Evaluation of effect of alcoholic extract of *Tinospora cordifolia* on learning and memory in alprazolam induced amnesia in albino mice


ABSTRACT

**Background:** *Tinospora cordifolia* is one of the most versatile shrub with cognitive enhancing effects could be beneficial for treatment of dementia and neurodegenerative diseases like alzheimer’s disease. The purpose of study was to evaluate the effect of *Tinospora cordifolia* on learning and memory in alprazolam induced amnesia in albino mice.

**Methods:** The study was carried out on albino mice, divided into 4 groups of 6 animals each (either sex, 3-4 months of age, weight 25-30g). Amnesia was induced by administering alprazolam (2 mg/kg body weight for 14 days) in all 4 groups for 14 days from 1st to 14th day. Group 1 was given alprazolam (2 mg/kg/p.o) alone for 14 days. In addition, group 2 was given piracetam (400 mg/kg p.o) from 8th to 15th day. Group 3 was given alcoholic extract of *Tinospora cordifolia* 140 mg/kg, p.o. from 1st to 15th day. Group 4 was given alcoholic extract of *Tinospora cordifolia* 280 mg/kg, p.o. from 1st to 15th day. The learning and memory of the animals was assessed by employing elevated plus maze (EPM) and step-down type passive avoidance model (SDA).

**Results:** Results were compared among the different groups using one way ANOVA followed by post hoc Tukey’s test. The measured parameters were compared with standard drug piracetam. *Tinospora cordifolia* at 140 mg/kg (p<0.02) and 280 mg/kg was significant in both models (p<0.02) and in EPM model, *Tinospora cordifolia* at 280 mg/kg showed highly significant result (P<0.01).

**Conclusions:** *Tinospora cordifolia*, Indian medicinal plant useful for treatment of various ailments can also be a useful alternative for treating dementia and associated diseases like alzheimer’s disease.

**Keywords:** *Tinospora cordifolia*, Piracetam, Alprazolam, Alzheimer’s disease, Elevated plus maze, Step-down type passive avoidance

INTRODUCTION

Dementia is defined as an acquired deterioration in cognitive abilities that impairs the successful performance of daily activities. Memory is the most common cognitive ability lost with dementia. Learning is defined as the acquisition of information and skills, and subsequent retention of that information is called memory. Memory is the ability of an individual to recall sensory stimuli, events, information etc. to retain them over short or long periods of time and recall the same at a later date when needed. One of the most common cause of dementia being alzheimer’s disease (AD) affecting 10% of elderly population. AD is associated with widespread neuronal degeneration involving hippocampus and cognitive changes begins with impairment of memory, spreading to language and visuospatial deficits. The progressive memory deficits and cognitive impairment affects patient’s quality of life.

*Tinospora cordifolia* is an Indian medicinal plant commonly known as Guduchi, has been used in Ayurvedic preparations throughout the centuries for the treatment of various ailments like general weakness, fever, dyspepsia, gonorrhoea, secondary syphilis, urinary diseases, impotency, gout, viral hepatitis, skin diseases, jaundice, rheumatoid arthritis, and diabetes.

Currently available treatment of AD includes cholinesterase inhibitors like donepezil, rivastigmine and galantamine. These drugs have only modest efficacy and side effects like GI distress, altered sleep with unpleasant
dreams, muscle cramping and bradycardia.⁵ There is a need for development of newer and effective drugs in order to improve patient’s quality of life. Ayurvedic literature recommends a rejuvenating recipe where Tinospora cordifolia being an important constituent to enhance the memory.⁶,⁷ With this background the present study has been taken to evaluate the effect of Tinospora cordifolia on learning and memory in alprazolam induced amnesia in albino mice.

The objective of this study was to evaluate the effect of Tinospora cordifolia on learning and memory in alprazolam induced amnesia in albino mice.

METHODS

Source of test drug

The whole plant of Tinospora cordifolia was collected from their natural habitat in Davangere city, Karnataka state, India.

Preparation of alcoholic extract of Tinospora cordifolia

The whole plant was shade dried for one month and then coarse powder was obtained using a mechanical grinder. Coarse powder of 300 g was successively extracted with 1.5 L of alcohol, in a soxhlet apparatus at 70-75°C each for 10-12 hours consecutively. Alcohol was removed from the extract and a semisolid mass was obtained using water bath. The yield of alcoholic extract was weighed and used for experiment.

Selection of animals

Albino mice of either sex, weighing around 25-30 g were employed in the present study. They were exposed to alternate light and dark cycle of 12 hours and had a free access to food and water. They were procured from animal house of J. J. M. Medical College, Davangere.

Inclusion criteria

Healthy albino mice weighing 25-30 gm with normal behaviour and activity. The mice previously unused for any experiment.

Exclusion criteria

Pregnant and diseased animals were not included in the study.
- Duration of study: 2 months

Drugs and chemicals used

- Standard drug- Piracetam
- Amnesic drug- Alprazolam
- 1% Carboxy methyl cellulose.

Drugs and chemicals were procured from local market and were of analytical grade.

Instruments required

- Elevated plus maze
- Step-down avoidance apparatus
- Oral feeding tube
- Stop watch

Procedure

Albino mice of either sex, weighing around 25-30 gm were randomly selected. All the animals were kept under laboratory condition for acclimatization period of seven days before carrying out the experiments. Each group of mice was housed separately in a metallic cage and provided with adequate food and water. They were maintained under standard laboratory conditions with natural light and dark cycle, under room temperature.

Study was conducted for 16 days. Experiment was carried out during the 15th and 16th day between 9.00 am and 3.00 pm. Animals were divided into four groups of 6 mice each. Study drug piracetam was suspended in 1% Carboxy methyl cellulose.

Group 1 received amnestic agent alprazolam (2 mg/kg/p.o) alone for 14 days. Group 2 received alprazolam (2 mg/kg p.o) for 14 days with piracetam (400 mg/kg p.o) from 8th to 15th day. Group 3 received alprazolam (2 mg/kg alone p.o) for 14 days with alcoholic extract of Tinospora cordifolia 140 mg/kg, p.o. from 1st to 15th day. Group 4 received alprazolam (2 mg/kg alone p.o) for 14 days with alcoholic extract of Tinospora cordifolia 280 mg/kg, p.o. from 1st to 15th day.⁷,⁸

Piracetam is a nootropic drug, has been used as a standard in many research articles.⁹

Group 1- Treated with alprazolam 2 mg/kg alone p.o.

Group 2- Alprazolam (2 mg/kg alone, p.o) + piracetam 400 mg/kg p.o (standard group).

Group 3- Alprazolam (2 mg/kg alone, p.o) + alcoholic extract of Tinospora cordifolia 140 mg/kg, p.o. (test group 1).

Group 4- Alprazolam (2 mg/kg alone, p.o) + alcoholic extract of Tinospora cordifolia 280 mg/kg, p.o. (test group 2).

On 15th day, 90 minutes after the administration of the last dose of drugs in the respective groups, mice were exposed to elevated plus maze and passive avoidance task for acquisition (learning) and retention (memory) was recorded 24 hours later, on the 16th day.
Laboratory models for testing learning and memory enhancing activity.

**Elevated plus maze**

Mazes are the traditional tools in assessing learning and memory performance in laboratory animals. These are used to measure the cognitive performance, notably to evaluate the spatial long term memory in mice and rats.

The apparatus consists of two open arms measuring 16×5 cm for mice and two enclosed arms measuring 16×5×12 cm with an open roof, arranged so that the two open arms and two enclosed arms are opposite to each other. The maze is elevated to a height of 25 cm. Transfer latency (TL) is a parameter of memory. The transfer latency was defined as the time in seconds taken by the animal to move into one of the enclosed arms. The experiment was performed in 2 stages. On day 15, the day of acquisition testing, each mice was placed at the end of an open arm facing away from the centre. The time taken to enter any one of the closed arms was recorded as transfer latency (TL).

All four legs inside the closed arm were counted as an entry. Cut off time allotted for each mice would be 90s. Those animals which could not enter the closed arms within the cut off time were excluded from the study. Retention testing was conducted 24 hours after the first trial and transfer latency was recorded in a similar manner as mentioned before. Shortened transfer latency on next day was considered as an index of improvement of memory.2

**Step-down type passive avoidance test**

The step-down type of passive avoidance task has been used to examine the long term memory based on negative reinforcement. The apparatus consists of a transparent acrylic cage (30×30×40 cm high) with a grid floor, inserted in a semi-soundproof outer box (35×35×90 cm). The cage was illuminated with a 15 W lamp during the experimental period. A wooden platform (4×4×4 cm) was fixed in the centre of the grid floor. Electric shocks (1 Hz, 500 msec, 40 V DC) were delivered to the grid floor with a commercially available isolated pulse stimulator. The training was carried out in two similar sessions. Each mouse was placed on the platform in the centre of the cage. When the mouse steps down and places all its paws on the grid floor, shock was delivered for 15 seconds. Step down latency (SDL), and the number of flinching reactions and vocalizations were measured. Animals showing SDL of 3-30 seconds during the first training session were preselected for second and retention trials. The second session was carried out 90 minutes after the first. Animals staying 60 seconds on the platform were considered as remembering the task and no longer received electric shocks. The retention test is carried out 24 hours after training, in a similar manner, except that electric shocks were not applied to the grid floor. Each mouse was again placed on the platform, and the SDL was recorded with an upper cut-off time of 300 seconds. Amnestic drugs are expected to decrease the SDL in subsequent trails indicating the impairment of learning and retention tasks.2

**RESULTS**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drugs</th>
<th>15th day Mean±SD</th>
<th>16th day Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Alprozolam only</td>
<td>72.5±30.62</td>
<td>65.17±29.8</td>
</tr>
<tr>
<td>Group B standard</td>
<td>Alprozolam + Piracetam</td>
<td>70.0 ±17.03</td>
<td>52.5±18.9</td>
</tr>
<tr>
<td>Group C test</td>
<td>Alprazolam + Tc 140 mg/kg</td>
<td>81.67±16.02</td>
<td>49.33±24.22</td>
</tr>
<tr>
<td>Group D test</td>
<td>Alprazolam + Tc 280 mg/kg</td>
<td>66.67±22.29</td>
<td>33.83 ± 22.96</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SD, p value<0.05 considered significant.

<table>
<thead>
<tr>
<th>Groups</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A versus B</td>
<td>p&gt;0.12</td>
</tr>
<tr>
<td>A versus C</td>
<td>p&lt;0.000</td>
</tr>
<tr>
<td>B versus C</td>
<td>p&lt;0.02</td>
</tr>
<tr>
<td>B versus D</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>
Both doses of alcoholic extract of *Tinospora cordifolia* showed significant result compared to standard drug (p<0.02). Results are given in Table 2 and Figure 2.

**DISCUSSION**

The present study was undertaken to study the effect of *Tinospora cordifolia* (Tc) on learning and memory in alprazolam induced amnesic mice. Alcoholic extract of Tc has enhanced cognition in elevated plus maze and step-down type passive avoidance task. Alprazolam has been used as an amnesic agent. It belongs to the class of Benzodiazepines and has been shown to impair cognitive process.\(^8\)

*Tinospora cordifolia* is a plant with diverse medicinal properties like anti-diabetic, anti-inflammatory, anti-arthritis, anti-oxidant, anti-stress, anti-allergic, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities.\(^3,10,11\) Various phytochemical constituents have been identified like alkaloids, diterpenoid lactones, glycosides, sesquiterpenoid, aliphatic compounds, phenolics, and polysaccharides, steroids like tinosporine, tinosporaside, cordifolide, cordifol, heptacosanol, furanolactone tinosporidine, columbin and β-sitosterol. Choline is one of the important constituent among alkaloids.\(^4\)
In our study both the doses of alcoholic extract of *Tinospora cordifolia* produced significant results compared to standard drug piracetam. A study by Agarwal A, Malini S, Bairy KL, et al. Effect of *Tinospora cordifolia* on learning and memory in normal and memory deficit rats suggested that Tc may bear a potential use in neurodegenerative disease affecting cerebral neurons and immusuppression induced memory changes. Another study by Malve HO, Raut SB, Marathe PA, et al. Effect of combination of *Phyllanthus emblica* (Pe), *Tinospora cordifolia* (Tc) and *Ocimum sanctum* on spatial learning and memory in rats suggested that 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. 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. This proposed mechanism of action could be due to supplementation of choline which is an important active constituent of Tc. Citicholine is a compound derived from choline and cytidine, used as cognitive enhancer, as studies have demonstrated that it causes short-term improvement in memory and behaviour in cerebrovascular disorders it is a supporting report for the proposed mechanism of action of Tc. Further studies are needed to explore the exact mechanism of action of Tc.

**CONCLUSION**

*Tinospora cordifolia* an Indian medicinal plant with various properties can be a useful alternative and natural source drug for treatment of defective learning and memory and also to other geriatric diseases. Further studies exploring the mechanism of action of *Tinospora cordifolia* are required to support these observations.

**Funding:** No funding sources  
**Conflict of interest:** None declared  
**Ethical approval:** The study was approved by the Institutional Ethics Committee

**REFERENCES**


