ABSTRACT
BR-16A is a herbal (non allopathic) medication used in India to enhance cognition. In experiment 1, 28 Wistar rats received either BR-16A (200 mg/kg/day) or vehicle alone for 3 weeks. During the third week, the rats were tested for learning in the Hebb Williams complex maze. BR-16A treated rats showed significantly better learning than did controls. Experiment 2 was conducted identically except that during the second week all of 32 rats additionally received six once-daily electroconvulsive shocks (ECS). An advantage for learning was again demonstrated for the BR-16A group. It is concluded that BR-16A facilitates learning, and that this effect extends to a protection against ECS-induced anterograde amnesia. Cognitive deficits induced by electroconvulsive therapy are a major disadvantage of the treatment and to-date, no drug has been found to offer satisfactory protection against such deficits. It is suggested that BR-16A may hold promise in the containment of electroconvulsive therapy (ECT)-induced cognitive compromise.

Key Words: Electroconvulsive therapy; electroconvulsive shock; amnesia; learning; BR-16A, rats.

INTRODUCTION
Electroconvulsive therapy (ECT) is a potent force in the psychiatrist’s therapeutic armamentarium: cognitive deficits induced by the treatment, however, are a major disadvantage associated with its use. A number of strategies have been proposed to limit ECT-induced cognitive deficits (Andrade 1990). Various drug therapies such as adrenocorticotropic hormone (ACTH) (Fredericken et al., 1985), naloxone (Nasrallah et al., 1986) and glycopyrrolate substitution for atropine in ECT premedication (Sommer et al 1989) have failed to prove effective whereas piracetam (Ezzat et al., 1985) ergoloid mesylates (Sachs et al., 1989) vasopressin (Mattes et al., 1990) and tri-iodothyronine (Stern et al., 1991) have offered tentative but unconfirmed promise.

BR-16A (Mentat: The Himalaya Drug Company) is a herbal medication derived from Ayurveda, a system of healing in India’s rich culture in traditional medicine. It contains over 20 different ingredients, the exact formulation differing between pediatric and adult presentations of the composite. Important ingredients of BR-16A suggested to improve memory function include the following: Jal-brahmi (Bacopa monnieri), Mandookaparni (Centella asiatica), Ashwagandha (Withania somnifera). Shankapushpi (Evolvulus alsinoides), Jatamansi (Nardostachys jatamansi). Vach (Acorus calamus) Malkangni (Celastrus paniculatus) and Sonth (Zingiber officinale).
Other ingredients of BR-16A claimed to be “nerve tonics” include Tagar (*Valeriana wallachii*), Badam (*Prunus amygdalus*), Salap (*Orchis mascula*), Lavang (*Syzgium aromaticum*) and Pearl (*Mukta pishti*). The remaining ingredients are putative general tonics and vitalizers (Iyengar 1981; The Himalaya Drug Company 1991; Satyavati 1993; Andrade *et al*., 1993a).

Each ingredient is a plant extract that contains a variety of psychotropic and other compounds. The formulation of BR-16A is in accordance with Ayurvedic principles – different components of the formulation mutually complement each other’s properties. BR-16A is claimed in Ayurveda to enhance cognition and to ameliorate various forms of deficits in organic brain states.

The present study sought to assess whether BR-16A indeed enhances cognition, and, if so, whether these beneficial effects extend to an attenuation of ECT-induced cognitive deficits. Specifically, experiment 1 addressed the effect of BR-16A on learning, whereas experiment 2 evaluated the effect of BR-16A on anterograde amnesia induced by electroconvulsive shocks (ECS). An animal model was used for both experiments.

**METHOD**

The experiments were conducted on adult male Wistar rats, weighing 180-200 g at intake. The rats were housed four per cage with free access to tap water and standard laboratory diet, and were kept in a 12-hr light-dark cycle (lights on at 6 a.m.) room during the period of the study.

In each cage, the rats were randomized two by two to receive either BR-16A in a freshly prepared aqueous suspension (200 mg/ml) or water alone. Administration was effected using an intragastric tube in a volume of 1 ml/kg, once a day (circa 3 PM) for 3 weeks.

**Experiment 1**

During the first 2 weeks, 28 rats received BR-16A or vehicle alone. On the first day of the third week, all rats were familiarised with the Hebb Williams complex animal maze (Techno Electronics, Lucknow, India). From the second to the fifth day (circa 9 PM) the rats received five consecutive trials of training per day in the maze.

The maze consists of a completely enclosed hexagonal 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. In each trial, the rat is placed in the entry chamber, a timer is activated immediately once the rat leave the chamber. A pressure-sensitive plate detects the entry of the rat into the reward chamber, and the timer is immediately deactivated.
The five-trail total of the times taken by the rat to reach the reward (food pellet) chamber was taken as its learning score for the day. Because animals showing more efficient learning reach the reward chamber faster, higher scores indicate poorer learning or, in rats receiving ECS, greater ECS-induced anterograde amnesia (Rao et al., 1991).

To ensure adequate motivation during learning assessments in the third week the animals were exposed to (ad lib) food for just 1 hr a day, after the maze exposure for the day was completed. The learning assessments were conducted under a single, zero-watt dim red bulb so as to minimally disturb the rats nocturnal cycle.

**Experiment 2**

This experiment was conducted as was experiment 1, except as follows: during the last 6 days of the second week, 32 rats received six once-daily ECS. ECS were administered (circa 6 PM) through saline-soaked earclip electrodes using Electrocon MK II 50 Hz sinusoidal wave ECT device (Associated Electronics Engineering, Bangalore, India) at stimulus settings of 120 v x 0.5 secs. The resultant seizures, timed by a single observer using a stopwatch, were at least 18 sec in motor duration. The motor seizure was defined as extending from the commencement of passage of current to the cessation of motor activity or the onset of asymmetrical/asynchronous limb movements, whichever occurred earlier.

**Analysis**

The data were separately analyzed for each experiment as the experiments were conducted on different batches of rats at different points in time. A one-way repeated measures analysis of variance (ANOVA) was applied to identify changes across time in each group A two-way repeated measures ANOVA was applied to compare learning performances in BR-16A and vehicle-treated groups.

**RESULTS**

**Experiment 1**

Table 1 presents the M ± SD learning scores in BR-16A and vehicle-treated rats on days 1-4 of assessment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR-16A (n=14)</td>
<td>87.2 ± 3.2</td>
<td>85.8 ± 3.4</td>
<td>81.5 ± 3.3</td>
<td>80.9 ± 3.9</td>
</tr>
<tr>
<td>Vehicle (n=14)</td>
<td>94.1 ± 5.9</td>
<td>94.1 ± 6.1</td>
<td>94.4 ± 4.0</td>
<td>93.8 ± 5.9</td>
</tr>
</tbody>
</table>

(Results of the ANOVAs are presented in the text).

A one-way repeated measures ANOVA revealed a significant effect for time in the BR-16A group (F3, 39=18.51, p<0.001), indicating a significant attenuation of learning scores across time. There was, however, no significant effect for time in the vehicle group (F3, 39=0.05, NS).
A two-way repeated measures ANOVA revealed a significant main effect for groups (F1, 26=56.02, p<0.001), indicating that, overall, learning scores were significantly smaller in the BR-16A as compared with the vehicle group. The group × time interaction was also significant (F3, 90=9.44, p<0.001), indicating that the two groups differed significantly in the pattern of change of scores across time.

**Experiment 2**

Table 2 shows the M ± SD learning scores on days 1-4 of assessment in ECS-treated rats receiving BR-16A or vehicle.

A one-way repeated measures ANOVA revealed a significant decrease in scores in BR-16A-treated rats between days 1 and 4 (F3, 45=6.01, p<0.001); vehicle-treated rats in contrast showed a significant increase in scores (F3, 45=26.70, p<0.001).

A two-way repeated measures ANOVA revealed a significant main effect for groups (F1, 30=191.83, p<0.01), indicating that, overall, learning scores were significantly smaller in rats treated with BR-16A. The group x time interaction was also significant (F3, 90=9.44, p<0.001), indicating that the two groups differed significantly in the pattern of change of scores across time.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR-16A (n=16)</td>
<td>75.4 ± 8.6</td>
<td>75.5 ± 7.7</td>
<td>73.6 ± 2.9</td>
<td>70.6 ± 4.1</td>
</tr>
<tr>
<td>Vehicle (n=16)</td>
<td>91.6 ± 8.5</td>
<td>96.0 ± 12.9</td>
<td>101.0 ± 11.1</td>
<td>107.4 ± 8.5</td>
</tr>
</tbody>
</table>

(Results of the ANOVAs are presented in the text).

**DISCUSSION**

There is presently a lot of interest: in India in the investigation of traditional systems of medicine; scientists hope to identify herbal ingredients with potential allopathic applications, much as reserpine was isolated over half a century ago from the root of *Rauwolfia Serpentina*.

BR-16A was addressed as a candidate drug in this study: the term drug is used for convenience, for BR-16A as indicated earlier, is actually a combination of substances.

In experiment 1, rats pretreated with BR-16A were found to show significant learning while control rats failed to learn within the same time span. In experiment 2, rats pretreated with BR-16A again showed significant learning in contrast, control rats surprisingly deteriorated. The reason for the deterioration in learning performance is unclear perhaps with this model of learning ECS-induced cognitive toxicity may cumulate transiently even after the termination of the ECS course.
The learning paradigm employed presents a confound between recall of the previous day’s learning and fresh learning on the current day’s trials. Impairments in either or both aspects of learning post-ECS however, are subsumed under the general rubric of ECS-induced retrograde amnesia (impaired recall of material acquired pre-ECS).

It thus appears from the two experiments in this study that BR-16A enhances learning, and that this effect offers protection against ECS-induced anterograde amnesia in rats. There is some support in literature for the beneficial effect of BR-16A on cognition.

In normal volunteers, BR-16A was found to improve memory parameters and decrease anxiety parameter after 12 weeks of administration in the dose of 350 mg twice daily (Agrawal et al., 1990a, 1990b).

Verma and Kulkarni (1991) studied mice in an elevated plus maze. Transfer latency, the time taken by the animal to leave the open arm and enter the closed arm, was used as a measure of learning. Pretreatment with BR-16A (50-100 mg/kg) was found to produce a dose-dependant reversal of scopolamine-induced prolongation of the transfer latency.

Kulkarni and Verma (1992a) studied mice using an avoidance paradigm. The apparatus consisted of a grid through which an electric shock could be delivered with an elevated, shock-free zone in the center of the grid. Latency to reach the shock-free zone was recorded as were the number of descents onto the grid over a period of 15 min. These two variables operationalised the efficiency of recall of the electric shock offered by the grid during training. In separate experiments, scopolamine, single ECS and 6 once daily ECS were found to induce amnesia as evidenced by increases in latency and increases in the number of descents. Pretreatment with BR-16A (50 to 500 mg/kg) was found to result in dose-dependant attenuations of these indices of amnesia.

Andrade et al., (1993a) used a reward-oriented paradigm to study the baseline learning behavior of rats in a T maze and in a complex maze. Number of trials to satisfactory learning and number of wrong arm entries were recorded as the learning variables in the T maze experiment, whereas time taken by the animal to reach the reward chamber was recorded as the learning variable in the complex maze experiment. After establishing baselines, rats were treated with BR-16A (200 mg/kg/day) or vehicle for 3 weeks. During the second week of treatment, all rats received 6 once daily ECS. Learning assessments were resumed during the third week of treatment and were compared with baseline scores. In both experiments, BR-16A (200 mg/kg/day) was observed to attenuate ECS-induced learning deficits.

The mechanism of action of BR-16A (200 mg/kg/day) was observed to attenuate ECS-induced learning deficits.
The mechanism of action of BR-16A is unclear. Cholinergic facilitation may be involved, as the drug reverses scopolamine-induced memory deficits (Verma and Kulkarni 1991; Kulkarni and Verma 1992a). BR-16A may also influence opioid neurotransmission: Kulkarni and Verma (1992b) found the drug to prevent the development of tolerance and dependence to morphine in mice. Preliminary work indicates no effects on adrenergic or dopaminergic neurotransmission (Andrade et al., 1993b).

BR-16A has a high therapeutic index with no mortality observed in mice even at a dose of 1600 mg/kg: compare this with the recommended human dose of 15-30 mg/kg/day. The drug does not influence the course or outcome of pregnancy, nor does it produce teratogenic effects in Wistar rats even at 2000 mg/kg/day for 20 days. In humans, chronic use of BR-16A does not produce impairments in cardiophysiological, hematological and biochemical indices (Himalaya Drug Company 1991). Several years of experience with the drug postmarketing has not resulted in the record of any adverse effects of note (The Himalaya Drug Company, unpublished data, 1994).

Systematic testing of BR-16A in allopathic contexts is presently under way. There is hope that BR-16A may be of use in the attenuation of the cognitive deficits induced by electroconvulsive therapy. A study testing this is currently being conducted.

REFERENCES


