Nootropic Activity of Saponins obtained from Tinospora Cordifolia Stem in Scopolamine induced Amnesia

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ABSTRACT
The present study was designed to evaluate the nootropic property of n-butanol (TBF) fraction of ethanolic extract of Tinospora cordifolia stem which contain saponin. The TBF was administered at 100 mg/kg and 200 mg/kg. The nootropic effect was evaluated using different models like elevated plus maze in mice to determine the transfer latency on day-2 and Day-9. The passive avoidance test was carried out in mice to evaluate the step down latency on day-2 and day-9. The object recognition test carried out to determine the recognition index. Scopolamine (1 mg/kg) was used as amnesic agent and piracetam (250 mg/kg) was used as a standard nootropic drug. The results of the present study showed significant increase in transfer latency by TBF on day -2 and Day-9. The TBF also increases step down latency, object recognition index significantly. Scopolamine was used to produce amnesia significantly decreased transfer latency, step down latency, object recognition index. The TBF antagonised the amnesic effects of scopolamine in the above experimental models. Anti-cholinesterase activity of TBF was evaluated by estimation of acetylcholinesterase (AchE) concentration in mice brain after 7-days treatment with TBF. The result showed decreased in AchE concentration indicating involvement of cholinergic system in nootropic activity of TBF.

Keywords: Acetyl choline esterase, nootropic, saponin, Tinospora cordifolia

INTRODUCTION
Memory is ability of an individual to record event, information and retains them over short or long periods of time and recalls the same whenever needed. Age, stress and emotion are conditions that may lead to memory loss, amnesia, dementia, anxiety, and other complications to more ominous threat like schizophrenia and Alzheimer’s diseases. Alzheimer’s disease is progressive neurodegenerative disease that primarily affects the elderly population, and is estimated to account for 50-60% of dementia cases in persons over 65 years of age. Nootropics are agents that enhance the cognitive skills, and “amnestics” are agent that disrupts the learning and memory processes [1]. Learning and memory can be conceived as both a psychological process, as well as a change in synaptic neural connectivity. Cognitive deficits have long been recognized as severe and consistent neurological disorders associated with numerous psychiatric and neurodegenerative states. Indian systems of medicine emphasize the use of herbs, nutraceuticals or life style changes for controlling age related neurodegenerative disorders. Plants like Bacopa monniera, Azadirachta indica, Withania somnifera, Hypericum perforatum, Albizzia lebbeck, Vitis vinifera, Panax ginseng as well as Ocimum sanctum have been investigated for their effect on cognitive functions [2-3]. The plant Tinospora cordifolia used for the treatment of various diseases and disorders, in the folk medicine of different cultures. Tinospora cordifolia reported to contains saponins. There are number of studies available regarding the nootropic activity of saponins [4-6]. Hence the present
study designed to investigate the nootropic activity of saponins obtained from n-butanoic fraction of \textit{Tinospora cordifolia} stem.

**MATERIALS AND METHODS**

**Plant material**
The whole plant of \textit{Tinospora cordifolia} collected from local area of Aurangabad in the month of August-September. Authentication of the plant was done at Botany department of Dr. Babasaheb Ambedkar Marathawada University Aurangabad (voucher specimen no. 0732). The whole plant was shade dried and coarse powdered.

![Figure 1: Tinospora cordifolia](image)

**Figure 1: Tinospora cordifolia**

**Extraction and isolation of saponins**
Coarse powder of shade dried stem of \textit{Tinospora cordifolia} were defatted with petroleum ether (60–80°C) in Soxhlet’s extractor. The marc was dried and again extracted with ethanol in Soxhlet’s extractor. The ethanolic extract was evaporated to dryness in vacuum. The residue was suspended in water, extracted with ethyl acetate and n-butanol to get ethyl acetate, n-butanol, and water soluble portions. n-butanol soluble portion then evaporated to dryness in vacuum. The n-butanol soluble fraction (TBF) was tested for the presence of saponins using haemolysis test and foam test [6-7] and used for the study.

**Experimental animals**
Albino mice (Swiss, 25–30 g) were used in this study. The animals were allowed to acclimatize to the laboratory conditions for 10 days after their arrival. The animals were housed under standard housing conditions. Animals were fasted overnight prior to drug administration and during the experiment. All experiments were carried out during the light period (09:00–17:00 h). Separate groups of mice were used for various tests. The mice were randomized into experimental and control groups and housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water \textit{add libitum}. Animals were habituated to laboratory conditions for 48 hr prior to experimental protocol to minimize if any of non-specific stress. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) constituted as per the direction of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under ministry of Animal Welfare Division, government of India, New Delhi IAEC. (CPCSEA/IAEC/P’COL-03/21).

**Acute toxicity test**
The Acute Toxicity of TBF was performed as per OECD guideline 425 using albino mice of female sex (20-25g) maintained under standard dietary conditions. The animals were fasted for 3hr before experiment. Animals were administered with single dose of TBF. Maximum dose of TBF administered was 3000 mg/kg.

**Preparation of drug solution**
Accurately the weighed quantity of TBF, Piracetam and scopolamine was dissolved in the distilled water to prepare the appropriate stock solutions. The doses administered orally by selecting the appropriate concentration of the stock solution as per the individual body weight of the mice.

**Elevated Plus Maze**
The EPM consisting of two open arms (35x6 cm) and two enclosed arms (35x6x15 cm) was elevated to the height of 25 cm. On the first day, each mouse was placed at the end of open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by mouse to move into one of the closed arm with all its four legs. TL was recorded on the first day. The mouse was allowed to explore the maze for
10 sec and then returned to its home cage. Memory retention was examined 24 h (day-2) after the first day trial on the day-9 [9-10]. Mice received vehicle, piracetam (250 mg/kg) or TBF (100 mg/kg and 200 mg/kg), Scopolamine (1mg/kg), 30 min before their placement on the elevated plus maze as before and TL was noted. Scopolamine (1mg/kg) was also given 30 min before piracetam and TBF. The “inflexion ratio (IR)” using the formula, 
\[ IR = \frac{(L_1 - L_0)}{L_0} \]
Where, \( L_0 \) = transfer latency on day-2/day-9 in sec.
\( L_1 \) = initial transfer latency in sec.

**Passive shock avoidance test**

The apparatus consisted of a box (27 X 27 X 27 cm) having three walls of wood and one wall of Plexiglas, featuring a grid floor (3 mm stainless steel rods set 8 mm apart), with a wooden platform (10 X 7 X 1.7 cm) in the center of the grid floor. Electric shock (1Hz, 500 msec, 40V DC) was delivered for 15 sec. to the grid floor. The individual mouse was placed on the elevated platform, i.e. the shock free zone (SFZ), and the step down latency (SDL) was noted [11-12].

\[ \text{Inflexion ratio} = \frac{(L_1 - L_0)}{L_0} \]
Where, \( L_0 \) is the SDL on the Day-2/ Day-9 in sec.
\( L_1 \) is the initial SDL in sec.

**Object recognition test**

A plastic chamber (35cm×35cm×35 cm) was used in low light condition during the light phase of the light/dark cycle. The general procedure consisted of three different phases: a habituation phase, an acquisition phase, and a retention phase. On the 1st day (habituation phase), mice were individually subjected to a single familiarization session of 10 min, during which they were introduced in the empty arena, in order to become familiar with the apparatus. On the 2nd day (acquisition phase) animals were subjected to a single 10-min session, during which floor-fixed two objects (A and B) were placed in a symmetric position in the central line of the arena, 10 cm from each and 8 cm from the nearest wall. The two objects, made of the same material with the similar color and smell, were different in shape but identical in size. Mice were allowed to explore the objects in the open field. The exploration time on each object was shown (as seconds) to indicate the exploring activity of mice. On the 3rd day (retention phase), mice were allowed to explore the open field in the presence of two objects: the familiar object A and a novel object C in different shapes but in similar color and size of A. Recognition index (for retention session), calculated for each mouse, was expressed as the ratio \((TC \times 100)/(TA + TC)\), where TA and TC are the time spent during retention phase on object A and object C, respectively. The time spent exploring any object (nose pointing toward the object at a distance ≤1 cm, but not mounting on the object or playing with the object) was recorded [13-14].

**Estimation Acetyl cholinesterase**

The TBF was administered for 7-days. On day-7, 1 h after the last doses, all mice were quickly decapitated by guillotine and brain was isolated from the skull immediately. The dissection for discrete regions of brain (prefrontal cortex, hippocampus and hypothalamus) was carried out. AChE inhibitory activity of MAR was measured in above brain regions [15]. Briefly, the discrete brain regions were homogenized in ice cold 0.1 M phosphate buffer (pH 8.0) using Remi homogenizer. The homogenates were centrifuged at 1000g for 10 min at 4°C, and supernatant was used as a source of enzyme in AChE assay. The total acetyl cholinesterase activity in the aliquot of the homogenate was estimated. The aliquot (0.3ml) was mixed with phosphate buffer (2.6ml) (pH7.0). The substrate acetyl thiocoline iodide and di-thio-bis-nitrobenzoic acid (DTNB) reagent were added. Acetyltiocholine iodide was hydrolyzed to thiocoline and acetate by AChE. Thiocoline reacted with DTNB reagent to produce a yellow colour. The rate of formation of thiocoline from acetylcholine iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The rate of colour development is the measure of the AChE activity at 412 nm.

**Statistics**

The observations are given as means ± S.E.M. The data was analyzed by student’s \( t \) test, \( P < .05 \) was considered significant.
RESULTS

Acute Toxicity
Animals treated with n-butanol fraction of Tinospora cordifolia (TBF) were shown no signs of any toxicity or mortality at the dose of 3000 mg/kg.

Elevated Plus Maze
The retention of learned task was studied after 24 h as transfer latency on the elevated plus maze. The effect on transfer latency was expressed by IR. Increase in IR after 24hr. indicated improved retention of learned task. Scopolamine shows significant reduction in the IR indicating impairment in retention of learned task. Piracetam showed significant increase in IR compared to vehicle treated group and antagonized the effect of scopolamine significantly (P<0.01). The TBF in the doses of 100 and 200 mg/kg increased the IR and also antagonized the amnesic effect of scopolamine significantly (P<0.01). The results are shown in [Table 1].

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Day-1 Transfer latency(s)</th>
<th>Day-2 Transfer latency(s)</th>
<th>Day-9 Transfer latency(s)</th>
<th>I.R.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>222.33±7.79</td>
<td>95.66±6.20</td>
<td>51.33±9.79</td>
<td>1.37</td>
</tr>
<tr>
<td>II</td>
<td>Scopolamine (1mg/kg)</td>
<td>279.00±5.89</td>
<td>163.0±13.5**</td>
<td>44.33±7.03**</td>
<td>0.76</td>
</tr>
<tr>
<td>III</td>
<td>Piracetam (250 mg/kg)</td>
<td>143.17±8.32</td>
<td>21.83±1.93**</td>
<td>15.33±2.09**</td>
<td>5.65</td>
</tr>
<tr>
<td>IV</td>
<td>TBF (100 mg/kg)</td>
<td>176.00±4.56</td>
<td>30.66±4.06**</td>
<td>14.33±0.80**</td>
<td>5.17</td>
</tr>
<tr>
<td>V</td>
<td>TBF (200 mg/kg)</td>
<td>125.5±5.90</td>
<td>44.83±6.25**</td>
<td>9.83±1.35**</td>
<td>2.56</td>
</tr>
<tr>
<td>VI</td>
<td>Scop. + Pira.(250 mg/kg)</td>
<td>154.50±7.30</td>
<td>22.66±1.70**</td>
<td>20.33±3.56**</td>
<td>5.30</td>
</tr>
<tr>
<td>VII</td>
<td>Scop.+ TBF (100 mg/kg)</td>
<td>120.8±40.8</td>
<td>34.16±11.4**</td>
<td>25.16±1.85**</td>
<td>3.85</td>
</tr>
</tbody>
</table>

The step down latency was assessed as inflexion ratio (IR). Vehicle-treated mice exhibited IR of 9.68±0.28 and 20.78±0.85 on day-2 and day-9 respectively. Scopolamine (1 mg/kg) decreased the IR to 7.21±6.7 and 6.43±1.01 day-2 and day-9 respectively indicating impairment of retention. The IR was significantly (P<0.01) increased by treatment of piracetam on day-2 and day-9. Piracetam also antagonized the effect of scopolamine. TBF in doses of 100 mg/kg increased the IR significantly but the higher dose (200 mg/kg) exhibited decreased IR on day-2 as well as on the day-9. TBF 100 mg/kg antagonized the effect of scopolamine significantly (P<0.01). The observations are given in [Table 2].

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Step down latency(s) (IR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day-2</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
</tr>
<tr>
<td>V</td>
<td>Scopolamine (1mg/kg)</td>
</tr>
<tr>
<td>II</td>
<td>Piracetam (250 mg/kg)</td>
</tr>
<tr>
<td>III</td>
<td>TBF (100 mg/kg)</td>
</tr>
<tr>
<td>IV</td>
<td>TBF (200 mg/kg)</td>
</tr>
<tr>
<td>VI</td>
<td>Scop. + Pira.(250 mg/kg)</td>
</tr>
<tr>
<td>VII</td>
<td>Scop.+ TBF (100 mg/kg)</td>
</tr>
</tbody>
</table>

The exploring time to the novel object as expressed by recognition index. The recognition index of normal control group was 52.40±3.86. Scopolamine shows significant decreases in the recognition index as 41.82±4.02. The piracetam and TBF 100 and 200 mg/kg show significant (P<0.01) increases in the novel object
exploration indicated as increased recognition index. The piracetam and TBF 100 mg/kg also antagonist effects of scopolamine by increasing novel object exploration time. The observations are shown in [Table 3].

**Table 3: Effect of TBF On Object Recognition Test In Mice**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Recognition Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>52.40±3.86</td>
</tr>
<tr>
<td>II</td>
<td>Scopolamine (1mg/kg)</td>
<td>41.82±4.02</td>
</tr>
<tr>
<td>III</td>
<td>Piracetam (250 mg/kg)</td>
<td>80.88±3.63**</td>
</tr>
<tr>
<td>IV</td>
<td>TBF (100 mg/kg)</td>
<td>64.51±4.11*</td>
</tr>
<tr>
<td>V</td>
<td>TBF (200 mg/kg)</td>
<td>70.56±1.57**</td>
</tr>
<tr>
<td>VI</td>
<td>Scop. + Pira.(250 mg/kg)</td>
<td>63.49±3.79</td>
</tr>
<tr>
<td>VII</td>
<td>Scop. + TBF (100 mg/kg)</td>
<td>54.71±3.85</td>
</tr>
</tbody>
</table>

n = 6, *P < 0.05, vs. respective control (Student’s t test)

The whole brain AChE activity with Scopolamine (1 mg/kg) demonstrated significant rise in AChE activity as compared to control, piracetam (250 mg/kg, i.p.) and TBF treated groups. TBF (100 and 200 mg/kg) significantly (P<0.05) lowered AChE. Results are shown in [Figure 2].

**Figure 2: Effect of TBF on Ache Concentration In Mice Brain.**

**DISCUSSION**

Nootropic drugs belong to the category of psychotrophic agents with selective facilitatory effect on intellectual performance, learning and memory [4]. Nootropic drugs are the potential tools in the study of behavioral and neurobiological basis of learning and memory which may provide critical data for understanding and treating disorders of cognitive dysfunctions.
A number of drugs including Piracetam, Aniracetam etc. have now been introduced in therapy to ameliorate cognitive deficits. Medicinal plants have been used to treat many psychotropic and behavioral conditions, such as dementia, Alzheimer’s disease, poor memory, anxiety, seizures, insomnia, depression and drug intoxication. The stems of *Tinospora cordifolia* possess saponins, hence potential to study nootropic activity.

The elevated plus maze is a widely accepted model to study nootropic activity. This observation has been strengthened by the finding that TBF has shortened the transfer latency in the elevated plus maze model indicating improvement in memory. In the Elevated plus maze test, mice show natural aversion to open and high spaces and therefore, spend more time in enclosed arms [10]. The TL might be shortened if the animal had previous experience of entering the open arm and it could be related to memory. TLs on day 1st and 2nd are taken as acquisition and retrieval, respectively [16]. The increase in the IR by TBF meets a major criterion for nootropic activity, i.e. improvement of memory [10]. Both TBF (100 and 200 mg/kg) and piracetam (250 mg/kg) reduced TL on day 2, 9 and significantly reversed scopolamine induced amnesia, suggesting an underlying cholinergic mechanism. Piracetam is known to reverse scopolamine induced amnesia [16]. The drug-induced increase in cortical muscarinic acetylcholine receptor capacity might partly explain the cognition enhancing and memory improving effects of TBF observed in mice. The increase in muscarinic receptors with cholinergic antagonists like scopolamine, represent receptor up regulation [17] as a part of the physiological response to overcome decreased cholinergic activity. Many anticholinergic drugs, such as scopolamine induce a transient disruption of memory in humans and experimental animals by blocking postsynaptic muscarinic receptors [18].

The present study demonstrates that in a passive avoidance paradigm a model for short-term memory, TBF produces improvement in passive avoidance acquisition and memory retrieval. The TBF has shown increase in step down latency and also antagonized effect of scopolamine. This model is predictive of aversion induced motivation. The increasing of step down latency by TBF as well as piracetam indicated improvement in memory in the absence of cognitive deficit [19]. The TBF increased occupancy in the shock free zone (SFZ) of the paradigm and also exhibited diminished preference to the proffered shock zone.

The object recognition test suggests that spontaneous tendency of rodents to explore the novel object (situation) in the test trial based on memory of previous experience from the acquisition trial. In present study the object recognition index is determine with mice and demonstrate its characteristic as spatial memory work [20]. Mice treated with TBF 100 and 200 mg/kg after experiencing an acquisition trial and spent more time exploring the novel object. Thus from results and observation the present study indicated that the n-butanol fraction of *Tinospora cordifolia* possesses nootropic activity.

The present study indicates that *Tinospora cordifolia* is a potential anti-cholinesterase agent. It also possesses nootropic activity in view of its facilitator effect on retention of acquired learning. Central cholinergic system plays an important role in learning and memory. Scopolamine is known to reduce hippocampal ACh concentration and cause cognitive impairment [21]. In the present study, Scopolamine (1mg/kg) significantly elevated brain AChE activity. Piracetam (250 mg/kg) and TBF (100, 150 and 200 mg/kg), on the other hand, significantly (P<0.05) lowered this activity. To find out whether TBF has any central cholinergic activity, the effect of TBF was evaluated for anti-AchE activity in brain. The prefrontal cortex, hippocampus and hypothalamus which are reported to be important regions involved in processing of memory and are profusely endowed with cholinergic neurons. The hippocampus is permanently involved with tasks in which memories have to be acquired and retrieved. In addition, the hippocampus it could be temporarily involved in memory consolidation and an area for the temporary storage of consolidated information. Then,
theses information is transferred into prefrontal cortex where short-term memory is converted into long term memory, the process is called consolidation. It has been suggested that stimulation of hypothalamus facilitates hippocampus dependent learning and memory processes in a wide variety of paradigms, in both young and aged rats. In the present study, TBF pre-treatment for 7 days (100, 150 and 200 mg/kg) inhibited AChE activity in Brain. These results suggest that TBF by virtue of its anti-cholinesterase may significantly enhance cholinergic neurotransmission in these distinct brain regions and thus enhance learning and memory functions. Thus, it is concluded that the TBF (n-butanol fraction of T. cordifolia), which contained saponins, possessed nootropic activity.

CONCLUSION
In the present study we have evaluated the nootropic activity of n-butanol fraction of Tinospora cordifolia (TBF) using elevated plus maze, passive avoidance and object recognition test. The result proved that TBF possesses nootropic activity. TBF also decreased the AChE activity in mice brain and hence indicates the involvement of cholinergic system in its mechanism.

REFERENCES
passive avoidance task, J. of Psychopharmacology, 1982; 77:66.