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Comparative In Vivo Study Of Two Different Routes: Oral And Transcranial For The Effect Of Polyphyto Nootropic Formulation For Its Memory Enhancing Properties

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Abstract

The present study objective is to comparing the effectiveness of the medhya (polyphyto) formulations for its learning and memory activity in two different routes oral and transcranial;could be delivered and targeted to the brain by transcranial delivery route. The unique anatomical arrangement of blood vessels and sinuses in the human skull and the brain, the prevalence of a high density of skin appendages in the scalp, extra cranial vessels of the scalp communicating with the brain via emissary veins and most importantly, the way that the scalp is used in the Ayurvedic medical system in treating diseases associated with the brain show that a drug could be transcranially delivered and targeted to the brain through the scalp. The medhya formulation FMLM; compose of Bacopa monnieri (51.8%), Oscimum sanctum (26.7 %), and Withania *somnifera* (21.5%) on memory acquisition and retention was studied using Elevated plus maze, Morris water maze (MWM) and Pole Climbing apparatus (PCA) in rat using two different routes, one is per oral ($FMLM^O$) and other one is transcranial ($FMLM^T$). As per the result, on administration of polyherbal formulations $FMLM^O$ and $FMLM^T$ showed significant ($p<0.01$) reduction in transfer latency in EPM, MWM and escape latency in PCA test as compared with the control per oral as well as transcranial delivery; whereas trancranially applied $FMLM^T$ is equally effective in comparison to standard drug but it is shown an extremely significant ($p<0.001$) effect compared to control group.

Keywords: Medhya or Intelligence, Improving Learning and memory, Transcranial application, Pole climbing test for memory, Elevated Plus maze test of memory,

Introduction

In general, learning is defined as the acquisition of information and skills, and subsequent retention of the information is called memory. Memory function is vulnerable to a variety of pathologic processes including neurodegenerative diseases like Alzheimer's disease, stroke, tumors, hypoxia, cardiac surgery, malnutrition, depression, anxiety, side effects of medication and normal ageing [1]. Memory loss is often the most disabling feature of many disorders, impairing the normal daily activities of the patients and profoundly affecting their families [2].

Herbal remedies for Alzheimer's disease or use to improving memory have become more and more popular in the recent years and not without a reason that there is a possibility to slow down the brain degeneration caused by different pathophysiology with natural treatments and it has shown the promising effect in the treatment of memory loss. The herbs that promote the intelligence are called Medhya [3] herbs. According to Ayurveda the intelligence is a triad of three powers of the mind - the acquisition, the retention and the recollection. The Power of Acquisition: It is the capacity to grasp some topic or something new. It is the capacity to understand or analyze. The Power of Retention: It is the capacity to retain what has been grasped or understood. This capacity also deals with short-term memory. The Power of Recollection: This is the capacity to retrieve the information after some time. It can be compared to long-term memory.

Many natural herbal treatments have been researched and the benefits derived from using herbal treatments for Dementia have been very promising and the use of some medicinal herbs has been touted to extend beyond that of modern prescription drugs. With so many natural and healthy compounds, it's no wonder that these medicinal herbs may hold the key to the cure to this severe disease. The herbs are inexpensive and can be easily obtained. Clinical research is being conducted world over to test the efficacy of herbal medicines vis-a-vis prescription medicines in treating Alzheimer's or dementia patients. The results are promising, as herbal products have been found to be not only as effective as prescription drugs but also with fewer side effects. Herbal supplements may be used as a substitute for pharmaceutical drugs or can be used in conjunction with the latter.

Bacopa monniera has been shown to be very useful in improving learning and memory [4-5]. *Bacopa monniera*, a member of the

Scrophulariaceae family, is a small, creeping herb with numerous branches, small oblong leaves, and light purple flowers [6]. It has been used in Ayurvedic medicine and traditional treatments for a number of disorders, particularly those involving anxiety, intellect, and poor memory [7]. The plant has prominent action on the central nervous system, where it improves understanding, memory, intellect, and speech, and corrects aberrations of emotions, mood, and personality of an individual. Animal studies have found *Bacopa monniera* attenuates scopolamine-induced dementia, and anticholinesterase activity has been demonstrated [8]. Preclinical and clinical studies have shown that *Bacopa monniera* improves memory and mental function [9]. The plant, plant extracts and isolated bacosides have been investigated for nootropic activity. A recent study reveals B. monniera extract is able to reduce amyloid levels in PSAPP mice which is a transgenic mice expressing the "Swedish" amyloid precursor protein and M146L presenilin-1 mutations [10].

O.sanctum posses nootropic activity in view of its facilitatory effect on retention of acquired learning. *O. sanctum* also reversed the scopolamine-induced impairment in learning and memory, when assessed on the elevated plus maze and passive avoidance paradigms. Therefore, it seems that *O. sanctum* improved learning and memory probably because of enhanced cholinergic transmission [11]. Antiinflammatory action of *O. sanctum* may also contribute to the memory enhancing activity [12]. *O. sanctum* is reported to possess antioxidant [13] and antistress [14] properties as well which is responsible for the development of Alzheimer's disease in elderly [15]

Withania somnifera (Ashwagandha) is a plant used in medicine from the time of Ayurveda, the ancient system of Indian medicine. Ashwagandha has been used as an aphrodisiac, liver tonic, anti-inflammatory agent, astringent, and to treat bronchitis, asthma, ulcers, emaciation, insomnia, and senile dementia. Clinical trials and animal research support the use of Ashwaganda for anxiety, cognitive and neurological disorders, inflammation, and Parkinson's disease [16]. The drug-induced increase in cortical muscarinic acetylcholine receptor capacity might partly explain the cognition- enhancing and memory-improving effects of WS extracts in animals and in humans. Effects of sitoindosides VII-X and withaferin isolated from aqueous methanol extract of roots of cultivated varieties of WS were studied on brain cholinergic, glutamatergic and GABAergic receptors in male Wistar rats [17].

The unique anatomical arrangement of blood vessels and sinuses in the human skull and the brain, the prevalence of a high density of skin appendages in the scalp, extra cranial vessels of the scalp communicating with the brain via emissary veins and most importantly, the way that the scalp is used in the Ayurvedic medical system in treating diseases associated with the brain show that a drug could be transcranially delivered [18] and targeted to the brain through the scalp. The drugs are effective if it's delivered to the site of action, In the Ayurvedic system of medicine practices oil therapies to the head to treat diseases of the central nervous system [19]

The oil therapies of Ayurveda using the head include Shirodara, Shiroabyanga, Shiropitchu, Shirovasthi and Shiropralepa in which drugs are delivered by the transcranial route [18]. Trans Cranial Routes means drugs are delivered to the brain through transcranial route, it was stated that the passage of an oil solubilized drug moiety across the skin of the scalp, including appendages of the skin such as sebaceous glands, walls of the hair follicles and sweat glands, through the cranial bones along with the diploe, the cranial bone sutures, the meninges and specifically through the emissary veins into the brain. The emissary veins draining blood from extracranial sites into the intracranial sinuses pierce a series of foramina present in the cranial bones. Scalp veins communicate with the sinuses of the brain via emissary veins. There are thirteen emissary veins connecting extracranial sites of the head with intracranial sinuses [20].

Materials and Methods

Plant material and preparation of formulation

Different parts of all plants were obtained from local sources and were identified and authenticated by department of botany, Sri Venkateswara University, Tirupati. Various Plant parts were air dried in the dark and grounded into a fine powder and pass it through a # 100 sieve, then prepared two different formulations one for oral drug delivery (FMLMO) and other one is transcranial drug delivery (FMLMT), according to their composition (Table No. 1). For preparing FMLMT the powder after mixing was further treated with sesame oil and subjected to heat for 10 minutes, then ultrasonication for 3 cycles at 3 mins each cycle, finally it centrifuged to separate the clear supernatant oil formulation and finally added two drops of rose oil.

Table No. 1: Medhya Formulation FM001

Botanical Name	Family	Plant Parts	Weight (g)	Voucher No
Bacopa monnieri	Scrophulariaceae	Herbs	51.8	1052
Oscimum sanctum	Labiatae	Leaf	26.7	1592
Withania somnifera	Solanaceae	Root	21.5	709

Experimental Animals

Adult albino Wistar strain rats (120 ±20 gm) of either sex were procured and were grouped randomly. The rats were acclimatized for one week in the animal house facility. They were housed in polypropylene cages in an ambient temperature of 25±1 C with a natural dark-light cycle. The animals had been provided standard pellet diet and water given *ad libitum.* All experiments were conducted in the daytime (9:30 AM to 5:00 PM). The study was approved by the institutional ethics committee (CPCSEA registration no. 1156/ac/07/CPCSEA).

Treatment groups

All the groups received the vehicle, standard drug and the test drug one hour prior to each experiment. Animals were selected and divided into groups (n=6). It was studied for Elevated Plus Maze test (EPM), Morris Water Maze Test (MWM), Pole Climbing Test (PCT). Group classifications are shown in a Table No. 2

Table No. 2: Animal Group Specification

GROUP	DOSE	ANIMAL USE	NAME OF TEST		
			EPM	**MWM**	**PCT**
Control		Rat (Wistar)	1	2	3
Standard	50 mg/kg		4	5	6
	100 mg/kg		7	8	9
	Transcranial		10	11	12
FMLMO	50 mg/ kg		13	14	15
	100 mg/kg		16	17	18
FMLMT	Transcranial		19	20	21

Method of transcranial drug administration

The hair of the scalp of animal was trimmed without injuring the skin for the transcranial application. The 19,20 and 21 group consider as a test group was treated with FMLMT transcranial oil by applying on to the hair trimmed bald area of the scalp followed by 'rubbing in' for 1 min with gentle massage by which it was to facilitate the oil solution come into contact with the skin and its appendages of the scalp properly. Similarly 10, 11, and 12 group consider as a standard oil containing *Bacopa monnieri* applied transcranially.

Acute Toxicity Study

To evaluate the acute toxicity of drugs after a single oral dose, Swiss albino rats were fasted for 6 hours with only water provided ad libitum. Rats were divided into experimental groups (n=6) and were treated orally at doses of 300, 1000 and 2000 mg/kg. The animals were then allowed free access to food and water. The animals were observed for any abnormal behavior, changes of body weight and mortality was noted for 14 days after the oral administration of formulation for the acute toxicity [21]. The control group was treated with normal saline (1ml/kg, i.p.). Both FMLMO and FMLMT were found to be safe.

Experimental Procedure

Elevated plus Maze test

The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in rats. The apparatus consisted of two open arms (50 cm 10 cm) and two covered arms (50 cm 40 cm 10 cm). The arms extended from a central platform (10cm 10cm) and the maze was elevated to a height of 50 cm from the floor. With little modification transfer latency (TL) was noted as the time taken by the rat to move into any one of the covered arms enter with all its four legs where opposite gender of rat is kept in the covered place to observe retention memory of test rat to come faster toward that area. On the first day, each rat was placed at the end of open arm, facing away from a central platform [22-24].TL was recorded on the first day for the each animal for both the formulations FMLM O AND FMLMT including control and standard. The rat was allowed to explore the maze for another 2 min and returned to its home cage. Retention of this learned task was examined 24 h after the first day trial.

Morris Water Maze Test

The Morris water maze consisted large circular pool, 1.50 m across and 0.60 m high filled with water, which was made opaque by adding milk. Water provided a uniform intramaze environment, thus eliminating any olfactory interference. A 28x10 cm rectangular escape platform was constructed of water resistant material and covered with material that allows the animal to remain on top when it is submerged. The platform was 28 cm in height so that it could be submerged 2 cm below the level of water surface. The water temperature was maintained at 26 ±2 C [25]. After treating with FMLMO, and FMLMT of respective group all the animals were

given a daily session of three trials per day and tested for seven days . Latency time to reach the platform was recorded in each trial. Significant decrease in latency times from that of the first session was considered as successful learning.

Pole Climbing Test

Cook's Pole Climbing Apparatus use to study cognitive function, mainly a response to conditioned stimuli during learning and its retention. The apparatus has an experimental chamber (25 25 25 cm) with the floor grid in a soundproof enclosure. Scrambled shock (6mA) is delivered to the grid floor of the chamber composed of stainless steel rods. A pole, 2.5 cm in diameter, hangs inside the chamber through a hole in the upper center of the chamber. The study rat was placed in the chamber and allowed to explore the chamber for 45 seconds. Conditioned stimulus (CS) i.e buzzer signal was turned on and unconditioned stimulus (US) i.e electric shock delivered through grid floor for 45 Sec. Animal learned to associate the buzzer with the impending foot shock and was capable of avoiding the foot shock by climbing the pole after buzzer signal. Avoidance response was defined as climbing reaction time <10 sec only; and escape response was climbing after applying reaction time >10 sec. Every rat was subjected to maximum 05 trials on 1st day, and 24 hrs later, rat was subjected to Relearning trials (2nd day 3 trials and on 3rd day one trial) and transfer latency was noted to check the retention of Conditioned Avoidance Response (CAR) and escape response. Animals were screened by using this model and those who demonstrated at least one escape response either on day one or two were included in the study [26-27].

Statistical Analysis

All results were expressed as mean ± standard error of mean (S.E.M.). Data was analyzed using one way ANOVA and two way repeated measures followed by Tukey's multiple comparisons and student's unpaired t test using the graphpadprism statistic software. $P < 0.05$ was set as statistically significant.

Results

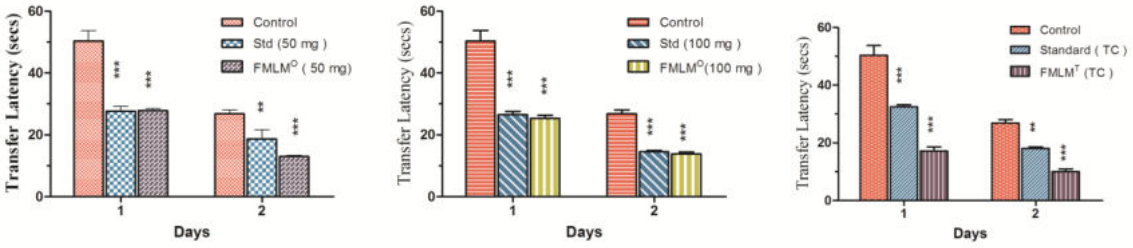
Effect of Transfer Latency using Elevated Plus Maze

Transfer latency was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all its four legs. With little modification an opposite gender of rat was placed in any one of the covered place to observe retentive memory of test rat to come faster toward that area. Significant decrease in TL value of retention indicated improving memory. Test formulation of 50 mg and 100 mg doses of FMLMO showed decrease in TL on the second day (after treatment) in rat when compared to control groups indicating significant($p<0.001$) memory improvement (Figure. 1). Transcranially applied FMLMT showed a significant effect ($p<0.01$) of memory improvement comparing Standard group containing *Bacopa monnieri*. Results of mean±SEM were shown in table no.3.

Table No. 3: Effect on EPM: Values are mean ± SEM of 6 animals per group

Treatment	Transfer Latency (seconds)					
	50 mg Dose		100 mg Dose		Transcranial	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Control	50.33 ± 3.403	26.8 ±1.249	50.33 ± 3.403	26.8 ±1.249	50.33 ± 3.403	26.8 ±1.249
Standard	27.66 ± 1.498	18.67 ± 2.985	26.5 ± 1.057	14.5 ± 0.428	32.5 ± 0.671	18 ± 0.577
FMLM	27.83 ± 0.703	13 ± 0.365	25.33 ± 1.54	13.83 ± 0.601	25.33 ± 1.54	13.83 ± 0.601

Figure 1: Bar Graph of mean Transfer Latency in elevated plus maze using rat



Statistical significance test was analyzed by one way ANOVA and two way repeated measures followed by Tukey's multiple comparison test (n=6) and students unpaired t Test; *** denotes P values <0. 001 was considered as statistically significant vs control; Test group FMLMO at 50 mg, 100 mg and FMLMT on TC

application found to be highly significant for its improving learning and memory ability.

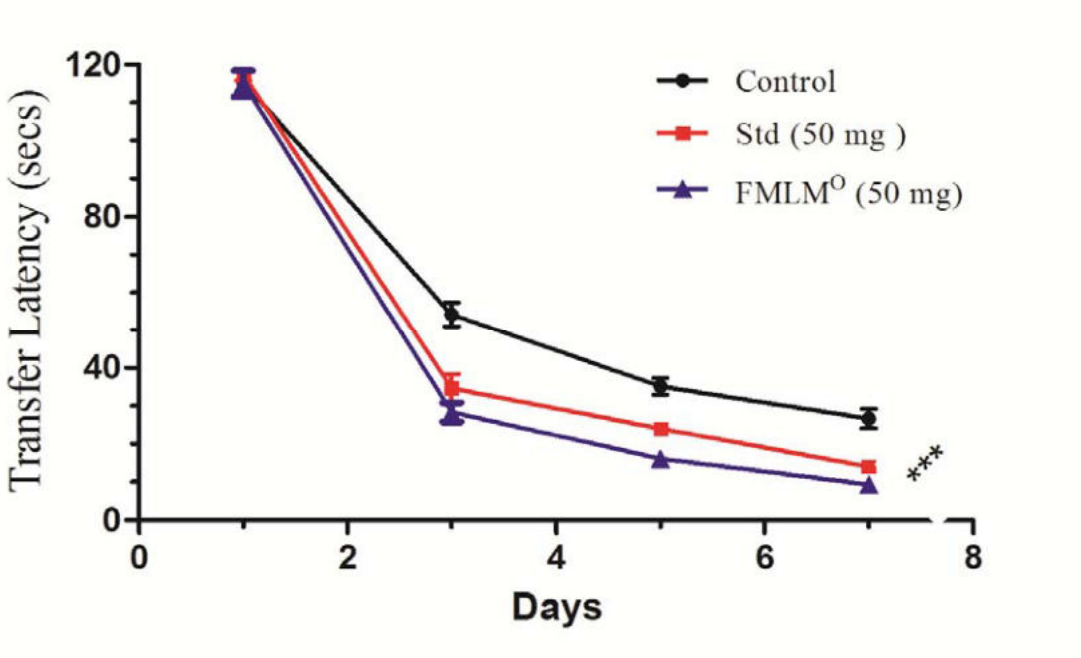
Water maze test

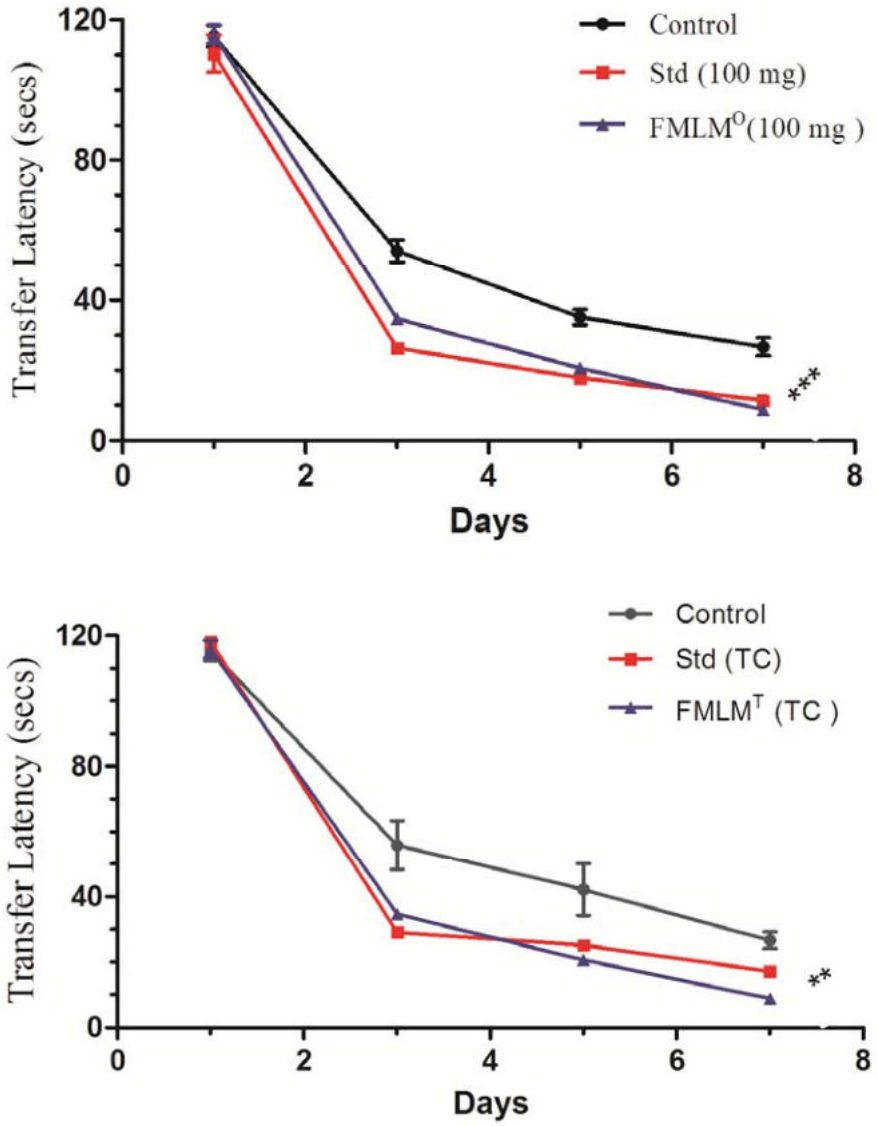
The transfer latency on a water maze test of rat was studied using a circular pool (diameter 70 cm; height 28 cm) and a platform (diameter 3.8 cm) was placed 1.5 cm below the water level in the middle of a fixed quadrant. The differences in the transfer latency were noted in control, standard, and test group. Results indicated that test group animals at 50mg and 100 mg dose, FMLMO showed less transfer latency time in seconds during the study and found to be an extremely significant decrease in transfer latency ($p<0.001$) when compared to respective control groups and found to be comparatively better than standard (*Bacopa monnieri*) per oral route. The test formulation FMLMT also found to be significant ($p<0.01$) on brain targeted transcranial application (Figure.2). Results of mean±SEM were shown in table 4:

Table No. 4: Effect on MWM: Values are mean ± SEM of 6 animals per group

Treatment GP	Dose	Transfer Latency (seconds)			
		Day 1	Day 3	Day 5	Day 7
Control		115.3 ± 3.127	54.167 ± 3.17	35.167 ± 2.21	26.6 ± 2.539
Standard	50 mg	117.5 ± 1.708	34.66 ± 3.765	24 ± 1.155	14 ± 0.577
	100 mg	110.3 ± 5.33	26.33 ± 1.2	17.83 ± 1.078	11.5 ± 0.428
	TC	118 ± 1.366	29 ± 1.528	25.167 ± 0.946	17.167 ± 0.946
FMLMO	50 mg	115 ± 3.416	28.33 ± 2.486	16.167 ± 1.4	9.33 ± 0.33
	100 mg	115.83 ± 2.713	34.667 ± 1.687	20.667 ± 1.33	8.83 ± 0.601
FMLMT	TC	115.83 ± 2.713	34.66 ± 1.687	20.66 ± 1.33	8.33 ± 0.601

Figure 2: The transfer latency of polyphyto formulations in rat in secs using MWM





Statistical significance test was analyzed by one way ANOVA and two way repeated measures followed by Tukey's multiple comparison test (n=6) and students unpaired t Test; *** denotes P values <0. 001 was considered as statistically significant vs control; Test groups FMLM was extremely significant comparing control and better than standard for its learning improvement ability at 50mg, 100 mg dose per oral route and on TC route application.

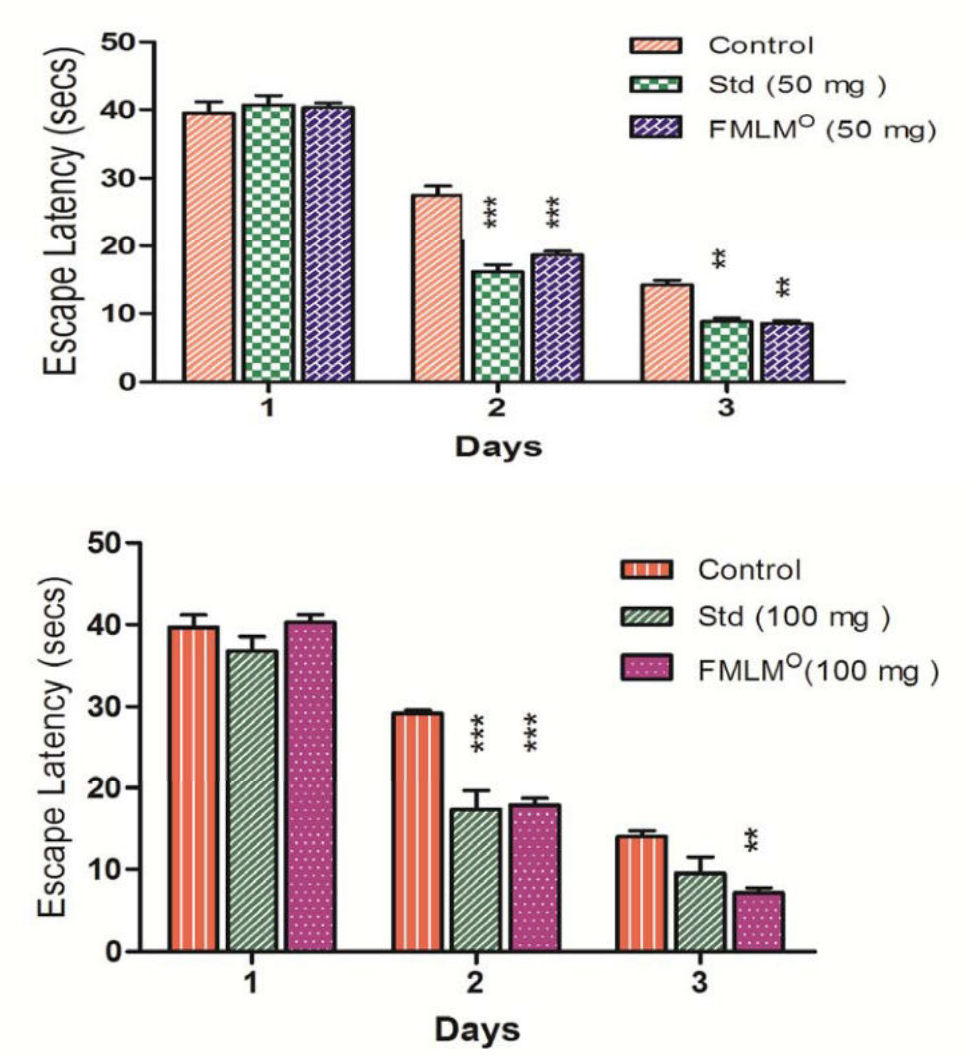
Pole Climbing test

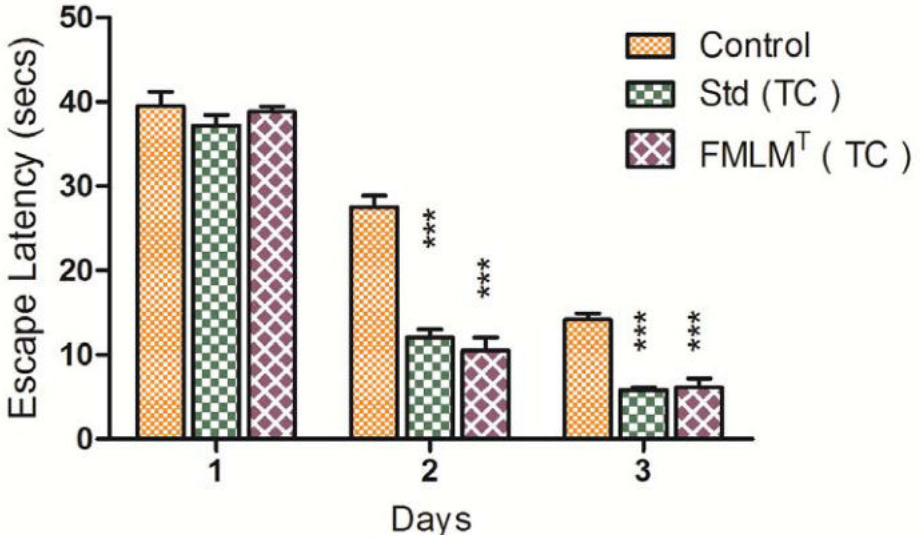
To study the escape latency in seconds - rat placed inside the Pole climbing apparatus, a shock for controlled duration of 200V AC 50 Hz single phase - 0.2 mA was applied. The Test group FMLMO at 50 mg and 100 mg doses revealed a statistically significant ($p<0.01$) and on Transcranial application was highly significant ($p<0.001$) decrease in escape latency in pole climbing test as compared to the control groups. The test group at 100 mg doses observed better than the standard group on day 3, whereas at 50 mg and TC application showed the same effect comparing standard group (Figur.3). The results were shown mean ± SEM in table no. 5.

Table No. 5: Effect on PCT: Values are mean ± SEM of 6 animals per group;

Treatment Group	Dose	Avoidance or Escape Latency (sec) [Mean ± SEM]		
		Day 1	Day 2	Day 3
Control		39.5 ± 1.708	27.5 ± 1.408	14.167 ± 0.749
Standard	50 mg	40.66 ± 1.498	16.167 ± 1.014	8.833± 0.477
	100 mg	36.83 ± 1.72	17.33 ± 2.348	9.5 ± 2.078
	TC	37.167 ± 1.276	12.00 ± 1.00	5.833 ± 0.307
FMLMO	50 mg	40.33 ± 0.667	18.66 ± 0.558	8.50 ± 0.428
	100 mg	40.33 ± 0.919	17.83 ± 0.910	7.167 ± 0.601
FMLMT	TC	38.83 ± 0.601	10.50 ± 1.522	6.167 ± 1.014

Figure 3: Bar Graph of escape latency of rat in secs using Pole Climbing Apparatus





Statistical significance testing was analyzed by one way ANOVA and two way repeated measures followed by Tukey's multiple comparison test (n=6) and students unpaired t Test; *** denotes P values <0. 001 statistically highly significant vs control; Test group FMLMO and standard at 50mg, and 100 mg were significant ($p<0.01$) on day3. FMLMT * P values < 0.001 denote test formulations are highly significant as compared with control on TC application.

Discussion

Poor memory, lower retention, and slow recall are common problems in today's stressful and competitive world. Herbal drug has shown the promising effect in the treatment of memory loss. The oil therapies of Ayurveda using the head include Shirodara, Shiroabyanga, Shiropitchu, Shirovasthi and Shiropralepa in which drugs are delivered by the transcranial route [18]. Trans Cranial Routes means drugs are delivered to the brain through transcranial route, it was stated that the passage of an oil solubilized drug moiety across the skin of the scalp, including appendages of the skin such as sebaceous glands, the walls of the hair follicles and sweat glands, through the cranial bones along with the diploe, the cranial bone sutures, the meninges and specifically through the emissary veins into the brain. The emissary veins draining blood from extracranial sites into the intracranial sinuses pierce a series of foramina present in the cranial bones. Scalp veins communicate with the sinuses of the brain via emissary veins. There are thirteen emissary veins connecting extracranial sites of the head with intracranial sinuses [28]

Many experimental models are currently available for the evaluation of agents that affect learning and memory process. Morris Water Maze is a traditional tool in assessing learning and memory performance in laboratory animals. Originally designed to evaluate the antianxiety agents, elevated plus maze has also been recently extended to measure the spatial long-term memory in animals. Passive avoidance behavior is used to examine the long term memory based on negative reinforcement [29].

The herbs acting on the brain are called as Nootropic herbs (Nootropic is derived from Greek and means acting on the mind) and their isolated constituents referred to as smart drugs. Memory enhancer herbs enhance the memory and increase blood circulation in the brain. The herbs act either by improving memory or preventing neurodegeneration by antioxidant and anti inflammatory activity. The medhya formulation; FM001 (*Bacopa monnieri, Oscimum sanctum, Withania somnifera*), on memory acquisition and retention was studied using Elevated plus maze, Morris water maze (MWM) and Pole Climbing apparatus (PCA) in rat.

In an Elevated plus maze study test formulation of 50 mg and 100 mg dose FMLMO showed decrease in TL of the second day (after treatment) in rat ($p<0.001$) when compared to control groups, indicating significant memory improvement, whereas transcranially applied FMLM T showed a significant effect ($p<0.01$) of memory improvement comparing Standard group containing *Bacopa monnieri*.

In MWM test observed that test group animals at 50mg and 100 mg dose FMLMO showed less transfer latency time in seconds during the study and found to be an extremely significant decrease in transfer latency ($p<0.001$) when compared to respective control groups and found to be comparatively better than standard (*Bacopa monnieri*) per oral route. The test formulation FMLMT also found to be significant ($p<0.01$) on brain targeted transcranial application.

The Test group FMLMO at 50 mg and 100 mg doses revealed a statistically significant ($p<0.01$) and FMLMT on Transcranial application was highly significant ($p<0.001$) decrease in escape latency in pole climbing test as compared to the control groups. The test group at 100 mg doses observed better than the standard group on day 3, whereas at 50 mg and TC application showed the same effect comparing standard group

In conclusion on administration of polyherbal formulations FMLMO and FMLMT showed significant ($p<0.01$) reduction in transfer latency in EPM, MWM and escape latency in PCA test as compared with the control per oral as well as transcranial delivery;

whereas trancranially applied FMLM^T is equally effective in comparison to standard drug but it is shown an extremely significant ($p<0.001$) effect compared to control group.

Authors Contribution

SK:Participated in literature search, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, editing and review.

NVSM: Conceived of study and Design
CS: Participated in study approval.

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