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ORIGINAL RESEARCH ARTICLE EXPERIMENTAL

Effect of combination of *Phyllanthus emblica*, *Tinospora cordifolia*, and *Ocimum sanctum* on spatial learning and memory in rats

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ABSTRACT

Background: There has been a steady rise in number of patients suffering from dementia including dementia associated with Alzheimer's disease. Effective treatment of Alzheimer's disease dementia is an unmet medical need. **Objective:** To evaluate effects of formulation containing combination of *Phyllanthus emblica (Pe)* and *Tinospora cordifolia (Tc)* with and without *Ocimum sanctum (Os)* on learning and memory performance of normal and memory impaired rats in complex maze and compare with effects of *Tinospora cordifolia* and *Phyllanthus emblica* alone. **Materials and Methods:** Wistar rats; either sex (100–150 g) were divided in seven groups Control, Piracetam, Rivastigmine, *Tc*, *Pe*, Formulation 1 (*Tc* + *Pe*), and Formulation 2 (*Tc* + *Pe* + *Os*).The study was divided in four parts: In part 1 memory enhancement was tested in normal rats. In part 2, 3, and 4 the effects of drugs were tested in Scopolamine-, Diazepam-, and Cyclosporine-induced amnesia. Hebb–Williams maze was used to test for learning and memory. Time required to trace food and number of errors in maze were noted. **Results:** In normal rats, all test drugs showed significant reduction in time required to trace the food and number of errors after 24 h compared with vehicle control. Formulations 1 and 2 reduced the time required to trace food and number of errors and the results were comparable with positive control groups and comparators *Tc* and *Pe*. Formulations 1 and 2 reversed amnesia produced by Scopolamine, Diazepam, and Cyclosporine when compared with vehicle control and showed comparable results with those of positive control groups and comparators *Tc* and *Pe*. **Conclusion:** Formulations 1 and 2 demonstrated nootropic activity and both the formulations showed comparable nootropic activity with that of *Tc* and *Pe* alone.

Key words: Alzheimer's disease, Dementia, Hebb-William's maze, *Medhya, Rasayana, Ocimum sanctum, Phyllanthus emblica, Tinospora cordifolia*

INTRODUCTION

There has been a steady rise in the number of patients suffering from dementia all over the world. An estimated 35.6 million people worldwide were living with dementia in 2010. This number is estimated to nearly double every 20 years, to 65.7 million in 2030 and 115.4 million in 2050.[1] There are various causes of memory loss (dementia), which include aging, Alzheimer's and other neurodegenerative diseases, Parkinson's disease, ischemic brain damage, head injuries, alcoholism, drug intoxication, vitamin deficiencies, chronic infections, and psychiatric and psychological disorders; the most common being Alzheimer's disease.[2] Once set in and especially if associated with neuronal loss, dementia is difficult to cure and the available therapies such as piracetam and newer anticholinesterases are far from satisfactory. Hence research is ongoing all over the world to explore neurobiology of learning and memory and to investigate the agents that can prevent progression of memory loss or improve the existing capacity of brain for learning and memory.

As plants have always been the major source of medicines, we decided to evaluate plant drugs from Ayurveda. Ayurveda recommends certain drugs, which are classified as *Rasayana*. They are claimed to have memory-enhancing and antiaging

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Received: 14-Nov-2013
Revised : 20-Jan-2014
Accepted: 27-Jan-2014

Access this article online	
Quick Response Code:	Website: www.jaim.in
	DOI: 10.4103/0975-9476.146564

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properties.[3,4] *Phyllanthus emblica* (*Pe*, Amala), *Tinospora cordifolia* (*Tc*, Gulavela) and *Ocimum sanctum* (*Os*, Tulsi) are the examples of *rasayana* drugs.[5] Ayurveda advocates use of these plants for variety of conditions that are seemingly different in their pathophysiology, for example, liver diseases, inflammatory conditions like arthritis, diabetes mellitus, acid-peptic disorders.[6] Their properties described in Ayurvedic textbooks have been validated by researchers from modern medicine and are described in the reviews available in the literature. For example, *Pe* and *Tc* have been evaluated for their effects on learning and memory performances and have shown promising results.[7,8] *Os* has also shown memory enhancing properties.[9,10]

They are used as monotherapy or in combination as seen from number of marketed Ayurvedic formulations for the neuropsychiatric disorders.[5] However, no study has been done to evaluate effects of combination of these drugs on learning and memory. Therefore, the present study was undertaken to evaluate effects of *Pe* and *Tc* used in combination on learning and memory performance compared in normal rats and rats with memory impairment induced by scopolamine, diazepam and cyclosporine. As recommended in Ayurvedic literature, *Os* is administered either as fresh juice of leaves or is used to coat other plant drug particles (process described as *Bhavana*).[6] Also it is claimed that *bhavana samskara* (process) potentiates the efficacy of the coated drug/s.[6] It was decided therefore to coat the formulation of *Tc* and *Pe* with *Os* and to explore whether *bhavana* of *Os* potentiates effect of the combination on learning and memory.

MATERIALS AND METHODS

Permission from the Institutional Animal Ethics Committee was obtained prior to initiation of study.

Animals

A total of 156 wistar rats of either sex, weighing between 100 and 150 g, were procured from the Institutional Centre for Animal Studies. Throughout the study period, the rats were maintained in accordance with laboratory guidelines for the care and use of laboratory animals laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Four rats were housed in each cage during the study period and were air conditioned with 12-15 filtered fresh air changes, at temperature 22 ± 3°C, relative humidity 30-70%. Rodent feed in form of pellets was provided. Water was provided *ad libitum*.

Test drugs

The aqueous extracts of *Tc* and *Pe* were procured from Natural Remedies Pvt. Ltd., Bangalore, India along with their authentication certification. These were prepared from the dried stem of *Tc* and dried fruit of *Pe*, respectively, and were available in powder form as dried aqueous extracts. The doses of *Tc* and *Pe* were selected as advocated in Ayurveda and are expressed in terms of the crude powder of the dried stem/fruit, respectively.[6] The combinations were prepared at Shri Dhootpapeshwar Ltd., Panvel, Mumbai. Dried stem of *Tc* and dried fruits of *Pe* were pulverized. A total of 2 kg of *Tc* and 6 kg of *Pe* were mixed. Juice obtained from 1 kg fresh leaves of *Os* was added and mixed thoroughly. The combination of *Tc* + *Pe* contained dried aqueous extract of these plants in a ratio of 1:3, respectively, and was designated as H1. The same combination given coating with juice of fresh leaves of *Os* was designated as H2. The extractive values of both the formulations were 24.5%. All these drugs were given by gavage feeding.

Rivastigmine (Sun Pharmaceutical Industries Ltd., Ahmednagar, Maharashtra) and Piracetam (UCB India Pvt. Ltd., Vapi, Gujarat) were used as positive controls. Rivastigmaine was used only in normal animals and in rats with scopolamine-induced amnesia. Distilled water served as the vehicle.

Amnestic insults

The amnesic agents were scopolamine (Sigma-Aldrich, USA as a hydrobromide salt), diazepam (Ranbaxy Ltd, India), and cyclosporine (Novartis India Ltd).

Experimental protocol

The study was conducted in the following parts:

1. Part 1: Effects of test drugs on learning and memory performances in normal animals
2. Part 2: Effects of the test drugs on learning and memory performances in rats with scopolamine-induced memory impairment
3. Part 3: Effects of the test drugs on learning and memory performances in rats with diazepam-induced memory impairment
4. Part 4: Effects of the test drugs on learning and memory performances in rats with cyclosporine-induced memory impairment.

In each part, the rats were divided into seven experimental groups, each comprising of six rats, which received specific drugs as follows: Group 1(vehicle control): distilled water (1 ml p.o. for 15 days); Group 2 (positive control 1) distilled water for first 7 days followed by piracetam (200 mg/kg/day ip for 8 days; Group 3 (positive control 2): rivastigmine (2.4 mg/kg day p.o. for 15 days); Group 4: *Pe* (300mg/kg/day p.o. for 15 days); Group 5: *Tc* (100 mg/kg/day p.o. for 15 days); Group 6: H1 (Pe + Tc:

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300 + 100 mg/kg/day for 15 days) and Group 7: H2 (*Pe* + *Tc* with coating of *Os*: 300 + 100 mg/kg/day for 15 days).

On 15th day, the rats received the last dose of test drug/distilled water. Ninety minutes later, they were subjected to the complex maze test to evaluate their learning performance as described below. Memory was tested 24 h later. The experiment was conducted at 8 pm on 15th and 16th day. To induce memory impairment with either scopolamine (3 mg/kg) or diazepam (1 mg/kg), rats received respective amnesic agents intraperitoneally as a single dose, 45 min after the last dose of the test drugs/distilled water.[11,12] The complex maze test was done 45 min later for learning and was repeated after 24 h. Cyclosporine (25 mg/kg/day) was given intraperitoneally alternating with distilled water injections for five doses.[6,7] From Day 11 onwards, the test drug/distilled water was given orally for next 15 days. On 15th day, 90 min after the last dose of test drug/distilled water complex maze test was carried out to evaluate learning. Memory was tested 24 h later.

Assessment of learning and memory

For this Hebb-William Maze was used.[12] It is an "incentive-based exteroceptive behavioral test" based on spatial working memory of the animal. It consists of a large platform with a series of vertical walls. In this model, the animal learns the path to trace the food-the incentive. With training it requires less time and commits less number of errors to reach the food.[12]

On the day of assessment of learning performance, the rats were kept fasting for 18 h and allowed access to food for only 1 h at the end of the day's training. A rat was placed in the entry chamber and allowed to reach the food chamber. In each trial, the rat had to learn to eliminate inappropriate navigational movements to derive the shortest path to the goal box, in which there was a food reward. An inter-trial interval (30-45 s) was used to clean the maze and allow the rat to finish eating the food. An error was considered if the rat enters the wrong alley. The time taken for each rat to reach the food chamber was noted with the help of a stop watch and the number of errors done while tracing the food was counted. The maximum time for which each animal was allowed to be in the chamber per trial was 300 s. Five such trials were given per rat on the day of assessment of learning. Average number of errors and time taken to trace the food in these five training sessions were computed. This performance indicates learning. Performance in repeat trial 24 h later reflects retention of learning, that is, memory.

Statistical analysis

The data was expressed as mean ± SD for each group. The results of training phase were compared with the retention phase for each group using paired *t*-test. In addition, inter-group comparison was done using one-way analysis of variance (ANOVA) followed by *post hoc* test. The level of significance was at $P < 0.05$.

RESULTS

Effects on learning and memory performances in normal animals

The time taken to trace the food on Day 15 was comparable in all the groups [Table 1]. A significant reduction in time was observed for the rats given test drugs when tested 24 h later. The extent of reduction found on Day 16 was comparable in all the groups. The rats given H2 formulation took least time to trace the food. When the values on Day 16 were compared with those found for vehicle on Day 16, only Rivastigmine-treated rats showed significant reduction in time ($P < 0.001$). For other groups, a trend toward reduction was observed but the difference was not significant compared with the values of the vehicle control. The values observed for the combinations, *H1* and *H2*, were comparable to those of individual agents.

The number of errors done on Day 15 was comparable in all the groups. In all the test groups, there was significant reduction in number of errors done on Day 16; the least errors were observed in the rats receiving H2. The errors committed by the animals receiving test drugs were significantly less than observed in the vehicle treated group ($P < 0.01$). The number of errors observed in the combination treated groups was comparable to the positive controls (Piracetam and Rivastigmine) as well as the comparators *Tc* and *Pe*.

Effects on learning and memory performances in rats with scopolamine-induced memory impairment

The time taken to trace the food on Day 15 by the animals given scopolamine (Table 1; 158.83 ± 12.50 min) was significantly more compared with the normal animals (Table 2; 136.33 ± 25.52 s). The values in the former group were comparable to the values observed for rats given test drugs prior to scopolamine insult. All the treated groups exhibited significant reduction after repeat testing 24 h later. All these groups had comparable values, which were significantly less than that observed for vehicle control ($P < 0.001$).

The errors observed in the scopolamine treated animals (5.5 ± 0.84) were significantly highcompared to normal rats (4.17 ± 0.41) on Day 15. In all the treated groups given scopolamine the number of errors was comparable to the vehicle control group on Day 15. However, when test was done after 24 h, a significant reduction in number of errors was observed in the treated

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groups as compared to the readings on Day 15. These errors on Day 16 were also significantly less compared with values observed for vehicle group. The number of errors in the combination groups was comparable to the positive controls, (Piracetam and Rivastigmine) and single plant drug-*Tc* and *Pe*.

Effects on learning and memory performances in rats with diazepam-induced memory impairment

The time taken to trace the food on Day 15 by the animals given diazepam (Table 3; 162.5 ± 17.28 s) was significantly more compared with the normal animals (Table 1; 136.33 ± 25.52 s). This time was comparable in all the groups of animals receiving allocated treatment and given diazepam. Though the rats from control group did not show any change in the time taken to trace the food on Days 15 and 16, all the drug treated groups showed a significant reduction in time after 24 h. This time noted on Day 16 was also significantly lesser than the vehicle control. However, there was no significant difference among the treated groups.

The number of errors observed was comparable for normal animals (4.17 ± 0.41) and those injected diazepam (5.83 ± 1.47) on Day 15. The drug treated groups given diazepam also showed comparable number of errors on the same day. On Day 16, the vehicle treated control animals injected diazepam committed errors comparable to Day 15. In the drug treated group, however, a significant reduction in number of errors was observed on Day 16 compared with their basal records as well as diazepam control group. The values among drug treated group did not differ significantly from each other.

Table 1: Effects on time taken to trace the food and number of errors committed in rats with Scopolamine induced memory impairment

Rats treated with vehicle/drug	Time taken (s)		Number of errors	
	Day 15 (learning)	Day 16 (memory)	Day 15 (learning)	Day 16 (memory)
Distilled water (15 days)	158.83±12.50	158.33±14.21	5.5±0.84	5±0.89
Rivastigmine (2.4 mg/kg for 15 days)	153.±13.42	100.67*$^{\#\#\#}$±13.11	5.67±0.82	3.5*$^{\#}$±0.55
Distilled water (7 days) followed by Piracetam (200 mg/kg for 8 days)	151.83±13.89	101.17**$^{\#\#\#}$±17.38	5.5±1.05	2.83*$^{\#\#}$±1.17
Tc (100 mg/kg for 15 days)	149.67±12.97	92.33*****$^{\#\#}$± 11.81	4.83±0.75	3*$^{\#\#}$±0.63
Pe (300 mg/kg for 15 days)	147.5±15.02	92.33*****$^{\#\#\#}$±13.56	5.33±0.82	3.17*$^{\#\#\#}$ ± 0.98
H1 (*Tc*+*Pe*) (400 mg/kg for 15 days)	140.17±12.16	90.17*****$^{\#\#\#}$±10.21	4.67±0.82	2.67*****$^{\#}$±0.82
H2 (*Tc*+*Pe*+*Os*) (400 mg/kg for 15 days)	138.33±15.85	88.33*****$^{\#\#\#}$±8.26	4.5±0.84	2.5*****$^{\#\#}$±0.55

N=6/group. All values represent mean±SD; Paired *t* test; *P<0.01, **P<0.001. ANOVA followed by *post hoc* Tukey's test: $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001

Table 2: Effects on time taken to trace the food and number of errors committed in normal rats

Normal rats treated with vehicle/drug	Time taken (s)		Number of errors	
	Day 15 (learning)	Day 16 (memory)	Day 15 (learning)	Day 16 (memory)
Distilled water (15 days)	136.33±25.52	101.5±24.21	4.17±0.41	3.83±0.98
Rivastigmine (2.4 mg/kg for 15 days)	132.83±21.02	45.17****$^{\#\#\#}$±15.74	3.67±0.52	2.33***$^{\#}$±0.52
Distilled water (7 days) followed by Piracetam (200 mg/kg for 8 days)	136.33±23.53	73.17**±32.89	4.17±0.75	2*****$^{\#\#}$±0.63
Tc (100 mg/kg for 15 days)	127.67±28.6	71.5**±20.22	3.5±0.55	2.17***$^{\#}$±0.41
Pe (300 mg/kg for 15 days)	121.33±16.74	71*±25.91	3.33±0.52	2.17***$^{\#}$±0.41
H1 (*Tc*+*Pe*) (400 mg/kg for 15 days)	128.67±12.68	64.17***±16.15	3.67±0.52	2*****$^{\#\#}$±0.63
H2 (*Tc*+*Pe*+*Os*) (400 mg/kg for 15 days)	116.5±13.05	63.17**±20.38	3.83±0.75	1.83*****$^{\#\#}$±0.75

N=6/group. All values represent mean±SD; Paired *t* test; *P<0.05, **P<0.01, ***P<0.001. ANOVA followed by *post hoc* Tukey's test: $^{\#}P$<0.01, $^{\#\#}P$<0.001

Table 3: Effects on time taken to trace the food and number of errors committed in rats with Diazepam induced memory impairment

Rats treated with vehicle/drug	Time taken (s)		Number of errors	
	Day 15 (learning)	Day 16 (memory)	Day 15 (learning)	Day 16 (memory)
Distilled water (15 days)	162.5±17.28	163.83±16.17	5.83±1.47	5.67±1.21
Distilled water (7 days) followed by Piracetam (200 mg/kg for 8 days)	155±14.85	98.5*****$^{\#\#\#}$±7.53	5.83±1.17	3.5***$^{\#}$±1.05
Tc (100 mg/kg for 15 days)	142.83±20.97	93.33*****$^{\#\#\#}$±11.66	5.83±0.75	3.33*$^{\#}$±1.21
Pe (300 mg/kg for 15 days)	143.83±14.05	86.5*****$^{\#\#\#}$± 14.60	5.33±0.52	3.33***$^{\#}$±1.03
H1 (*Tc*+*Pe*) (400 mg/kg for 15 days)	139.33±18.27	84.33*****$^{\#\#\#}$±12.23	5.33±1.03	3.17*****$^{\#\#}$±1.17
H2 (*Tc*+*Pe*+*Os*) (400 mg/kg for 15 days)	133.17±17.83	83.33*****$^{\#\#\#}$±9.64	4.83±0.75	2.67*****$^{\#\#\#}$±0.82

N=6/group. All values represent mean±SD; Paired *t* test; *P<0.01, **P<0.001; ANOVA followed by *post hoc* Tukey's test: $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001

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Effects of the test drugs on learning and memory performances in rats with cyclosporine-induced memory impairment

Cyclosporine treated rats showed a significant increase in the time to trace food on the first day of the test (Table 4; 170.17 ± 18.02, $P < 0.05$ *vs* normal). All the drug treated groups given cyclosporine showed values comparable to vehicle control on the first day of the test but there was a significant reduction in time taken to trace the food next day compared with the basal values. Significant difference was also observed compared with vehicle control except for those treated with piracetam. In the latter, no significant difference was observed when compared with vehicle control. The values in the plant drug treated groups were significantly less than those observed for piracetam, the positive control. There was no difference among individual plant drugs or their combinations.

The number of errors done was comparable for normal animals (4.17 ± 0.41) and those injected cyclosporine (6.5 ± 1.23) on Day 25. In case of the latter group, no significant difference was found in these values and the values obtained on Day 26. On Day 25, all the drug treated groups exhibited comparable number of errors as for vehicle treated groups. However, there was a significant reduction in number of errors done by the rats from these groups on Day 26 ($P < 0.001$) as compared with their readings on Day 25. Piracetam treated rats did not show any significant reduction compared with vehicle control on Day 26 but all the plant drug treated groups committed significantly less errors than the vehicle control. The numbers of errors were not significantly different for single plant drugs and their combinations.

DISCUSSION

The present study, contrary to our hypothesis that the combinations of plant drugs produce better effects than the individual agents, has revealed that there is no significant difference in the effects of combination and individual agents in normal animals and in rats with memory impairment. However, it has proved that effects of the plant drugs; individual as well as in combination are comparable or even better sometimes compared with the currently used modern medicinal drugs.

It was decided to study the effects in normal rats using Hebb-Williams Maze, which is a complex type of learning requiring integration of multiple cortical areas and then restoration of learning as memory. The present study demonstrated that rivastigmine and piracetam administered to normal rats did not show any effect on learning but they exhibited retention of learning about the path to be traced to reach the food in the complex maze. This indicates memory enhancing effects of these drugs. Since the purpose of the study was to compare the effect of combination of *Pe, Tc* and *Os* with monotherapy, we decided to include individual plant groups in the study. Pretreatment with plant drugs, single as well as combinations also did not show any effect on learning but significantly enhanced memory. Their effects were found to be comparable to Rivastigmine and Piracetam. Though the time taken to trace the food and number of errors in the repeat maze test were comparable for both the plant drug combinations and the individual agents, these values were found to be the lowest in the rats administered the three drug combination of *Tc* and *Pe* with *Os*.

It was decided to use three different models of memory impairment. Two of these models using scopolamine and diazepam are standard and commonly used.[12] The duration of treatment was selected in case of modern medicinal drugs (rivastigmine and piracetam) based on the reports in the literature[6,13] and for plant drugs arbitrarily to ensure adequate drug concentrations.

In the third model, cyclosporine was selected to induce amnesia as it has been shown to cause degeneration of hippocampal neurons.[7] The dose and regime of pretreatment with cyclosporine was as described by

Table 4: Effects on time taken to trace the food and number of errors committed in rats with Cyclosporine induced memory impairment

Amnesic rats treated with vehicle/drug	Time taken (s)		Number of errors	
	Day 25 (learning)	Day 26 (memory)	Day 25 (learning)	Day 26 (memory)
Distilled water (15 days)	170.17±18.02	166.17±15.06	6.5±1.23	6.17±1.17
Distilled water (7 days) followed by Piracetam (200 mg/kg for 8 days)	171±11.05	153*^{NS}±9.61	7.17±0.75	5*NS±0.89
Tc (100 mg/kg for 15 days)	158±9.36	102.5*$^{\#\#\#\$}$±13	6.5±0.55	3.67*$^{\#\#}$±1.03
Pe (300 mg/kg for 15 days)	160.33±11.91	100.67*$^{\#\#\#\$}$±5.75	6.83±1.47	4.17*$^{\#}$±0.98
H1 (*Tc*+*Pe*) (400 mg/kg for 15 days)	161.5±8.89	98.33*$^{\#\#\#\$}$±6.86	7±0.89	4.33*$^{\#}$±0.82
H2 (*Tc*+*Pe*+*Os*) (400 mg/kg for 15 days)	161.17±13.63	97*$^{\#\#\#\$}$±9.34	7±1.1	3.83*$^{\#\#}$±0.75

N=6/group. All values represent mean±SD; Paired *t* test; *P<0.001. ANOVA followed by *post hoc* Tukey's test: NS=Not significant, $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001 compared with control, $^{\$}P$<0.001 compared with Piracetam

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Agarwal *et al.*[7] In this study, the authors have demonstrated that cyclosporine causes impairment in learning and memory by immunosuppression and degeneration of hippocampal neuron.[7] As against the earlier models, insult with cyclosporine was given before initiating drug therapy and hence this model tested the ability of test drugs to reverse the changes induced by the cyclosporine.

The memory impairment was detected by testing the rats on Day 16. Both rivastigmine and piracetam were found to reduce the time taken to trace the food and number of errors compared with basal values and values seen in the vehicle control group. The plant drugs differ from the positive controls as far as their effect on the learning is concerned. They showed a trend toward better learning activity but the values were not statistically significant from positive control. The effect of these agents on the memory impairment was also comparable to the positive controls except the H2 formulation. Its effect on memory was significantly more than seen with piracetam. In a study done earlier, pretreatment with pulverized *Pe*prevented memory deficits induced by scopolamine in this test.[8,14] There was no report available in the literature regarding effects of *Tc* or *Os*inscopolamine-induced amnesia.

Difference observed in the effects on learning between modern medicinal agents and these plant drugs can be attributed to the possible effect of the latter on a different neurotransmission than on the cholinergic system. Moreover, *Tc*, *Pe* and *Os* have been shown to be immuonostimulants in variety of experiments.[15,16] It may be possible through immune mechanisms they may be modulating neurotransmitters for learning. Presence of all the three plant drugs in H2 formulation may be responsible for the least time taken to trace the food and the least errors committed by the rats from this group.

In our study, diazepam was found to impair learning as well as memory. The positive control used in this model was piracetam, a nonspecific nootropic, which acts through multiple mechanisms.[17]It was felt that rivastigmine would have no role against diazepam-induced memory dysfunction. Piracetam in our study showed memory retention but did not improve learning ability. Rats pretreated with the plant drugs exhibited effects were comparable to those of piracetam. A similar study done in the past has shown that 15 days pretreatment with pulverized *Pe* prevented memory deficits induced by diazepam.[7] Extract from leaves of *Os* has also reported to prevent memory impairment due to diazepam.[9] The H2 formulation has both these ingredients. The rats treated with this formulation, like in the earlier model, needed the least time to trace the food and committed the least errors. But the reported values did not differ statistically and hence there does not appear any significant difference in the effects of individual plants and their combination.

In the model of cyclosporine-induced learning-memory deficit, piracetam was selected as a positive control based on the claims regarding its neuroprotective effects.[7] All the plant drugs have shown reversal of the memory impairment induced by cyclosporine and also shown a distinctly better effect as compared with piracetam. Among all, again H2 formulation showed better values but statistically all the plant drugs were comparable.

Thus the present study proves memory enhancing effect of *rasayana* drugs from Ayurveda. This effect was observed in the animals pretreated with plant drugs and then subjected to the amnestic agents as is done in routine learning-memory testing models. However, it is important to note that the plant drugs could reverse the memory impairment induced by cyclosporine. The latter finding is of significance because in clinical practice, treatment always follows the diagnosis of memory deficit. The effects of the plant drugs were also found to be comparable to the modern agents and in the model of cyclosporine induced amnesia better than piracetam. Our study did not prove that combination of plant drugs exert better effect than the individual ingredient. However, in normal animals as well as in all the three models, H2 formulation has shown promising results as indicated by the lowest time reported by the rats treated with this combination to trace the food and the least number of errors committed by them. Hence it is worthwhile to evaluate this combination further to develop an indigenous nootropic.

Although in this study no attempt has been made to elucidate the mechanism of action of these agents, it appears that these drugs are modulating psychoneuroimmune (PNI) axis. As per Ayurveda, they are medhya rasayanas and are supposed to act on central nervous system (CNS). These drugs are also used in various anti-stress formulation and have shown adaptogenic effects[15] indicating their ability to modulate PNI axis. Learning and memory is influenced or modified by the immune system. In one study, it is reported that cyclosporine administration caused cognitive deficits in chicks.[18] Freund's adjuvant in doses of active immunization led to improvement in the learning and memory process in rats,[19] supporting the evidence further that cognition is influenced by immunomodulation.[20] *Pe* has been shown to have immunomodulatory and antioxidant action.[21] *Tc* is a well-researched immunomodulator.[16,22] *Os* has antistress, antioxidant properties and immunomadulatory activities.[23,24] Thus it is possible that these three medicinal plants by virtue of their immunomodulatory activity improve learning and memory.

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It would be worthwhile exploring effects of *Pe*, *Tc* and *Os* on individual neurotransmitters associated with learning and memory and delineate the link between immune system modulation and memory enhancement. This would provide a scientific basis for their effects observed in the present study. For example, the beneficial effect of the plant drugs against scopolamine-induced amnesia is suggestive of effects of these plant drugs on the cholinergic system. Hence brain cholinesterase activity or assay of ACh concentration need to be performed. Similarly it would be of interest to study whether the plant drugs exhibit GABA inhibitory effects.

CONCLUSION

In the present study, combination of *Phyllanthus emblica* and *Tinospora cordifolia* with and without *Ocimum sanctum* demonstrated nootropic activity in normal and memory impaired rats and both the formulations showed comparable nootropic activity with that of *Tc* and *Pe* alone.

ACKNOWLEDGMENTS

The authors acknowledge the support from Dr. Reena Karkhele during the study conduct. The authors also acknowledge the support of Dr Ashwinikumar Raut in critical appraisal of manuscript.

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How to cite this article: Malve HO, Raut SB, Marathe PA, Rege NN. Effect of combination of *Phyllanthus emblica*, *Tinospora cordifolia*, and *Ocimum sanctum* on spatial learning and memory in rats. J Ayurveda Integr Med 2014;5:209-15.

Source of Support: Postgraduate student Fund by Diamond Jubilee Society Trust., Seth G.S. Medical College and K.E.M. Hospital, **Conflict of Interest:** Nill.