Interaction of hydroalcoholic extract of *Acorus calamus* Linn. with sodium valproate and carbamazepine

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Anticonvulsant property of *Acorus calamus* is known. Since combination therapy can lower the dose of individual drug and dose related toxicities, in this study, the effect of co-administration of hydroalcoholic extract of *A. calamus* (HAEAC) on conventional antiepileptic drugs (AEDs), sodium valproate and carbamazepine was determined using pentylenetetrazole-induced seizures model in rats. On combining the subanticonvulsant doses of HAEAC with sodium valproate and carbamazepine, greater protection as compared to either drug alone was observed. This was not related to change in levels of the AEDs. Thus, the results further substantiate anticonvulsant effect of HAEAC and suggest a potential for add on therapy with AEDs.

**Keywords :** *Acorus calamus*, Carbamazepine, Pentylentetrazole-induced seizures, Sodium valproate

Epilepsy is commonest neurological disorder incidence of which is reported as 5-10 cases per 1000 persons. Several antiepileptic drugs (AEDs) are available which act through different mechanisms. However, their adverse reactions, tetratogenesisity, drug interactions, remain the major limitations. Further, in spite of several newer anti-epileptic drugs such as lamotrigine, vigabatrin, tiagabine and gabapentin, that have been added to antiepileptic armamentarium, it is estimated that about 15-30% of epileptics remain refractory. Thus, the need for safer and more efficacious AEDs.

There is a wide use of herbals as complementary system in management of epilepsy and many herbal drugs have been evaluated for their antiepileptic effect. *Acorus calamus* Linn. (commonly known as Sweet Flag in English, or calamus in Latin and Bach in Hindi, Family: Araceae) has been shown to be effective in prevention and delaying seizures induced by pentylenetetrazole (PTZ), maximal electroshock (MES) and ferric-chloride induced spike-wave discharges. However, depending on the extract used, both an anticonvulsant and proconvulsant effect have been reported.

In Ayurvedic system of medicine, *Acorus calamus* rhizome is used in the treatment of neurosis, epilepsy, insomnia, melancholia, hysteria and memory loss. Various scientific studies have also evaluated and proven potential of *A. calamus* as antimutagenic, antibacterial, antifungal, antispasmodic, antitubercular, immunosuppressant, antidiarrhoeal, anti-arrhythmic and significant anti-inflammatory activity in acute, chronic and immunological models of inflammation.

In spite of its efficacy in diverse conditions, *A. calamus* has a limited potential. This is owing to genotoxicity and mutagenicity with beta-asarone and a dose-dependent mutagenicity with alpha-asarone, the two major components of *A. calamus*. It has therefore been suggested that preparations free from or with a low content of beta-asarone should be used. The composition of *A. calamus* extracts can be controlled by using different extraction procedures and specifically, methanolic extraction is reported to lower the beta–asarone content.

Since combination therapy can lower the dose of individual drug as well as the dose related toxicities, the effect of a hydroalcoholic extract of *Acorus calamus* (HAEAC) has been studied in combination with sodium valproate and carbamazepine, two first line anti-epileptic drugs (AEDs), in pentylenetetrazole(PTZ)-induced seizures in rats and on levels of these AEDs.
Materials and Methods

Plant extract—The standardized hydroalcoholic extract of A. calamus (HAEAC) was procured from Ranbaxy Research Foundation, Gurgaon, India. For preparation of extract, each kg of coarsely powdered authenticated A. calamus rhizomes, purchased from Khari Baoli, New Delhi, India, was macerated with five times volume (5 litres) of methanol: water (50:50) for 24 h at room temperature. The macerated liquid was filtered out through muslin cloth and the remaining material (marc) was again subjected to maceration twice on two successive days. The filtrate obtained was mixed with first-obtained macerate. The total macerate obtained was dried in Rotavapour under vaccum, at 40°C, till completely dry. The yield of the extract was 4.05% (81.8 g) from 2.5 kg of authenticated rhizomes. The HAEAC was diluted with normal saline on the day of administration.

Drugs and chemicals—Pentylenetetrazole (PTZ) and carbamazepine (CBZ) were purchased from Sigma Inc., USA, while sodium valproate (SV) was a gift from Sun Pharmaceuticals Ind Ltd., India. All drugs were dissolved in normal saline except carbamazepine which was suspended in a mixture containing 10% ethanol, 40% propylene glycol and 50% water. Control experiments were performed with the respective vehicles.

Experimental animals—Male Wistar rats, weighing 150–200 g, were procured from the Central Animal Facility of All India Institute of Medical Sciences, New Delhi and were maintained in the departmental animal house. The rats were housed in polycrylic cages (38x23x10 cm) with not more than four animals per cage and maintained under standard laboratory conditions with natural dark and light cycle. They were allowed free access to standard dry rodent diet (Ashirwad Industries, Chandigarh, India) and tap water ad libitum. All procedures described were reviewed and approved by the Institutional Animal Ethics Committee, AIIMS, (approval no. 303/IAEC/05). All the study-related activities conformed to Indian National Science Academy Guidelines for Use and Care of Experimental Animals in Research.

Pentylenetetrazole-induced seizures—Pentylenetetrazole (PTZ) was dissolved in normal saline. PTZ (60 mg/kg body weight, ip) consistently produced seizures with least mortality as standardized previously and therefore was used in the entire study to see the effect of different drug treatments. Rats were observed for 30 min after PTZ administration. The incidence and latency for myoclonic jerks were noted. Additionally, incidence and duration of generalized clonic seizures were also recorded.

Per se effect of HAEAC—In order to determine the dose-dependency of the effect of HAEAC on PTZ-induced seizures and to determine the subanticonvulsant dose for further experimentation, rats were pretreated with HAEAC at 25, 50, 100, 150, and 200 mg/kg body weight, ip doses, 30 min before injecting PTZ (60 mg/kg).

Combination experiments with sodium valproate (SV)—On the basis of earlier experiments, SV (150 mg/kg body weight), was selected as the subanticonvulsant dose, and administered concurrently with the subanticonvulsant dose of HAEAC, i.e., 100 mg/kg, before the convulsant (PTZ 60 mg/kg, ip) challenge. The pretreatment time for SV and HAEAC was 30 min.

Combination experiments with carbamazepine (CBZ)—Carbamazepine (10mg/kg body weight) was given along with HAEAC at a dose of 100 mg/kg, 30 min before PTZ. The dose of CBZ was selected as subanticonvulsant dose based on earlier studies.

Effect on AED levels—Rats (72) were divided into 4 groups of 18 each and administered the AEDs (SV 150 mg/kg and CBZ 10 mg/kg) alone and in combination with HAEAC (100 mg/kg). Blood was collected by direct cardiac puncture under ether anesthesia at 30 min, 1 h, and 2 h post drug treatment. For each time point, separate animals were used (n=6). The serum was separated from collected blood by centrifugation at 7200 rpm for 8 min and the blood levels of SV and CBZ were estimated by the EMIT assay method using kits from Cedia, Microgenics GmbH (Germany) in the Automated Analyzer MCG, Version 1.50, Microgenics Corporation European Headquarters, Germany.

Statistical analysis of data—The data are represented as mean±SE. All statistical analyses were performed using SPSS statistical software version 13.0 and one way analysis of variance (ANOVA) with Bonferoni post hoc test was performed. P<0.05 was taken as the level of significance.

Results

Effect of HAEAC on PTZ-induced seizures—In control experiments, PTZ (60 mg/kg, ip) consistently...
produced myoclonic jerks and generalized clonic seizures in all animals. HAEAC, (200 mg/kg) showed complete protection against generalized clonic seizures (GCS) while the incidence of myoclonic jerks was reduced significantly (1 out of 6 vs. 6 out of 6 rats in vehicle control group). With 100 mg/kg HAEAC, while all the rats experienced myoclonic jerks, 5 out of 6 rats showed GCS. It however afforded protection in terms of an increased latency and decreased duration of GCS. The 150 mg/kg dose reduced the incidence of GCS with 3 out of 6 rats showing GCS and 4 out of 6 rats showing myoclonic jerks. The two lower doses of HAEAC used (i.e., 25 and 50 mg/kg), had no protective effect on any parameter (Table 1).

Apart from this, a significant increase (P<0.001) was observed in the mean latency of PTZ-induced seizures with increasing doses of HAEAC, while duration of GCS tended to decline.

**Evaluation of combination of HAEAC and sodium valproate (SV) in PTZ-induced seizures**—There was a significant decrease in the incidence of myoclonic jerks and an increase in latencies with combination doses of SV (150 mg/kg) and HAEAC (100 mg/kg), as compared to SV alone (P<0.001; Table 2).

**Pharmacokinetic interaction of HAEAC with sodium valproate and carbamazepine**—Mean serum levels with combination treatment remained unchanged at all points of time, i.e., 30 min, 2 h, and 4 h (Table 3).

Table 1—Effect of different doses of HAEAC in PTZ induced seizures (n=6)

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Incidence of myoclonic jerks(%)</th>
<th>Incidence of clonic seizures(%)</th>
<th>Latency of myoclonic jerks/sec.</th>
<th>GCS duration (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (N.S.)</td>
<td>100</td>
<td>100</td>
<td>38.8 ± 3.5</td>
<td>21.7 ± 2.5</td>
</tr>
<tr>
<td>2</td>
<td>HAEAC 25 mg/kg</td>
<td>100</td>
<td>100</td>
<td>55.5 ± 5.4</td>
<td>19 ± 2.3*</td>
</tr>
<tr>
<td>3</td>
<td>50 mg/kg</td>
<td>100</td>
<td>100</td>
<td>86.7 ± 9.0*</td>
<td>11.5 ± 0.8**</td>
</tr>
<tr>
<td>4</td>
<td>100 mg/kg</td>
<td>100</td>
<td>83.3</td>
<td>106.7 ± 11.7*</td>
<td>11.7 ± 0.7**</td>
</tr>
<tr>
<td>5</td>
<td>150 mg/kg</td>
<td>66.7</td>
<td>50</td>
<td>250.3 ± 17.9*</td>
<td>No GCS observed</td>
</tr>
<tr>
<td>6</td>
<td>200 mg/kg</td>
<td>16.7</td>
<td>0</td>
<td>118#</td>
<td>No GCS observed</td>
</tr>
</tbody>
</table>

P values: *<0.05; **<0.01; # only one animal

Table 2—Effect of different doses of HAEAC in combination with sodium valproate (SV) and carbamazepine (CBZ) in PTZ induced seizures.

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Incidence of myoclonic jerks (%)</th>
<th>Incidence of clonic seizures (%)</th>
<th>Latency of myoclonic jerks/sec.</th>
<th>GCS duration (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N.S.)</td>
<td>100</td>
<td>100</td>
<td>38.8 ± 3.5</td>
<td>21.7 ± 2.5</td>
</tr>
<tr>
<td>SV (150 mg/kg)</td>
<td>83.33</td>
<td>0</td>
<td>116.2 ± 2.1*</td>
<td>No GCS</td>
</tr>
<tr>
<td>HAEAC (100 mg/kg)</td>
<td>100</td>
<td>83.3</td>
<td>106.7 ± 11.7*</td>
<td>11.7 ± 0.7**</td>
</tr>
<tr>
<td>HAEAC (100 mg/kg) + SV (150 mg/kg)</td>
<td>50</td>
<td>0</td>
<td>219.5 ± 12.3**</td>
<td>No GCS</td>
</tr>
<tr>
<td>Vehicle control for CBZ</td>
<td>100</td>
<td>100</td>
<td>32.7 ± 1.3</td>
<td>19.7 ± 1.8</td>
</tr>
<tr>
<td>CBZ (10 mg/kg)</td>
<td>83.3</td>
<td>0</td>
<td>119.2 ± 3.5*</td>
<td>No GCS</td>
</tr>
<tr>
<td>HAEAC (100 mg/kg) + CBZ (10 mg/kg)</td>
<td>83.3</td>
<td>0</td>
<td>210.2 ± 24.6**</td>
<td>No GCS</td>
</tr>
</tbody>
</table>

P values: *<0.05; **<0.01; *<0.005; **<0.001
Discussion

Nowadays, herbal medicines are being increasingly used to supplement the conventional medicines worldover and epilepsy is no exception. This, however, necessitates the scientific scrutiny of these herbal medicines. The therapeutic potential of A. calamus which has been mentioned in Ayurveda to possess an antiepileptic effect have been evaluated.

Depending on the preparation/extract used, an anticonvulsant as well as proconvulsant effect has been reported for A. calamus. Thus, the steam volatile fractions exacerbated the tonic seizures induced by metrazol, while Acorus oil as well as the aqueous and alcoholic extracts were protective. Dandiya and Sharma worked with two active principles of A. calamus, namely asarone and beta-asarone, and reported an opposite profile against different seizure models for the two components. In yet another study, pre-inhalation of an essential oil from A. gramineus increased seizure latency in PTZ seizures in mice. In this study with HAEAC, a dose-dependent anticonvulsant effect was observed.

Different workers have demonstrated different constituents and unique activities for different extracts from Acorus species. While no phytochemical analysis, was carried out in the present study, a hydroalcoholic (methanol) extract was used and methanolic extraction has been reported to decrease the beta-asarone content. Beta-asarone is associated with carcinogenic activity and is also reported to possess a proconvulsant effect. Other major component of A. calamus, alpha-asarone has also been associated with a dose-dependent mutagenic effect. Thus the therapeutic viability of A. calamus per se as treatment option for epilepsies becomes doubtful.

In the present study, an effort was made to assess the potential of A. calamus as an add-on agent with other AEDs. To the best of our knowledge, till date there is only one report of such a study which provides a positive interaction between A. calamus and the conventional antiepileptic drugs, phenobarbitone and phenytoin in maximal electroshock (MES) and PTZ–induced seizures. However, these workers do not comment on the mechanistic aspects i.e. whether there is a pharmacokinetic component in this pharmacodynamic interaction.

The effect of combination of subanticonvulsant doses of the other two first line conventional AEDs, SV (150 mg/kg) and CBZ (10 mg/kg), was evaluated with the subanticonvulsant dose of HAEAC (100 mg/kg). The dose of HAEAC used was derived from dose response study. Attempt was made to use as low a dose as possible of A. calamus. The better protection observed against PTZ-induced seizures as compared to either drug alone, at doses used, would suggest a potential as add on therapy with other AEDs. Though the reason for this enhanced protection is obscure it can be presumed that it is due to either pharmacokinetic changes or a pharmacodynamic interaction.

Whether a pharmacokinetic component is involved was assessed in the present study by determining the blood levels of SV and CBZ alone and in combination with HAEAC, at different time points, in a separate set of experiments. Results of the present pharmacokinetic interaction study show that the serum level of either drug is not significantly altered when given in combination and hence no pharmacokinetic interaction. Thus, the potentiation of anticonvulsant activity should have a pharmacodynamic basis.

In this study the pharmacodynamic basis of potentiation has not been explored but it is tempting to speculate that since an increase in GABAergic activity and decrease in glutamate has been demonstrated by Acorus species, this could account for potentiation.

Conclusions

The results of the present study substantiate a potentiation of anticonvulsant effect on concurrent administration of subanticonvulsant doses of AEDs and HAEAC. This potentiation is unlikely to be due to a pharmacokinetic interaction and suggests a potential for add-on therapy with A. calamus. However, further studies need to be carried out to determine whether doses of A. calamus used are low enough to obviate toxic side-effects and the possible mechanism.

Conflict of interest: The authors declare that there is no conflict of interest.

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