

Efficacy of *Tinospora cordifolia* on Learning and Memory in Healthy Volunteers: A Double-Blind, Randomized, Placebo Controlled Study

K. LAXMINARAYANA BAIRY, YESHWANTH RAO and K. BALACHANDER KUMAR

Department of Pharmacology (K.L.B., Y.R.), Department of Clinical Psychology (K.B.K.), Kasturba Medical College and Hospital, Manipal, India.

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ABSTRACT

Background: *Tinospora cordifolia*, an Indian medicinal plant, has been reported to have beneficial effects on disorders like peptic ulcer, hepatobiliary disorders, rheumatism, infectious diseases etc. It enhances cognition in normal rats and successfully overcomes cyclosporine induced memory deficit. **Methods:** Thirty healthy volunteers of age 18-30 years received *Tinospora cordifolia* (500 mg of pure aqueous extract) or a matching placebo for 21 days in a double blind, randomized and placebo controlled design. Learning and memory was assessed by subjecting the volunteers to a battery of psychological tests that aimed at studying visual memory, logical memory, verbal memory, attention span and concentration. **Results:** *Tinospora cordifolia* showed a significant ($p < 0.05$) increase in the test scores for 'verbal learning and memory' (control -1.2 ± 1.9 , drug 6.9 ± 2.5) and 'logical memory' (control 5.1 ± 6.1 , drug 26.6 ± 6.7). No significant untoward effects were reported during *Tinospora cordifolia* treatment. **Conclusion:** *Tinospora cordifolia*, 500 mg daily, enhances verbal learning and memory and logical memory (of immediate and short term type) compared to placebo in healthy volunteers.

Keywords: Learning, Memory, *Tinospora cordifolia*

Learning is the process of acquiring knowledge about the world and memory is the retention of the acquired knowledge, which can be retrieved as and when, required. This process is not an independent process, but is influenced or modified by the immune system [1].

Tinospora cordifolia, an Indian medicinal plant, is known to be beneficial for the treatment of disorders like peptic ulcer, hepatobiliary disorders, rheumatism, infectious diseases etc [2, 3]. It has an immunostimulant activity [4]. It improves intelligence, power of retention and memory [5]. It enhances cognition in normal rats and successfully overcomes cyclosporine induced memory deficit [6].

The present study was undertaken to evaluate the effects of *Tinospora cordifolia* on cognitive function in healthy volunteers using a battery of psychological tests that aimed at studying visual memory, logical memory, verbal memory, attention span and concentration.

METHODS

Thirty healthy volunteers (male-22 and female-8) aged 18-30 years (mean 22.5 ± 0.28) were studied in a

randomized, double-blind, placebo controlled design. The protocol was approved by the institutional ethical committee and all participants gave informed consent.

Exclusion criteria

Individuals with history of smoking, alcoholism, head injuries in the past, convulsions, psychotic disorders, and on those medications like anticonvulsants, antipsychotics, hypnotics or cognitive enhancers were excluded.

Volunteers were randomized into two groups of 15 each and were provided with 21 days of medication (coded box) containing *Tinospora cordifolia* 500 mg, or matching placebo. One tablet of *Tinospora cordifolia* or placebo was taken once daily orally in the morning along with breakfast.

Procurement of the tablets and placebo

Pure aqueous extracts of *Tinospora cordifolia* (500 mg tabs) were obtained from Sami labs, Bangalore. Placebo (Lacose-450 mg + starch 50 mg) similar in size, shape and taste was obtained from KMC Pharmacy manufacturing unit.

Table 1. The effect of *Tinospora cordifolia* on various tests for verbal memory and logical memory.

Tests	Pre-treatment Scores (Mean ± S.E.M)		Post-treatment Scores (Mean ± S.E.M)		Raw Score % Difference ¹ (Mean ± S.E.M)		95% Confidence Interval		
	Control	Drug	Control	Drug	Control	Drug	Control	Drug	p-value ²
VERBAL MEMORY									
RVLMT ¹	13.44±0.47	13.67±0.28	13.27±0.56	14.25±0.23	-1.2±1.9	6.9±2.5	-5.3-2.9	1.4-12.5	0.02
RVLMTDR ²	12.47±0.61	13.2±0.42	13.06±0.48	13.83±0.46	3.9±2.7	4.8±2.2	5.3-2.9	-0.06-9.6	0.25
LOGICAL MEMORY									
LMT ³	14.27±1.05	14.06±0.76	16±1.02	16.53±0.58	16.06±5.8	22.8±8.7	3.57-28.6	6.7-42.6	0.50
LMTDR ⁴	14.73±1.05	13.6±0.73	15.87±1.36	16.67±0.54	5.1±6	26.6±6.8	-9.9-16.8	12.1-41.2	0.02

¹ Post treatment score – Pretreatment score / Pretreatment score × 100.

² Based on Mann-Whitney non parametric test.

¹ Rey's Verbal learning & memory test.

² Rey's Verbal learning & memory test – delayed response.

³ Logical memory test.

⁴ Logical memory test – delayed response.

Efficacy assessment

This was done once before the administration of medication as the baseline values and once after the treatment. Learning and memory assessment was carried out in the department of Clinical Psychology.

Learning and Memory Assessment Tests

The learning and memory tests [7] can be classified as follows:

Tests for Visual Memory. Benton's visual retention test (tests visual memory only) (BVRT) and Rey's complex figure test (RCFT) includes RCFT, RCFTDR & RCFC (tests visual memory & retention, DR–delayed response and C–copy).

Tests on Logical Memory. Logical memory test (LMT). Includes LMT & LMTDR (tests immediate recall, delayed recall & verbal learning, DR – delayed response), involves narrating a story – involves logic hence the term 'logical'

Tests on Verbal Memory. Rey's verbal learning and memory test (RVLMT). Includes RVLMT and RVLMTDR – tests learning curves and strategies.

Tests on Attention Span & Concentration. Digit Forward and Digit Backward (DF & DB) plus Associated learning – 'easy' and 'hard' (ALE & ALH).

All the above tests assess the immediate and short term memory. The individual tests were carried out as follows:

BVRT. This was comprised of 10 card series test with each card having 3 figures, 2 large and 1 small, with the small figure always to one side or the other. Each card was shown for 10 sec and then the subject was asked to reproduce the card (1 correct reproduction card = 1 score).

RCFT. Consisted of reproduction of a complex figure. This test investigated visual memory and retention. Both immediate and delayed recall trials were given. The figure was divided into 18 units and maximum score was 22 points. The same test was carried out after a lag period (RCFT-DR delayed response) and by looking (RCF-C copy).

RVLMT. This test measured immediate memory span, provided learning curves and revealed learning strategies. It consisted of 5 presentations with recall of a 15-word list, one presentation of a second 15-word list

and a sixth recall trial that altogether takes 10-15 mins. The score for each trial is the number of words correctly recalled. The same test was carried out after a lag period (RVLMT-DR delayed response).

LMT. This was tested by narrating a story that had 21 units. Three trials were given. This test measured both immediate and delayed recall and verbal learning. The same test was carried out after a lag period (LMT-DR delayed response)

ALE & ALH. The format of paired associate tests consisted of word pairs which were read to the subject with one or more recall trials in which the first of the pair is presented to the subject to give the associated word. The format consists of 10 word pairs, six forming easy associations (baby-cries) and the other hard pairs that are not associated (cabbage-pen). The test was read 3 times with a memory trial following each reading. Total score was one-half the sum of all correct associations to the easy pairs plus the sum of all correct associations to the hard pairs made within 5 sec after the stimulus word was read. Thus, the highest possible score was 21.

Immediate Recall (Digit Forward and Digit Backward). This test measured attention span and concentration. It was carried out by recalling the digits in forward and backward direction. The number of digits recalled was equal to the score. Normal score was DF-5 and DB-3.

Possible untoward effects related to the medication were noted by 'open questionnaire method' and neurological side effects by the 'Udvalg for Kliniske, Undersegelser (UKU) Side Effect Rating Scale [8].

Statistical analysis

Statistical analysis was carried out using Mann-Whitney test' using SPSS computer software package. Level of significance (p-value) was lower than 0.05.

RESULTS

Demographic details of the volunteers

Of the 30 (Males-22; females-8) volunteers enrolled in the study all 30 completed the study. Average age of the volunteers is 22.5±0.28 yrs. Sixteen volunteers were from North India, 8 from Karnataka, 4 from Kerala and 2 from Maharashtra.

Table 2. The effect of *Tinospora cordifolia* on various tests for visual memory.

Tests	Pre-treatment Scores (Mean ± S.E.M)		Post-treatment Scores (Mean ± S.E.M)		Raw Score % Difference ¹ (Mean ± S.E.M)		95% Confidence Interval		
	Control	Drug	Control	Drug	Control	Drug	Control	Drug	p-value ²
VISUAL MEMORY									
BVRT ¹	8.53±0.23	8.2±0.4	9.06±0.24	9.27±0.27	5.07±2.4	15.8±5	1.59-11.2	5-26.5	0.39
RCFT ²	15.67±1.3	16.06±1.09	19.87±0.9	20.4±0.69	25.4±8.4	29.3±9.7	7.4-43.5	11.6-64	0.5
RCFTDR ³	16.6±1.3	16.6±1.04	19.67±0.95	20.4±0.7	29±14.4	28±8.5	-1.8-59.9	9.7-46.3	0.5
RCFC ⁴	14.5±0.7	14.98±0.94	14.78±0.67	15.03±0.68	1.25±0.6	0.984±0.7	0.06- 2.4	-0.5-2.5	0.6

¹ Post treatment score – Pretreatment score / Pretreatment score × 100.

² Based on Mann-Whitney non parametric test.

³ Benton's Visual Retention test.

⁴ Rey's Complex Figure test.

⁵ Rey's Complex figure test – delayed response.

⁶ Rey's Complex figure – Copy.

Drop outs

No dropouts recorded in the study.

Compliance

Volunteer compliance was noted by asking the volunteers to return the empty boxes in which the tablets were dispensed. None of the boxes given back by the volunteers contained any drug or placebo. Hence the compliance was assumed to be 100%.

Efficacy Assessment

Analysis of the scores by taking the % difference in the tests for verbal learning and memory (RVLMT) and logical memory (LMT) (post-treatment score - pre-treatment score / pre-treatment score × 100) in the control and the drug treated group showed a significant increase (p < 0.05) in the drug treated group when compared to the control group (control -1.2±1.9, drug 6.9±2.5 for RVLMT) & (control 5.1±6.1, drug 26.6±6.8 for LMTDR, Table 1). However, there is no significant difference in the scores of tests for visual memory (BVRT, control: 5.0±2.4; drug:15.8±5; RCFT, control: 25.4±8.4, drug: 29.3±9.7; RCFTDR, control: 29±14.4, drug: 28±8.5 and RCFC, control: 1.25±0.6, drug: 0.9±0.7, Table 2) and attention span and concentration (DF, control: -0.49±7.9, drug: 7.7±7.5; DB, control: 0.89±6.1, drug: 2.8±5.3; ALE, control: 2.7±1.8, drug: 0.4±2.53 and ALH, control 37.2±19.5, drug: 26.6±10.4, Table 3).

Untoward Effects

No volunteer complained of any adverse effects during and after the period of drug intake. No neurological changes were observed at the given dose and for the given duration.

DISCUSSION

Tinospora cordifolia has been reported to improve intelligence, power of retention and memory [5]. We had previously reported that *Tinospora cordifolia* improves learning and memory in normal and immunodeficient rats [6]. However, there was no scientific study regarding its efficacy in humans. We felt the need for a critical evaluation of the cognitive effects of this traditional remedy in a well designed, scientific study which might open new vistas for future research in this field.

Analysis of data by taking the percentage difference in scores, showed a significant(p < 0.05) increase in the raw score for verbal learning and memory (RVLMT) and logical memory test in the treated group as compared to control group (Table 1). Many volunteers had felt an increased freshness after taking the drug. These results indicated for the first time that *Tinospora cordifolia* is associated with beneficial effects on learning and memory (of short term type) in humans (visual and logical memory). However, perusal of the result of Table 2 and 3 shows that *Tinospora cordifolia* did not affect visual memory and attention span and concentration. These differential effects on various types of memories may be due to brain asymmetry. There are reports that the right and left hemispheres of the brain process qualitatively different information [9]. In particular, the left hemisphere was thought to be involved in processing of verbal material, signs, and symbols, whereas the right hemisphere was thought to be involved in handling non-verbal material, images, melodies, and spatial information. Further, it is suggested that right hemisphere functioning is closely associated with the limbic system and the left hemisphere functioning is responsible for maintenance of semantic memories [10] There is evidence for a greater interconnection between the limbic system and right hemisphere than the left hemisphere [11]. *Tinospora cordifolia* may be acting at the hemisphere level and not on limbic system and hence the differential effect on various types of memory. This is our hypothesis and further studies are necessary in this direction.

With regards to its precise mechanism of the beneficial effects little is known. It probably acts by modulating the immune mechanism. There is a good deal of interaction between central nervous system and immune system [12-14]. Diminished intellectual functions and motor impairment are seen in patients with AIDS and psychiatric illnesses associated with immunological abnormalities [14]. Viral or cyclosporine (an immunosuppressant) induced immunosuppression is found to produce learning and memory impairment [15, 16]. Cyclosporine is thought to act on its receptor immunophilin in the brain and reduce the synthesis of IL-1 [17]. Low levels of IL-1 has also been found to produce both cognitive defects and lowered pain threshold in animals [18].

The impairment of cognition was absent when cytokines, interleukine-2 and lymphokine activated killer

Table 3. The effect of *Tinospora cordifolia* on various tests for attention span and concentration.

Tests	Pre-treatment Scores (Mean ± S.E.M)		Post-treatment Scores (Mean ± S.E.M)		Raw Score % Difference ¹ (Mean ± S.E.M)		95% Confidence Interval		
	Control	Drug	Control	Drug	Control	Drug	Control	Drug	p-value ²
ATTENTION SPAN & CONCENTRATION									
DF ¹	6.73±0.28	6.53±0.33	6.67±0.32	6.84±0.28	-0.49±7.9	7.7±7.5	-6.8-9.2	-11.5-21.7	0.98
DB ²	5.42±0.38	5.33±0.43	5.57±0.32	5.53±0.35	0.89±6.1	2.8±5.3	-1.6-5.8	-5.3-6.23	0.59
ALE ³	9.86±4.01	2.83±0.24	3.43±0.64	3.84±0.94	2.7±1.8	0.4±2.53	-1.23-6.6	-5-5.9	0.59
ALH ⁴	2.67±0.27	2.8±0.24	3.27±0.25	3.13±0.17	37.2±19.5	26.6±10.4	-4.6-79.1	4.3-48.9	0.8

[†] Post treatment score – Pretreatment score / Pretreatment score × 100.

[‡] Based on Mann-Whitney non parametric test.

¹ Digit forward.

² Digit backward.

³ Associated learning easy.

⁴ Associated learning hard.

cells were administered to experimental animals treated with immunosuppressants [19].

Levamisole, an immunostimulant enhanced learning and memory in mice and cyclosporin impaired learning and memory supporting the above studies [20]. From the foregoing discussion it is clear that immune system also influences the learning and memory

Therefore *Tinospora cordifolia*, used for its immunomodulatory effect in our study could have enhanced cognition by increasing the synthesis of IL-1 [14]. The second mechanism may be due to increased synthesis of acetylcholine (an important neurotransmitter in learning and memory) by the supplementation of choline, which is an important active constituent of *Tinospora cordifolia* [21]. There are also reports that supplementation of choline enhances the cognitive function in animals [22, 23]. Further studies are needed to know the exact mechanism of action.

We believe that there is a strong need for further evaluation *Tinospora cordifolia* in patients with cognitive dysfunction. Our study has opened up a new direction for future large scale clinical research to find an alternative and inexpensive drug for defective learning and memory.

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Address correspondence to: Prof. KL Bairy, Department of Pharmacology, Kasturba Medical College, Manipal 576104, Karnataka, India. E-mail: klbairy@yahoo.com