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LEARNING AND MEMORY ACTIVITY OF *BRAHMI VATI* BY USING MORRIS WATER MAZE MODEL – AN IN-VIVO STUDY

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ABSTRACT

Introduction: Learning and memory are vital cognitive functions that depend on *Dhi*, *Dhriti* and *Smriti* as described in *Ayurveda*. Modern lifestyle stress and psychological disturbances adversely affect these functions, leading to cognitive impairment. *Medhya Rasayana* drugs are traditionally advocated for enhancing intellect and memory. *Brahmi Vati*, a classical Ayurvedic formulation is commonly prescribed for cognitive enhancement, yet experimental evidence supporting its efficacy is limited. The present study was designed to evaluate the learning and memory enhancing activity of *Brahmi Vati* in experimental study. *Brahmi Vati* is one among the *Medhya dravya* mentioned by *Ayurveda Sara Sangraha* in *Gutika Vati Prkarana.* **Materials and Methods:** *Brahmi Vati*, Piracetam, Carboxy Methyl Cellulose, drugs as Test drug, Standard drug, Control drug, And for Memory impairment Scopolamine Hydrobromide and 18 male Albino rats form the materials for the study. In Three groups, as Standard group- Piracetam (17.28mg / 200g body wt + 1mL CMC), in Test group- *Brahmi Vati* (13.5mg/ 200g body wt + 1mL CMC) and in Control group- Carboxy Methyl Cellulose (1mL/ 200g body wt) was administered for 6 days period. Analysis was done by using Morri's water maze model and results of ITL (Initial Transverse latency), RTL (Retention Transverse latency) were used for Statistical analysis. **Results:** The result indicates the *Brahmi Vati* might play a significant role to enhance spatial learning and memorizing behaviour of rat which has shown improvement trend towards memory and learning. **Discussion:** The study indicates that use of *Brahmi Vati* had significant encouraging effect for cognitive enhancing by the Enhancement of Spatial learning and memory in scopolamine treated rat, possibly because of the combined action of ingredients of *Medhya Rasayana* which were well known for antioxidant and neuro-protective activity.

KEYWORDS: Learning & Memory, *Brahmi Vati*, Piracetam, Scopolamine hydrobromide, Morris Water maze model.

INTRODUCTION

Learning is the acquisition of new information, behaviours or abilities after practice, observation or other experiences, as evidenced by change in behaviour, knowledge or brain function. Learning involves attending to relevant aspects of incoming information, organizing the information into a coherent cognitive representation and integrating it with relevant existing

knowledge from long-term memory. Memory is the ability to retain information or a representation of past experience, based on the mental processes of learning or encoding, retention across some interval of time and retrieval or reactivation of the memory, includes specific information or a specific past experience that is recalled.[1] Due to any cause the Satvika state of mind is disturbed or lowered, then administration of *Medhya* drugs and formulations will have great work on it. The mind-body connection is very important in *Ayurveda. Ayurveda* gives prime importance to positive mental and physical health. The Physical imbalances can disturb the mental state while mental illness leads to disruption of body functions.[2]

In modern era due to stress, disturbed emotion, unhealthy lifestyle there is increased incidence of psychosomatic disease where *Dhi, Dhriti* and *Smriti* of person are affected. *Medhya Rasayanas*, a category of herbal tonics, significantly enhance memory and cognitive functions. Their use in Ayurvedic treatments supports mental clarity and retention abilities. *Medhya* pertains to qualities that promote intelligence and mental acuity.

In *Ayurveda*, substances classified as *Medhya* improve cognitive functions, including memory and comprehension. Incorporating *Medhya* elements into diets or therapies supports brain health and enhances an individual's capacity for learning and information retention.[3] Considering all the factors which are needed to improve and effective in the memory *Brahmi Vati*.[4] is more useful in the memory retention and with the act of learning which is very much useful in the healthy individual to promote and boosting up the memory by ancient Acharya's.

MATERIALS AND METHODS

Materials

A. Materials for Pharmaceutical Study

Brahmi Vati have *Brahmi, Shankapushpi, Vacha, Krishnamaricha* and *Gojihva Churna*[5], *Jatamamsi Kwatha*[6], *Swarnamakshika Bhasma* and *Rasasindura.*[7]

B. Materials for Experimental Study

Materials

a. Albino rats
b. *Brahmi Vati* (Test drug)
c. Piracetam (standard drug)
d. Carboxy methyl Cellulose (control/vehicle)
e. Scopalamine Hydrobromide (for memory impairment)

Equipment's and Glass wares

1. Morri's water maze model with camera and PC connected (for swim training and trial to albino rats)
2. Milk powder (for making water opacity)
3. Towel (to dry and clean the rats after training and trial session)
4. Glass beakers
5. 18 G Needles
6. Disposable syringes
7. Stop watch
8. Hand gloves
9. Glass rod

IAEC approval was taken to conduct study on animals before to start the study with letter reference no. BLDEA/AVS/AMV/143/A/2024-25 Dated 29/05/2024 from BLDEA's AVS Ayurveda Mahavidyalaya, Hospital and Research Centre, Vijayapura. CCSEA approved with registration number 533/CCSEA.

Selection of animals

- **Inclusive Criteria:** Healthy male Albino rats, 90 to 120 days old, Weight ranges in between 150 to 200gm.
- **Exclusive Criteria:** Animals which are not according to inclusive criteria will be excluded.

1) **Drugs for Experimental Studies:** *Brahmi Vati*, Piracetam, Carboxy Methyl Cellulose, Scopolamine Hydrobromide
2) **Animals for Experimental Study:** 18 Male Albino rats were taken for the study, 6 in each group and maintained under standard laboratory protocol.

Experimental Protocol: Learning and memory activity using Morri's water maze Model (Scopalamine hydrobromide as memory impairment drug)
Sample size: 18 albino rats were taken for the experimental study, distributed 6 in each.
Group. Three groups were taken for Learning and memory activity.

Study groups

Group 1: Control (Carboxy methyl Cellulose)
Group II: Standard drug (Piracetam)
Group III: Test drug (*Brahmi Vati*)

Dosage and mode of drug administration

Animal dose= Human dose x 0.018.

- Carboxy methyl Cellulose (1mL).
- *Brahmi Vati* (13.5mg/ 200g body wt).
- Piracetam (17.28mg / 200g body wt).

Methods

A. Method of preparation of *Brahmi Vati*[4]

Brahmi Vati is prepared as per the reference Anonymous, *Ayurveda Sara Sangraha*, *Gutika-Vati-Prakrana*. *Brahmi Vati* was prepared in the Pharmacy, Department of PG and Ph.D. studies in Rasashastra & Bhaishajya Kalpana, BLDEA's AVS Ayurveda Mahavidyalaya, Hospital & Research Centre, Vijayapura.

B. Method of Experimental study

Tests for Learning and Memory: This Experimental study is done by using model Morri's water maze used to evaluate the effect of *Brahmi Vati* on learning and memory in Rats.

Study Procedure

Eighteen albino rats were randomized into control, Standard and test groups consisting of six rats in each. The animals were housed in groups with paddy husk as bedding. Animal were acclimatized at room temperature for 5 days prior to the experiment. The animals had free access to food and water. The control group received 1ml of Carboxymethyl cellulose (CMC). The Standard group received Piracetam, 17.28mg/200gm BW of Rat given orally. *Brahmi Vati* 13.5mg/200gm BW of Rat is suspended in 1ml of CMC was used at test drug. This suspension was administered orally every day for 7 days. And for memory impairment Scopolamine drug given Intraperitonially 0.4mg/200gm BW of Rat.

Model: Morri's Water maze apparatus: - A modified version of Morris water maze 120cm diameter 50cm height, filled with opaque water just 1cm above the hidden movable platform was used.

a) Morris Water Maze Test[8]

- Method used is Morri's water maze test - consist of a large circular tank (120cm diameter ×50cm height) with black coloured round platform (8cm diameter × 29cm height, ~1cm below the surface of the opaque water).
- Scopolamine hydro bromide induced method was followed
- Albino rats were taken, then rats divided into 3 groups. Each group consisting of 6 rats.
- Rats were pre- trained in all 4 quadrants of water maze tank to find the platform. The platform was kept one inch above the surface of water. Maximum time of 90sec was given to reach the platform.
- During experimental trial, the platform was kept one inch below the surface of water. Dry milk powder was added to the water to make water opaque and to mask the platform.
- Then standard and test drug are administered to the animals before 30 min of Scopolamine Intra peritoneally 2mg/kg body weight respectively.
- Then rats were left in water maze tank & observed time duration taken by the individual rat to reach the hidden platform.
- If rat fails to identify the hidden platform within 90 sec were removed out from the water maze tank.
- Then the animals were Monitored until reaches the platform and time will be recorded.
- Latency time (Time to find the platform) and Swim distance was recorded in each trial.
- Average over 3 trials Latency time for 7 days were used for statistical analysis.

Study drug and Dosage

Table No 01: Showing Study Drugs and Dosage.

Sl No	GROUP	No. of Animals	DRUG	Dosage form	Dose/per kg body weight
1	Control group	6	Carboxy methyl Cellulose	Suspension	1mL
2	Standard	6	Piracetam	Suspension	86.4mg in 1mL
3	Test group	6	*Brahmi Vati*	Suspension	67.5mg in 1mL

OBSERVATION AND RESULTS

Table No. 02: Showing Comparison of ITL to Last Day of RTL of MWM (In Sec)

MWM	Group	N	Mean± Std. Deviation	Kruskal Wallis H test value	p-value	Remarks
ITL	Control	6	73.166±6.098	0.502	0.778	Not Significant
	Standard	6	75.555±9.749			
	Test	6	74.666±11.805			
	Total	18	74.462±8.995			
6^{th} RTL	Control	6	36.555±32.730	3.716	0.156	Not Significant
	Standard	6	5.611±2.004			
	Test	6	9±5.785			
	Total	18	17.05556±23.009			

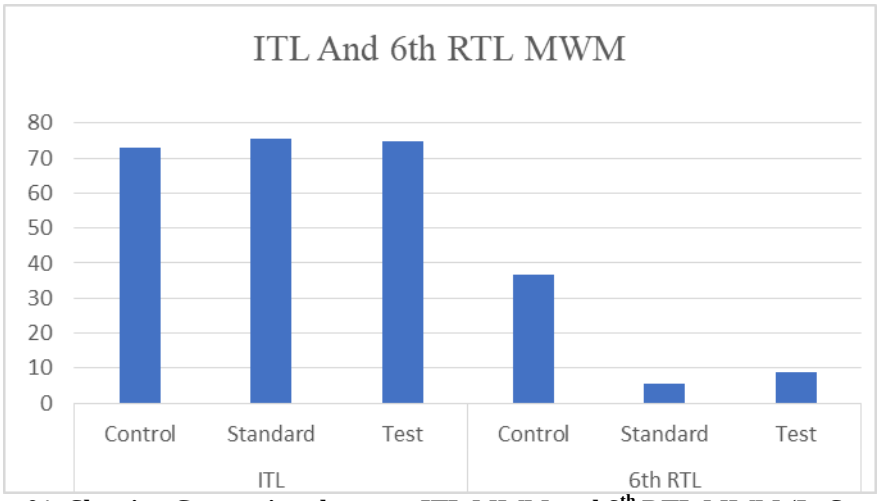

Figure 01: Showing Comparison between ITL MWM and 6th RTL MWM (In Seconds).

Kruskal-Wallis test results showed that there were no statistically significant difference among Control, Standard, and Test groups for both ITL and 6th RTL. The descriptive statistics was shown below. Figure ITL (mean scores were 73.17 ± 18.50, 75.56 ± 19.49, 74.67 ± 19.33; H=0.502; p=0.778). 6th RTL (mean scores were 36.56 ± 23.11, 5.61 ± 6.55, 9.00 ± 6.48; H=3.716; p=0.156). In summary, no significantly different of the ITL and 6th RTL was observed for the three groups.

Table No. 03: Showing Comparison between 2 groups in control, standard and test group in ITL MWM (In Seconds).

Variable	Descriptive Statistics			Mann-Whitney U test p-value	Remarks
	Comparing Groups	Mean	SD		
ITL MWM	Control	73.17	6.10	1	Not Significant
	Standard	75.56	9.75		
	Control	73.17	6.10	1	Not Significant
	Test	74.67	11.81		
	Standard	75.56	9.75	1	Not Significant
	Test	74.67	11.81		

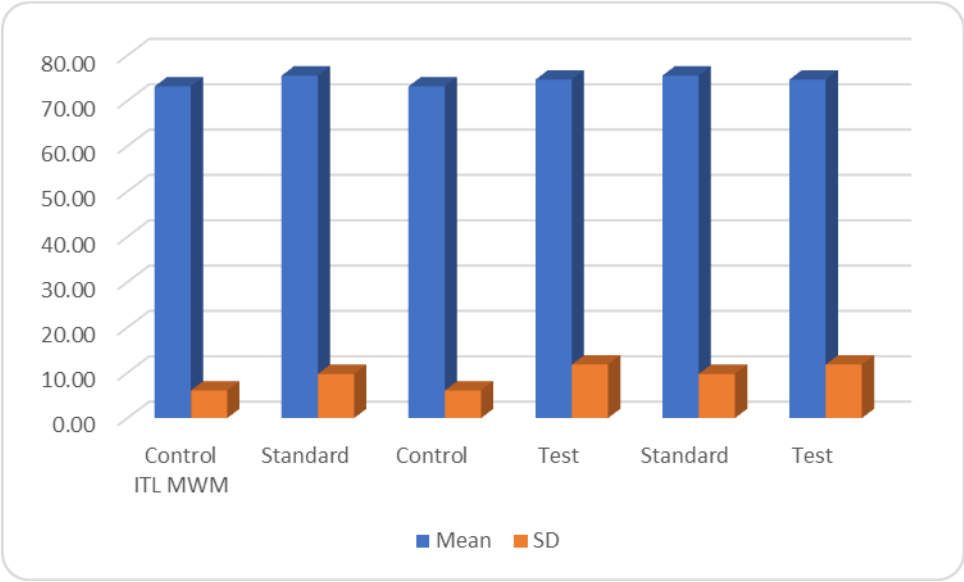

Figure 02: Showing Comparison between 2 groups of ITL MWM (In Seconds).

Table No. 04: Comparison between 2 groups in control, standard and test group in 6th RTL MWM (In Seconds).

Variable	Descriptive Statistics			Mann-Whitney U test p-value	Remarks
	Comparing Groups	Mean	SD		
6th RTL MWM	Control	36.56	32.73	0.041	Significant
	Standard	5.61	2.00		
	Control	36.56	32.73	0.076	Not Significant
	Test	9.00	5.79		
	Standard	5.61	2.00	1	Not Significant
	Test	9.00	5.79		

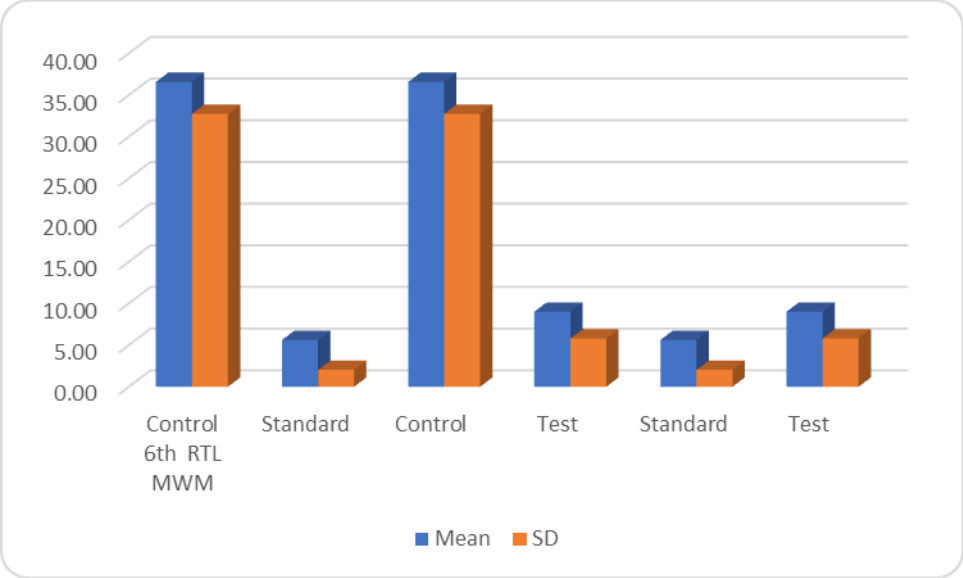

Figure 03: Showing Comparison between 2 groups of 6th RTL MWM (In Seconds).

Results in The Morris water maze MWM: In the 1st RTL, the average latencies for control group was (41.33 ± 38.92), with that of the standard (25.44 ± 17.43) and test groups (27.17 ± 17.95). Upon analyses with Mann Whitney U test the resulting p values were 0.958 and 1.000 respectively, thus $p > 0.05$. This meant both of the treatment has nothing special in their ability to learn and remember the location in first learning and memory trial RTL in the water.

In 6th RTL, the average value for the control group was (36.56 ± 32.73), whereas for the standard and test groups it was significantly lower (5.61 ± 2.00 and 9.00 ± 5.79) respectively.

The p value for the standard group was 0.041, which revealed it was statistically significant and possessed spatial learning and memory enhancing property, whereas for the test group, though the mean latency for test group was lower than that of the control, it was not statistically significant ($p = 0.076$). It may just reveal the trend towards enhancing memory property. From the graph as well, one could see that there was less variation between the groups in 1st RTL whereas during 6th RTL, a very marked reduction of average time to escape was found in the standard and test groups.

DISCUSSION

The present study evaluated the learning and memory enhancing activity of *Brahmi Vati* in scopolamine-induced cognitive impairment using Morris Water Maze (MWM) and models. These experimental paradigms are widely accepted for assessing spatial learning, memory acquisition, and retention in rats. The effects of *Brahmi Vati* were compared with a standard drug Piracetam, and a control group.

In present experimental study we assessed memory consolidating and enhancing activity of *Brahmi Vati* using Morris Water Maze (MWM) model for producing scopolamine induced memory impairment in albino rats. MWM has been a good behavioural model to assess spatial memory and learning by the measured escape latency to find hidden platform in water. Using muscarinic receptor blocker named as Scopolamine which is responsible to produce memory impairment is used in albino rats. The ITL, the latency required by the animals for navigating the maze is more likely representing immediate ability of learning and in between the MWM trials one would observe initial training latency as ITL on day 1.

In terms of Initial Training Latency, there were no statistical significant differences among control, standard (Piracetam) and test group (*Brahmi Vati*) (p=0.778),

while using pairwise comparison by using Mann Whitney U test it was found to be that there was no significant difference in the First retention latency in any of the groups which clearly implies that there was no immediate improvement in the learning ability due to these drugs. Possibly short duration exposure might also be responsible for this. Memory enhancing drugs took 6 to 10 doses for producing effect.

But by the time of sixth retention latency, we found out a decrease in the escape latency in standard group; significant (p=0.041) as compared to the control group. This result with the standard drug Piracetam proved its *Medhya* effect, with which the test group of animals treated with *Brahmi Vati* found to have a reduce escape latency as compared to the control group but not significant (p=0.076), Although we found some promising effect but this could not reach up to statistically significant limit. Because the small number of animals (n= 6) in group.

Possible active constituents of *Brahmi Vati* in *Medhya Rasayana* class namely *Brahmi, Shankhapushpi, Vacha, Jatamamsi, Gojihva* known from ayurvedic classics for increasing intellect, memory, attention, concentration. *Brahmi, Shankhpushpi* is known for their anti-oxidant, neuro-protection, cholinergic properties which increase neurotransmission that improve learning and memory. Therefore, the result of our study indicates that *Brahmi Vati* have positive and promising effect on memory, learning behaviour in rats.

CONCLUSION

The study Learning and Memory enhancing activity of *Brahmi Vati* was assessed on the Morris Water Maze model using scopolamine induced memory impaired albino rats. In *Brahmi Vati* reduced the latency to reach the platform for the 6th day retention trials, suggesting its efficiency in improvement of spatial learning and memory. However, this reduction of the latency was not statistically significant compared to Standard. Where Piracetam a standard memory enhancing had shown significant reduction of escape latency of the 6th day of the retention and confirmed its established property as a nootropic.

The *Brahmi Vati* showing reducing tendency of escape latency, though it was not statistically significant mean while it indicates the favourable tendency towards cognition enhancement may occur on combination of *Medhya Rasayana* components such as *Brahmi, Shankhpuspi, Vacha, Jatamamsi, Gojihva* which is known for their intellect & learning capacity and to improve physical as well as mental functions and efficiency on animal models may be attributed towards their combined effect.

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