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Evaluation of Nootropic activity of *Bacopa monnieri* and *Emblica officinalis* combination

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Abstract

Bacopamonnieri, a plant belonging to family Scrophulariaceae, has been used in the traditional system of Ayurvedic medicine to improve intelligence & memory for long time. *Emblica officinalis*, commonly known as amla, is an important medicinal plant of India. Thefruits have potent antioxidant activity due to presence of tannins, vitamin C & flavonoids. The present investigation was undertaken to assess the pharmacological effects of *Bacopa monnieri* and *Emblica officinalis* as nootropic agent in mice. Morris Water Maze task, Elevated plus maze task and estimation of Acetylcholinesterase was done to evaluate the effects. Combination of Ethanolic Extract of *Bacopa monnieri and* Hydroalcoholic Extract of *Emblica officinalis* showed significant decrease in Transfer Latency (TL). Combination of Ethanolic Extract of *Bacopa monnieri and Hydroalcoholic Extract of Emblica officinalis* significantly decreases the level of acetylcholinesterase activity. Combination of Ethanolic Extract of *Bacopa monnieri and* Hydroalcoholic Extract *of Emblica officinalis* in dose 100mg/kg+100mg/kg significantly opposed the memory loss caused by scopolamine (0.4mg/kg, i.p.) and sodium nitrite (75mg/kg, i.p.). Piracetam (200mg/kg,i.p.) was utilized as standard Nootropic drug. Hence, Combination of Ethanolic Extract of *Bacopa monnieri and Hydoalcoholic extract of Emblica officinalis* show nootropic activity and it would be beneficial for the treatment of Alzheimer's Disease.

Key words : Ethanolic Extract of *Bacopa monnieri* (EEBM), Hydroalcholic Extract *Emblica officinalis* (HAEEO), Morris Water Maze(MWM), Elevated Plus Maze(EPM), Acetylcholinesterase (AchE) and Alzheimer's Disease (AD).

Neurodegenerative diseases :

Neurones are functional unit of nervous system which is also known as nerve cells. Nerve cells cannot divide like other cells. Loss of neuronal function may lead to

neurodegenerative diseases. The term "Neurodegenerative" split into two words – "Neuro" means brain "degeneration" means dysfunction or damage of neurons. Neuronal dysfunction may occur due to several factors such as increased oxidative stress, defective protein aggregation and degradation, genetic factors, increased heavy metals (Cu, Zn, Pb) and pesticides, and mitochondrial dysfunction.

Memory is one of the most essential roles of the brain. Because memory is a process through which organisms are able to store their observations and use this information to change their reactions to the environment, memory is essential for survival. The main characteristics of Alzheimer's disease (AD) include diminished brain functioning. Acetylcholine is present in sufficient amounts in the neo cortex toimprove memory loss and learning deficiencies. Memory loss has been linked to altered cholinergic activation in the brain, increased oxidative stress, hypercholesterolemia, and neuro-inflammatory reactions. For both humans and animals, the central cholinergic system is involved in cognitive processes and is crucial for learning and memory[1].

Alzheimer's disease (AD) :

The most frequent cause of dementia is Alzheimer's disease (AD), a progressive neurological condition that primarily affects elderly people[2].

Dementia refers to a generalised cognitive disability that has been developed, without conscious or physical dysfunction that affects cognition, memory, comprehension, and the ability to recognise people[2].

Collection and Authentication :

A. Bacopa monnieiri (Brahmi): The standardized ethanolic extract of *Bacopa-monnieri* was procured from Herbo Nutra extract Pvt. Ltd., Kasna Surajpur Industrial Area, Gautam Budh Nagar, UP-201308, India. *B. Emblica officinalis* (Amla): The standardized hydroalcoholic extract of *Emblica officinalis* was procured from HerboNutra extract Pvt.Ltd., KasnaSurajpur Industrial Area, Gautam Budh Nagar, UP-201308, India.

Route of administration :

The Eebm And Haeeo were administered by oral route (p.o.) by using oral feeding needle in a volume of 5 ml/kg and standard Piracetam was given by intraperitoneal route (i.p.); inducers Scopolamine and sodium nitrite were given by intraperitoneal route (i.p.) in volume of 1 ml/kg.

Standard phytochemical tests for evaluation:

Ethanolic extract of *Bacopa monnieri* :

The various phytochemical tests were carried out to detect the presence of the chemical constituents such as alkaloid, glycoside, tannins, proteins, saponins and amino acids[8].

Test Dose selection :

Bacopa monnieri [9]

The selection of test doses for current study was based on the results of toxicity-studies of *Bacopa monnieri* extract. The extract was tolerated by mice to a level of

1000mg/kg, hence we calculated and selected dose as per quantity of *Bacopa monnier* is hows 140mg as higher test dose, 100 mg/kg as middle dose and 60mg/kg a low-test dose.

Emblica officinalis [10]

The selection of test doses for current study was based on the results of toxicitystudies of *Emblica officinalis* extract. No mortality and no signs of any toxicity were evidence after the administration of limit dose of 1000 mg/kg in acute oral toxicity test. Thus, the selective dose for *Emblica officinalis* is reported as 140 mg as higher test dose. 100 mg/kg as middle dose and 60mg/kg show as low test dose.

In vivo methods for memory enhancing activity :

Procedure :

Animals were divided into eleven groups, each group comprised of six animals. Plus maze and Morris Water maze task was used in mice and to study their behavioural parameters such as learning, memory escapes latency and will be determined by Acetylcholine esterase. Scopolamine is considered as main agent which causes memory impairment in animals by antagonizing the effect of acetylcholine at muscarinic receptors.

Morris *water maze task :*

MWM testing was conducted in a sphere-shaped deep pool. The pool was filled to a depth of 30 cm with non-toxic white water-based chalk paint, which was utilized to make the water opaque. In order to maintain the pool at 25 ± 5°C, warm water was supplied. A 25 cm^2 glass square serving as the escape platform was placed in the centre of a pool's quadrant, 15 cm away from the edge, and 1 cm below the water's surface[41].

Parameters :

Measurement of Escape Latency:

The amount of time it takes an animal

Table-1. Morris water maze task

Group	Name of Group	Dose	Route
1	Normal control	1ml/kg	p.o
2	Positive group treated with piracetam	200mg/kg	i.p
3	Negative control treated with scopolamine	0.4mg/kg	i.p, i.p
4	EEBM	200mg/kg	p.o,
5	HAEEO	300mg/kg	i.p
6	Piracetam+scopolamine	200mg/kg+0.4mg/kg	i.p
7	EEBM+scopolamine	150mg/kg+0.4mg/kg	i.p
8	HAEEO+scopolamine	150mg/kg/p.o+0.4mg/kg	p.o., i.p
9	EEBM+HAEEO	140mg/kg+70mg/kg	p.o
10	EEBM+HAEEO	70mg/kg+140mg/kg	p.o
11	EEBM+HAEEO	100mg/kg+100mg/kg	p.o

to walk from the starting quadrant to the target quadrant where the hidden platform is located is known as the "escape latency" (EL). EL was noted for each animal from the sixth to the ninth day. Each animal performed training trials for four days in a row, with the target quadrant remaining constant during the training period while the beginning position was altered for each exposure[11].

Elevated plus Maze Task :

An elevated plus maze with two open and two closed arms was used to assess learning and memory in mice. Animals were divided into six groups and treated with plant extracts, a vehicle, or Piracetam. One hour post-treatment, each mouse was placed in the center of the maze. Over five minutes, the number of entries and time spent in open and closed arms were recorded. Transfer latency (TL), the time taken to move from an open to a closed arm, was used as a measure of memory performance[12-14].

Table-2. Elevated plus Maze Task

Group	Name of Group	Dose	Route
1	Normal control	1ml/kg	p.o
2	Positive group treated with piracetam	200 mg/kg	i.p
3	Negative control treated with sodium nitrite	75 mg/kg	i.p
4	EEBM	200 mg/kg	p.o,
5	HAEEO	300 mg/kg	i.p
6	Piracetam+sodium nitrite	200mg/kg+75mg/kg,	i.p
7	EEBM+sodium nitrite	150mg/kg+75mg/kg	i.p
8	HAEEO+sodium nitrite	150mg/kg 75mg/kg	p.o., i.p
9	EEBM+HAEEO	140mg/kg+70mg/kg	p.o
10	EEBM+HAEEO	70mg+140mg/kg	p.o
11	EEBM+HAEEO	100mg/kg+100mg/kg	p.o

Parameters :

Measurement of transfer latency :

TL was discovered on either the first day or the tenth day after the animal got thedrug. The TL was set at 90 seconds and the animal was gently moved into one of the two covered arms if it had not entered after that time. The mouse was given permission to depart and was transported back to its own cage after spending another two minutes crossing the maze. After the initial trial, this memorized task was tested 24 hours later (on the eleventh day)[15].

Ex vivo methods for memory enhancing activity :

Tissue preparation :

Tissues were washed with ice-cold PBS (pH 7.0–7.2), weighed (300–500 mg), and minced for homogenization in 500 µL PBS on ice. Cell membranes were disrupted by freeze-thaw cycles or ultrasonication. The

homogenate was centrifuged at 3000 rpm for 15 minutes, and the supernatant was either analyzed immediately or stored at –20°C or –80°C.

Acetyl cholinesterase estimation by ELISA method :
Acetylcholineesterase kit (ELAB SCIENCE)
Principle of assay :

Brain homogenate (100 µL) was added to each well and incubated at 37°C for 90 minutes. After washing, 100 µL biotinylated AChE antibody was added and incubated for 60 minutes. Then, 100 µL streptavidin-HRP was added and incubated for 30 minutes. After a final wash, 90 µL TMB substrate was added and incubated for 10 minutes. A blue color appeared, and 50 µL stop solution was added to turn it yellow. Absorbance was measured at 450 nm within 15 minutes.

Statistical analysis :

The data were presented as mean ± SD (n=6). One-way analysis of variance (ANOVA) was used to determine statistical significance, followed by Dunnett's test, $p<0.05$, $p<0.01$ and $p<0.001$ were declared statistically significant.

Physical properties :

1. Ethanolic Extract of *Bacopa monieri*

Colour	: Brown
Odour	: Characteristics
Taste	: Natural
Appearance	: Powder

2. Hydroalcoholic Extract of *Emblica officinalis*

Colour	: Brown
Odour	: Characteristics:
Taste	: Natural
Appearance	: Powder

Preliminary phytochemical evaluation :

Table-3. Phytochemical evaluation of ethanolic extract of *Bacopa monnieri and Emblica officinalis*

Sr.no.	Phytochemicatests	Inference
1.	**Alkaloids**	
	Dragondroff'stest	+
	Hager'stest	+
	Mayer'stest	+
	Wagner'stest	+
2.	**Flavonoids**	
	Leadacetatetest	+
	Shinodatest	+
3.	**Carbohydrates**	
	Molisch'test	-
	Fehling'stest	-
	Barfoed's test	-
	Benedict'stest	-
4.	**Saponins**	
	Frothtest	+
5.	**Tannins**	
	Ferric chloride test	+
	Lead acetate test	+
6.	**Proteins & Amino acids**	
	Biuret test	-
	Millon'stest	-
	Ninhydrin test	-

In vivo methods for memory enhancing activity:

- Effect of EEBM & HAEEO on scopolamine induced amnesia in mice using Morris water maze. (Escape Latency):

Table-4. Measurement of escape latency (in secs)

Group	Name of Group	LEARNING (in secs)	RETENSION (in secs)
1	Normal control	34.67± 1.033*	31.83± 3.312*
2	Positive group treated with piracetam	23.83± 1.722***	16.83±1.722***
3	Negative control treated with scopolamine	51.33± 2.160#	59.67± 1.211#
4	EEBM	28.83± 2.927**	23.67±2.251**
5	HAEEO	31.67± 0.633**	26.33±2.658**
6	Piracetam+scopolamine	24.83± 0.753***	17.33± 1.033***
7	EEBM+scopolamine	33.33± 1.211**	28.33±0.816**
8	HAEEO+scopolamine	24.50± 1.378***	15.67±0.816***
9	EEBM+HAEEO	37.17± 1.472**	26.33±1.751**
10	EEBM+HAEEO	37.00± 1.265**	27.33±1.633**
11	EEBM+HAEEO	36.17± 1.722**	27.50±0.837**

- ✓ Group 1 (Normal Control): Shows moderate escape latency, indicating normal learning and memory ability.
- ✓ Group 2 (Piracetam-treated): Significantly reduced escape latency (***$p<0.001$), showing strong memory enhancement.
- ✓ Group 3 (Scopolamine-treated): Shows highest escape latency, indicating severe memory impairment.
- ✓ Group 4 (EEBM-treated): Significant improvement in learning and memory (**$p<0.01$) compared to negative control.
- ✓ Group 5 (HAEEO-treated): Also improves memory (**$p<0.01$), similar to EEBM.
- ✓ Group 6 (Piracetam + Scopolamine): Escape latency significantly reduced (***$p<0.001$), showing protection against scopolamine-induced memory loss.
- ✓ Group 7 (EEBM + Scopolamine): Improved performance (**$p<0.01$), indicating EEBM's protective effect.
- ✓ Group 8 (HAEEO + Scopolamine): Shows excellent memory improvement (***$p<0.001$), better than EEBM + scopolamine.
- ✓ Groups 9–11 (EEBM + HAEEO in various doses): All show significant memory enhancement (**$p<0.01$), indicating the potential synergistic effect of combined extracts.

Effect of Eebm & Haeeo &Piracetam on scopolamine induced amnesia in mice using Elevated plus maze(Transfer latency):

Table-5. Measurement of transfer latency

Group	Nameof Group	Mean Transfer Latency (Seconds) Before	MeanTransfer Latency (Seconds) After
1	Normal control	39.167± 0.983*	34.667 ±2.658*
2	Positive group treated with piracetam	19.500 ± 1.049***	12.000±1.265***
3	Negative control treated with sodium nitrite	44.000 ±1.549	37.833±1.602
4	EEBM	18.333 ± 1.033***	18.333 ±1.033***
5	HAEEO	19.333 ±0.816 ***	17.167 ±1.169***
6	Piracetam+sodium nitrite	25.500 ± 3.619**	22.500 ± 1.049**
7	EEBM+sodium nitrite	25.500 ± 2.429**	22.833 ± 1.472**
8	HAEEO+sodium nitrite	26.167 ± 1.941*	30.333 ± 1.033*
9	EEBM+HAEEO	26.500 ± 1.871**	21.833 ± 0.753**
10	EEBM+HAEEO	26.167 ± 2.483**	22.667 ± 1.366**
11	EEBM+HAEEO	25.333 ± 1.966**	15.333 ± 1.033**

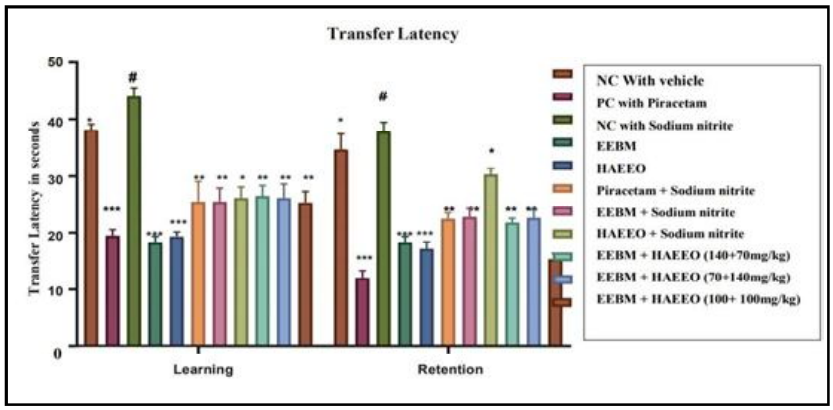

Figure 1. Measurement of transfer latency in secs

- ✓ Group 1 (Normal Control): Shows moderate TL, indicating normal learning and memory.
- ✓ Group 2 (Piracetam-treated): Shows very low TL both before and after (***p<0.001), indicating strong memory enhancement.
- ✓ Group 3 (Sodium Nitrite – Negative Control): Has the highest TL, suggesting memory impairment.
- ✓ Group 4 (EEBM): Shows significantly improved TL (***p<0.001), similar to piracetam, confirming strong memory-boosting effect.
- ✓ Group 5 (HAEEO): Also shows excellent improvement in memory (**p<0.001).
- ✓ Groups 6 & 7 (Piracetam/EEBM + Sodium Nitrite): Both reduce TL significantly (**p<0.01), indicating protective effects against memory loss.
- ✓ Group 8 (HAEEO + Sodium Nitrite):

Shows mild improvement (*p<0.05), slightly less effective than others.

- ✓ Groups 9–11 (EEBM + HAEEO combinations): All show significant TL reduction (**p<0.01), with Group 11 having the best post-treatment retention (15.33 sec).

2) Ex vivo methods for memory enhancing activity:

Effect of EEBM & HAEEO and piracetam on scopolamine induced amnesia in mice using estimation of Acetylcholinesterase activity of brain tissue.

Morris Water Mazetask :

Table-6. Estimation of AchEby Morris water maze task

Group No.	Name of Groups	Final Conc. (µg/ml)
1	Normal control	4.149±0.10*
2	Positive groupt reated with piracetam	2.753±0.38****
3	Negative control treated with scopolamine	9.160±0.14#
4	EEBM	3.707±0.311****
5	HAEEO	3.408±0.29****
6	Piracetam+scopolamine	3.422±0.29****
7	EEBM+scopolamine	5.011±0.27****
8	HAEEO+scopolamine	4.341±0.4247****
9	EEBM+HAEEO	3.255±2.79****
10	EEBM+HAEEO	3.187±0.24****
11	EEBM+HAEEO	2.764±0.42****

Values are mean ± SD, n = 6 in each group. As compare to Negative control with Scopolamine 9.160±0.14. All groups except scopolamine induced group showed significant decrease in AchE concentration. (p<0.0001) results were found to be significant.

Elevated plus Maze task :

Table-7. Estimation of AchE by Elevated plus maze task

Group No.	Name of Groups	Final Conc. (µg/ml)
1	Normal control	4.003±0.3248*
2	Positive group treated with piracetam	2.803±0.3670****
3	Negative control treated with sodiumnitrite	8.472±0.4930#
4	EEBM	3.650±0.2460
5	HAEEO	3.262±0.2932**
6	Piracetam+sodium nitrite	3.130±0.30201***
7	EEBM+sodium nitrite	4.754±0.3613
8	HAEEO+sodium nitrite	4.327±0.3484
9	EEBM+HAEEO	3.155±0.3439***
10	EEBM+HAEEO	3.056±0.1762****
11	EEBM+HAEEO	2.739±0.1477****

- ✓ Group 1 (Normal Control): AchE reduced to 4.003 µg/ml (*p<0.05), indicating normal cognitive activity.
- ✓ Group 2 (Piracetam-treated): AchE dropped significantly to 2.803 µg/ml (****p<0.0001), showing strong cognitive enhancement.
- ✓ Group 3 (Negative Control): Highest AchE level (8.472 µg/ml), confirming severe memory impairment.
- ✓ Group 4 – EEBM: AchE = 3.650 µg/ml, Moderate reduction, improved memory, though not statistically significant in this data set.
- ✓ Group 5 (HAEEO): Moderately reduced AchE (3.262 µg/ml) (**p<0.01), indicating memory-improving potential.
- ✓ Group 6 (Piracetam + Sodium Nitrite): AchE lowered to 3.130 µg/ml (***p<0.001), showing protection against memory loss.
- ✓ Group 7 & 8 (EEBM/HAEEO + Sodium Nitrite): Slightly elevated AchE (around 4.3–4.7 µg/ml), still improved vs. negative control, but less effective alone.
- ✓ Groups 9–11 (EEBM+HAEEO combinations): AchE significantly reduced (~3.1 to 2.7 µg/ml), with Group 11 showing the lowest level (2.739 µg/ml) (****p<0.0001), indicating best memory restoration effect.

Alzheimer's disease (AD) is the leading cause of dementia in older adults and is characterized by progressive memory loss and cognitive impairment. Current nootropic medications used to manage AD symptoms often come with adverse effects. Therefore, natural alternatives such as Bacopa monnieri

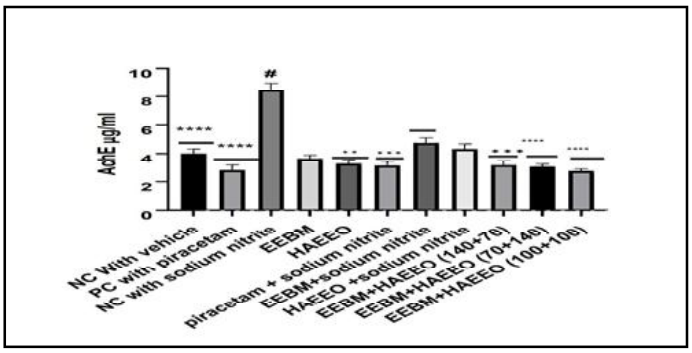

Figure 2. Estimation of AchE by Morris water maze task

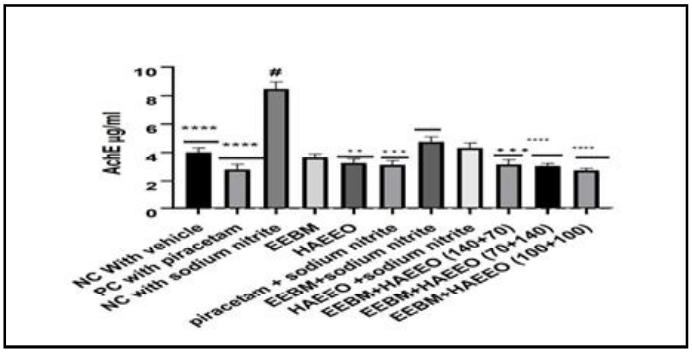

Figure 3. Estimation of AchE by Elevated plus maze task

and Emblica officinalis are gaining attention for their neuroprotective and cognitive-enhancing properties. These herbs are rich in phytochemicals like bacosides and emblicanins, which contribute to their antioxidant and acetylcholinesterase (AChE) inhibitory activities. In the present study, the ethanolic extract of Bacopa monnieri (EEBM, 200 mg/kg) and hydroalcoholic extract of *Emblica officinalis* (HAEEO, 300 mg/kg) were administered to mice for 7 days. Behavioral assessments using the elevated plus maze and Morris water maze showed that both extracts significantly improved learning and memory. Combination treatment (100 mg/kg each) further enhanced performance and reversed memory deficits induced by sodium nitrite and scopolamine. Biochemical analysis revealed a significant reduction in brain AChE levels, suggesting enhanced cholinergic transmission. These findings confirm the cognitive-enhancing potential of both extracts, especially in combination, and support their use as a natural therapeutic strategy against AD. Their mechanisms include antioxidant effects, modulation of neurotransmitters, and inhibition of neurodegeneration.

From the results it can be concluded that combination of ethanolic extract of *Bacopa monnieri* and *Hydroalcoholic* extract of *Emblica officinalis* at a dose 100mg/kg+100mg/kg,p.o. possess nootropic activity which is comparable to piracetam. Both the extracts reversed the action of scopolamine and sodium nitrite. Bacoside and antioxidants may be responsible for the nootropic activity.

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