuvette. Hydrogen peroxide was introduced, and the change in absorbance was recorded. The results were expressed as µmol of H_2O_2/min/mg wet tissue. This procedure allowed for the quantitative determination of catalase activity in the brain homogenate, serving as an indicator of antioxidant status.

Lipid peroxidation assay[15]

75 mg of Thiobarbituric acid (TBA) was dissolved in 15% trichloroacetic acid (TCA), followed by the addition of 2.08 ml of 0.2 N Hcl. The total volume was adjusted to 100 ml using 15% TCA. Then, 3.0 ml of this reagent was mixed with 0.75 ml of brain homogenate. The resulting mixture was placed in a boiling water bath for 15 minutes, cooled, and then centrifuged for 10 minutes at 10,000 rpm. At 535 nm, the supernatant's absorbance was

measured in relation to a blank. The results were expressed in mol/mg of protein, providing a quantitative assessment of the tested parameter.

Acetyl cholinesterase activity[16]

The acetyl cholinesterase inhibitory activity of the plant extract was evaluated by the Ellman‘s reagent. Concisely, 0.1 M Na3PO4 buffer (pH 8.0,150 µL), 10 µL of test i.e. extract at the concentrations (10- 50 µg/mL) and enzyme solution (brain homogenate of mice) (0.1units/mL, 20 µL) were assorted and reared for 15 min at 25°C. 10 µL of DTNB (10 mM) will be added and response will be started by the adding the substrate (10 µL of Acetyl thiocholine iodide, 14 mM solution). Acetyl thiocholine iodide hydrolysis can be estimated through the creation of the coloured product 5-thio-2-nitrobenzoate anion formed by the reaction of DTNB (5, 5-dithio-bis-[2-nitro benzoic acid] and thiocholine, which is free via the cleavage of enzyme. The creation of the tinted produce will be determined at 410 nm wave length after 10 min. In the assay Donepezil at concentrations of 10 µM will be used as a standard with the same procedure as for the test extract. AChE % inhibition will be estimated using the formula:

Inhibition activity (%) = (1 − Absorbance of sample/ Absorbance of control) × 100

Protein estimation.[17, 18]

The total protein content in brain homogenates was evaluated using Lowry method. 100 µl of brain homogenate sample will be taken in a test tube. 1.0 ml of Lowry stock reagent will be added to each tube and will be incubated for 30 min at room temperature. 100 µl of Folin's reagent will be further added to each tube and incubated for 30 min at room temperature. At 595 nm, the absorbance will be measured. The protein content in brain tissue will be expressed as µg/mg of tissue.

Statistical Analysis:

The data obtained from the above findings was subjected to statistical analysis using one-way ANOVA followed by Tukey’s Kramer Multiple Comparison Test to assess the statistical significance of the result.

RESULTS:

The percentage yield of the ethanolic extract from *Mangifera indica* L leaves, obtained through continuous Soxhlet extraction using 95% ethanol as a solvent, was found to be 20.5%. The extract exhibited a Dark green and Thick Semi-solid.

Preliminary phytochemical screening

Preliminary phytochemical screening of EEMI leaves confirms the presence of carbohydrates, alkaloids, glycosides, flavonoids, tannins, triterpenes, and saponins, while steroids and resins were found to be absent.

Behavioral Parameters Results

Elevated plus maze model:

The results demonstrated that scopolamine induced amnesia in the positive control group, as evidenced by increased entries and time spent in closed arms, along with reduced time in open arms and the centre. In contrast, Donepezil (10 mg/kg) and 250 mg/kg of EEMI showed

a significant increase in entries and time spent in open arms and the centre, while reducing entries and time spent in closed arms compared to the positive control group. Moreover, 500 mg/kg of EEMI showed an extremely significant increase in entries and time spent in open arms and the centre, further emphasizing its effectiveness. The impact of EEMI on cognitive behaviour via the elevated plus maze has been summarized in Table No.01.

Table No.01: Effect of EEMI on cognitive behavior by Elevated plus maze

Treatment	Number of Entries			Time Spent (Sec)		
	Open Arm	Closed Arm	Center	Open Arm	Closed Arm	Center
Negative Control	6.83 ± 1.04	9.66 ± 1.28	3.16 ± 0.94	40 ± 10.16	200 ± 11.75	37.33 ± 9.06
Positive Control	2.83 ± 0.60	14.66 ± 1.40	3.16 ± 0.47	30.16 ± 8.03	249 ± 12.93	33 ± 10.0
Standard Group	8.5 ± 1.17 ***	4.3 ± 0.49 ***	6.8 ± 1.01 **	127 ± 16.0 ***	127 ± 15.9 ***	51.83 ± 10.31
Low Dose of EEMI (250mg/kg)	6.3 ± 0.98 *	4.1 ± 1.23 *	2.5 ± 0.79 **	131.3 ± 9.94 ***	113.6 ± 4.41 ***	70.3 ± 12.17
High Dose of EEMI (500mg/kg)	6.5 ± 1.41 ***	3.8 ± 0.88 ***	1.8 ± 1.07 ***	132.1 ± 5.57 ***	86.1 ± 4.41 ***	71.6 ± 5.54 ***

Values were expressed as Mean ± SEM (n=6); Significance values are: *P < 0.05, **P<0.01 and ***P < 0.001. Stress group vs all groups.

Rota rod model:

Muscle grip strength was assessed using a rota-rod apparatus, with mean fall-off time as the measure of muscular rigidity. The positive control group exhibited reduced fall-off time, indicating muscle incoordination. However, the Donepezil-treated group (10 mg/kg) and 500mg/kg of EEMI showed extremely significant and 250 mg/kg of EEMI showed significant increase in mean fall-off time compared to the positive control group, displaying dose-dependent effects. The impact of EEMI on muscle grip strength, as measured by the rota-rod apparatus, as detailed in Table no: 02.

Table N.02: Effect of EEMI on muscle grip strength by Rota rod apparatus

Sl. No.	Treatment	Fall of time (sec)
I	Negative Control group	141.5 ±7.214
II	Positive control group	76.66 ± 5.67
III	Standard group (Donepezil HCl) 10 mg/kg	131.1 ± 8.04***
IV	Low dose of EEMI (250 mg/kg)	100.6 ± 4.160 **
V	High dose of EEMI (500 mg/kg)	154.5 ± 7***

Values were expressed as Mean ± SEM (n=6); Significance values are: *P < 0.05, **P < 0.01 and ***P < 0.001. Stress group vs all groups.

Actophotometer model:

All animals underwent locomotor activity assessment using an actophotometer, which records ambulatory scores based on beam-crossing. Donepezil-treated (10 mg/kg) and EEMI-treated 250 mg/kg and 500 mg/kg showed a significant and extremely significant increased locomotor activity compared to the positive control group. The activity in all drug-treated and test groups showed a progressive increase over the entire 5-minute period, with dose-dependent effects observed. The impact of EEMI on locomotor activity, as measured by the actophotometer, as summarized in Table no. 03.

Table No. 03: Effect of EEMI on Loco-Motor activity by Actophotometer

Sl. No	Treatment	Ambulatory Score
I	Negative Control group	173.6±3.282
II	Positive control group	77.833±5.510
III	Standard group (Donepezil HCl)10 mg/kg	155±15.220***
IV	Low dose of EEMI (250 mg/kg)	129.5±5.761 **
V	High dose of EEMI(500 mg/kg)	156±15133**

Values were expressed as Mean ± SEM (n=6); Significance values are: *P < 0.05, **P < 0.01 and ***P < 0.001. Stress group vs all groups.

Open field model:

In open field apparatus, locomotion (rearing, assisted rearing) and exploratory (number of squares traversed i.e., central, and peripheral). Locomotion and exploratory activities of Positive control group were compared with standard group (Donepezil 2.5 mg/kg), low dose

of EEMI (250 mg/kg) and high dose of EEMI (500 mg/kg). Donepezil treated group shows highly significance value (***P<0.001) increase in locomotion and exploratory activity compared to remaining four group of animals. In a low dose of EEMI (250 mg/kg) it shows less significance value (*P<0.05) when compare with control group. In a high dose of EEMI (500 mg/kg) it shows moderately increase in significance value (**P<0.01) when compare with control group. Dose dependent activity was showed by animals in open field model. The results are summarized in table no.04

Table No 04: Effect of EEMI leaves on Open field model

Sl.No.	Treatment	Rearings	Assisted rearings	Number of square traversed in 5 mins		
				Central	Peripheral	Total
1	Negative Control	5.166 ± 0.980	7.166 ± 0.600	2.5 ± 0.428	81 ± 5.88	83.5 ± 5.71
2	Positive control	4 ± 0.966	3.5 ± 0.428	1.5 ± 0.428	58 ± 7.66	59.5 ± 8.048
3	Standard group (Donepezil) 2.5mg/kg	8.666 ± 1.855	9.5 ± 1.727	4.833 ± 0.73***	95.33 ± 6.275***	100 ± 6.493***
4	Low dose of EEMI (250 mg/kg)	5.6 ± 1.22*	11.8 ± 0.881	3.3 ± 0.6**	85.8 ± 8.095**	89 ± 8.139**
5	High dose of EEMI (500mg/kg)	6 ± 1.021	5 ± 0.881**	4.5 ± 0.428**	79 ± 4.524**	85.66 ± 2.616**

Values were expressed as Mean ± SEM (n=6); Significance values are: *P < 0.05, **P < 0.01 and ***P < 0.001. Stress group vs all groups

Biochemical Markers:

Catalase assay

The breakdown of hydrogen peroxide indicates the level of catalase present in brain homogenate, assessed using the endogenous antioxidant catalase assay. The data revealed an extremely significant elevation in CAT levels in the standard Donepezil Hcl group, as well as the high dose of EEMI (500 mg/kg) and the low dose of EEMI (250 mg/kg) showed significant increase in CAT, compared to the positive control group. The effect of EEMI on the catalase assay is depicted in Table no.05.

Table No. 05: Effect of EEMI on Catalase assay

Sl.No.	Treatment	Catalase (µmol/min/mg of protein)
I	Negative Control group	26.184 ± 0.508
II	Positive control group	17.148 ± 0.335
III	Standard group (Donepezil HCl) 10 mg/kg	37.368 ± 0.297***
IV	Low dose of EEMI (250 mg/kg)	32.77 ± 2.624**
V	High dose of EEMI (500 mg/kg)	35.40 ± 0.962***

Values were expressed as Mean ± SEM (n=6); Significance values are: *P < 0.05,**P < 0.01 and ***P < 0.001. Stress group vs all groups

Lipid peroxidation assay

Malondialdehyde (MDA) serves as a reliable peroxidation indicator. Increased free radical activity leads to MDA overproduction, assessed by reacting brain homogenate samples with Thiobarbituric acid (TBA) in the lipid peroxidation assay. The data demonstrated that standard Donepezil Hcl and EEMI at doses of 250mg/kg and 500mg/kg significantly reduced MDA levels compared to the positive control group. The impact of EEMI on the LPO assay is presented in Table no. 06.

Table No. 06: Effect of EEMI on LPO assay

Sl.No	Treatment	MDA (nmol /mg of protein)
I	Negative control	2.379±0.031
II	Positive control	3.503±0.014
III	Standard Donepezil(2.5mg/Kg)	2.187±0.040***
IV	Low dose of EEMI (250mg/Kg)	2.085±0.0147***
V	High dose of EEMI (500mg/Kg)	2.201±0.01336***

Values were expressed as Mean ± SEM (n=6); Significance values are: *P < 0.05,**P < 0.01 and ***P < 0.001. Stress group vs all groups

Acetyl Cholinesterase assay (AChE):

AChE activity was estimated in rat brain using Ellman‘s assay. Scopolamine treated group exhibited a substantial rise in brain AChE activity when compared to the other groups.

Administration of EEMI (250 and 500 mg/kg, bd.wt, p.o.) and standard drug Donepezil (2.5 mg/kg, bd.wt. p.o) significantly decreased the AChE levels when compared to Positive control. The effect of EEMI on Acetyl Cholinesterase assay is presented in Table No.07

Table No. 07: Effect of EEMI on Acetyl Cholinesterase assay

Sl.No	Treatment	AChE µmol/min/mg protein
I	Negative control	0.361±0.039
II	Positive control	0.927±0.034
III	Standard Donepezil(2.5mg/Kg)	0.571±0.024***
IV	Low dose of EEMI (250mg/Kg)	0.6898±0.06699**
V	High dose of EEMI (500mg/Kg)	0.5510±0.03358***

Values were expressed as Mean ± SEM (n=6); Significance values are: *P < 0.05, **P< 0.01 and ***P < 0.001. Positive group vs all groups.

Protein estimation

The result indicated that, the Scopolamine treated animals significantly decreases the level of protein, whereas the Donepezil-treated group (2.5 mg/kg) and EEMI (250 mg & 500mg/kg) showed a significantly increases the level of Proteins in the brain, the effect of EEMI on protein estimation is showed in Table no. 08.

Table No. 08: Effect of EEMI on protein estimation.

Sl.No	Treatment	µg/mg of tissue
I	Negative control	4.443±0.301
II	Positive control	1.374±0.129
III	Standard Donepezil(2.5mg/Kg)	4.388±0.176***
IV	Low dose of EEMI (250mg/Kg)	2.754±0.09001***
V	High dose of EEMI (500mg/Kg)	3.728±0.1849***

Values were expressed as Mean ± SEM (n=6); Significance values are: ***P < 0.001, **P < 0.01 and *P < 0.05 and nsP > 0.05. Control group vs all groups. (By one-way ANOVA followed by Tukey-Kramer Multiple comparison tests)

Discussion:

Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defence system, can damage neuronal structures and disrupt brain function. Neuro-inflammation, involving the activation of immune-like cells in the brain (microglia) and the production of proinflammatory cytokines, can also contribute to neuronal damage and alterations in neurotransmitter systems. Impairments in neuroplasticity, the ability of the brain to adapt and reorganize in response to experiences, can further exacerbate these effects and lead to the manifestation of depressive symptoms.[19]

Alzheimer's disease is a multifaceted and intricate neurodegenerative condition that poses significant challenges. It manifests with a pronounced decline in cognitive function and various motor dysfunctions across its spectrum. This study's exploration of Alzheimer's pathophysiology highlighted two distinct categories: familial and sporadic cases. Familial cases, often associated with genetic anomalies in presenilin and amyloid precursor protein genes, lead to early-onset dementia, typically around the age of 65. In contrast, sporadic Alzheimer's disease predominantly affects individuals over the age of 65, with a prevalence exceeding 95%. Despite its high incidence, the precise etiology of sporadic Alzheimer's remains elusive, pointing to a complex interplay of genetic and environmental factors.[20]

The present research study has been designed to screen the Anti-amnesic activity of ethanoic extract of *Mangifera indica* L Leaves against Scopolamine induced memory impairments in Rats. The results of the phytochemical analysis of *Mangifera indica* L Leaves showed that the ethanolic extract contained alkaloids, glycosides, flavonoids, tannins, steroids, saponins, triterpenes, resins, amino acids, and carbohydrate. Behavioral tests revealed that rats treated with EEMI exhibited significant improvements increase the number of entries into the open arm and spent more time exploring the open arms (measured by Elevated plus maze), improved motor coordination (measured by Rotarod apparatus), increased locomotor activity (measured by Actophotometer), increase the number of rearing, assisted rearing, and number of squares crossed (measured by open field). The EEMI also significantly increased catalase activity, reduced the lipid peroxidation level, reduced the AChE level, increased Protein level in brain.

Conclusion:

The study found that the EEMI leaves showed significant potential against scopolamine induced amnesia in rats. Using various behavioral and oxidative stress assessment models, the leaf extract demonstrated effectiveness in reducing anxiety, enhancing muscle grip strength, and increasing locomotor activity and increased catalase activity and reduced lipid peroxidation, reduced the AChE level, increased Protein level in brain. This suggests the extract has notable antiamnesic properties, likely due to compounds like flavonoids, tannins, steroids, glycosides, and triterpenoids. However, further research is needed to isolate active constituents and understand the exact mechanisms involved, enhancing its potential as a Amnesic treatment.

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