Anticonvulsant Activity of Methanolic Extract of Acorus Calamus Leaves in Albino Mice

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Abstract: Acorus calamus, commonly known as sweet flag, has a long history of use in the treatment of a variety of ailments including inflammation, chest pain, digestive disorders and some mental illnesses. Its effects on the neurological conditions have been well documented for axinolytic and antidepressant activities. With this background, the aim of the present study is evaluate the anticonvulsant activity of methanolic extract of Acorus calamus leaves in albino mice. The study included albino mice divided into 8 groups of 6 mice each. Maximum electroshock induced seizures (MES) and Pentylenetetrazole (PTZ) tests were performed on the animal models to evaluate the antiepileptic activity (4 groups were allocated to MES and 4 to PTZ). The methanolic extract of Acorus calamus leaves exhibited a significant reduction in the duration of hind limb extensor phase in MES model (7.116±0.501 seconds for control and 9.116±0.527 seconds for extract) and delayed the latency of seizures induced by PTZ (485.500±14.941 seconds) when compared with that of the control group (297.000±21.918 seconds). In addition, the groups administered with the extract and sodium valproate in combination exhibited significant results in both MES and PTZ models (T2- 92.61% and T4- 21.95 %, respectively). Preliminary phytochemical screening performed in several studies has shown the presence of triterpenoids, flavonoids, saponins and tannins. The anticonvulsant activity of Acorus calamus may be mediated by its GABA potentiating activity. It can thus be concluded that the observed anticonvulsant effects could be the resultant of a synergistic action of these phytochemicals. Further studies should be undertaken to substantiate these results on various animal models along with a thorough phytochemical analysis and in silico studies to understand the mode of action of these phytochemicals on various GABA receptor-mediated signaling.

Keywords: Anticonvulsant, Electroshock induced seizures (MES), Pentylenetetrazole (PTZ), methanolic extract, Acorus calamus.
1. INTRODUCTION

Epilepsy is a common neurological disorder. An epileptic seizure has been defined as a paroxysmal discharge of cerebral neurons accompanied by clinical phenomena apparent to the patient or to an observer. The phenomena can be motor, sensory, or autonomic and there may also be impairment or complete loss of consciousness.\textsuperscript{1} The incidence of epilepsy is \textasciitilde{}0.3–0.5% in different populations throughout the world, and the prevalence has been estimated to be 5–10 persons per 1000.\textsuperscript{2} The causes of epilepsy are many, namely, idiopathic, infection, neoplasm and head injury. In some cases, heredity has proven to be a predominant factor.\textsuperscript{3} Epilepsy is a serious health concern due to its effect on the disruption of the normal activities of the brain cell. Despite the introduction of several new therapeutic options, a significant fraction of the patients with epilepsy continue to live with uncontrolled seizures.\textsuperscript{4} There is still a need for an ideal antiepileptic agent with properties like broad spectrum activity, rapid onset of action, least side effects, good oral bioavailability and low costs.\textsuperscript{5} Currently existing drugs entail adverse effects including central nervous system depression, ataxia, megaloblastic anemia, cardiac arrhythmias, hepatic dysfunction and teratogenicity.\textsuperscript{6} There is still a need for broad spectrum anticonvulsant drugs possessing multiple mechanisms of action with decreased adverse effect and marked efficacy against all types of seizures, preferably originating from natural products.\textsuperscript{7} Over the last few years, researches have aimed at identifying and validating plant derived substances for the treatment of various diseases. Indian medicinal plants are considered a vast source of pharmacologically active compounds that are commonly used as home remedies against multiple ailments.\textsuperscript{8} \textsuperscript{9} Herbal medicines are widely used due to their easy availability, applicability and efficacy coupled with least side effects, which in turn has accelerated the scientific research regarding the antiepileptic activity. \textit{Acorus calamus} Linn. (Family: Araceae) is an aromatic semi-aquatic perennial marshy herb commonly known as Bach and Ugragaranti in Sanskrit, Baje in Kannada and sweet flag in English. It is a well known medicinal plant used in ayurvedic medicine.\textsuperscript{10} All parts of the plant contain volatile oil which contains asaronaldehyde, terpenoids, calamine, calamenone, eugenol, camphene and pipene. Its roots and rhizomes are used in the treatment of various ailments like appetite loss, bronchitis, chest pain, diarrhoea, rheumatism, vascular disorders and many mental disorders such as hysteria, insanity, insomnia, melancholia and neurasthenia.\textsuperscript{11} All the reported literature on \textit{Acorus calamus} for its pharmacological activities was done mainly by using roots and rhizomes extracts. Methanol and acetone extracts of \textit{Acorus calamus} leaves were studied for CNS activity in mice. Result of this work provides evidence that it may contain inhibitory psychoactive substance (alpha asarone) that is depressant in nature.\textsuperscript{12} The essential oil, alcoholic and aqueous extract of this rhizome have been found to be pharmacologically active. Experimental studies with essential oil and alcoholic extracts of this plant in experimental seizures are ambiguous and results vary with the model used.\textsuperscript{9} \textsuperscript{10} Therefore the present study was carried out to scientifically evaluate the antiepileptic activity of methanolic extract of \textit{Acorus calamus} leaves, as experimental studies with this extract is insufficient.

2. MATERIAL AND METHODS

2.1 Collection and Extraction of plant material

The fresh and matured leaves of \textit{Acorus calamus} were collected from Chandravana, Mysore and authenticated by the Department of Horticulture, Mysore with the voucher specimen number 3948224. The leaves were dried under shade and were coarsely powdered. 70 grams of the powder was wrapped in a filter paper and put into a thimble, with 500 ml of methanol in a round bottom flask and subjected to Soxhlation for 6 – 8 hrs. Dark green solution of extract with alcohol was collected. Paste of dark green extract was obtained after evaporation of alcohol.\textsuperscript{13}

2.2 Animals

Swiss albino mice (48 numbers for 8 groups of 6 animals each) weighing around 25 g – 30 g of either sex were randomly selected from central animal facility, J S S Medical College, Mysore. Animals were provided free access to tap water and commercial food, and were maintained under standard laboratory conditions with a natural light and dark cycle, under room temperature. The animals were acclimatized for 24 hours before the start of experimentation. Animals were administered drugs both standard and test orally. The study was conducted after obtaining institutional animal ethical committee clearance (JSSMC/IAEC/011/December – 2014) and conducted as per the guidelines of CPCSEA, Chennai, India.

2.3 Experimental protocol

Animals were divided into 8 groups of 6 mice each. Test animals were divided into two groups such that one group was subjected to electroshock of 50 mA intensity for 0.2 seconds, through auricular electrodes, (covered with cotton wool moistened with saline). A majority of mice showed tonic flexion of fore and hind limbs with tail erection, tonic extension of both fore and hind limbs, clonus, stupor followed by postictal depression, and recovery. Only those mice showing the convulsive responses were used for experiment and divided into 4 groups of six each. Remaining group of mice were used for chemoshock (pentylenetetrazol) and divided into 4 groups of six each.

2.4 Grouping

2.4.1 Maximal electro shock (MES) method groups

The grouping and the treatment were carried out based on the method given by Pushpa et al., 2020.\textsuperscript{14} Group 1(C1) - the control group was treated with propylene glycol (0.1ml/100g) Group 2(S1) – Standard group was treated with 200mg/kg Sodium Valproate dissolved in sodium valproate Group 3 - T1 group was treated with 200 mg/kg of methanolic extract of \textit{Acorus calamus} leaves dissolved in propylene glycol. Group 4 – T2 group was treated with 100 mg/kg of methanolic extract of \textit{Acorus calamus} leaves dissolved in propylene glycol and 100 mg/kg of Sodium Valproate in distilled water.

2.4.2 Pentylenetetrazol method groups

Group 5(C2) - control group was treated with 0.5 ml/100g of propylene glycol Group 6(S2) – Standard group was treated with 200mg/kg of Sodium Valproate dissolved in propylene glycol. Group 7 - T3 group was treated with 200mg/kg of methanolic extract of \textit{Acorus calamus} leaves dissolved in propylene glycol. Group 8 – T4 group was treated with 100mg/kg of methanolic extract of \textit{Acorus calamus} leaves and 100mg/kg of Sodium Valproate dissolved in propylene glycol.
All the preparations except PTZ were administered orally. PTZ was administered intraperitoneally.

2.4.3 Maximal electro shock (MES) method

The MES model evaluates the ability of drugs to prevent electrically induced Tonic Hind Limb Extension (THLE) in mice or rats. Drugs that are active in the MES test often have a phenytoin like effect on voltage dependent Na+ channels.

Method

Adult albino mice (20-25 gms) grouped into six animals per drug dose/vehicle were used. A number of factors alter seizure susceptibility such as endocrine, nutritional, temperature etc., Therefore, to reduce variability all experiments were carried out on male animals, with similar age and weight with treatments induced around the same time of the day. All animals are maintained on an adequate diet and allowed free access to food and water, except during testing, as pre-test starvation modifies the MES induced seizure pattern (shortens tonic flexion & prolongs tonic extension). Transauricular electrodes (applied to the pinna with small crocodile clips covered with saline-moistened cotton wool) were used. Pre-test saline moistening is mandatory to ensure better contact & to reduce fatalities resulting from MES induced seizures. Maximal seizures are evoked by supramaximal electroshock stimulation of 50mA, 50HZ, for 0.2 seconds by using conventional electroconvulsiometer. The abolition of the Hind Limb Tonic Extension is taken as an index of anticonvulsant activity.\textsuperscript{15}

2.4.4 Pentyleneetetrazol method

The PTZ model evaluates the ability of potential antiepileptic agents to prevent clonic seizures may correlate with activity against absence seizures. Activity of a drug in this model may affect GABAergic brain systems, either by enhancing GABA levels or by altering sensitivity of postsynaptic GABA receptors.\textsuperscript{16}

Method

PTZ at a dose of 75 mg/kg IP (dissolved in distilled water) was used, which produces excitement, myoclonic jerks & clonic seizures. An occasional fatal tonic seizure may occur, which was observed in at least 97% of the animals. PTZ is given 30 minutes after the test drug. The PTZ response occurs about 5 to 10 minutes after administration. The mice were observed for half an hour after the administration of the drug. Prolongation of duration of seizure latency was taken as an index of protection & anticonvulsant activity of the test compound.\textsuperscript{17,18}

3. STATISTICAL ANALYSIS

Results were presented as Mean ± SEM. One way ANOVA was used for multiple comparisons followed by Scheffe’s post hoc test for comparison between groups. For all the tests a ‘P’ value of 0.05 or less was considered for statistical significance.\textsuperscript{19,20}

4. RESULT

4.1 MES Model

The mean duration of Tonic Hind limb Extension (THLE) in the control group was 9.116±0.527 seconds. Methanolic extract of Acorus calamus leaves at a dose of 200 mg/kg (T1) reduced the duration of Tonic Hind limb extension to 7.116±0.501 seconds. Combination of the standard drug, sodium valproate at a dose of 100 mg/kg and methanolic extract of Acorus calamus leaves at a dose of 100mg/kg (T2) reduced the duration of Tonic Hindlimb extension to 3.700 ± 0.596 seconds which is statistically significant when compared to control as shown in Table 1. Assuming that protection offered by sodium valproate against Tonic Hindlimb Extension is 100%, the percentage protection of methanolic extract of Acorus calamus leaves at the dose of 200 mg/kg (T1) and combination of the standard drug, sodium valproate at a dose of 100 mg/kg with methanolic extract of Acorus calamus leaves at a dose of 100mg/kg (T2) against THLE were 59.38 and 21.95 %, respectively (Table 2).

4.2 PTZ Model

The mean duration of Seizure Latency in the control group was 297.000±21.918 seconds. Standard drug sodium valproate at a dose of 200mg/kg increased the duration of seizure latency to 485.500±14.941 seconds which was statistically significant when compared to that of the control. Combination of the standard drug, sodium valproate at a dose of 100 mg/kg and methanolic extract of Acorus calamus leaves at a dose of 100mg/kg (T4) showed statistically significant increase in the duration of seizure latency to 449.666±19.793 seconds when compared to control as shown in Table 3. Assuming that Sodium valproate showed 100 % protection against seizure latency, the percentage protection of methanolic extract of Acorus calamus leaves at the doses of 200 mg/kg (T3) and combination of the standard drug, sodium valproate at a dose of 100 mg/kg with methanolic extract of Acoruscalamus leaves at a dose of 100mg/kg (T4) against seizure latency were 65.67% and 92.61%, respectively (Table 4).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hind limb Tonic Flexion (sec)***</th>
<th>Hind limb Tonic Extension (sec)***</th>
<th>Clonus (sec)***</th>
<th>Stupor (sec)***</th>
<th>Postical Depression (sec)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>8.350±0.513</td>
<td>9.116±0.527</td>
<td>18.450±0.826</td>
<td>316.672±34.318</td>
<td>349.666±17.704</td>
</tr>
<tr>
<td>S1</td>
<td>3.916±0.291</td>
<td>1.817±0.2007</td>
<td>12.266±1.960</td>
<td>95.000±3.559</td>
<td>116.333±8.969</td>
</tr>
<tr>
<td>T1</td>
<td>5.633±0.972</td>
<td>7.116±0.501</td>
<td>16.216±0.849</td>
<td>263.502±15.968</td>
<td>247.500±39.053</td>
</tr>
<tr>
<td>T2</td>
<td>4.833±0.454</td>
<td>3.700±0.596</td>
<td>11.550±0.371</td>
<td>115.502±2.974</td>
<td>201.500±14.582</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM of six observations. *** P<0.001

Statistical significant test for comparison was done by ANOVA, followed by Scheffe’s post hoc test.
Table 2. Percentage protection against Tonic hindlimb extension by the various treatment groups in maximal electroshock seizure model in comparison to standard.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Percentage Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Group</td>
<td>100 %</td>
</tr>
<tr>
<td>Test Group 2</td>
<td>59.38%</td>
</tr>
<tr>
<td>Test Group 1</td>
<td>21.95%</td>
</tr>
</tbody>
</table>

Table 3. Effect of Acoruscalamus leaves extract on PTZ induced convulsions in mice.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>seizure atency(sec)***</th>
<th>Myoclonic jerks (sec)***</th>
<th>Generalized Clonic seizures (sec)***</th>
<th>postictal depression (sec)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2</td>
<td>297.000±21.918</td>
<td>4.700±0.822</td>
<td>10.833±0.600</td>
<td>342.000±3.454</td>
</tr>
<tr>
<td>S2</td>
<td>485.500±14.941</td>
<td>1.650±0.210</td>
<td>7.783±0.470</td>
<td>265.333±19.032</td>
</tr>
<tr>
<td>T3</td>
<td>318.833±10.864</td>
<td>4.016±0.612</td>
<td>9.650±0.408</td>
<td>314.666±11.485</td>
</tr>
<tr>
<td>T4</td>
<td>449.666±19.793</td>
<td>1.950±0.076</td>
<td>7.700±0.350</td>
<td>276.500±16.451</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM of six observations.***p<0.001
Statistical significant test for comparison was done by ANOVA, followed by Scheffe's post hoc test.

Table 4. Percentage protection in seizure latency among various treatment groups in Pentylenetetrazole Model in comparison to standard.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Percentage Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Group</td>
<td>100 %</td>
</tr>
<tr>
<td>Test Group 4</td>
<td>92.61%</td>
</tr>
<tr>
<td>Test Group 3</td>
<td>65.67%</td>
</tr>
</tbody>
</table>

DISCUSSION

Significant advances are being made in recent years to treat epilepsy using second-generation drugs. Poly pharmacy is often advocated to 30% of all epileptic patients for refractory partial or generalized tonic clonic seizures. However, none of the new drugs fulfill the ultimate goal of drug treatment for epilepsy, i.e., complete control of seizures. Therefore, despite the beneficial effects of the currently available drugs, there is still a need for broadly acting anticonvulsant drugs possessing multiple mechanisms of action with decreased adverse effect, preferably originating from natural products. Acorus calamus is a traditional herb with over 145 constituents isolated and identified for medicinal properties to date. Their effects on neurological disorders such as neuroprotective, antidepressant, as well as cardio protective, antihypertensive, immune modulatory activities are well documented. With this background, the present study evaluated the anticonvulsant activity of Acorus calamus was against MES- and PTZ- induced convulsions. Recent studies have demonstrated that the plant possesses anxiolytic activity on albino mice, which was evaluated using the ethanol extract showing an effective dose at 200 mg/kg body treatment. In line with this and several other previous studies, the test doses for the administration were chosen to be 100 and 200 mg/kg body weight. In the MES model at dose of 200 mg/kg of methanolic extract of Acorus calamus leaves (T1) there was no significant anticonvulsant activity when compared to that of the control. But combination of the standard drug, sodium valproate at a dose of 100 mg/kg and methanolic extract of Acorus calamus leaves at a dose of 100mg/kg (T2), showed statistically significant reduction in the duration of Tonic Hindlimb Extension when compared to that of the control. Hence Acorus calamus leaves extract
when given along with the standard drug Sodium Valproate in reduced doses, might possess anticonvulsant activity. Several studies have reported the Tonic Hindlimb Extension as an efficient end point assessment for anti-epileptic activity in tonic-clonic seizure models. Recent studies from our lab on the effects of lacidipine on the clonic seizures also demonstrated that 51.3% protection was exerted against the MES induced seizures. In the PTZ model, a combination of the standard drug sodium valproate at a dose of 100 mg/kg and methanolic extract of *Acorus calamus* leaves at a dose of 100mg/kg (T4) showed significant anticonvulsant activity when compared to that of the control. Hence *Acorus calamus* leaves extract when given along with the standard drug Sodium Valproate in decreased doses might possess anticonvulsant activity. It is proposed that PTZ induces convulsion by either inhibiting gamma amino butyric acid (GABA) pathway in CNS or by increasing the central noradrenergic activity. The effect of extract in this model can therefore suggest its involvement in GABA-ergic or noradrenergic pathways. On comparing the percentage protection offered by *Acorus calamus* against both MES and PTZ model as shown in Figure 1, a combination of the standard drug, sodium valproate at a dose of 100 mg/kg with methanolic extract of *Acorus calamus* leaves at a dose of 100mg/kg was evaluated in the present study showing greater effectiveness in the PTZ model than in the MES model. Recent studies have shown the improved efficacy of combination therapy over only the extract primarily owing to a synergistic action of the various phytocomponents present in the plant extract and its interaction with the drugs. Similarly, in our study the effect of the combination was higher in comparison with the treatment of plant extracts alone. This indicates that the anticonvulsant activity of methanolic extract of *Acorus calamus* leaves when given as a monotherapy is less efficacious when compared to the standard drug, sodium valproate, but when given with the standard drug in a decreased dose it possesses significant anticonvulsant activity when compared with that of the control. This indicates that the test drug *Acorus calamus* can be used as an adjuvant along with the standard drug for treating epilepsy. Hence by reducing the dose of sodium valproate, its adverse effects can also be limited.

Phytochemical investigations revealed that methanolic extract of *Acorus calamus* leaves contain triterpenoids, flavonoids, saponins and tannins. A number of scientific reports indicate that triterpenoids produce CNS depressant action. Therefore, the observed CNS depressant activity and in turn its anticonvulsant activity could be primarily due to the presence of these terpenoids.

6. CONCLUSION

In conclusion, the methanolic extract of *Acorus calamus* leaves possess a remarkable anticonvulsant effects, which may be mediated by its GABA potentiating activity. Further studies are required to substantiate the results. In addition, studies on the isolation of the active principle/principles that contribute to the anticonvulsant activity should be performed. The active principle/principles when isolated can be evaluated using in silico methods to evaluate the specific targets for the observed anticonvulsant activities. The study thus proves to be the basis for identification of a prospective broad spectrum anticonvulsant agent, either alone or in combination with the already available anticonvulsant drugs.

7. ACKNOWLEDGEMENT

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8. AUTHOR'S CONTRIBUTION STATEMENT

Concept and design: Kalabharathi H. L; Data acquisition: Pragathi Balakrishna, Patali Snehalatha N & Ashwini V; Critical revision of the manuscript: RamithRamu. The manuscript was completed with equal contributions from all the authors in terms of preparing the manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none


