The Pharma Innovation

ISSN: 2277- 7695
CODEN Code: PIHNBQ
ZDB-Number: 2663038-2
IC Journal No: 7725

Received: 18-02-2014
Accepted: 19-04-2014

Vol. 3 No. 3. 2014
Online Available at www.thepharmajournal.com

THE PHARMA INNOVATION - JOURNAL

Evaluation of nootropic activity of smrithi: a polyherbal formulation

Jyothi Vadthya [1*], Satyavati. D [2], Pradeep Kumar .C [3], Ch. Maheshwara Reddy [4]

1. Department of Pharmacology, Teegala Krishna Reddy College of Pharmacy, Medbowli, Meerpet, Saroornagar, Hyderabad, Andhra Pradesh, India- 500097. [Email: jyothivadhtya@gmail.com, Tel: +91-8985940435]
2. Principal, Senior Professor, Department of Pharmacology, Sree Dattha Institute of Pharmacy, Sheriguda, Ibrahimpatnam, Ranga Reddy, Andhra Pradesh, India.
   [Email: satyavati.dhulipala@gmail.com, Tel: +91-9966430580]
3. Assistant Professor, Department of pharmacology, Teegala Krishna Reddy College of Pharmacy, Medbowli, Meerpet, Saroornagar, Hyderabad, Andhra Pradesh, India- 500097.
   [Email: pharmacologypradeep@gmail.com, Tel: +91-8121952454]
4. Assistant Professor, Department of pharmacology, Teegala Krishna Reddy College of Pharmacy, Medbowli, Meerpet, Saroornagar, Hyderabad, Andhra Pradesh, India- 500097.
   [Email: pharma.mahesh@gmail.com, Tel: +91-9963031123]

Author for correspondence: Jyothi Vadthya, Email: jyothivadhtya@gmail.com; Tel: +91-8985940435

In the present study 'Smrithi' selected for evaluation of its nootropic activity in different experimental animal models like Scopolamine induced cognitive deficits in mice on Elevated plus maze (EPM) and Morris water maze (MWM) tasks. Smrithi was administered for seven days at the dose of 100 and 200 mg/kg body weight, scopolamine (0.3 mg/kg) was used to induce amnesia, piracetam (50 mg/kg) and Mentat (1and 2 ml/kg) served as reference standards. Smrithi treated animals significantly ($p<0.01$) reduce the Transfer latency on Elevated plus maze and Escape latency in Morris water maze when compared with that of standard nootropic Piracetam, standard polyherbal formulation Mentat and a control group of animals. The probable mechanism of action of Smrithi might be due to its ability to elevate Acetylcholine levels by significant reduction of Acetylcholinesterase enzyme activity in the brain and ultimately improved memory. In the light of above, it may be worthwhile to explore the potential of this formulation in the management of Alzheimer's patients.

Keyword: Smrithi, Nootropics, Mentat, Piracetam, Elevated plus maze, Morris water maze.

1. Introduction

Memory is the ability of an individual to record the event, information and retains them over short or long periods of time. The different conditions such as age, stress and emotion may lead to memory loss, amnesia, anxiety, high blood pressure, dementia to more threat like schizophrenia and Alzheimer's disease [1]. Dementia is the name for progressive loss of memory and other aspects of thinking that are severe enough to interfere with the ability to function in daily activities. Although there are many causes of dementia, including blood vessel disease, drug or alcohol abuse, or other causes of damage to the brain the most common and familiar is Alzheimer's disease [2]. Alzheimer's disease is characterized by a progressive neurodegenerative disease that primarily affects the elderly population, and is estimated to

account for 50-60% dementia cases in persons over 65 years of age [3].

1.1 Neurophysiology of learning and memory

Being able to acquire and retain salient information about the environment to enable survival is a crucial ability ubiquitous to all organisms. Thus, understanding how the brain is able to retain lifelong memories while simultaneously remaining plastic to new information is fundamental to understanding how the brain works. Research into the neurophysiological basis of learning and memory has shown that in addition to synaptic plasticity, the brain can also be modified at the neuronal and global level. Considered to be the basic structural and functional unit of the central nervous system, the synapse can be strengthened or weakened in an activity-dependent manner in long-term potentiation (LTP) and depression (LTD), respectively. Changes in the probability of neurotransmitter release from the pre-synaptic terminal and the density of glutamate receptors and dendritic spines in the post-synaptic terminal have all been implicated in modulating the strength of the transmission at a synapse. At the neuronal level, intrinsic plasticity, which refers to the neuron's ability to adapt its intrinsic propensity to generate action potentials, has been shown to occur in a learning-dependent manner. Modulation of the neuron membrane excitability directly contributes to the likelihood of NMDA receptor activation and induction of synaptic plasticity[4]. Finally, metaplasticity refers to the brain's ability to reset the LTP and LTD induction thresholds globally after a period of stimulation. This prevents previously potentiated synapses from being further excited and reaching saturation, or depressed synapses from being driven into extinction. By maintaining neurons in a dynamic range of activity, metaplasticity ensures that the brain is continually susceptible to plasticity and that learning can always occur. Thus, an understanding of how plasticity at these three levels changes the brain in an experience-dependent manner is crucial to elucidating the neurophysiological basis of learning and memory [5, 6].

Cognitive enhancers are drugs, supplements, nutraceuticals and functional foods that enhance concentration and memory. Based on the above neurophysiology it is learn that memory can be enhanced by inducing long term potentiation in the brain. A number of drugs are available for the treatment of dementia, but clinical evaluation of these drugs has shown the incidence of relapses, side effects, and drug interactions. This has been the rationale for the development of new nootropics, which includes herbal drugs. Indian medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders including depression, Alzheimer's disease. Herbal products are often perceived as safe because they are "natural". In recent years, there is increased research on traditional Ayurvedic herbal medicines on the basis of their known effectiveness in the treatment of ailments for which they have been traditionally applied. Herbal medicine is a major component in all traditional medicine systems, and a common element in Siddha, Ayurvedic, Homeopathic, Naturopathic, Traditional Chinese medicine, and Native American medicine.

1.2 Polyherbal formulations showing nootropic activity

There are polyherbal formulations available in the market showing nootropic activity. Some of them are: *BR-16A (Mentat)* tablets, Brahmi Plus Capsules, Brahmivita Granules, Braintab Tablets, Saraswatharishtham Syrup, Gingcopa Tablets, Mind Power Tablets, Bramhi Grita Tablets. It has been observed that the main ingredients of these preparations include: *Withania somnifera, Ginkgo biloba, Ocimum sanctum, Asparagus racemosus Willd., Emblica officinalis Gaertn., Panax ginseng, Nardostachys jatamansi DC., Evolvulus alsinoides* Linn., *Valeriana jatamansi Jones, Acorus calamus* Linn., *Tinospora cordifolia Miers, Celastrus paniculatus Willd., Saussurealappa C.B. Clarke, Terminalia chebula Retz., Terminalia bellirica* Roxb., *Sasakurinensis Makino et Sibata, Pinusdensiflora Sieb.et Zucc, Tribulus terrestris Linn and Piper nigrum* [7].

The aim of the present study is to evaluate the nootropic activity of Smrithi, a polyherbal

formulation. Each 500 mg of Smrithi contains: *Bacopa monniera* 100 mg, *Hydrocotyle asiatica*-100 mg, *Acorus calamus*-100 mg, *Asparagus racemosus* 100 mg, *Emblica officinalis* 100 mg. The data obtained with Smrithi is compared with that of Piracetam, a nootropic agent, Mentat, a standard polyherbal formulation and control group of animals.

2. Materials and Methods

2.1 Drugs and chemicals

Smrithi (IMIS pharmaceuticals private limited)
Mentat (Himalaya herbal healthcare)
Piracetam (Micro labs limited)
Scopolamine (Cadila healthcare limited)

2.2 Drug treatment

To evaluate nootropic activity, the Smrithi is suspended in 2% Gum Acacia in doses 100 and 200 mg/kg body weight intraperitoneally. The doses were fixed, based on an acute toxicity study on mice.

2.3 Animals

Albino Swiss mice of either sex (18-30 grams) were used in the present study. Animals were housed in plastic cages at an ambient temperature (25±2 ^{o}C) and relative humidity of 45-55%. A 12:12 hour light-dark cycle was followed. The animals had free access feed with balanced rodent pellet diet and water *ad libitum* throughout the experimental period. The mice were acclimatized to laboratory conditions for 10 days before behavioral studies. All the readings were taken during the same time of the day i.e.; between 9 AM to 12 AM. The Institutional Animal Ethics Committee (IAEC) had approved the experimental protocol, and care of animals was taken as per the guidelines of CPCSEA no GNIP (TKR)/CPCSEA/IAEC/2013/11, Department of Animal welfare and Government of India.

2.4 Acute toxicity test

The procedure was followed by using OECD guidelines (Organization of economic cooperation and development) 423 (Acute toxic class method).Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the best substance. The acute toxic class method is a stepwise procedure with these animals of a single sex per step [8]. The method used to define doses (30, 100, 300, 1000, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of the chemical, which cause acute toxicity.

Mice weighing about 20-25 grams were selected and divided into groups of six animals each for acute toxic studies. Smrithi is suspended in 2% gum acacia and administered to mice in the doses as mentioned above. The animals were observed for 3-4 hrs after administration of the drug and up to 14 days to assess toxicity.

The mice were observed before and after the administration of the sample for behavioural, neurological and autonomic activities. The onset and signs of toxicity, the overnight mortality were recorded as it indicates toxicity. It is observed that there is no mortality up to 2000 mg/kg body weight and there are no signs of toxicity, 100 mg/kg and 200 mg/kg doses of Smrithi were selected to carry out nootropic activity.

In the present study the nootropic activity of 'Smrithi' is evaluated using:

- Scopolamine induced cognitive deficits in mice on elevated plus maze.
- Scopolamine induced cognitive deficits in mice on Morris water maze.

2.5 Effect of Smrithi on scopolamine induced cognitive deficits in mice on elevated plus maze

The elevated plus-maze was introduced by Pillow *et al*., 1985 [9] for rats and by Lister *et al*., 1987 [10] for mice is based on the apparent aversion of rodents to open and high spaces. The plus-maze consists of two open arms & two closed arms of 50 x 10 x 40 cm dimensions facing each other with an open roof. Two open arms are opposite to each other and maze elevated at 50 cm height.

Mice were divided into seven groups of 6 animals each
Group -I: - Control (2% gum acacia).
Group-II: - Scopolamine 0.3 mg/kg.

Group-III: - Scopolamine, 0.3 mg/kg +Smrithi, 100mg/kg.
Group- IV: - Scopolamine, 0.3 mg/kg+ Smrithi, 200 mg/kg.
Group- V: - Scopolamine, 0.3 mg/kg + Mentat, 1 ml/kg.
Group- VI: - Scopolamine, 0.3 mg/kg + Mentat, 2 ml/kg.
Group- VII: - Scopolamine, 0.3 mg/kg + Piracetam, 50 mg/kg

Scopolamine is administered for a period of seven days to induce amnesia. Smrithi, scopolamine and piracetam were given intraperitoneally, Mentat administered as orally for seven days to evaluate Nootropic activity. The mice were placed individually at the end of open arm of the elevated plus maze facing away from the center. The time taken by the mouse to move into the enclosed arm was noted as transfer latency. On the first day mice were allowed to explore the maze for 20 seconds after recording the Transfer latency (TL) and returned to home cages. TL measured on 1st (before drug administration) and 2nd day (after drug administration) served as parameters for acquisition and retrieval respectively. The TL was noted after 30 minutes of administration of the drug given intraperitoneally and TL time recorded after 60 minutes in case drug is administered orally. TL was recorded for seven days and expressed as Mean±SEM. TL is measured on the 1st and 2nd day for all mice and tabulated. Results were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's test and presented in the Table 1.

2.6 Effect of Smrithi on scopolamine induced cognitive deficits in mice on morris water maze [11]

Morris water maze task was developed where rodents learn to swim in a water tank to find an escape platform hidden under the water (Morr's, 1984) [12].The Morris water maze consists of large circular tank 1.0-1.2 m in diameter and 0.2-0.4 m height. The pool is filled with water (25 °C) and rendered opaque by the addition of a small quantity of titanium dioxide suspension. Four quadrants equally distributed along the perimeter of the tank served as starting locations and an escape platform (5 cm width) was located in the center (Achliya *et al*., 2004) [13].

Mice were divided into eight groups of 6 animals each.
Group –I: - Control (5% gum acacia suspension).
Group –II: - Smrithi, 100 mg/kg.
Group –III: - Standard Polyherbal Formulation (Mentat, 1 ml/kg)
Group -IV: - Scopolamine, 0.3 mg/kg
Group -V: - Scopalamine, 0.3 mg/kg + smrithi, 100 mg/kg.
Group -VI: - Scopalamine, 0.3 mg/kg + smrithi, 200 mg/kg.
Group-VII: - Scopolamine, 0.3 mg/kg + mentat, 1 ml/kg.
Group- VIII: - Scopolamine, 0.3 mg/kg + piracetam, 50 mg/kg.

Smrithi (2% gum acacia suspension), scopolamine and Piracetam were given intraperitoneally, Mentat administered orally. The mice were explored on MWM and allowed 90 sec to find the platform. Animals received 4 trials per day with 5 min inter-trial interval for 8 days until the performance was stable and the latency to find the platform was low (<10 sec). The drugs were administered 30 min prior to the first trial daily. Time to find the hidden platform is considered as escape latency. The platform in the water maze was kept at the same position throughout the test to assess the effect of test compounds on spatial reference memory. Escape latencies for each animal in all groups for seven days were noted. The mean±SEM for each group was calculated and reported in the Table 2. The time required to reach the hidden platform in the water maze (Escape latency) is illustrated in figure. Results were analyzed using one-way analysis of variance (ANOVA) followed by Dennett's test.

3. Results and Discussion

Nootropic drugs are the class of psychotropic drugs that enhances learning, acquisition and reverse learning impairments in experimental

animals, and are likely to be clinically effective in memory dysfunctions and also improve memory in the absence of cognitive deficit [14, 15]. Many experimental models are currently available for the evaluation of agents that affect learning and memory process. Mazes are traditional tools in assessing learning and memory performance in laboratory animals. Originally designed to evaluate the antianxiety agents, elevated plus maze also been recently extended to measure the spatial long-term memory in animal [16, 17].

3.1 Elevated plus maze

The Transfer latency (TL) on the 2nd day was found to be shorter than that on the 1st day. Scopolamine (0.3 mg/kg), an anticholinergic agent, injected 30 min prior to the 1st trial, produced a significant elevation of TL on 1st day. Scopolamine; through delayed the TL on 2nd day, the effect was not statistically significant. Smrithi, Mentat, Piracetam when administered concomitantly with scopolamine (0.3 mg/kg), the transfer latency was shorted and the results were statistically found significant on 1st and 2nd day (Table 1, figure 1).

3.2 Morris water maze

There was a significance difference in Escape Latency (EL) between smrithi (100 mg/kg) and vehicle treated animals on days 3, 4, 5, 6&7. The mice treated with Mentat(1 ml/kg) showed significant decrease in escape latency on days 2, 3, 4, 5, 6 & 7 as compared with the control group. The mice underwent combined administration with scopolamine (0.3 mg/kg) showed a significant increase in escape latency on days 4, 5, 6 & 7 as compared with the control group (Table 2, figure 2). Group V (scopolamine 0.3 mg/kg + smrithi 100 mg/kg) showed a significant decrease in EL on days 2, 4, 5, 6 & 7, group VI (scopolamine 0.3 mg/kg+ smrithi 200 mg/kg) showed a significant decrease in EL on days 2-6 & 7, Group VII (scopolamine 0.3 mg/kg+ Mentat 1 ml/kg) showed a significant decrease in EL on days 3-6 & 7, Group VIII (scopolamine 0.3 mg/kg + piracetam 50 mg/kg) showed a significant decrease in EL on days 3, 4, 5 & 6 as compared with group 4(scopolamine 0.3 mg/kg) (Table 2, figure 3).

4. Conclusion

In the present study, based on the findings of the results, Smrithi enhanced significantly the memory in normal mice in EPM and MWM, which indicates that Smrithi shown to possess nootropic effect. The precise mechanism by which Smrithi elicits its nootropic effect is not known.

Table 1: Effect of drugs (alone or in combination) on transfer latency on elevated plus maze

Group No.	Treatment, mg/kg	Transfer Latency in (TL) seconds (Mean ± SE)	
		1st day	2nd day
1	Control (2% gum acacia)	32.57±1.757	25.57±2.759
2	Scopolamine (0.3 mg/kg)	62.42±4.556**	30.286±2.87
3	Scopolamine, 0.3 mg/kg + sample, 100 mg/kg	14.00±2.478**	10.57±1.395**
4	Scopolamine, 0.3 mg/kg + sample, 200 mg/kg	12.14±2.017**	10.28±1.539**
5	Scopolamine, 0.3 mg/kg + Mentat, 1 ml/kg	15.57±2.680**	13.429±2.75**
6	Scopolamine, 0.3 mg/kg + Mentat, 2 ml/kg	15.28±2.17**	13.143±2.16**
7	Scopolamine, 0.3 mg/kg + Piracetam, 50 mg/kg	13.571±2.148**	12.429±2.057**

p<0.05 and **p<0.01 control Vs treated groups using one way ANOVA followed by Dunnett's test.

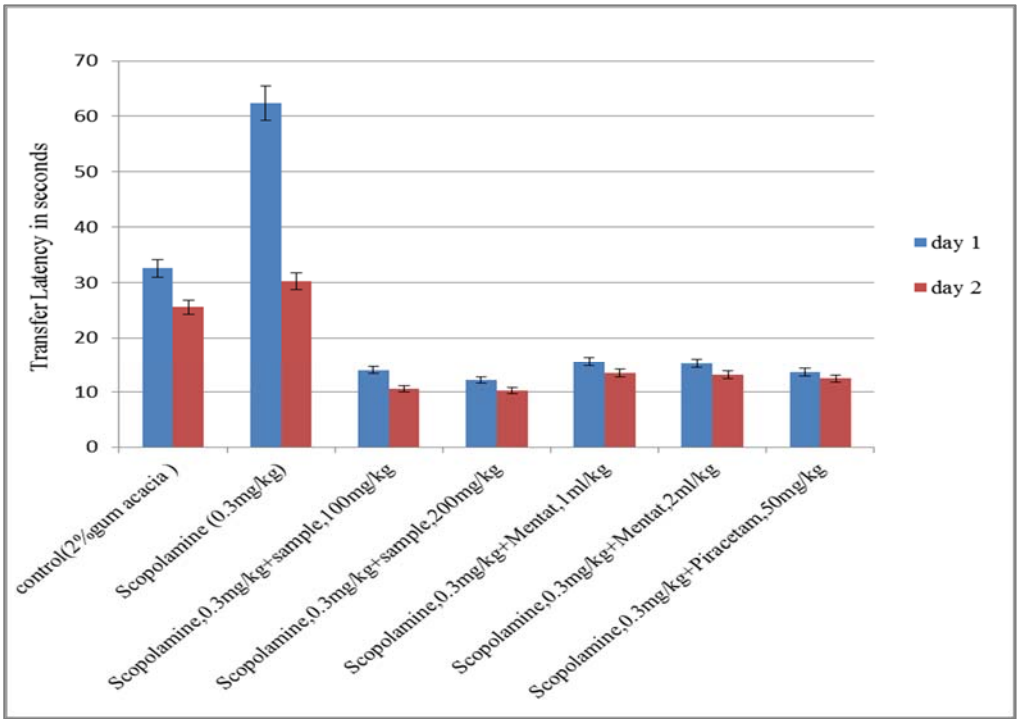

Fig 1: Effect of test drugs on spatial memory in elevated plus-maze
*p<0.05 and **p<0.01 control Vs treated groups using one way ANOVA followed by Dunnett's test

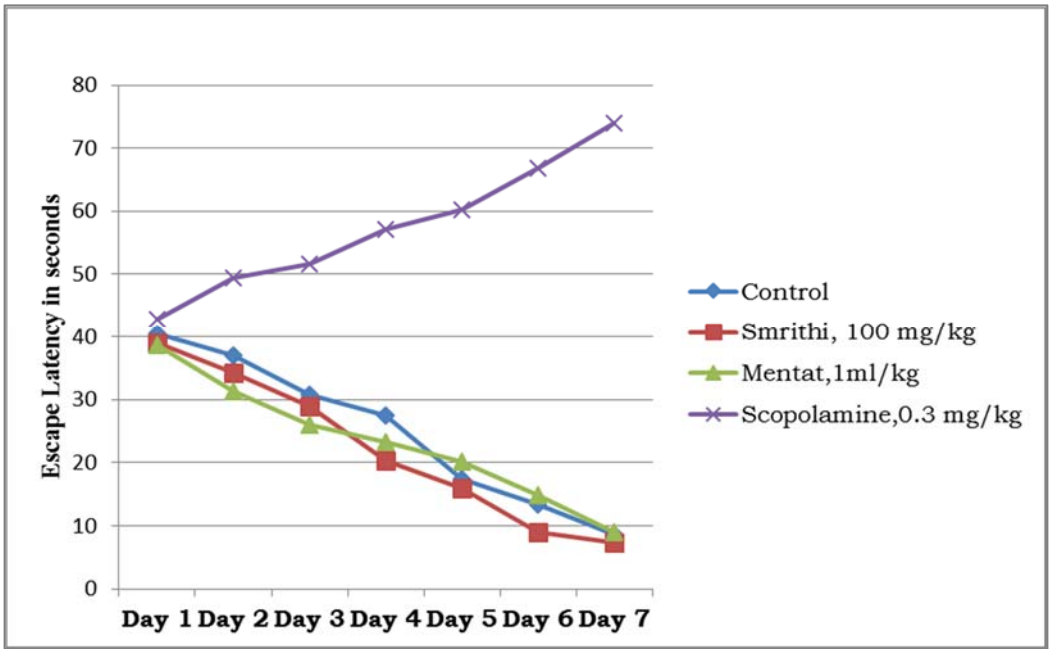

Fig 2: Effect of drugs (individual) on spatial memory in Morris water maze

Table 2: Effect of Smrithi on scopolamine induced cognitive deficits in mice on morris water maze

Group	Treatment, mg/kg	Escape Latency in seconds (MEAN ± SE)Values)						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
I	Control	40.4±2.159	37.0±1.99	30.8±1.281	27.4±1.281	17.4±1.281	13.4±1.66	8.6±1.030
II	Smrithi, 100	39.0±2.025	34.6±1.749	29.0±0.836**	20.4±0.748**	16.0±1.703**	9.02±2.289**	7.4±0.6782**
III	Mentat,1ml/kg	38.6±1.249	31.4±1.600**	26.00±1.095**	23.2±1.095**	20.2±0.9695**	14.8±1.020**	9.0±1.140**
IV	Scopolamine,0.3	42.8±1.744	49.4±3.341	51.6±3.995	57.2±2.577*	60.2±3.865**	66.8±3.426**	74.00±3.209**
V	Scopalamine,0.3 +smrithi 100	40.2±2.874	23.4±1.806**	27.2±5.553	17.4±3.311**	16.6±3.356**	12.8±2.223**	11.2±1.463**
VI	Scopalamine,0.3 +smrithi 200	39.4±1.806	28.0±2.345**	21.2±3.826**	15.0±2.345**	14.0±2.345**	9.6±0.8713**	6.4±0.400**
VII	Scopolamine,0.3 +mentat 1ml/kg	39.8±4.66	28.4±3.389	24.8±3.023**	23.4±2.482**	21.6±2.088**	15.6±1.368**	11.8±1.855**
VIII	Scopolamine,0.3 +piracetam 50	36.6±3.945	27.6±3.855	24.10±4.561	17.4±4.802**	17.2±3.813**	13.0±2.205**	8.6±1.939**

p<0.05 and **p<0.01 control Vs treated groups using one way ANOVA followed by Dunnett's test

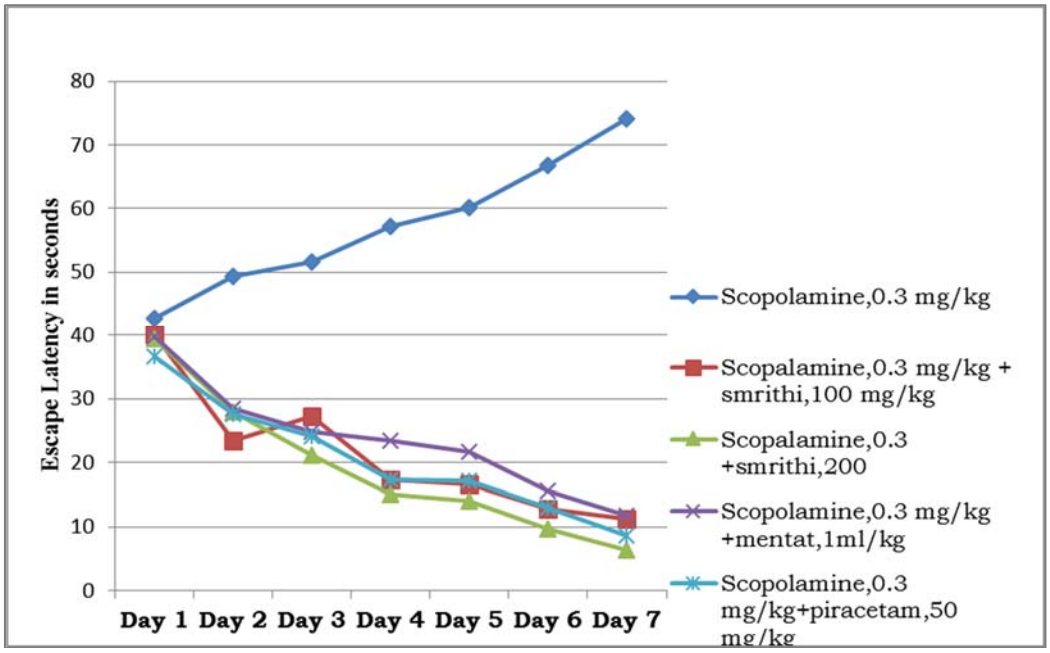

Fig 3: Effect of drugs (alone or combination) on spatial memory in Morris water maze
*p<0.05 and **p<0.01 control Vs treated groups using one way ANOVA followed by Dunnett's test.

5. Acknowledgement
The authors like to thank the management members of Teegala Krishna Reddy College of Pharmacy, Hyderabad. Special thanks to IMIS Pharmaceuticals private limited for supplying polyherbal formulation.

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