BR-16A (Mentat), A Herbal Preparation, Improves Learning and Memory Performance in Mice

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ABSTRACT
The effectiveness of BR-16A, a herbal psychotropic preparation, was investigated in short-term memory paradigms in mice. The passive avoidance task consisted of an electric grid with a centrally located shock-free zone (SFZ). BR-16A (50-500 mg/kg) improved passive avoidance acquisition as well as retrieval in mice. BR-16A reversed scopolamine (0.3 mg/kg)-induced disruption of learning and memory. Physostigmine (0.5 mg/kg) enhanced the effectiveness of BR-16A (50 and 100 mg/kg) against scopolamine-induced deficits. The amnesia-induced by acute treatment with electroconvulsive shock (ECS) was also significantly reversed by BR-16A (50 and 100 mg/kg). When administered in combination with GABA (50 mg/kg) and aniracetam, respectively BR-16A (50 mg/kg) treated mice showed improved learning and memory retrieval. In another model, using transfer latency as a parameter employing elevated plus-maze, similar observations were recorded in scopolamine-treated mice following administration of BR-16A along or its combination with aniracetam. The results suggest for a possibly nootropic action of BR-16A involving cholinergic and GABAergic modulation.

INTRODUCTION
BR-16A (Mentat), a herbal psychotropic preparation has been reported to possess a beneficial effect in cases of mental retardation, cerebral deficit, behavioural disorders following postnatal organic lesions of central nervous system and in cases having organic loss of bladder function. BR-16A contains the following indigenous plant ingredients reputed in the ancient system of Ayurvedic medicine to be of value in the management of nervous disorders. Brahmi (Hydrocotyl asiatica), Bach (Acorus calamus), Ashwagandha (Withania somnifera), Shankpushpi (Evolvulus alsinoides), Jatamansi (Nardostachys jatamansi), Jalabrahmi (Harpestis monniera), Amla (Embelica officinalis), Giloi (Tinospora cordifolia) and Triphala. Preliminary toxicity studies have shown it to be a safe preparation and no adverse effect ensued the chronic use of BR-16A. The LD₅₀ value of BR-16A has been reported to be 2400 mg/kg by oral route of administration (Personal communication).

In the present study the effectiveness of BR-16A in improving short-term memory and reversing the amnestic effect of scopolamine or electroconvulsive shock (ECS) was studied using animal models. The effect of BR-16A on acquisition and memory retrieval was investigated employing a passive avoidance paradigm and an elevated plus-maze. Pharmacological interventions such as scopolamine-dementia and the role of cholinergic or
γ-amino-butyric acid (GABAergic) interactions in the facilitatory action of BR-16A were studied in mice.

METHODS

Animals:
Balb/c albino mice of either sex in the ratio of 1 female: 5 male (bred in Central Animal House facility of Punjab University), weighing 20-25 g were used. The animals were housed under standard light/dark cycle with food and water provided ad libitum. All the experiments were performed between 8.00 and 12.00 hrs.

Drugs:
The drugs used in the present study were obtained from the following sources: BR-16A (Mentat; The Himalaya Drug Co., Bombay, India), scopolamine HBr (Merck and Co. Inc., NJ, USA), physostigmine (C.H. Boehringer Sohn Ingelheim Am Rhein, Germany), GABA (Sigma, U.S.A.) and aniracetam (F. Hoffman-La Roche, Basel, Switzerland).

BR-16A powder was suspended in distilled water and administered orally while the remaining drugs were given intraperitoneally in a constant volume of 1 ml/100 g of body weight as aqueous solutions.

Apparatus:

Passive avoidance paradigm - The method described by Sharma and Kulkarni was used. In brief, the apparatus consisted of an electric grid (24 x 30 cm) with a shock free zone (SFZ; 2 x 3 x 1 cm) in the centre and the entire grid having a perflex enclosure.

Elevated plus-maze - An elevated plus-maze consisting of two open arms (16 x 5 cm) and two enclosed arms (16 x 5 x 12 cm) was used in the present study. The maze was elevated to a height of 25 cm.

Electroconvulsive shock (ECS)
ECS (10mA, 0.2 sec) was applied through ear clip electrodes. The animals received either a single shock (acute treatment) or a series of six shocks at 24 hr interval (chronic treatment).

Procedure:

Experiment 1 - Passive avoidance training was done as follows. The mice were put individually on the electric grid and allowed to explore for 1 min. The stimulus (20v) was then applied and latency to reach SFZ was recorded three consecutive times as basal readings. Animals that reached the SFZ in 2 min in the first trial were selected for the study. After 1 hr of training, each animal was put on the electric grid again and the latency to reach SFZ and the number of mistakes (descents) the animal made in 15 min were recorded as parameters for acquisition and memory retention, respectively. Thirty minutes after the first trial mice received vehicle or BR-16A (50-500 mg/kg) either alone or in combination with GABA and aniracetam, respectively.
Experiment 2 - The procedure and the apparatus were identical to those described in experiment 1. Two groups of mice were used to study the effect of vehicle or scopolamine (0.3 mg/kg). Four groups of mice were used for studying the effect of BR-16A (50-500 mg/kg) on amnesia produced by scopolamine (0.3 mg/kg) and three groups were used to study the effect of physostigmine or its combination with BR-16A (50 and 100 mg/kg) on scopolamine-induced amnesia.

Experiment 3 - The procedure and the apparatus were identical to those described in experiment 2, except that instead of number of mistakes (descents) in 15 min, the latency to reach SFZ measured 24 hr after training served as a parameter for retention. The animals were divided into four groups. One group received ECS through the ears and another group was subjected to the same procedure but without ECS (non-ECS: control group). In the ECS experiment, ECS was applied immediately after the training. Latency to reach SFZ was then recorded 1 hr and 24 hrs after training. BR-16A (50 and 100 mg/kg) or vehicle was administered 30 min following the application of ECS.

Experiment 4 - Elevated plus-maze was employed for the measurement of transfer latency (TL). The mice were placed individually at the end of one open arm facing away from the central platform and the time it took to move from open arm to either of the enclosed arms (TL) was recorded. Transfer latency was the time elapsed between the time the animal was placed on the open arm and the time when it fully entered (all the four paws in) the enclosed arm. On the 1st day the mouse was allowed to explore the plus-maze for 20 sec after the measurement of TL. The mice were returned to their home cages after the 1st trial. Twenty-four hours later, the mice were placed on the elevated plus-maze individually as before and TL was recorded again. Transfer latency measured on 1st and 2nd day served as parameters for acquisition and retrieval, respectively. All the drugs were administered 30 min prior to the 1st trial, either alone or in combination and each treatment group consisted of 6-9 animals.

Statistical analysis:
The data was analysed by one-way analysis of variance (ANOVA) followed by Dunett's t-test. \( p < 0.05 \) was considered significant.

RESULTS
Experiment 1:
Control (vehicle-treated) mice, when placed on the grid, showed training latency of 3.68 ± 0.37 sec (n=17). 1 hour later, when again placed on the electric grid, mice reached SFZ in 9.00 ± 2.25 sec and showed 23 ± 4.00 mistakes (descents) in 15 min (Fig. 1).

Latency to reach SFZ measured 1 hour after training was not significantly affected by BR-16A (50 and 100 mg/kg). The latency was reduced by the higher doses (250 and 500 mg/kg), the effect being significant only at 500 mg/kg (Fig. 1).
BR-16A (100-500 mg/kg) produced a significant decrease in the number of mistakes (descents) as compared with the vehicle treated control. However, 50 mg/kg dose failed to elicit any significant reduction in the number of mistakes (Fig. 1). GABA (100 mg/kg) produced a significant decrease in the latency to reach SFZ, measured 1 hour after training as well as the number of mistakes (n=5) as compared with the vehicle-treated control group. A lower dose of GABA (50 mg/kg) failed to produce a significant effect on latency measured 1 hr after training but significantly reduced the number of mistakes as compared with the control (n=5) (Fig. 2).

When BR-16A (50 and 100 mg/kg) was administered in combination with GABA (50 mg/kg) a significant decrease in the number
of mistakes as compared with BR-16A or GABA alone was obtained (n=4-6). The combination of BR-16A (100 mg/kg) and GABA (50 mg/kg) also significantly reduced the retention latency as compared with the effect of BR-16A or GABA alone (Fig. 2).

Aniracetam (50 and 100 mg/kg) significantly increased the latency to reach SFZ but the higher dose of aniracetam (300 mg/kg) failed to influence the latency measured 1 h after training (n=5-6). Aniracetam (100 and 300 mg/kg) also produced a significant decrease in the number of mistakes as compared with the control group (Fig.3).

The combination of BR-16A (50 and 100 mg/kg) plus aniracetam (50 mg/kg) produced significant reduction in the number of mistakes as compared with the effect of BR-16A or aniracetam alone. The latency to reach SFZ, measured 1 h after training, was also significantly reduced by the combination of BR-16A (50 and 100 mg/kg) and aniracetam (50 mg/kg) in comparison with the effect of aniracetam alone (n=5-6) (Fig.3).

**Experiment 2:**

Scopolamine (0.3 mg/kg) significantly increased the latency to reach SFZ and the number of mistakes as compared with the vehicle-treated control (n=10) (Table 1).

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment, mg/kg</th>
<th>Latency to reach shock-free-zone (Sec ± SEM)</th>
<th>Number of mistakes in 15 minutes (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vehicle</td>
<td>9.00 ± 2.25</td>
<td>23.00 ± 4.00</td>
</tr>
<tr>
<td>2.</td>
<td>Scopolamine, 0.3</td>
<td>36.30 ± 3.00***</td>
<td>71.00 ± 9.00*****</td>
</tr>
<tr>
<td>3.</td>
<td>Scopolamine, 0.3 + BR-16A, 50</td>
<td>40.00 ± 8.12</td>
<td>65.00 ± 8.00</td>
</tr>
<tr>
<td>4.</td>
<td>Scopolamine, 0.3 + BR-16A, 100</td>
<td>29.00 ± 6.00</td>
<td>52.00 ± 9.00*</td>
</tr>
<tr>
<td>5.</td>
<td>Scopolamine, 0.3 + BR-16A, 250</td>
<td>21.08 ± 5.03*</td>
<td>38.00 ± 6.01**</td>
</tr>
<tr>
<td>6.</td>
<td>Scopolamine, 0.3 + BR-16A, 500</td>
<td>17.21 ± 5.11**</td>
<td>25.00 ± 4.01***</td>
</tr>
</tbody>
</table>
BR-16A (100-500 mg/kg) reversed scopolamine-induced delay in latency to reach SFZ and reduced the number of mistakes in mice pretreated with scopolamine. The lower dose of BR-16A (50 mg/kg) failed to produce any significant reversal effect (Table-1).

Physostigmine (0.5 mg/kg) significantly reduced scopolamine (0.3 mg/kg) induced increase in number of mistakes but further delayed retention latency in scopolaine-treated mice (n=5). The combination of BR-16A (50 and 100 mg/kg) with physostigmine (0.5 mg/kg) produced a further reduction in the latency measured 1 h after training and the number of mistakes as compared with the effect of physostigmine alone in scopolamine-treated mice (n=5) (Table2).

Table 2: Effect of combination of BR-16A with physostigmine on retention latency and number of mistakes (descents) in 15 minute in scopolamine-treated mice

<table>
<thead>
<tr>
<th>Group No.</th>
<th>N</th>
<th>Treatment (mg/kg)</th>
<th>Retention latency in seconds (Mean ± SEM)</th>
<th>Number of mistakes (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>10</td>
<td>Control</td>
<td>9.00 ± 2.25</td>
<td>23.00 ± 4.00</td>
</tr>
<tr>
<td>2.</td>
<td>10</td>
<td>Scopolamine (0.3)</td>
<td>36 ± 3.30*</td>
<td>71.6 ± 4.2*</td>
</tr>
<tr>
<td>3.</td>
<td>5</td>
<td>Scopolamine (0.3) + Physostigmine (0.5)</td>
<td>50.6 ± 10.6*</td>
<td>12.5 ± 1.5*</td>
</tr>
<tr>
<td>4.</td>
<td>5</td>
<td>Scopolamine (0.3) + Physostigmine (0.5) + BR-16A (50)</td>
<td>25 ± 2.0*</td>
<td>1.5 ± 0.5*</td>
</tr>
<tr>
<td>5.</td>
<td>5</td>
<td>Scopolamine (0.3) + Physostigmine (0.5) + BR-16A (100)</td>
<td>14 ± 5.0*</td>
<td>1.5 ± 0.5*</td>
</tr>
</tbody>
</table>

*<p<0.05 as compared between groups 2 : 1  
**<p<0.05 as compared between groups 3 : 2  
***<p<0.05 as compared between groups 4: 3 and 5 : 3.

Experiment 3:
ECS, applied immediately after training, produced a significant increase in latency to reach SFZ, measured 1 hr and 24 hr after training, as compared with the non-ECS control. Pretreatment with BR-16A (50 and 100 mg/kg) significantly reduced the latency to reach SFZ, measured 1 hr and 24 hr after training as compared with the ECS-treated group (Table 3).

Table 3: Effect of BR-16A on disruption of passive avoidance acquisition and retention produced by acute treatment with ECS (10 mA, 0.2 sec)

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment, mg/kg</th>
<th>Latency (sec ± SEM) measured after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 hour</td>
</tr>
<tr>
<td>1.</td>
<td>Non-ECS</td>
<td>5.30 ± 0.53</td>
</tr>
<tr>
<td>2.</td>
<td>ECS</td>
<td>9.13 ± 0.75*</td>
</tr>
<tr>
<td>3.</td>
<td>BR-16A, 50</td>
<td>10.15 ± 1.24</td>
</tr>
<tr>
<td>4.</td>
<td>BR-16A, 100</td>
<td>5.70 ± 0.12*</td>
</tr>
</tbody>
</table>
All the values are mean ± SEM. n=6-7.

\*p<0.05 as compared with non-ECS control
\**p<0.05 as compared with the ECS-treated group.

**Experiment 4:**
The TL on the 2\textsuperscript{nd} day was not significantly different than that on 1\textsuperscript{st} day in the vehicle-treated control group.

Scopolamine (0.3 mg/kg) produced a significant increase in TL on 1\textsuperscript{st} day but not on the 2\textsuperscript{nd} day as compared with the control. Scopolamine-induced increase in TL was, however, reversed by the prior treatment with BR-16A (50 and 100 mg/kg). Aniracetam (50 mg/kg) produced a significant elevation of TL on 1\textsuperscript{st} day as compared with the control but significantly reduced the TL on the 1\textsuperscript{st} day in scopolamine-treated mice. The effect on TL on 2\textsuperscript{nd} day was not statistically significant. The combination of BR-16A (50 mg/kg) and aniracetam produced a further reduction in TL on 1\textsuperscript{st} day in scopolamine-treated mice as compared with the effect of either drug alone (Table 4).

<table>
<thead>
<tr>
<th>Table 4: Effect of the combination of BR-16A with scopolamine and Aniracetam on transfer latency (TL) as studied in the elevated plus-maze in naïve mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group No.</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>1.</td>
</tr>
<tr>
<td>2.</td>
</tr>
<tr>
<td>3.</td>
</tr>
<tr>
<td>4.</td>
</tr>
<tr>
<td>5.</td>
</tr>
<tr>
<td>6.</td>
</tr>
<tr>
<td>7.</td>
</tr>
</tbody>
</table>

\*p<0.05 as compared between groups 2 : 3, 1 : 5 and 5 : 6;
*** p<0.001 as compared between groups 1 : 2 and 2 : 4.

**DISCUSSION**
The present study suggests the effectiveness of BR-16A in improving passive avoidance acquisition and memory retention in mice. The reduction in latency measured 1 hr after training (acquisition) and number of mistakes (descents) the animals made in 15 min (retrieval) following administration of BR-16A support the above proposal.

A deficient cholinergic system is implicated in the progressive loss of memory in several neuropsychiatric disorders\textsuperscript{9}. Scopolamine an, anticholinergic-induced amnesia has been used as a pharmacological tool in various clinical and experimental paradigms\textsuperscript{10,11}. In the present study scopolamine delayed the latency to reach SFZ and increased the number of mistakes suggesting disruption of passive avoidance learning as well as retention, respectively.
BR-16A significantly reversed scopolamine-induced impairment of short-term memory. Physostigmine, an anticholinesterase, enhanced the reversal effect of BR-16A against scopolamine-induced amnesia. Hence, the improvement of short-term memory following the administration of BR-16A could be attributed to a possible modulation of cholinergic neurotransmission.

The role of other neurotransmitter systems particularly GABA has also been speculated in learning and memory. The combination of BR-16A with GABA (50 mg/kg) or aniracetam (50 mg/kg) showed enhanced effectiveness in reducing the latency to reach SFZ and the number of mistakes. This suggests an improvement in passive avoidance acquisition and retrieval in naïve as well as scopolamine-treated mice following administration of BR-16A. There is general agreement that the pyrrolidinone class of nootropic compounds (particularly piracetam and aniracetam) do not interact directly with any of the major neurotransmitter systems, although there have been some speculations about their indirect effects on both cholinergic and GABAergic systems.

In another study in which latency measured 24 hr after training served as a parameter for memory retention, acute treatment with ECS immediately after training produced a significant increase in latency measured 1 hr and 24 hr after training. The suggestion that ECS impairs acquisition, as well as retention of a learned task. To substantiate the claim for the improvement in memory retention, the effect of BR-16A on two different parameters i.e. the number of mistakes (descents) in 15 min and latency to reach SFZ measured 24 hr after training was studied. Since treatments such as ECS or scopolamine cause retrograde amnesia by interfering with memory consolidation process, the above studies suggests an effectiveness of BR-16A in improving short-term memory in naïve as well as amnesic mice.

To substantiate the claim for the nootropic effect of BR-16A, the performance of mice on elevated plus-maze was also evaluated. In plus maze, mice show natural aversion to open and high spaces and therefore, spend more time in enclosed arms. Itoh et al. suggested that transfer latency (TL) might be shortened if the animal has previously experienced entering the open arms. The shortened TL could be related to memory. In our study, a shortened TL was obtained on the 2nd day in the control group but the effect was not statistically significant due to large variation in animal behaviour. Scopolamine (0.3 mg/kg) injected 30 min prior to the 1st trial, produced deficit in learning as it causes significant elevation in TL on 1st day. However, the TL of the amnesic mice was shortened by BR-16A (50 and 100 mg/kg). The reversal effect of BR-16A (50 mg/kg) on TL measured on 1st day was enhanced by its concomitant administration with aniracetam in scopolamine-treated mice. This study demonstrates that BR-16A reverses scopolamine-induced deficit in acquisition; an effect being enhanced following its co-administration with aniracetam. The lack of any significant effect of any of the drugs on TL on 2nd day could be attributed to the possible lack of any forceful motivation for learning.
Thus based on three independent observations i.e. studies on passive avoidance paradigm, elevated plus-maze and ECS-amnesia. BR-16A has been found to possess a nootropic action in mice. A modulatory role of cholinergic and GABAergic systems is speculated in the efficacious role of BR-16A in improving short-term memory.

ACKNOWLEDGMENT
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REFERENCES