EFFECT OF *TINOSPORA CORDIFOLIA* ON LEARNING AND MEMORY IN NORMAL AND MEMORY DEFICIT RATS

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**ABSTRACT**

**Objective:** To study the effect of *Tinospora cordifolia* (Tc) on learning and memory in normal and cyclosporine induced memory deficit rats.

**Methods:** Alcoholic and aqueous extracts of the whole plant of *Tinospora cordifolia* was administered orally for 15 days in two groups of rats. Cyclosporine 15, 25 mg/kg, *i.p.* was administered on alternate days for 10 days. Combination of cyclosporine 25 mg/kg, *i.p.* for 10 days and Tc alcoholic 200 mg/kg and Tc aqueous 100 mg/kg were administered in two different groups of rats. At the end of treatment, learning and memory was assessed using Hebb William maze and passive avoidance task. The locomotor activity was assessed using open field chamber. The immune status was studied using DNCB skin sensitivity test. Histopathological examination of hippocampus was done.

**Results:** Both alcoholic and aqueous extracts of Tc produced a decrease in learning scores in Hebb William maze and retention memory indicating enhancement of learning and memory. However, cyclosporine at both the doses increased the learning scores in Hebb William maze and decrease in retention time in the passive avoidance task suggesting a memory deficit. The combination of cyclosporine and Tc produced a decrease in learning scores in Hebb William maze and increase latency in passive avoidance task compared to cyclosporine alone treated rats. The histopathological examination of hippocampus in cyclosporine treated rats showed neurodegenerative changes which were protected by the Tc.

**Conclusion:** Tc enhances cognition (learning and memory) in normal rats. Cyclosporine induced memory deficit was successfully overcome by Tc.

**KEYWORDS** Cyclosporine learning memory *Tinospora cordifolia*

**INTRODUCTION**

Dementia is a syndrome of failing memory and other intellectual functions with little or no disturbance in consciousness¹. Degeneration of the cerebral neurons is one of the commonest and vital causes for dementia with increasing age⁰, there by leading to deterioration in quality of life in elderly.

Hence a greater research is required in early diagnosis of the condition and development of newer effective drugs to prevent or halt the progression of the disease. This is possible by basic understanding of learning and memory process.

Learning is a process of acquiring knowledge about the world and memory is its retrieval². This process is not an independent process, but is influenced or modified by the immune system. There are reports that administration of cyclosporine in chicks produced cognitive deficits³. Immunosupression induced by virus in mice impaired learning and memory process⁴. Similar cognitive impairment was also observed in patients following cyclosporine administration⁵. Animals treated with Freund's adjuvant in doses of active immunization enhanced the learning and memory process⁶, evidencing further that immunomodulation affects the cognition⁷.
Tinospora cordifolia has been extensively studied and reported to have potent immunostimulant action\textsuperscript{8-10}. In addition Tinospora cordifolia also found to have antistress\textsuperscript{11} effect. Ayurvedic literature recommends a rejuvenating recipe where Tinospora cordifolia being an important constituent to enhance the memory function\textsuperscript{12}. However, there are no experimental evidence to support memory enhancing property of Tinospora cordifolia.

Hence the present work was undertaken with the objective of studying the effect of Tinospora cordifolia on (a) learning and memory in normal rats, (b) on cyclosporine induced memory deficits.

**MATERIALS AND METHODS**

**Animals:** Healthy male in-bred albino rats of Wistar strain, aged between 70-90 days, weighing 120-160 gms were used in the present study. The rats were housed two per cage under an alternate 12 h light:dark cycle in temperature and humidity controlled environment with free access to food and water. The study was conducted following local ethical committee clearance.

**Method of Tinospora cordifolia extraction**

**Alcoholic extract:** The whole plant of Tinospora cordifolia was obtained from the local Ayurvedic store in Udupi (Karnataka) and identification of the plant was done at the College of Pharmaceutical Sciences, Manipal.

The whole plant was shade dried for one month and then coarse powdered. Soxhlet extraction method was used for obtaining alcoholic extracts (13.12% yield).

**Aqueous extract:** The dried coarse powdered crude plant (100 gms) was transferred to a round bottom flask and 2 litre of distilled water was added to the round bottom flask and soaked for 2 h. This was then boiled for 4 h. The extract so obtained was decanted in a beaker and then concentrated to 1/6\textsuperscript{th} of the total volume on a water bath. This was preserved by adding a few drops of chloroform and kept in the refrigerator. This extract was administered to the animals by making the concentration required by weighing the water-evaporated extract (14.2% yield).

**Experimental protocol**

Eleven groups of rats, eight in each group were used in the present study.

- **Group I** Normal control (A) equivolume of gum acacia p.o.
- **Group II** Alcoholic extract of Tinospora cordifolia 100 mg/kg, p.o. for 15 days.
- **Group III** Alcoholic extract of Tinospora cordifolia 200 mg/kg, p.o. for 15 days.
- **Group IV** Aqueous extract of Tinospora cordifolia 100 mg/kg, p.o. for 15 days
- **Group V** Normal control (B) equivolume/olive oil i.p.
- **Group VI** Cyclosporine 15 mg/kg, i.p. 5 doses on alternate days
- **Group VII** Cyclosporine 25 mg/kg, i.p. 5 doses on alternate days
- **Group VIII** Normal control (C) olive oil i.p. for 10 days followed by equivolume of gum acacia p.o. for 15 days
- **Group IX** Cyclosporine 25 mg/kg, i.p. 5 doses on alternate days followed by equivolume of gum acacia p.o. for 15 days
- **Group X** Cyclosporine 25 mg/kg, i.p. 5 doses on alternate days followed by alcoholic extract of Tc 200 mg/kg, p.o. for 15 days.
- **Group XI** Cyclosporine 25 mg/kg, i.p. 5 doses on alternate days followed by aqueous extract of Tc 100 mg/kg, p.o. for 15 days.

Acute toxicity study was done for Tinospora cordifolia up to 3000 mg/kg in rats. Doses of Tinospora cordifolia were selected based on the previous study which had immunostimulant activity\textsuperscript{8,9}. Dose of cyclosporine was computed from human dose.

**Assessment of learning and memory**

**Hebb William Maze:** The Hebb William Maze (Allwin manufacturers, New Delhi) was used for learning assessment. The maze consists of completely enclosed rectangular box with an entry and a reward chamber appended at opposite ends. The box is partitioned with wooden slats into blind passages leaving just
one twisting corridor leading from the entry to the reward chamber.

The learning assessment for control and treated rats was conducted at end of treatment under zero watt red coloured bulb so as to minimize the nocturnal cycle disturbances. On the first day all the rats were familiarized with Hebb William maze for a period of 10 min. From 2\textsuperscript{nd} to 5\textsuperscript{th} day, the rats received four consecutive trials of training per day in the maze. In each trial the rat was placed in the entry chamber and the timer was activated as soon as the rat leaves the chamber. The time taken for the rat to reach the reward chamber was taken as the learning score of the trial. The average of four trials was taken as the learning score for the day. Lower scores of assessment indicate efficient learning while higher scores indicate poor learning in animals. During learning assessment, the animals were exposed to food and water ad libitum only for 1 h after the maze exposure for the day was completed to ensure motivation towards reward area.

**Two compartment passive avoidance apparatus:**
The method of Bures et al., was used to assess the retention of learned behavior\textsuperscript{13}. The apparatus consists of a square box with a floor grid of 50 x 50 cms and wooden walls of 35 cms height. This box was illuminated with 100 watts bulb. In the centre of one of the walls there is an opening of 6 x 6 cms which can be opened or closed using a transparent plexy glass sliding door. This opening leads to a small (15 x 15 cms) dark compartment provided with an electrified floor, that can be connected to a shock source. Stimulator obtained from (Hugo Sachs Electronics, Germany) having a maximum output of 100 mA. The retention test was conducted blind to the treatment. The animals were placed in the illuminated chamber facing away from the entrance to the dark compartment. The door was closed after the rat entered the dark compartment and 1 mA foot shock was delivered for a period of 2 sec. Then the animal was returned to the home cage. 24 h later each animal was placed again in the illuminated chamber as before for a maximum period of 180 sec. The latency taken by the animal to enter the dark compartment was measured. Animals not entering the dark compartment within this period received a latency time of 180 sec. Absence of entry into the dark compartment indicated positive retention.

**Assessment of motor activity**

**By open field chamber:** Motor activity was measured using large rectangular box (100 x 100 x 40 cm). The floor consists of finely knit wiremesh divided into 25 equal squares (5 x 5 cm). Illumination was provided with 100 watts bulb fixed 60 cm above the centre of the field as described by Bures et al., 1983\textsuperscript{13}.

Control and drug treated animals were placed in open field chamber and motor activities were measured as described by (Bures et al., 1983)\textsuperscript{13} for a period of 5 min. Total number of central and peripheral square crossings were recorded for each animal.

**Assessment of immune status (Cell-mediated immunity)**

**Dinitro chloro benzene (DNCB) skin sensitivity test:** The cell-mediated immunity was quantitatively assessed by the contact sensitization test with 1-chloro, 2, 4 dinitro benzene (DNCB). DNCB was dissolved in acetone to obtain a stock solution of 30 mg/kg and 0.1 ml of stock solution of DNCB was applied locally on the shaved anterior abdominal region and allowed to spread over an area of 3 cm\textsuperscript{2}. Evaporation of solvent was hastened using a hair drier. The applied site examined after 24 h for any irritative reaction and on the 7\textsuperscript{th} day for erythema and/or induration. The induration was measured using centimeter scale\textsuperscript{14}.

**Brain histopathological studies:** After the treatment and the behavioral studies, two animals in each group were sacrificed by excessive ether anesthesia and the brains were isolated and were kept in 10% formaldehyde solution. The brain was stained with cresyl violet, hippocampus region was studied under light microscope.

**Statistical analysis:** Statistical analysis of the data was done by one-way ANOVA, followed by Scheffe’s test using the SPSS computer package. The level of significance was fixed at $P<0.05$.

**RESULTS**

**Acute toxicity test:** There was no notable adverse effect and no death was recorded upto 3 gm/kg.

**Hebb William Maze:** The rats treated with equivolume of 2% gum acacia (P.O.) and olive oil (I.P.) had no significant difference in learning scores on day 1 to day 4 in Hebb William maze (Table 1 & 2). Alcoholic
### Table 1. Learning scores of rats on day 1-4 in Hebb William maze following vehicle, alcoholic (Alc) and aqueous (Aq) *Tinospora cordifolia* extracts treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (in mg/kg)</th>
<th>Learning scores (time in minutes)</th>
<th>n= 8/group</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control- A (2% gum acacia)</td>
<td>Equivolume</td>
<td>5.78 ± 0.665</td>
<td></td>
<td>6.31 ± 0.494</td>
<td>5.96 ± 0.474</td>
<td>4.80 ± 0.562</td>
<td></td>
</tr>
<tr>
<td><em>Tinospora cordifolia</em> Alc. extract</td>
<td>100</td>
<td>3.78 ± 0.412*</td>
<td></td>
<td>3.24 ± 0.440*</td>
<td>2.9 ± 0.495*</td>
<td>2.346 ± 0.471*</td>
<td></td>
</tr>
<tr>
<td><em>Tinospora cordifolia</em> Alc. extract</td>
<td>200</td>
<td>2.97 ± 0.297*</td>
<td></td>
<td>2.46 ± 0.308*</td>
<td>1.596 ± 0.219*</td>
<td>0.962 ± 0.080*</td>
<td></td>
</tr>
<tr>
<td><em>Tinospora cordifolia</em> Aq. extract</td>
<td>100</td>
<td>2.33 ± 0.368*</td>
<td></td>
<td>2.463 ± 0.258*</td>
<td>1.575 ± 0.144*</td>
<td>0.913 ± 0.081*</td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean ± SEM, *P< 0.05 Vs control- A

### Table 2. Learning scores of rats on day 1-4 in Hebb William maze following vehicle, cyclosporine alone and cyclosporine with *Tinospora cordifolia* (Tc) alcoholic (alc) and aqueous (aq) extracts treatment, respectively.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Learning scores (time in minutes)</th>
<th>n= 8/group</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control- B (Olive oil)</td>
<td>Equivolume</td>
<td>6.93 ± 0.610</td>
<td></td>
<td>4.9 ± 0.782</td>
<td>3.32 ± 0.269</td>
<td>3.22 ± 0.530</td>
<td></td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>15</td>
<td>7.69 ± 0.774</td>
<td></td>
<td>6.92 ± 0.841</td>
<td>7.28 ± 0.723*</td>
<td>6.89 ± 0.699*</td>
<td></td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>25</td>
<td>8.99 ± 0.349</td>
<td></td>
<td>9.15 ± 0.258*</td>
<td>9.14 ± 0.416*</td>
<td>8.78 ± 0.473*</td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean±SEM, *P< 0.05 Vs control- B, *P< 0.05 Vs control- C, *p< 0.05 Vs cyclosporine 25 mg/kg + 2% gum acacia
Figure 1. Photomicrographs of hippocampal CA1 neurons in control (A), treated with cyclosporine (B) and cyclosporine + Tc (C). Note the degenerative neurons (arrows) in B & C Cresyl violet stain. Scale bar=20 µ.
(alc) and aqueous (aq) extract of *Tinospora cordifolia* (Tc) treated rats had a significant decrease in the learning scores on day 1 and day 4 when compared to control-A (2% gum acacia treated) group indicating an enhancement of learning & memory. (Table 1)

The cyclosporine treated rats showed a significant dose dependent increase in the learning scores on day 3 and day 4. Whereas, cyclosporine 25 mg/kg showed significant increase in learning scores from day 2 to day 4 of learning in Hebb William maze indicating memory deficit.

In rats treated with cyclosporine (25 mg/kg) for 5 alternate days and 2% gum acacia for 15 days produced an increase in the learning scores on day 2 to day 4 when compared to control (treated with olive oil. I.P. and 2% gum acacia orally). However, the rats treated with alcoholic (200 mg/kg) and aqueous (100 mg/kg) extracts of Tc following cyclosporine administration decreased the learning scores significantly compared to the cyclosporine and 2% gum acacia treated rats on day 1 to day 4 in Hebb William maze indicating the overcome of memory deficit induced by cyclosporine. (Table 2)

**Two compartment passive avoidance test:** In the two compartment passive avoidance test there was no significant change in the latency to enter the dark compartment on day 0 among the groups. Following 24 h after shock the latency to enter the dark compartment in the retrieval trial (day 1) produced no significant alternation in retention memory among the Tc treated groups and control- A (180 sec).

Cyclosporine at 25 mg/kg dose significantly reduced the latency to enter the dark chamber in two compartment passive avoidance as compared to control- B (Olive oil) treated group (Table 3).

The rats treated with 2% gum acacia following cyclosporine had a reduced latency to enter the dark compartment in the retrieval trial compared to control-C (olive oil + 2% gum acacia) though not statistically significant. However, the rats treated with alcoholic and aqueous extract of Tc following cyclosporine administration had a significant increase in latency to enter dark compartment in retrieval trial compared to cyclosporine with 2% gum acacia treatment (Table 3).

**Assessment of motor activity**

**Open field chamber:** The alcoholic extract (200 mg/kg) and aqueous extract (100 mg/kg) of Tc treated rats had an increase in the peripheral and total square crossings in the open field chamber compared to control. However, there was no significant change in the central square crossing among the treated and control groups indicating an enhanced locomotor activity.

However, Tc along with cyclosporine administration continued to show an enhancement in locomotor activity in the open field chamber by increase in peripheral and total square crossing.

**Assessment of immune status**

**Dinitro chloro benzene (DNCB) skin sensitivity test:** In the DNCB skin sensitivity test, Tc alcoholic extract at both the doses (100, 200 mg/kg) and Tc aqueous extract (100 mg/kg) produced a significant increase in the induration of skin when compared to control-A (Table 4). In the DNCB skin sensitivity test cyclosporine at both the doses (15, 25 mg/kg) produced a significant reduction in induration of skin when compared to control-B (Table 5).

Cyclosporine 25 mg/kg with 2% gum acacia produced a significant decrease in induration, in the DNCB skin sensitivity test compared to control-C (Olive oil + 2% gum acacia). Cyclosporine (25 mg/kg) with Tc alcoholic extract (200 mg/kg) treated groups significantly increased the induration compared to cyclosporine (25 mg/kg) with 2% gum acacia and cyclosporine (25 mg/kg) with Tc aqueous extract (100 mg/kg). Whereas, cyclosporine (25 mg/kg) with Tc aqueous extract (100 mg/kg) has increased induration when compared to cyclosporine (25 mg/kg) with 2% gum acacia but the difference is not statistically significant. However, it has produced a statistically significant decrease in induration of the skin when compared to control-C (Olive oil + 2% gum acacia) (Table 5).

**Brain histopathological studies**

**Effect of *Tinospora cordifolia* on hippocampus:** There were no observable structural changes in all the subregions of hippocampus, dentate gyrus (DG) in the Tc extracts alone treated groups (Tc. Alcoholic extract 200 mg/kg and Tc aqueous extract 100 mg/kg) compared to control-A (2% gum acacia) treated group.
the present work was undertaken to study the effect of Tc on learning and memory in normal and cognition deficit rats. Both alcoholic and aqueous extract of Tc have enhanced the cognition in normal rats as seen in behavioural test-Hebb William maze and the passive avoidance task. However, microscopically there are no gross change in the hippocampal neurons. Hippocampus is one of the main centers for the learning and memory process.

Effect of cyclosporine on hippocampus:
Hippocampal subregion CA1, CA3, dentate gyrus showed neurodegenerative features in rats treated with 25 mg/kg cyclosporine (Figure 1B, 2B, 3B). There are no such changes in controls-B (olive oil treated) and cyclosporine 15 mg/kg treated groups (Figure 1A, 2A, 3A respectively).

Effect of combination of cyclosporine 25 mg/kg and Tc (alcoholic 200 mg/kg and aqueous 100 mg/kg) on hippocampus: There are neurodegenerative changes in CA1, CA3, and dentate gyrus in cyclosporine 25 mg/kg + 2% gum acacia treated but not in control-C (olive oil + 2% gum acacia), cyclosporine 25 mg/kg + Tc alcoholic 200 mg/kg and cyclosporine 25 mg/kg + Tc aqueous 100 mg/kg groups (Figure 1C, 2C, 3C).

DISCUSSION

The present work was undertaken to study the effect of Tc on learning and memory in normal and cognition deficit rats. Both alcoholic and aqueous extract of Tc have enhanced the cognition in normal rats as seen in behavioural test-Hebb William maze and the passive avoidance task. However, microscopically there are no gross change in the hippocampal neurons. Hippocampus is one of the main centers for the learning and memory process. Tc has increased the induration in DNCB test indicating immunostimulant activity, thereby supporting the previous reports.

Cyclosporine, a known immunosuppressant drug has caused immunosuppression in our study too, as shown by decreased induration in DNCB skin sensitivity. In the cognitive tests, cyclosporine has produced a significant impairment of learning and memory in rats. This cognitive impairment is associated with degeneration of hippocampal neurons.
Figure 2. Photomicrographs of hippocampal CA2 neurons in control (A), treated with cyclosporine (B) and cyclosporine + Tc (C). Note the degenerative neurons (arrows) in B & C. Cresyl violet stain, Scale bar =20 µ.
Figure 3. Photomicrographs of hippocampal dentate gyrus neurons in control (A), treated with cyclosporine (B) + Tc (C). Note the degenerative neurons (arrows) in B & C. Cresyl violet stain, Scale bar=20 μ.
**TINOSPORA CORDIFOLIA ON LEARNING AND MEMORY**

In conclusion, alteration of immune function affects learning and memory process and Tc is a potent immunomodulator and cognitive enhancer. The probable mechanism of cognitive enhancement by Tc could be by immunostimulation and increasing the synthesis of acetylcholine which is an important neurotransmitter in learning and memory process\(^{17}\). This central action could be due to supplementation of choline which is an important active constituent of Tc\(^{18}\). There are reports that supplementation of choline enhances the cognitive function in animals\(^{19}\) thereby supporting our hypothesis. Further studies are needed to explain the exact mechanism of action of Tc. This dual property of Tc may bear a potential use in neurodegenerative disease affecting the cerebral neurons and immunosuppression induced memory changes\(^{20}\).

**ACKNOWLEDGEMENT**

The authors acknowledge the support extended by the Dean, KMC, Manipal for carrying out the work. We also acknowledge Dr. Prathiba J an ex-colleague for helping in the study.

**REFERENCES**


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**Table 5.** Effect of vehicle, cyclosporine alone and cyclosporine with *Tinospora cordifolia* (Tc) alcoholic (alc) and aqueous (aq) extracts treated rats on the induration of skin in DNCB skin sensitivity test.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Induration of skin (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control- B (olive oil)</td>
<td>Equivolume</td>
<td>1.492 ± 0.134</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>15</td>
<td>0.144 ± 0.076(^*)</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>25</td>
<td>0.05 ± 0.033(^*)</td>
</tr>
<tr>
<td>Control- C (olive oil + 2% gum acacia)</td>
<td>Equivolume</td>
<td>1.386 ± 0.132</td>
</tr>
<tr>
<td>Cyclosporine + (2% gum acacia)</td>
<td>Equivolume</td>
<td>0.486 ± 0.111(^*)</td>
</tr>
<tr>
<td>Cyclosporine + Tc. alc. extract</td>
<td>25+200</td>
<td>1.311 ± 0.119(^*)</td>
</tr>
<tr>
<td>Cyclosporine + Tc. aq. extract</td>
<td>25+100</td>
<td>0.864 ± 0.095(^*)</td>
</tr>
</tbody>
</table>

Values represent mean±SEM. \(^*\)P< 0.05 Vs control- B, \(^\circ\)P< 0.05 Vs control- C, \(^\circ\)P< 0.05 Vs cyclosporine 25 mg/kg + 2% gum acacia, \(^\circ\)P< 0.05 Vs cyclosporine 25 mg/kg + Tc. aqueous extract 100 mg/kg.

histopathologically. Similar claims have been made by earlier workers following cyclosporine administration in chicks and in patients treated with cyclosporine\(^{3,5}\). However, Borlongan et al.,\(^{6}\) reported that chronic administration of cyclosporine at 10 mg/kg produced no change in the passive avoidance task. This effect could be due to low dose of cyclosporine used by the worker, where as we have found cognitive deficits at 15, 25 mg/kg dose level.

Memory impairment induced by cyclosporine lasted upto the 25\(^{th}\) day. This induced memory impairment by cyclosporine was successfully overcome by both the alcoholic and aqueous extract of Tc. Even histopathologically Tc has successfully reversed the hippocampal neuronal degeneration induced by cyclosporine. In conclusion, alteration of immune function affects learning and memory process and Tc is a potent immunomodulator and cognitive enhancer.

The probable mechanism of cognitive enhancement by Tc could be by immunostimulation and increasing the synthesis of acetylcholine which is an important neurotransmitter in learning and memory process\(^{17}\). This central action could be due to supplementation of choline which is an important active constituent of Tc\(^{18}\). There are reports that supplementation of choline enhances the cognitive function in animals\(^{19}\) thereby supporting our hypothesis. Further studies are needed to explain the exact mechanism of action of Tc. This dual property of Tc may bear a potential use in neurodegenerative disease affecting the cerebral neurons and immunosuppression induced memory changes\(^{20}\).


