Anticonvulsant and antioxidative activity of hydroalcoholic extract of tuber of *Orchis mascula* in pentylenetetrazole and maximal electroshock induced seizures in rats

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**A R T I C L E  I N F O**

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**A B S T R A C T**

Ethnopharmacological relevance: *Orchis mascula* tuber is used in many polyherbal formulations as a nerve tonic in India.

Aim of the study: In the present study, effect of hydroalcoholic extract of *O. mascula* (HEOM) tuber was evaluated against seizures, seizure-induced oxidative stress and cognitive deficit in pentylenetetrazole and maximal electroshock-induced seizures in rats.

Materials and methods: HEOM was administered orally 30 min before induction of seizures by pentylenetetrazole (PTZ; 60 mg/kg, i.p) or maximal electroshock (MES; 70 mA). Elevated plus maze and passive avoidance tests were used to assess the learning and memory. Oxidative stress was studied by estimation of reduced glutathione and lipid peroxidation. Whole brain total cholinesterase activity was also evaluated.

Results: HEOM produced 33.3%, 50% and 66.7% protection in PTZ model and 16.7%, 16.7% and 33.3% at 250, 500 and 1000 mg/kg, respectively, in MES-induced seizures. Pre-treatment with HEOM significantly decreased the retention transfer latency in elevated plus maze test, and an increase in the retention latency in passive avoidance test was observed. Oxidative stress induced by seizures was also attenuated as indicated by significant increase in GSH and decrease in MDA levels in HEOM treated groups. PTZ and MES caused a significant decrease in AChE and BChE activities, which was prevented by HEOM.

Conclusions: HEOM thus showed protection against seizures, prevented the associated memory impairment probably by modulating cholinergic status and reducing oxidative stress.

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1. Introduction

Pharmacotherapy is the mainstay of treatment for epileptic patients but currently available drugs are unable to provide adequate control of seizures in about one-third of patients and also do not prevent underlying epileptogenic changes (Cockerell, 1996). In addition to the disease itself, numbers of factors significantly affect the disease progression. Recently, oxidative stress has reported to play a contributing role in acquired epilepsies (Waldbaum and Patel, 2010). Moreover, memory impairment, mental slowing, and attention deficits are frequently associated with epilepsy, which in turn, may be due to the disease itself or the side effects of treatment (Aldenkamp, 2006). Recently, it has been reported that the impairments in learning and behaviour is associated with at least in part, by changes in cholinergic neurotransmission (Sales et al., 2010). Therefore, there is felt a need for a medication with improved anticonvulsant efficacy and side effects profile.

*O. mascula* has been used as a nerve tonic in traditional medicine in India (Joseph et al., 1994). It is one of the major ingredients of a polyherbal formulation which have been used to improve memory and cognitive deficit in the Ayurvedic system of medicine. Previous studies of a marketed formulation containing *O. mascula* have shown to improve acquisition and retention of learning in animal experiments (Bhattacharya et al., 1995; Bhardwaj and Srivastava, 1995). In the present study, the effect of hydroalcoholic extract of *O. mascula* (HEOM) tuber in experimental models of seizures and seizure induced oxidative stress, cognitive impairment and cholinesterase activity was evaluated.

2. Material and methods

2.1. Plant material

*O. mascula* roots were purchased from local market in Delhi and authenticated at the Department of Pharmacognosy, Hamdard University, Delhi (Voucher No.-PRL/JH/08/26). Hydroalcoholic extraction was done at a GMP certified laboratory according
to the method as described earlier (Pahuja et al., 2011). The semisolid form of extract was vacuum dried to get the powder form.

2.2. Animals

Male Wistar rats (150–200 g) were obtained from the Central Animal Facility of All India Institute of Medical Sciences, New Delhi, India for the experiments (6 rats per group). The rats were maintained under standard animal housing conditions (22 ± 5 °C, 40–70% relative humidity, natural light/dark cycle) and had access to food and water ad libitum. They were fasted for 24 h before tests. The study protocol was approved by the Institutional Animal Ethics Committee (507/IAEC/09).

2.3. Drugs and chemicals

Pentylenetetrazole (PTZ), diphenylhydantoin, 5′-5-dithiobis (2-nitrobenzoic acid) (DTNB) and reduced glutathione (GSH) were purchased from Sigma (Sigma Chemical Co., USA). Sodium valproate was a generous gift from Sun Pharma, Mumbai, India. All other reagents were of analytical grade and were obtained from Qualigens, India.

2.4. Experimental protocol

2.4.1. PTZ-induced seizures

Animals were randomly assigned into six groups. Group 1 was given distilled water and serves as vehicle control group, group 2 received pentylenetetrazole (60 mg/kg, i.p.). Group 3 served as positive control and received valproate (300 mg/kg, i.p.). Groups 4–6 received hydroalcoholic extract of Ocimum sanctum (HEOM) at the doses of 250, 500 and 1000 mg/kg orally by gavage, 30 min before seizure induction with PTZ. PTZ was prepared freshly by dissolving in normal saline. Vehicle/drugs were administered in a volume not exceeding 10 ml/kg. Animals were observed for myoclonic jerk latency and the occurrence of generalized tonic clonic seizures (GTCS) with loss of righting reflex up to 30 min after PTZ injection (Gupta et al., 2002).

2.4.2. MES-induced seizures

Animals were randomly divided into six groups. The first group was vehicle (distilled water) control, the second group was MES group and the third group was positive control group (phenytoin, 40 mg/kg, i.p.). Groups IV–VI received HEOM in the doses of 250, 500 and 1000 mg/kg, orally, respectively. Shock was given by sinus wave stimulus (current intensity-70 mA, duration 0.2 s) delivered via ear clip electrodes using ECT unit (Ugo Basile, Italy) (Reeta et al., 2011). Animals were observed for the occurrence of tonic hind limb extension (THLE).

2.5. Behavioural parameters

The elevated plus maze and passive avoidance tests were performed before induction of seizures with PTZ or MES. Elevated plus maze was performed before the passive avoidance test. Seizures were induced 30 min after the administration of HEOM. The retention latency was taken after 24 h of seizure induction by PTZ or MES.

2.5.1. Elevated plus maze test

Elevated plus maze was used to evaluate the acquisition and retention of memory processes as described previously (Reeta et al., 2010).
3.2. MES-induced seizures

All the animals in the MES group experienced THLE. HEOM pre-treatment produced 16.7%, 16.7% and 33.3% protection at 250, 500 and 1000 mg/kg, respectively, (Table 1). None of the animals exhibited THLE in the phenytoin (40 mg/kg) treated group.

3.3. Effect on behavioural parameters

3.3.1. Elevated plus maze

No significant difference was observed in the initial transfer latency amongst the groups in PTZ model, whereas a significant difference was observed in the retention transfer latency between the groups \([F(5,27)=8.298, P<0.001]\). Retention transfer latency was found to be significantly increased in the PTZ group \((P<0.001)\) as compared to vehicle control group, thus showing an impairment of memory in the PTZ group. Significant decrease in the retention transfer latency was observed in HEOM (250, 500 and 1000 mg/kg) treated groups (Table 2) as compared to the PTZ group, showing an improvement in memory retention. Similarly, the standard drug valproate also caused significant decrease in the retention transfer latency as compared to the PTZ group and the values were comparable to that of the HEOM pre-treated groups.

In MES-induced seizures, no significant difference was found in the initial transfer latency among the groups, whereas a significant difference was observed in the retention transfer latency \([F(5,30)=5.895, P<0.01]\). On post-hoc analysis, it was found that retention transfer latency in MES group was significantly increased \((P<0.001)\) in comparison to vehicle control group. Pre-treatment with HEOM significantly decreased \((P<0.05)\) the retention transfer latency at all the dose levels tested as compared to MES group, which were comparable to that of the phenytoin group (Table 2).

3.3.2. Passive avoidance test

In PTZ model, no significant difference was observed in the initial latency amongst the groups, whereas retention latency between the groups differed significantly \([F(5,27)=12.051, P<0.001]\). After Bonferroni post-hoc analysis, it was found that retention latency was decreased significantly in PTZ group \((P<0.001)\) in comparison to the vehicle control group. Both HEOM pre-treatment and valproate significantly increased the retention latency as compared to the PTZ group (Table 2).

There was no significant difference in initial latency amongst the groups in MES model, whereas retention latency between the groups shows significant difference \([F(5,30)=6.063, P<0.01]\). A significant decrease \((P<0.05)\) in the retention latency in MES group as compared to the vehicle control group was observed. However, as compared to the MES group, pre-treatment with 250, 500 and 1000 mg/kg doses of HEOM caused a significant increase in the retention latency, which was comparable to that of phenytoin group (Table 2).

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### Table 1

Effect of hydroalcoholic extract of Orchis mascula (HEOM) on myoclonic jerk latency, occurrence of GTCS, seizure score and occurrence of THLE in PTZ and MES-induced seizures, respectively.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Myoclonic jerk latency (s) (mean ± SEM)</th>
<th>% protection against occurrence of GTCS</th>
<th>Seizure score (mean ± SEM)</th>
<th>% protection against occurrence of THLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTZ (60 mg/kg)</td>
<td>50.1 ± 2.3</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>MES (70 mA)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sod. Valproate (300 mg/kg)</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Phenytoin (40 mg/kg)</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>HEOM (250 mg/kg)</td>
<td>88.3 ± 3.7***</td>
<td>33.3</td>
<td>0.42 ± 0.04***</td>
<td>16.7</td>
</tr>
<tr>
<td>HEOM (500 mg/kg)</td>
<td>105.8 ± 5.4***</td>
<td>50</td>
<td>0.51 ± 0.01***</td>
<td>16.7</td>
</tr>
<tr>
<td>HEOM (1000 mg/kg)</td>
<td>119.8 ± 8.4***</td>
<td>66.7</td>
<td>0.57 ± 0.03***</td>
<td>33.3</td>
</tr>
<tr>
<td>HEOM (1000 mg/kg) (14 days)</td>
<td>128.5 ± 18.9***</td>
<td>66.7</td>
<td>0.56 ± 0.08***</td>
<td>–</td>
</tr>
</tbody>
</table>

*** P < 0.001, as compared to PTZ group.

### Table 2

Effect of HEOM on initial and retention latencies in elevated plus maze test and passive avoidance test in PTZ and MES-induced seizure models.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Elevated plus maze test</th>
<th>Passive avoidance test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PTZ-induced seizures</td>
<td>MES-induced seizures</td>
</tr>
<tr>
<td></td>
<td>Initial transfer latency (s)</td>
<td>Retention transfer latency (s)</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>36.8 ± 4.9</td>
<td>16.8 ± 1.9</td>
</tr>
<tr>
<td>PTZ (60 mg/kg)</td>
<td>41.7 ± 4.6</td>
<td>44.3 ± 4.6**</td>
</tr>
<tr>
<td>MES (70 mA)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sod. Valproate (300 mg/kg)</td>
<td>43.3 ± 3.7</td>
<td>17.4 ± 3.1**</td>
</tr>
<tr>
<td>Phenytoin (40 mg/kg)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HEOM (250 mg/kg)</td>
<td>33.1 ± 5.7</td>
<td>25.0 ± 4.2*</td>
</tr>
<tr>
<td>HEOM (500 mg/kg)</td>
<td>32.8 ± 7.2</td>
<td>22.1 ± 4.6*</td>
</tr>
<tr>
<td>HEOM (1000 mg/kg)</td>
<td>35.9 ± 7.7</td>
<td>20.4 ± 3.3**</td>
</tr>
<tr>
<td>HEOM (1000 mg/kg) (14 days)</td>
<td>36.1 ± 7.0</td>
<td>19.8 ± 2.8**</td>
</tr>
</tbody>
</table>

Data represent mean ± SEM (n=6). *P < 0.05, **P < 0.01, ***P < 0.001. a—as compared to vehicle control group; b—as compared to PTZ group; c—as compared to MES group.
3.4. Biochemical parameters

3.4.1. Cholinesterase activity

In PTZ model, there was a significant difference in the AChE \([F(5,30)=17.240, P<0.001]\) and BChE \([F(5,30)=11.816, P<0.001]\) activity amongst the groups. AChE and BChE activities were significantly decreased \((P<0.001)\) in the PTZ group in comparison to vehicle control group. Pre-treatment with HEOM caused a significant increase in the AChE activity at all dose levels tested, which were comparable to that of the phenytoin group. However, no significant difference in BChE activity was found on pre-treatment with HEOM at all doses as compared to the MES group (Table 3).

In MES-induced seizures, a significant difference was found in AChE \([F(5,30)=13.554, P<0.001]\) and BChE \([F(5,30)=22.770, P<0.001]\) activity amongst the groups. AChE and BChE activities were decreased significantly \((P<0.001)\) in the MES group, as compared to the vehicle control group (Table 3). A significant reversal of the decreased AChE activity was observed with HEOM pre-treatment at all dose levels tested (Fig. 1). The increase in GSH in HEOM pre-treated groups was found to be comparable to that of the phenytoin group. However, HEOM pre-treatment with HEOM at all doses as compared to the MES group did not cause any significant change in BChE activity (Table 3).

3.4.2. GSH levels

In PTZ-induced seizure, a significant difference was observed in the reduced glutathione levels between the groups \([F(5,30)=12.208, P<0.001]\). A significant decrease \((P<0.001)\) in the GSH levels was observed in PTZ group as compared to vehicle control group. HEOM pre-treatment reversed the PTZ-induced decrease in GSH levels at all dose levels tested (Fig. 1). The increase in GSH in HEOM pre-treated groups was found to be comparable to that of the valproate group.

Similarly, there was a significant difference in the GSH levels amongst the groups \([F(5,30)=16.072, P<0.001]\) in MES seizures. GSH levels significantly decreased in MES group \((P<0.001)\) as compared to the vehicle control group. Pre-treatment with HEOM significantly reversed the decreased GSH levels, which were comparable to that of the phenytoin group (Fig. 1).

3.4.3. MDA levels

In PTZ model, a significant difference was found in the brain MDA levels in between the groups \([F(5,30)=8.975, P<0.001]\). Post-hoc analysis revealed that MDA level was significantly increased in PTZ group \((P<0.001)\) as compared to the vehicle control group. There was a significant difference in the brain MDA levels between the groups \([F(5,30)=17.240, P<0.001]\). A significant increase \((P<0.001)\) in the brain MDA levels was observed in PTZ group as compared to vehicle control group. HEOM pre-treatment reversed the PTZ-induced increase in brain MDA level at all dose levels tested (Fig. 2). The increase in MDA in HEOM pre-treated groups was found to be comparable to that of the valproate group.

Similarly, there was a significant difference in the brain MDA levels amongst the groups \([F(5,30)=11.816, P<0.001]\) in MES seizures. MDA levels in MES group \((P<0.001)\) as compared to the vehicle control group. Pre-treatment with HEOM significantly reversed the increased MDA levels, which were comparable to that of the phenytoin group (Fig. 2).

Table 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>PTZ-induced seizures</th>
<th>MES-induced seizures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AChE activity (µmoles of acetylthiocholine hydrolysed/mg protein/min)</td>
<td>BChE activity (µmoles of butyrylthiocholine hydrolysed/mg protein/min)</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>0.15 ± 0.005</td>
<td>0.070 ± 0.002</td>
</tr>
<tr>
<td>PTZ (60 mg/kg)</td>
<td>0.07 ± 0.004***</td>
<td>0.023 ± 0.001***</td>
</tr>
<tr>
<td>MES (70 mA)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sod. Valproate (300 mg/kg)</td>
<td>0.12 ± 0.005***</td>
<td>0.051 ± 0.003***</td>
</tr>
<tr>
<td>Phenytoin (40 mg/kg)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HEOM (250 mg/kg)</td>
<td>0.10 ± 0.007***</td>
<td>0.031 ± 0.004</td>
</tr>
<tr>
<td>HEOM (500 mg/kg)</td>
<td>0.11 ± 0.004***</td>
<td>0.034 ± 0.006</td>
</tr>
<tr>
<td>HEOM (1000 mg/kg)</td>
<td>0.11 ± 0.008***</td>
<td>0.044 ± 0.008</td>
</tr>
<tr>
<td>HEOM (1000 mg/kg) (14 days)</td>
<td>0.14 ± 0.014***</td>
<td>0.066 ± 0.005***</td>
</tr>
</tbody>
</table>

Data represent mean ± SEM \((n=6)\), *\(P<0.05\), **\(P<0.01\), ***\(P<0.001\). a—as compared to vehicle control group; b—as compared to PTZ group; c—as compared to MES group.
control group. Reversal of the increased MDA levels was observed in HEOM pre-treated groups in comparison to the PTZ group (Fig. 2).

In MES model, there was a significant difference in MDA levels amongst the groups \( r(5.30) = 35.559, P < 0.001 \). MDA levels were increased significantly in the MES group as compared to the vehicle control group. However, pre-treatment with HEOM significantly attenuated the increased lipid peroxidation as indicated by the decreased MDA levels, which were comparable to that of the valproate group (Fig. 2).

4. Discussion

In the present study, protective effect of hydroalcoholic extract of \( O. \) mascula against seizures and seizure-induced oxidative stress and cognitive deficit was evaluated. Administration of HEOM prolongs the latency to myoclonic jerks dose-dependently and also showed protection against PTZ-induced GTCS. The administration of HEOM for 14 days, showed further increase in myoclonic jerk latency, but no enhanced protection against PTZ-induced GTCS was observed. In MES model, the protection shown by HEOM against THLE was not much pronounced as in PTZ model. Protection against GTCS and THLE revealed the potential of \( O. \) mascula in increasing the seizure threshold. Thus, the results showed the anticonvulsant activity of \( O. \) mascula in both PTZ as well as MES-induced seizures.

Seizures causes imbalance in oxidant, antioxidant system of brain which leads to oxidation of lipids, protein and DNA resulting into neurodegeneration. Thus, in the present study the seizure-induced oxidative stress was also evaluated. PTZ and MES induced seizures caused a significant increase in MDA levels and a significant decrease in GSH levels in PTZ and MES groups as compared to the vehicle control group. HEOM pre-treatment prevented the oxidative stress as indicated by significant decrease in MDA levels and significant increase in GSH levels in comparison to PTZ and MES control groups. Previous studies have reported the antioxidant (Aziz et al., 2009), radical scavenging and preventing oxidative DNA damage activity (Kalim et al., 2010) of methanolic extract of \( O. \) mascula. In concordance with previous studies the present study showed the dose dependent antioxidant potential of HEOM in both PTZ and MES model, which may be at least in part is responsible for its anticonvulsant action.

A study has shown that a brief ictal activity results in learning impairment (Kleen et al., 2010). HEOM pre-treatment showed significant improvement in memory as evident by decrease in retention transfer latency in elevated plus maze test and increase in retention latency in passive avoidance test in comparison to PTZ and MES groups, at all dose levels tested. Earlier studies on polyherbal formulations containing \( O. \) mascula have reported to enhance learning, reverse cognitive deficits induced by colchicine and ibotenic acid and affords protection against electro-convulsive shock induced anterograde amnesia in rats (Bhattacharya et al., 1995; Bhardwaj and Srivastava, 1995; Joseph et al., 1994).

Decreased acetylcholine (ACh) levels in the brain are associated with cognitive deficit (Biegol et al., 1986; Perry et al., 1978; Giacobini, 2000). Decrease in AChE activity in hippocampus and mid brain, 30 min after a flurorothy-induced convulsion, PTZ induced seizures and 2 min after convulsion induced electrically have also been reported (Appleyard et al., 1986; Visweswari et al., 2010). In agreement with earlier studies, our results also showed decrease in AChE and BChE activity following PTZ and MES-induced seizure. HEOM pre-treatment brings AChE activity in whole brain to the levels that are comparable to vehicle control, with no significant changes in BChE activity. Thus, the results of the present study showed anticonvulsant activity of \( O. \) mascula against PTZ and MES-induced seizures and also its protective effect against seizure induced oxidative stress and cognitive impairment.

References


