Protective Effect of Mentat (BR-16A) A Herbal Preparation, on Alcohol Abstinence-Induced Anxiety and Convulsions

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ABSTRACT
Chronic administration of ethanol (2-5 g/kg p.o.) on days 1 to 6 and its withdrawal produced anxiogenic reaction in mice and rats as assessed on the elevated plus-maze. Daily administration of Mentat (100-mg/kg) prior to ethanol intoxication for 6 days prevented withdrawal-induced anxiety in both rats and mice. However, the acute administration of a single dose of Mentat to animals withdrawn from ethanol i.e. On the 7th day, elicited a significant anxiogenic response. Ethanol withdrawal also sensitized the convulsogenic-reaction to pentylenetetrazole (PTZ). A non-convulsive dose (40 or 60 mg/kg) of PTZ produced full-blown convulsions and increased mortality in ethanol-withdrawn rats and mice, respectively. Both acute and chronic administration of Mentat (100 mg/kg) exhibited significant protection against ethanol withdrawal-induced reduction of PTZ threshold in rats and mice. The results suggest the usefulness of this safe herbal psychotropic preparation in the management of ethanol withdrawal reactions.

INTRODUCTION
Physical dependence on ethanol is defined by the presence of an ethanol is withdrawal syndrome that becomes apparent following cessation of ethanol intake and elimination of ethanol from the system. The withdrawal syndrome, well characterised in humans and in animal models\(^1,2\), consists of an early reaction (tremor, diaphoresis, hallucinations, convulsions) and a delayed one (delirium tremens, increased autonomic activity, profound sweating, and profound disorientation). Alcoholism and withdrawal of chronic alcohol intake are grave social and medical problems. There is no single drug therapy that helps patients in overcoming alcoholism\(^3\).

Mentat (BR-16A), a herbal psychotropic preparation, contains the following main indigenous ingredients (besides others) reputed in the ancient system of Ayurvedic medicine to be useful in the management of nervous disorders: Brahmi (*Hydrocotyl asiatica*), Shatavari (*Asparagus racemosus*), Shatavari (*Asparagus racemosus*), Bach (*Acorus calamus*), Ashwagandha (*Withania somnifera*), Giloe (*Tinospora cordifolia*), Amla (*Emblica officianlis*), Shankhapushpi (*Evolulus alsinoides*) and Triphala. Preliminary toxicity studies have shown it to be a safe preparation and no adverse effect ensued its chronic use (unpublished data). Recent studies have demonstrated the effectiveness of Mentat in preventing the development of tolerance to and dependence on morphine in mice\(^4\). The present study has been undertaken to evaluate the de-addiction potential of this herbal preparation against alcohol. Alcohol
withdrawal reactions are determined as reduction in pentylenetetrazole (PTZ) threshold (convulsions) and increase in anxiety response as measured on elevated plus-maze.

**MATERIALS AND METHODS**

The effectiveness of Mentat in suppressing the symptoms of ethanol withdrawal was investigated in rats and mice. Balb/c strain, albino mice weighing 20-25 g and porton rats weighing 150-200 g of either sex, bred in the Central Animal House facility of the University were used. The animals were housed under standard light/dark conditions with food and water *ad libitum*. The experiments were performed between 0900 and 1700 hrs.

*Treatment schedule* - In acute studies, animals received ethanol (2 g/kg of 10% ethanol in mice and 5 g/kg of 20% ethanol in rats), intragastrically. After 30 min, its effect on elevated plus-maze was studied. In chronic studies, mice received 2 g/kg of 10% w/v ethanol, intragastrically, twice a day on the 1st day and once daily on successive days for a total of 6 days. In rats, 5 g/kg of ethanol (20% w/v) was administered intragastrically, three times a day for 6 days. On the 7th day, i.e. 24 hr after the last dose of ethanol, rats/mice were tested for withdrawal reactions. The other treatment groups included (pretreatment: treatment): (i) saline: saline (ii) saline: ethanol, and (iii) Mentat: (100 mg/kg): ethanol. Control experiments were performed on day 7 to determine whether Mentat prevented the development of withdrawal syndrome or it simply altered the behavioural expression of the withdrawal symptoms. On this day, the treatments were reversed so that the animals that had received Mentat, followed 30 min later, by ethanol on days 1 through 6, were challenged with saline. Similarly, the animals which received saline followed 30 min later by ethanol, received only Mentat. The group chronically treated with saline received the same (saline only). The withdrawal reaction was assessed by studying the anxiogenic reaction on elevated plus-maze or decreased threshold to pentylenetetrazole (PTZ: convulsive response to an otherwise non-convulsive dose in naïve animals).

*Elevated plus-maze: measurement of anxiety* - The elevated plus-maze used for rats and mice was the same as described earlier\(^5,6\). Briefly, the apparatus for mice consisted of two open arms (16 x 5 cm) and two enclosed arms (16 x 5 x 12 cm), and for rats it consisted of two open arms (50 x 10 cm) and two enclosed arms (50 x 10 x 40 cm). The maze was elevated to a height of 25 cm for mice and 50 cm for rats\(^7,8\). During the 5 min test session the following parameters were noted: (I) number of entries the animal made in open and enclosed arms (ii) the total time spent in each arm, and (iii) choice of open/enclosed arm as first entry. Animals were put individually at the centre of the plus-maze facing an open arm at the beginning of the test. An anxiogenic response was defined as decreased number of entries and time spent in open arm. There was reduction in percent preference for the open arm in anxiogenic animals.

*PTZ threshold* - The onset of body jerks, clonic convulsions followed by tonic convulsions and death were recorded following PTZ challenge in naïve and ethanol-withdrawn animals. Each animal was observed individually for 2 hr for acute response. Reduction in the dose of
PTZ to produce full blown convulsions in ethanol-withdrawn animals was considered as withdrawal-induced reduction in the convulsive threshold. The protective effect of Mentat following acute and chronic treatment was noted in rats and mice.

**Drugs** - The drugs used were Mentat (The Himalaya Drug Co., Bombay), pentylentetrazole (Sigma, USA) and ethanol (Bengal Chemicals & Pharmaceuticals Ltd., Calcutta).

Pentylenetetrazole and ethanol were prepared in distilled water. Mentat (100 mg/kg) powder was suspended uniformly in distilled water. Ethanol (2g/kg of 10% w/v in mice and 5 g/kg of 20% w/v in rats) and Mentat were administered orally, while PTZ (40-80 mg/kg) was injected i. p. All the drugs except ethanol were administered in a constant volume of 1 ml/100 g of body weight. Pentylenetetrazole was administered 24 hr after the last dose of ethanol (withdrawal animals) and Mentat was given 30 min prior to ethanol.

**Statistical analysis** - The data expressed as mean ± SE were analyzed by Student's 't' test. Probability levels <5% were considered significant.

**RESULTS**

**Effect of Mentat on ethanol withdrawal-induced anxiety** - Acute administration of ethanol (2 g/kg; 10% w/v) in mice produced a significant increase in the duration in open arms as compared with the vehicle-treated control mice (Table 1). Similarly, in rats ethanol (5 g/kg; 20% w/v) produced a significant decrease in the number of entries in closed arms and increase in the time spent in open arms (Table 2). Percent preference for closed arm entry was also reduced following acute treatment with ethanol as compared with the vehicle-treated group in both rats and mice.

<table>
<thead>
<tr>
<th>Table 1: Effect of Mentat on ethanol (EtOH) withdrawal-induced anxiety in mice (Values are mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>1</td>
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<td>2</td>
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<td>3</td>
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<td>4</td>
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<td>4</td>
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</table>

*p values: *<p<0.05 as compared with the vehicle-treated control.

#<p<0.05 as compared with the ethanol (EtOH)-withdrawal (W/D) group.

On the other hand, mice withdrawn from chronic ethanol treatment showed greater preference for closed arm entry and significant reduction in the duration of time spent in open
arms as compared with the vehicle-treated control group (Table 1). Rats withdrawn from chronic ethanol treatment also exhibited increase in percent preference for closed arms and the duration in closed arms as compared with the vehicle-treated controls. The duration and the number of entries in open arms was also reduced significantly (Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>n</th>
<th>% preference for closed arm entry</th>
<th>Closed arm</th>
<th>Open arm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Duration (sec)</td>
<td>Entries (mean)</td>
</tr>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>7</td>
<td>50</td>
<td>167.00 ± 8.25</td>
<td>7.82 ± 0.64</td>
</tr>
<tr>
<td>2</td>
<td>EtOH, 20% (5 g/kg; Acute)</td>
<td>5</td>
<td>20</td>
<td>154.80 ± 17.81</td>
<td>3.33 ± 0.75*</td>
</tr>
<tr>
<td>3</td>
<td>EtOH, 20% (5 g/kg; Chronic) W/D</td>
<td>6</td>
<td>66.6</td>
<td>272.00 ± 9.56*</td>
<td>1.00 ± 0.25*</td>
</tr>
<tr>
<td>4</td>
<td>EtOH, 20% (5 g/kg; Chronic) W/D + Mentat (100 mg/kg; Acute)</td>
<td>5</td>
<td>100</td>
<td>262.75 ± 27.62</td>
<td>1.25 ± 0.25</td>
</tr>
<tr>
<td>5</td>
<td>EtOH, 20% (5 g/kg; Chronic) W/D + Mentat (100 mg/kg; Chronic)</td>
<td>5</td>
<td>80</td>
<td>155.33 ± 6.76**</td>
<td>1.00 ± 0.33</td>
</tr>
</tbody>
</table>

*p values: p<0.05 as compared with *vehicle-treated control group; **ethanol (EtOH)-withdrawal (W/d) group.

Mice receiving chronic treatment with Mentat (100 mg/kg) followed by ethanol failed to show any withdrawal-induced anxiety. There was a significant decrease in the duration in closed arms. The duration and the number of entries in open arms increased significantly as compared with the ethanol-withdrawn group (Table 1). Daily administration of Mentat (100 mg/kg) prior to ethanol intoxication for 6 days in rats completely abolished entry into open arms. The duration and number of entries in closed arms were the same as those in the ethanol-withdrawn group (Table 2).

Mice treated repeatedly with saline followed by ethanol for 6 days and then challenged with Mentat on the 7th day, displayed a significant increase in preference for closed arms entry and time spent in closed arms. The duration in open arms reduced significantly as compared with the vehicle treated controls (Table 1). Acute administration of Mentat (100 mg/kg) in ethanol-withdrawn rats failed to exhibit any entry into the open arms. The duration in closed arms displayed a significant decrease. The duration in closed arms was not significantly different from that in the vehicle-treated control group (Table 2).

Effect of Mentat on PTZ threshold in ethanol-withdrawn animals - Pentylenetetrazole (80 mg/kg) induced severe clonic-tonic seizures followed by 100% mortality in naïve mice. A lower dose (60 mg/kg) induced mild clonic-tonic seizures with no mortality (Fig.1A), whereas a still lower dose failed to cause any observable behavioural change in naïve mice or rats. However, in ethanol-withdrawn mice PTZ (60 mg/kg) produced severe clonic-tonic seizures (Fig.2A) and 60% mortality (Fig.1A). Acute administration of Mentat (100 mg/kg) to ethanol-withdrawn mice showed protection against PTZ (60 mg/kg) convulsions. The
animals showed only mild clonic convulsions followed by recovery (Fig. 2A). Chronic administration of Mentat (100 mg/kg) followed by ethanol for 6 days exhibited only mild clonic seizures with delayed onset, and 25% mortality following administration of PTZ (60 mg/kg) on day 7, in mice (Figs. 1 and 2).

Fig. 1: Modification of pentylenetetrazole (PTZ)-induced convulsions by Mentat (BR-16A) in ethanol (EtOH)-withdrawal groups of mice (A) and rats (B)

In ethanol-withdrawn rats, severe tonic seizures (Fig.2B) and 100% mortality (Fig. 1B) were observed following administration of PTZ (40 mg/kg). Acute administration of Mentat (100 mg/kg) to ethanol-withdrawn rats produced complete protection against PTZ convulsions (Fig.1B). Mild jerks and clonus with delayed onset were observed following administration of PTZ (40 mg/kg; Fig.2B). Withdrawal of chronic treatment with Mentat (100 mg/kg) followed by ethanol over 6 days in rats also produced mild clonic-tonic convulsions with delayed onset following PTZ (40 mg/kg) administration (Fig. 2B). There was only 40% mortality in this group (Fig.1B). In addition to ethanol withdrawal-induced anxiety and convulsions, both animals generally exhibited various other abstinence signs such as body tremors, escape attempts, jumping and increased locomotor activity which were suppressed by prior treatment with Mentat.
Fig. 2: Effect of Mentat (BR16-A) on pentylenetetrazole (PTZ)-induced convulsions in ethanol (EtOH)-withdrawal groups of mice (A) and rats (B).

* $p<0.05$ as compared with respective control groups.
DISCUSSION
Chronic ethanol administration is known to produce behavioural and biochemical changes in humans and animals. Withdrawal from chronic intake of ethanol is also known to produce withdrawal reactions, which range from craving behaviour to severe seizures. Although the exact cellular and biochemical mechanism of development of tolerance to and dependence on ethanol is not fully understood, recent studies have, however, focussed attention to the involvement of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) in the action of ethanol. At pharmacologically active concentrations, ethanol produces some of its pharmacological effects through GABA-ergic transmissions. In the present study, acute administration of ethanol produced a significant increase in the time spent in open arms, thereby suggesting an anxiolytic profile of ethanol in rats and mice. Biochemical studies have provided evidence that enhancement of GABA-mediated neuronal chloride fluxes may contribute to the sedative and anxiolytic profile of ethanol. The observed anxiogenic action following ethanol withdrawal would, therefore, be consistent with the notion that the GABAergic system is down regulated during withdrawal. The animals treated chronically with Mentat followed by ethanol for 6 days displayed a significant reversal of withdrawal-induced anxiety on the 7th day. Acute administration of Mentat on the 7th day to ethanol-withdrawn animals also displayed considerable anxiety on the 7th day. The anxiety reaction in ethanol-withdrawn animals may be attributed to anxiogenic action of Mentat per se or to an acute interaction between Mentat and ethanol. Reversal of anxiogenic response following substitution of Mentat with saline in ethanol-withdrawn animals suggests that Mentat need not be present during testing to observe anxiety in chronically treated animals.

Pentylenetetrazole, a chemoconvulsant, is reported to induce seizures by depressing chloride channel function by binding to a picROTOXIN site on the GABA receptor complex. In ethanol-withdrawn animals lower doses of PTZ as compared to control animals was required so as to exhibit severe tonic seizures and mortality. Sensitization of an inverse agonistic site on the GABA benzodiazepine receptor complex following ethanol withdrawal has been shown to be responsible for the decrease in convulsive threshold to PTZ in ethanol-withdrawn animals. Mentat significantly inhibited PTZ convulsions in these animals when given repeatedly 6 days. Acute Mentat administration following ethanol-withdrawal also produced significant attenuation of PTZ convulsions. The reversal of ethanol withdrawal-sensitized convulsant response to PTZ suggests the effectiveness of this herbal preparation in suppressing the ethanol withdrawal syndrome whether given acutely or chronically. The above data suggest that the anticonvulsant effect of Mentat alone or its acute interaction with ethanol is unlikely to account for its deaddiction potential.

Mentat is a safe preparation having no apparent response per se on the reward system. Concurrent administration of this multicomponent herbal preparation may help prevent the development of tolerance to and dependence on ethanol and other psychotropic drugs. The preparation is also effective in combating withdrawal reactions to chronic administration of ethanol. The present study provides further evidence for the de-addiction potential of acute as well as chronic administration of Mentat against ethanol.
ACKNOWLEDGEMENT
This work was supported by a grant from The Himalaya Drug co., Bombay.

REFERENCES