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# EVIDENCE FOR NOOTROPIC EFFECT OF BR-16A (MENTAT®), A HERBAL PSYCHOTROPIC PREPARATION, IN MICE

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**Abstract:** BR-16A (Mentat® 50-500 mg/kg) improved acquisition and retention of a passive avoidance task in a step-down paradigm in mice. BR-16A (50-500 mg/kg) reversed scopolamine (0.3 mg/kg)-induced disruption of acquisition and retention. BR 16-A (50 and 100 mg/kg) attenuated amnesia produced by the acute treatment with electroconvulsive shock (ECS), immediately after training. Chronic treatment with ECS, for 6 successive days at 24 h interval, disrupted memory consolidation on the 7th day. Daily administration of BR-16A (50 and 100 mg/kg) for 6 days significantly improved memory consolidation in mice receiving chronic ECS treatment. BR-16A (20-500 mg/kg), administered on the 7th day, also attenuated the disruption of memory consolidation produced by chronic treatment with ECS. On elevated plus-maze, BR-16A (50 and 100 mg/kg) reversed scopolamine (0.3 mg/kg)- induced delay in transfer latency on the 1st day. The above data suggests a nootropic effect of BR-16A in naive and amnesic mice.

Key words : passive avoidanc, acquisition, retrieval, scopolamine, consolidation, elevated plus-maze, transfer latency, electroconvulsive shock, BR-16A

## INTRODUCTION

BR-16A (Mentat®), a hebral psychotropic preparation is claimed to be effective in improving learning ability and behavioural disturbances in mentally retarded children (1,2,3) and to exert a beneficial effect in the cases of cerebral deficit (4), behavioural disturbances following postnatal organic lesions of central nervous system and in cases having organic loss of bladder function *(Personal communication)*. The power preparation of BR-16A contains essentially the following herbal ingredients : Brahmi *(Hydrocotyl asiatica)*, Vach *(Acorus calamus)*, Ashwagandha *(Withania somnifera)*, Guduchi *(Tinospora cardifolia)*, Amla *(Embelica officinalis)*, Shankhpushi *(Evolvulus alsinoides)*, Jatamansi *(Nardostachys jatamansi)*, Tagar *(Valeriana wallichii)* and Triphala (5). The market preparation Mentat®, however, has several other components in addition to the above said ingredients, which help in the preparation of the product. Preliminary toxicity studies have shown it to be a safe preparation and no adverse effect ensued its chronic use. The $LD_{50}$ value has been reported to be 2400 mg/kg by oral route of administration (Personal communication).

Several existing models for the evaluation of learning and memory are based on positive or negative reinforcement behaviour. However, recently Itoh et al (6,7) have reported that elevated plus-maze introduced for the measurement of anxiety in rodents (8,9), could be used for the evaluation of learning and memory in mice, although this method is not bases on positive or negative reinforcers. Because of the disliking for the open arms, the animal spends more time in enclosed than the open arms and this aversive quality becomes apparent only when the animal enters them. Itoh et al (5,6), therefore, speculated that the time in which the animal moves from the open arm to the enclosed arms (transfer latency; TL) might be shortened if the animal had previously experienced entering the open arms. The shortened transfer latency was then related to memory.

The present study was undertaken to study the effectiveness of BR-16A in improving short-term memory in naive mice. The reversal effect of BR-16A

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against memory deficits induced by scopolamine and acute or chronic treatment with ECS was evaluated on passive avoidance paradigm as well as elevated plus-maze in mice.

## METHODS

**Animals :** Albino mice of either sex (LAKA strain, bred in Central Animal House facility of Panjab University), weighing 20-25g were used. The animals were housed under standard light/dark cycle with food and water provided *ad libitum*. The experiments were performed between 9.00 and 12.00 hr.

**Drugs :** BR-16A (Menta®); Himalaya Drug Co. Bombay, India) powder was suspended uniformly in deionized water and administered orally. Scopolamine HBr (Merck and Co., Inc., Rahway, NJ, USA) was given ip in a constant volume (1 ml/100 g) as aqueous solution.

**Electroconvulsive shock :** Electroconvulsive shock (ECS; 10 mA, 0.2 sec) was applied through ear clip electrodes. The animals received either a single shock (acute treatment) or a series of 6 shocks at 24 hr interval (chronic treatment).

### Apparatus

*Passive avoidance step-down paradigm*: The method described by Sharma and Kulkarni (10) was used. In brief, the apparatus consisted of an electric grid (24x30 cm) with a shock-free zone (SFZ; 2x3x1 cm) in the centre and the entire grid having a perflex enclosure.

*Elevated plus-maze*: An elevated plus-maze consisting of two open arms (16x5 cm) and two enclosed arms (16 x 5 x 12 cm) was used in the present study (11). The maze was elevated to a height of 25 cm.

### 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 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 the 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 retention respectively.

*Experiment 2*: The procedure and the apparatus was identical to those described in experiment 1. Animals were divided into 10 groups. Thirty minutes after the first trial the mice of 6 of these groups were given vehicle, BR-16A (50-500 mg/kg) or scopolamine (0.3 mg/kg). The other 4 groups were used for investigating the effect of BR-16A on amnesia produced by scopolamine. Scopolamine (0.3 mg/kg) was given immediately after passive avoidance training and 30 min later BR-16A (50-500 mg/kg) was administered.

*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 climb SFZ measured 24 hr after the training served as a parameter for retention. The animals were divided into 4 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 hr after the training. BR-16A (50 and 100 mg/kg) or vehicle was administered 30 min following the application of ECS.

*Experiment 4:* The procedure and the apparatus were identical to those described in experiment 3, except that the treatment with ECS was repeated every 24 hr over 6 successive days. 7 groups of mice were treated with ECS immediately after training. ECS treatment was given over 6 successive days at 24 hr interval. On the seventh day mice received BR-16A (20-500 mg/kg) or saline and returned to their home cages. 30 min later latency to reach SFZ was recorded. 2 other groups received BR-16A (50 and 100 mg/kg) 30 min prior to training on the 1st day. ECS was applied immediately after the training. Latency to reach SFZ was measured 1 hr and 24 hr after the training. On the second day, after the measurement of retention latency, administration of BR-16A was followed 1 hr later by the application of ECS. On the third and successive days the mice received BR-16A followed by ECS for a total of 6 days. On the seventh day, 24

hr after the last shock the latency to reach SFZ was measured.

*Experiment 5:* 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 (6). TL was the time elapsed between the time the animal was placed in 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 first trial. Twenty four hours later, the mice were placed on the elevated plus-maze individually as before and TL was recorded again. TL measured on 1st and 2nd day served as parameters for acquisition and retrieval respectively. All the drugs were administered 30 min prior to the first 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 followed by Dunett's t-test. P< 0.05 was considered statistically significant.

## RESULTS

### Experiment 1

*Performance of control mice on passive avoidance step-down paradigm*: Control (untreated) mice, when placed on the grid, showed training latency of 3.68 ± 0.35 (n=17) sec. 1 hr 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 (Table I).

### Experiment 2

*Effect of BR-16A on passive avoidance acquisition and retrieval:* Latency to reach SFZ, measured 1 hr 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.

BR-16A (100-500 mg/kg) produced a significant decrease in the number of mistakes (descents) as compared to vehicle treated control. However, 50 mg/kg dose failed to elicit any significant reduction in the number of mistakes (Table I).

TABLE I : Effect of various doses of BR-16A on a) latency to reach SFZ and b) number of mistakes (descents) made in 15 min in passive avoidance step-down paradigm in mice.

| *Treatment, mg/kg* | *Latency to reach SFZ in sec (a)* | *Number of mistakes (descents) in 15 min (b)* |
|---|---|---|
| Vehicle | 9.00±2.25 | 23.00±4.00 |
| BR-16A, 50 | 9.50±1.5 | 19.50±2.50 |
| 100 | 10.25±1.75 | 12.50±0.75 |
| 250 | 8.00±3.00 | 10.00±1.25 |
| 500 | 4.25±1.00* | 6.75±1.25* |

Results are expressed as Mean ±SEM.
Control n= 10 and n = 5-7 in other treatment groups.
*P <0.05 as compared with vehicle-treated control.
a) ANOVA, F - ratio (28, 110) = 76.00, P <0.05; b) ANOVA, F-ratio (28,110) = 15.03, P <0.05.

*Effect of BR-16A on passive avoidance performance in scopolamine-treated mice:* Scopolamine (0.3 mg/kg) significantly increased the latency to reach SFZ and the number of mistakes as compared to vehicle-treated control.

TABLE II : Effect of various doses of BR-16A on a) latency to reach SFZ and b) number of mistakes (descents) made in 15 min in passive avoidance step-down paradigm in scopolamine-treated mice.

| *Treatment, mg/kg* | *Latency to reach SFZ in sec (a)* | *Number of mistakes (descents) in 15 min (b)* |
|---|---|---|
| Vehicle | 9.00±2.25 | 23.00±4.00 |
| Scopolamine, 0.3 | 36.30±3.00*** | 71.00±9.00*** |
| Scopolamine, 0.3 BR-16Å, 50 | 40.00±8.12 | 65.00±8.00 |
| Scopolamine, 0.3 BR-16Å, 100 | 29.00±6.00 | 52.00±9.00* |
| Scopolamine, 0.3 BR-16Å, 250 | 21.08± 5.03* | 38.00±6.01** |
| Scopolamine, 0.3 BR-16Å, 500 | 17.21±5.11** | 25.00±4.01*** |

All the values are Mean±SEM. Control n = 10 and n = 6-10 in different treatment groups. *P <0.05, **P < 0.01, ***P<0.001 as compared with scopolamine-treated group. ***a P< 0.001 as compared with vehicle-treated control.
a) ANOVA, F - ratio (28, 110) = 76.00, P < 0.05.
b) ANOVA, F - ratio (28, 110) = 15.03, P < 0.05.

BR-16A (100-500 mg/kg) reversed scopolamine-induced delay in the 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 II).

**Experiment 3**

*Effect of BR-16A on passive avoidance performance in mice receiving acute treatment with ECS:* 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 III).

TABLE III : Effect of pretraining administration of BR-16A on amnesia produced by acute treatment with ECS (10mA, 0.2 S).

| *Treatment, mg/kg* | *Latency (sec) measured after* | |
|---|---|---|
| | *1 hr* | *24 hr* |
| Non-ECS | 5.30±0.53 | 3.13±0.62 |
| ECS | 9.13**±0.75 | 9.45**±0.75 |
| BR-16A, 50 | 10.15±1.24 | 8.83±1.25 |
| BR-16A, 100 | 5.70*b±0.12 | 3.90*b±0.55 |

Results are expressed as Mean±SEM, n = 6-7.
**P < 0.05 as compared with non-ECS control, *bP < 0.05 as compared with ECS-treated group. ANOVA, F-ratio (3, 23) = 10.39, P < 0.05.

**Experiment 4**

*Effect of BR-16A on passive avoidance performance in mice receiving chronic treatment with ECS:* Chronic treatment with ECS for 6 successive days at 24 hr interval produced a significant prolongation of latency to climb SFZ, measured on the 7th day after training as compared with non-ECS control.

BR-16A (20-500 mg/kg), administered on the 7th day, 24 hr after the last treatment with 6 successive shocks, produced significant reversal of ECS-induced delay in latency to reach SFZ as compared with the ECS-treated group (Table IV).

TABLE IV : Effect of pre-retention administration of various doses of BR-16A on latency to reach SFZ, measured on 7th day after training in mice chronically exposed to ECS (10 mA, 0.2 sec).

| *Treatment, mg/kg* | *Latency (sec) to reach SFZ on 7th day* |
|---|---|
| Non-ECS | 7.43±0.42 |
| ECS | 16.85±1.52***a |
| BR-16A, 20 | 13.66±2.33 |
| 50 | 10.00±1.04* |
| 100 | 8.00±0.89** |
| 250 | 6.50±0.35** |
| 500 | 3.00±0.35*** |

The data are expressed as Mean±SEM, n = 5-7.
***P < 0.01 as compared with non-ECS control, *P < 0.05, **P < 0.01, ***P < 0.001 as compared with ECS-treated control. ANOVA, F-ratio (6, 35) = 17.41, P < 0.05.

Daily administration of BR-16A (50 and 100 mg/kg), 30 min prior to the application of ECS, for 6 successive days, prevented any delay in latency to reach SFZ on the 7th day after training as compared with ECS-treated control. The latency of the non-ECS group on the 7th day was same as that in group receiving concurrent treatment with ECS and BR-16A (100 mg/kg; Table V).

TABLE V : Effect of daily administration of BR-16A on amnesia produced by chronic treatment with ECS (10 mA, 0.2 sec) over 6 successive days in mice.

| *Treatment, mg/kg* | *Latency (sec) to reach SFZ on 7 th day* |
|---|---|
| Non-ECS | 7.43±0.42 |
| ECS | 16.85±1.52***a |
| BR-16A, 50 | 10.66±1.02* |
| 100 | 6.59±0.68** |

The data are expressed as Mean ±SEM, n = 6-7.
***P < 0.01 as compared with non-ECS treated control, *P < 0.05, **P < 0.01 as compared with ECS-treated control.
ANOVA, F-ratio (3, 23) = 24.78, P < 0.05.

**Experiment 5**

*Effect of BR-16A on TL in scopolamine-treated mice:* The TL on the 2nd day was not significantly different than that on 1st day in the vehicle treated control group.

Scopolamine (0.3 mg/kg) produced a significant increase in TL on 1st day but not on the 2nd day as compared to control. Scopolamine-induced increase in TL was, however, reduced/reversed by the prior treatment with BR-16A (50 and 100 mg/kg; Table VI).

TABLE VI : Effect of BR-16A on transfer latency (TL) as studied on elevated plus-maze in scopolamine-treated mice.

| *Treatment, mg/kg* | *n* | *Transfer Latency (TL) in sec (Mean ± SEM)* | |
|---|---|---|---|
| | | *1st day* | *2nd day* |
| Vehicle | 8 | 36.25±9.50 | 28.87±3.56 |
| Scopolamine, 0.3 | 7 | 101.43±10.89*** | 39.97±7.04 |
| Scopolamine, 0.3 + BR-16A, 50 | 8 | 58.25±7.20* | 37.25±4.82 |
| Scopolamine 0.3 + BR-16A, 100 | 8 | 29.50±5.64*** | 25.50±3.06 |

***P < 0.001 as compared with the vehicle-treated control.
*P < 0.05, ***P < 0.001 as compared with scopolamine-treated control. ANOVA, F-ratio (6, 48) = 15.45, P < 0.05.

## DISCUSSION

The present study demonstrates that in a paradigm of short-term memory, BR-16A produces improvement in passive avoidance acquisition and memory retrieval. The memory improving effect of BR-16A manifested as decrease in latency to reach SFZ (acquisition) and number of mistakes (descents) the animal made in 15 min (retention) on passive avoidance paradigm.

A deficient cholinergic system has been implicated for the progressive decline of learning and memory in various neuropsychiatric disorders (12). Scopolamine-induced amnesia has been used as a pharmacological tool in various clinical and experimental paradigms (13,14, 15). In the present study scopolamine (0.3 mg/kg), an anticholinergic agent, produced deficits in learning as well as memory retention as the animals showed a delay in reaching SFZ and increase in the number of mistakes. BR-16A (50-500 mg/kg) reversed scopolamine induced deficits in acquisition and retrieval. In another study in which latency measured 24 hr after the 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 the training. This suggests that ECS impairs acquisition as well as retention of a learned passive avoidance task. To substantiate the claim for the improvement in memory retention, effect of BR-16A on two different parameters i.e. the number of mistakes (descents) in 15 min and latency to reach SFZ, 24 hr after training was studied. Pretraining administration of BR-16A (50 and 100 mg/kg) prevented increase in the latency to reach SFZ, measured 1 hr and 24 hr after the application of shock. Since the treatments such as scopolamine or ECS cause retrograde amnesia by interfering with memory consolidation process (16,17,18), the above studies suggest an effectiveness of BR-16A in improving short-term memory in naive as well as amnesic mice.

Additional evidence for the noortropic action of BR-16A was obtained from the studies on elevated plus-maze. In plus-maze, mice show natural aversion to open and high spaces and, therefore, spend more time in enclosed arms. Itoh et al (6) suggested that TL might be shortened if the animal had previously experienced entering the open arms. The shortened TL could be related to memory. In our study, the shortened TL was obtained on the 2nd day in control but the effect was not statistically significant due to large variation in animal behaviour. However, TL was significantly increased in the Ist trial in the experimental amnesic mice, in which amnesia was induced by scopolamine (0.3 mg/kg), injected 30 min before the 1st trial. TL of the amnesic mice was shortened by the administration of BR-16A (50 and 100 mg/kg). However, only the latency on the 1st but not 2nd day was affected. This study consolidates that BR-16A improves acquisition in amnesic mice. The results of this study are well in agreement with those of passive avoidance paradigm. The observation that the TL on the 2nd day in all the treatment groups was not significantly different from that of control suggests the failure to retain the learned task in the absence of any forceful motivation.

Chronic application of ECS for 6 days produced a significant increase in latency to reach SFZ on

the 7th day as compared with non-ECS control. This suggests that application of ECS disrupts acquisition, retention and consolidation of a learned task. Concurrent administration of BR-16A (50 and 100 mg/kg) and ECS for 6 days prevented any impairment in memory consolidation. Daily administration of BR-16A for 6 days produced significant attenuation of amnesic effect of chronic application of ECS. The latency measured on the 7th days was of same magnitude as in non-ECS group. The above results further supplement the effectiveness of BR-16A in improving cognitive functions in acute as well as chronic amnesic models in mice.

The above proposition is strenthened by the observation that single dose administration of BR-16A (20-500 mg/kg) on the 7th day, 24hr after the last treatment with ECS, produced a significant reduction in latency to reach SFZ. Thus, the present study suggests that BR-16A possesses nootropic action in naive as well as amnesic mice.

In conclusion, Mentat® (BR-16A), a herbal preparation with wide margin of safety, is useful in cognitive dysfunctions.

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