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Original Article

# SAFETY AND EFFICACY STUDY OF HERBAL POLYPHYTO FORMULATIONS: FOR ITS LEARNING AND MEMORY ENHANCING PROPERTIES

**SHIBNATH KAMILA[1*], N. V. SATHEESH MADHAV[2], C. N. SARKAR[3]**

[1]Research Scholar, Uttarakhand Technical University, Dehradun, Uttarakhand, [2]DIT University, Faculty of Pharmacy, Dehradun, UK, India, [3]Fortis Hospital, Anandpur, Kolkata
Email: shibnath007@gmail.com

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**ABSTRACT**

**Objective:** The present study was designed to evaluate the effectiveness of the poly photo formulation for its learning and memory activity.

**Methods**: The Indian origin test drug, FM7 phyto formulation compose of *Convolulus pluricaulis*, *Habiscus rosasinnsis*, *Withania somnifera*, *Terminalia arjuna* and *Emblica officinalis*, having the potential effect to improving memory studied at a dose of 50 mg and 100 mg/kg p. o. by using three different animal model like Elevated plus maze (EPM), Morris water maze (MWM) and Pole Climbing apparatus (PCA) for the effect of nootropic action; against standard drug *Bacopa monnieri* evaluated on the basis of transfer latency reduction on a rat, before and after drug administration.

**Results:** On treatment with polyherbal formulation FM7 showed a significant effect on enhancing learning and memory properties. It was observed that significant ($p<0.001$) reduction in transfer latency in Elevated Plus Maze, Morris Water Maze test and escape latency in Pole Climbing Apparatus test as compared with the control; as well as standard *Bacopa monnieri*.

**Conclusion**: The polyphyto formulation FM7 composed of (*Convolulus pluricaulis 20%*, *Habiscus rosasinnsis 20%*, *Withania somnifera 20%*, *Terminalia arjuna 20%* and *Emblica officinalis 20%*) found to be safe and effective in enhancing learning and memory properties.

**Keywords:** Learning and memory, *Convolulus pluricaulis, Phyllanthus Emblica, Withania somnifera, Habiscus rosasinnsis, Terminalia arjuna*



## INTRODUCTION

The loss of memory in Dementia or in Alzheimer's disease is the main challenges of treatment. Traditional Indian origin Nootropic drugs (smart drugs), use to improve human cognitive abilities and improve learning and memory function. Typically these are work by increasing the brain's supply of neurochemicals, increase perfusion of brain's oxygen or by activating nerve growth [1]. Memory is the ability of the individual to record the sensory stimulant information and events retain them over a short or a long period of time and recall the same at a later when it is needed. Poor memory, less attention, and slow learning are quite natural problems among students and old age people. Herbal medicine for improving memory and treat memory disorder have been urgent need of findings because natural remedies were shown promising effect believes in working by different pathophysiology and preventing brain's degeneration. Indian system of medicine reported that use of herbs, nutraceuticals of lifestyle changes important for controlling age-related neurodegenerative disorders [2].

The present study was focused upon exploring the potential of polyphyto formulation to improve memory; it includes ''Shankhpushpi'' (*Convolulus pluricaulis*), ''Japapushpa'' (*Habiscus rosasinnsis*), "Ashwagandha" (*Withania somnifera*), ''Arjuna'' (*Terminalia arjuna*) and "Amla" (*Emblica officinalis*). *C. pluricaulis* (Shankhpushpi) is used for the treatment of various disorders mainly nervous weakness, like insomnia, mental as well as physical fatigue, loss of memory, etc. *C. pluricaulis* is generally recommended as a brain tonic [3,4]. *Convolvulus pluricaulis* contain glycosides, coumarins, flavonoids and alkaloids whereas the alkaloid has been recognized as an active principle [5]. Flowers of *Habiscus rosasinnsis* use as refrigerant" emollient and emmenagogue, aphrodisiac; decoction given in bronchial catarrh; infusion of petals use as a demulcent in a cough and useful in strangury, cystitis, and other genitourinary troubles. It also reported to have a positive effect on learning and memory model [6] 3, 7-diglucoside, Quercetin-3-diglucoside, cyanidin-3-sophoroside-5-glucoside, and diglucoside have been isolated from deep yellow flowers; moreover, kaempferol-3-xylosylglucoside have been identified from ovary white flowers [7]**.** Withanolide A (WL-A) is a major constituent of Ashwagandha, especially reported for action like normal cortical neurons, predominant axonal outgrowth [8] and can be used for neurodegenerative diseases [9]. It stimulates the growth of axons and dendrites in human neuroblastoma cells and in rat neurons [10,11], and enhances cognition and improve memory effects [12, 13].

*Terminalia arjuna* used by Ayurvedic physicians for its curative properties in heart problems like angina, hypertension, and coronary artery disease. Terminalia arjuna is having antioxidant action as it contains flavonoids and oligomeric proanthocyanidins [14]**.** *Terminalia arjuna* provides strength to the nervous system and also strengthens the reflexes [15]. The fruits of *Emblica officinalis* are widely used in the Aryuveda and it increases defense against diseases in our body. It has its beneficial role in anemia, diabetes, liver treatment, cancer, heart trouble, ulcer, and various other diseases. It has recommended as an analgesic, antipyretic, antioxidant [16], antitussive, immunomodulatory, cytoprotective [17], and gastroprotective [18]. Amla is a natural remedy to improve memory function [19].

### Standard drug as *Bacopa monnieri* [20]

*Bacopa monnieri* has been shown to be very useful in improving learning and memory [21, 22]. *Bacopa monnieri*, a member of the Scrophulariaceae family, is a small, creeping herb with numerous branches, small oblong leaves, and light purple flowers [23]. It has been used in Ayurvedic medicine and traditional treatments for a number of disorders, particularly those involving anxiety, intellect, and poor memory [24]. 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 monnieri* attenuates scopolamine-induced dementia, and anticholinesterase activity has been demonstrated [25]. Preclinical and clinical studies have shown that *Bacopa monnieri* improves memory and mental function [26]. The plant, plant extracts and

isolated bacosides have been investigated for nootropic activity. A recent study reveals *B. monnieri* 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 [27]. The present study was designed to evaluate the effectiveness of the poly photo formulation having nootropic agents for its learning and memory activity.

## MATERIALS AND METHODS

### Plant material and preparation of formulation

Different parts of all plants were obtained from Dehradun, India and were identified and authenticated by the department of botany, Sri Venkateswara University, Tirupati. The voucher specimen number of *Bacopa monnieri* was 1052. Various Plant parts were air dried in the dark, and grounded into a fine powder and passes it through a # 100 sieve, then prepared a formulation FM7 according to their composition shown in table no. 1.

### Experimental animals

Adult albino wistar strain rats (120±20 Gms) of either sex were procured and were grouped randomly. The male and female rats were separated and 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) on 26th May' 2011.

**Table 1: The composition nootropic phyto formulation (FM7)**

| Plant | Sanskrit name | Family | Plant parts | 100 gm | Voucher specimen no. |
|---|---|---|---|---|---|
| *Convolulus pluricaulis* | Shankhpushpi | Convolvulaceae | Herbs | 20 | 1222 |
| *Habiscus rosasinnsis* | Japapushpa | Malvaceae | Flower | 20 | 436 |
| *Withania somnifera* | Ashwagandha | Solanaceae | Root | 20 | 709 |
| *Terminalia arjuna* | Arjuna | Combretaceae | Bark | 20 | 405 |
| *Emblica officinalis* | Amla | Euphorbiaceae | Fruit | 20 | 1441 |

### Treatment groups

After acclimatization, the animals were randomly divided into following groups consisting of n=6 rats each. All the groups received the vehicle, standard drug and the test drug one hour prior to each experiment. It was studied for Elevated Plus Maze test (EPM), Morris Water Maze Test (MWM), Pole Climbing Test (PCT).

Group1: Control (Normal saline 1 ml/rat per oral) for MWM test

Group2: Control (Normal saline 1 ml/rat per oral) for EPM test

Group3: Control (Normal saline 1 ml/rat per oral) for PCA test

Group4: Standard (*Bacopa monnieri* 50 mg/kg per oral) for MWM test

Group5: Standard (*Bacopa monnieri* 50 mg/kg per oral) for EPM test

Group6: Standard (*Bacopa monnieri* 50 mg/kg per oral) for PCA test

Group7: Standard (*Bacopa monnieri* 100 mg/kg per oral) for MWM test

Group8: Standard (*Bacopa monnieri* 100 mg/kg per oral) for EPM test

Group9: Standard (*Bacopa monnieri* 100 mg/kg per oral) for PCA test

Group10: Test FM7 (50 mg/kg per oral) for MWM test

Group11: Test FM7 (50 mg/kg per oral) for EPM test

Group12: Test FM7 (50 mg/kg per oral) for PCA test

Group13: Test FM7 (100 mg/kg per oral) for MWM test

Group14: Test FM7 (100 mg/kg per oral) for EPM test

Group15: Test FM7 (100 mg/kg per oral) for PCA test

### Acute toxicity study

To evaluate the acute toxicity of drugs after a single oral dose, Swiss albino rats fasted for 6 h 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 d after the oral administration of formulation for the acute toxicity. The control group was treated with normal saline (1 ml/kg, i. p.). FM7 was found to be safe [28].

### Experimental method

### Morris water mazes 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 into amaze environment, thus eliminating any olfactory interference. A 28x10 cm rectangular escape platform was constructed of water resistant material and covered with a 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. The animals were given a daily session of three trials per day. Latency time to reach the platform was recorded in each trial. A significant decrease in latency times from that of the first session was considered as successful learning [29]. The group 1, 4, 7, 10, and 13 were used as control, standard (50 mg/kg), standard (100 mg/kg), FM7 (50 mg/kg), and FM7 (100 mg/kg) respectively for MWM test.

### 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 (10 cm×10 cm) and the maze was elevated to a height of 50 cm from the floor. On the first the day, each rat was placed at the end of the open arm, facing away from a central platform [30, 31]. With little modification transfer latency (TL) was taken at 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 placed in any one of the covered places to observe retentive memory of test rat come faster toward that area. TL was recorded on the first day for the each animal. 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 [32]. The group 2, 5, 8, 11, and 14 were used as control, standard (50 mg/kg), standard (100 mg/kg), FM7 (50 mg/kg), and FM7 (100 mg/kg) respectively for EPM test.

### Pole climbing test

Cook's Pole Climbing Apparatus use to study cognitive function, mainly a response to conditioned stimuli during learning & 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 h later, the 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 [33]. The group 3, 6, 9, 12, and 15 were used as control, standard (50 mg/kg), standard (100 mg/kg), FM7 (50 mg/kg), and FM7 (100 mg/kg) respectively for PC test.

**Statistical analysis**

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**

**Water maze test**

The transfer latency on a water maze test 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 50 mg and 100 mg doses of FM7 showed lesser 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 test group found to be much better than standard (*Bacopa monnieri*) per oral route (fig. 1).

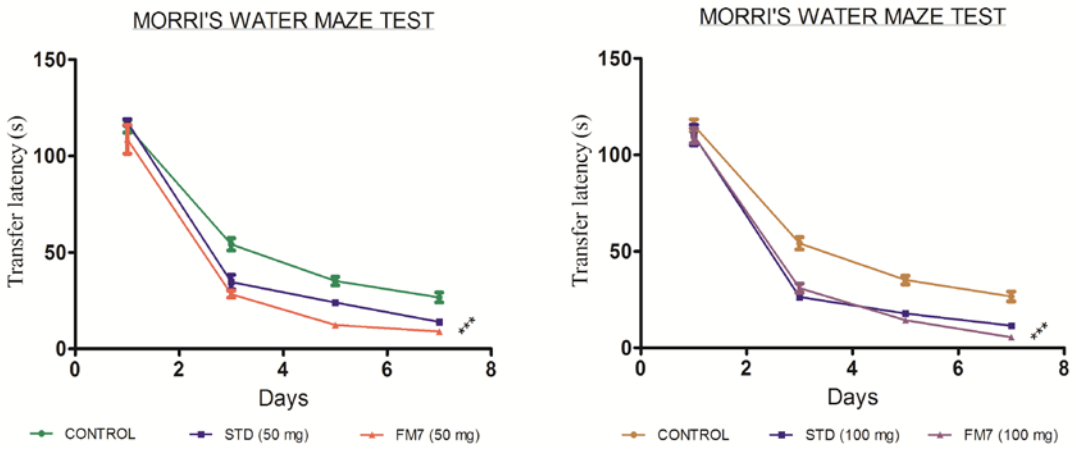


**Fig. 1: The transfer latency of polyphyto formulations FM7 in rat in secs using MWM; ***=p<0.001 vs control**

**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. A little modification has been made that an opposite gender of rat is placed in any one of the covered places to observe retentive memory of all group of animals tested for whether it come faster toward that area where a different sex of animal kept. A significant decrease in transfer latency (TL), the value of retention, indicated improving memory. Test formulation FM7 at 50 mg and 100 mg dose showed a decrease in TL on the second day when compared to control groups indicating significant (p<0.001) memory improvement (fig. 2). FM7 observed better than Standard drug in improving learning and memory.

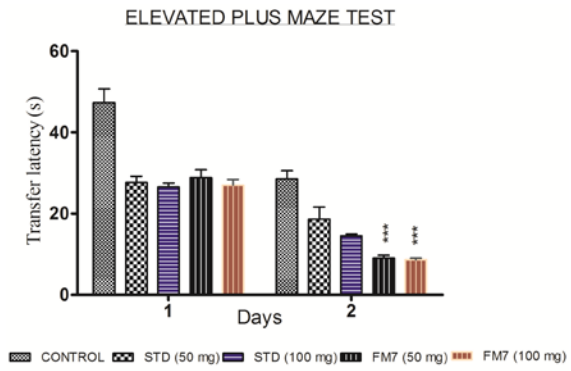


**Fig. 2: Bar Graph of mean transfer latency in elevated plus maze using rat; ***=p<0.001 vs positive and negative control**

**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 FM7 at 50 mg doses revealed a statistically significant (p<0.01) and at 100 mg dose highly significantly (p<0.001) decrease in escape latency in pole climbing test as compared to the control and standard group (Bacopa monnieri) treated group (fig. 3).

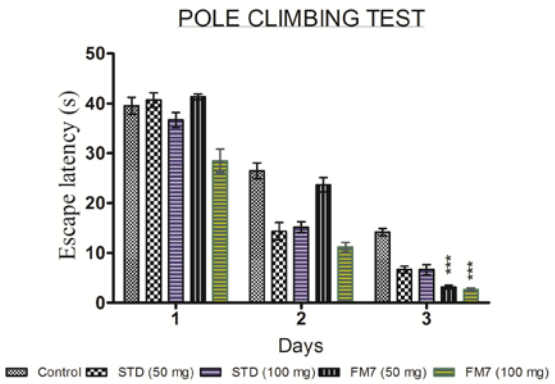


**Fig. 3: Bar Graph of escape latency of rat in secs using pole climbing apparatus; ***=p<0.001 vs positive and negative control**

## DISCUSSION

Poor memory, lower retention, and slow recall are common problems in today's stressful and competitive world. The herbal drug has shown the promising effect in the treatment of memory loss. 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 [34].

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 long-term spatial memory in animals. Passive avoidance behavior is used to examine the long-term memory based on negative reinforcement [35]. Elevated plus maze, Morris water maze (MWM) and Pole Climbing apparatus (PCA) were used to evaluate the effect of learning and memory improvement properties in the rat.

Dose selection for *in vivo* study was made on the basis of acute toxicity studies (300, 1000 and 2000 mg/kg body weight) result and in consideration of estimating the human equivalent dose (HED) for treating the patient. The dose 50 mg/kg in rats, means 8 mg/kg in humans calculated by division method:

mg/kg animal dose ÷ [Km human/Km animal]

The drug dose 50 mg/kg or 100 mg/kg was found to be safe as well as effective for two novel poly photo herbal formulations, so it can use clinically at a dose range of 500 mg to 1000 mg to a 60 kg adult human [20].

In MWM test observed that test group animal treated with 50 mg and 100 mg dose p. o of FM7 showed less transfer latency time (in seconds) during the study and found to be an extremely significant ($p<0.001$) when compared to control groups and much better than *Bacopa monnieri* for its nootropic action. In an Elevated plus maze test nootropic agent FM7 at 50 mg and 100 mg dose showed a decrease in transfer latency on the second day when compared to control and standard groups indicating significant ($p<0.001$) memory improvement. In another test model like pole climbing test revealed that the test group treated with FM7 (50 mg doses p. o) found to be statistically significant ($p<0.001$) compared to the control groups, while FM7 found that comparatively better than standard group.

The herbal drugs like *Convolulus pluricaulis*, *Habiscus rosasinnsis*, *Withania somnifera*, *Terminalia arjuna* and *Emblica officinalis* have been reported good for nervous disorder, example *C. pluricaulis* is generally recommended as a brain tonic [3,4]; *H. rosasinnsis good for* learning and memory [6], *W. somnifera* can be used for neurodegenerative diseases [9]; *T. arjuna* strengthens the nervous system and the reflexes [15]; *E. officinalis* is a natural remedy to improve memory function [19]. The different herbs containing different plant constituent and their action in the brain was different from each other, eg. Withanolide A (WL-A) is a major constituent of Ashwagandha, especially reported for action like normal cortical neurons, predominant axonal outgrowth [8], *T. arjuna* having antioxidant action as it has flavonoids and oligomeric proanthocyanidins [14] reported for neurological action; *Convolvulus pluricaulis* contain glycosides, coumarins, flavonoids and alkaloids where the alkaloid has been recognized as active principle [5] etc. In the present study observed that the combination of traditionally use herbal drugs shown a synergistic effect in improving learning and memory.

## CONCLUSION

The nootropic polyphyto FM7 formulation composed of (*Convolulus pluricaulis 20%*, *Habiscus rosasinnsis 20%*, *Withania somnifera 20%*, *Terminalia arjuna 20%* and *Emblica officinalis 20%*), having promising significant effect for enhancing learning and memory properties on per oral administration on a rat. The herbal plants such as (*Convolulus pluricaulis*, *Habiscus rosasinnsis*, *Withania somnifera*, *Terminalia arjuna)* its action are due to affinity of their phytoconstituents towards nervous system and neuron, *Emblica officinalis* act by anti-inflammatory activity in brain cell. Further research required on phytoconstituent and receptor model to understand the drug action. The FM7 having a synergistic effect can be used for memory loss or dementia and prophylactically use to prevent neuro-degeneration.

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## CONFLICT OF INTERESTS

Declared none

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