STUDY ON ANTICONVULSANT AND ANXIOLYTIC ACTIVITIES OF LEAVES EXTRACTS OF ACORUS CALAMUS LINN. IN MICE

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ABSTRACT

Many synthetic drugs are available for treatment of convulsions and anxiety but they have also many side effects. Therefore new anticonvulsant and anxiolytic drugs from traditional system of medicine have to be developed with less or no side effects. Among various medicinal plants Acorus calamus Linn. has been used to cure various illnesses including psychopathy. But it lacks scientific validation. Therefore the present study undertaken to evaluate the anti-convulsant and anti-anxiety activity using Picrotoxin induced convulsions model and Open-Field test respectively by using the petroleum ether, Methanolic and aqueous extracts of leaves of Acorus calamus Linn. along with acute toxicity tests. A dose of 200 mg/kg body weight of the all three extracts i.e. Petroleum ether, Methanolic and Aqueous extract of leaves of Acorus calamus Linn. showed significant anti-convulsant and anti-anxiety activity when compared to control group. But Aqueous extract having much greater anticonvulsant effect than methanolic and petroleum ether extracts, where as methanolic extract showed much anxiolytic activity than aqueous and petroleum ether extracts and acute oral toxicities test performed revealed that LD₅₀ of the test drug was found to be greater than 2000 mg/kg body weight. The results suggest that Acorus calamus Linn. can be used as a potential anti-convulsant and anti-anxiety drug in future.
KEYWORDS: Acorus calamus Linn., Anticonvulsant, Anxiolytic, Petroleum Ether Extract, Methanolic Extract, and Aqueous Extract.

INTRODUCTION
Epilepsy[1] is the commonest serious disorder of the brain, and epileptic fit or seizure is caused by brief, excessive and abnormal discharge of nerve cells in the brain, like a small "electrical storm" or "short circuiting" in the brain. The symptoms of epilepsy are the seizures that occur at unpredictable moments. These may vary from frequent brief lapses of consciousness to short periods of automatic subconscious behavior or convulsions of the whole body that make the person fall over and lose of consciousness completely. A majority those with a seizure disorder (66 percent) were given a prescription of Phenobarbital or phenytoin, and most (65 percent) of those with a psychiatric diagnosis who were given a prescription for an anticonvulsant received carbamazepine[2]. But, these agents having many side effects.

Anxiety related disorders such as generalized anxiety, panic, obsessive-compulsion, phobias or post traumatic stress disorders are common and a major cause of disability[3] and 1/8th of the total population worldwide is affected with the anxiety. It has become a very important area of research interest in psychopharmacology[4]. Anxiety is also an obvious component of many psychiatric and medical conditions[5]. Benzodiazepines have been used as anxiolytics for more than 3 decades but have many side effects including psychological and physical dependence, withdrawal symptoms fatigue, muscle weakness etc. BZD’s also said to have adverse prenatal effect[6]. Studies have been carried out to find out an alternative traditional medicine for treating convulsions and anxiety as they have no or less side effects, easily available and are cost effective. Many pharmaceutical companies are in search of more safe plant derived anxiolytics.

Acorus calamus Linn. belongs to the family Araceae and commonly known as Vacha. Sweet flag or buch plant widely used for its antipyretic, antiperiodic, neuroprotective, anticonvulsant and antiparalytic activities. The literature survey on Acorus calamus Linn. reveals that the plant having neuroprotective activity so the present study was undertaken to explore the possible anticonvulsant and anxiolytic activities of leaves extracts of Acorus Calamus Linn. in experimental animals, mice[7].
MATERIALS

Collection and Authentication of leaves of Acorus calamus Linn.
Leaves (10 kg) was collected from shree shail medifarms, Nagpur in the month of August, and authenticated by taxonomist Mr. Rajasamarsen K Modi, Assistant Profesor, Dept. of Botany, Govt. Degree College, Kalaburagi, Karnataka and deposited herbarium to same department, with Ref NO.: GCK/Bot/Herbarium/2015-16/02 and HGCG NO.: 39. Authenticated leaves were shed dried and powdered for extraction.

Preparation of extracts of Acorus calamus Linn.
Shed dried leaves of Acorus calamus Linn., powdered and extracted using solvent such as petroleum ether, methanol and water. Each time before extracting with next solvent, marc was dried in shed at room temperature. Finally the marc was macerated with 1% chloroform/water v/v for 24 hrs to obtain the aqueous extract. Each extract were concentrated by distillation of the solvent and then evaporated to dryness in rotary evaporator and stored in air tight container in cool and dry place.

Preliminary phytochemical screening\[8\]
The phytochemical investigations was carried out with the leaves extracts of A. calamus Linn. for qualitative identification of phytoconstituents.

Animals
Swiss albino mice of either sex weighing between 20-30 gms, were procured from central animal house, M.R. Medical College, Kalaburagi for experimental purpose. Ethical clearances for conducting animal experiments were obtained from the Institutional Animal Ethics Committee (IAEC) HKES’s MTRIPS Kalaburagi. IAEC no.- HKE COP/IAEC/67/2014-2015 and CPCSEA registration no.- 142/1999, CPCSEA. 5th July 1999. All the animals were acclimatized for seven days under standard husbandry conditions i.e.; room temperature of 24\(^0\) ± 10\(^0\) C; relative humidity 45-55% and a 12:12 hrs light/dark cycle. The animals had free access to standard rat/mice pellet diet (Amrut laboratories, Pranava Agro
Industries Ltd., Sangli, Maharashtra, India) and water *ad libitum* under strict hygienic conditions.

**Pharmacological Activities**

**Determination of LD₅₀ of leaves extract of *A. calamus* Linn**[9]

The acute toxicities of extracts of *A. calamus* Linn. were determined by using female albino mice of (20-25 g), those maintained under standard husbandry conditions. The animals were fasted overnight prior to the experimental procedure. OECD guidelines no. 425 method of CPCSEA was adopted for toxicity studies.

**Preparation of doses**

From acute oral toxicity studies, it revealed that the leaves extracts of *A. calamus* Linn. did not showed any toxic effect at dose of 2000 mg/kg, so 1/10 of this dose, i.e. 200 mg/kg were selected for experimental procedure.

- **PEEAC** - Petroleum ether extract of *A. calamus* Linn. (200 mg/kg)
- **MEAC** - Methanolic extract of *A. calamus* Linn. (200 mg/kg)
- **AEAC** - Aqueous extract of *A. calamus* Linn. (200 mg/kg)

**Determination of Anticonvulsant activity**

**Picrotoxin-induced convulsions**[10],[11]

Albino mice of either sex weighing between 20-30 g each group consists of six animals were divided into five groups.

- **Group A** - Normal control (0.5% sod. CMC, 10 ml/kg, p.o.)
- **Group B** - Standard (Diazepam, 2 mg/kg, i.p.)
- **Group C** - Petroleum Ether extract of leaves of *A. calamus* Linn. (200 mg/kg, P.o.)
- **Group D** - Methanolic extract of leaves of *A. calamus* Linn. (200 mg/kg, P.o.)
- **Group E** - Aqueous extract of leaves of *A. calamus* Linn. (200 mg/kg, P.o.)

**Experimental procedure**

Albino mice of either sex with body weight 20-30 g were divided into five groups of 6 animals in each. Group A were served as control and treated with 0.5% Sodium CMC, (10 ml/kg, P.o.), Group B as standard and treated with diazepam (2 mg/kg, i.p.), Group C, D and E were treated with petroleum ether, methanolic, and aqueous extract of leaves of *A. calamus* Linn. (200 mg/kg, P.o.). After 30 min, all animals were treated with picrotoxin (10 mg/kg,
Each animal were placed individual into plastic cage and were observed initially for the 30 min and later up to 24 hrs. The following parameters were recorded during test session.

- Latency (onset of tonic seizure)
- Percentage protection

**Determination of anxiolytic activity**

**Open-field test**\(^{(12) , (13)}\)

Albino mice of either sex weighing between 20-30 g, divided into five groups, 6 mice in each.

- **Group A** - Normal control (0.5% Sod. CMC, 10 ml/kg, p.o.)
- **Group B** - Standard (Diazepam, 2 mg/kg, i.p.)
- **Group C** - Petroleum Ether extract of leaves of *A. calamus* Linn. (200 mg/kg P.o.)
- **Group D** - Methanolic extract of leaves of *A. calamus* Linn. (200 mg/kg P.o.)
- **Group E** - Aqueous extract of leaves of *A. calamus* Linn. (200 mg/kg P.o.)

**Experimental procedure**

This method is used to evaluate exploratory activity and emotionality of animal. The open field consisted of a dark colored box (60x60 x30cms) with its floor curved into 16 equal sized squares (15 x 15cms) and 40 W lamp appended 100 cms above for illumination was used. Before dropping the individual mice in one of the corner of box (i.e.; 30 min prior), Group A were served as normal control and treated with 0.5% Sodium CMC, (10 ml/kg, p.o.), Group B i.e. standard and treated with Diazepam (2 mg/kg, i.p.); and Group C, D and E were treated with petroleum ether, methanolic, and aqueous extract of leaves of *A. calamus* Linn. (200 mg/kg, P.o.). The following parameters were observed.

- No. of crossing in periphery square
- No. of crossing in centre square
- Rearing frequency (number of times the animal stood on its hind leg)
- Immobility time and
- Grooming time

**Statistical Analysis**

The all values obtained from anticonvulsant and anxiolytic models were expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using ANOVA followed by Dunnett’s- t – test to calculate the significance difference if any among the groups. \( P< 0.05 \) was considered as significant.
RESULTS

Preliminary phytochemical screening
It was found that the leaves extracts of *Acorus calamus* Linn. contains carbohydrates, glycosides, flavonoids, saponins, proteins, tannins and phenolic compounds.

Acute toxicity studies
The PEEAC, MEAC and AEAC were studied for acute oral toxicities at a dose of 2000 mg/kg p.o. in female mice. It did not shown any toxic effect in mice, so dose were increased to 5000 mg/kg and at this dose showed mortality of animals. So, 1/10 of 2000 mg/kg dose were selected for experimental procedure according to OECD guideline no. 425. Hence 200 mg/kg dose were fixed for all experimental procedure.

Assessment of Anticonvulsant activity
Picrotoxin induced convulsion model
All three extracts i.e. PEEAC, MEAC and AEAC (200 mg/kg) were subjected for anticonvulsant activity using picrotoxin induced convulsions in mice. All extracts and diazepam administered 30 min prior to picrotoxin in all animals. After picrotoxin administration it was found that two extracts i.e. PEEAC and MEAC significantly increased the latency of tonic seizure where as AEAC abolished the tonic convulsion and also offered high percentage of protection of the animals i.e. 83.33%, 100% and 100% respectively, when compared to control group and exhibited significant anticonvulsant activity. Standard drug diazepam (2 mg/kg) also had significant anticonvulsant activity and it was also abolished the onset of convulsion and offered 100% protection (Table No.: 01, fig.: 01, 02).

Table No.: 01, showing effects of PEEAC, MEAC and AEAC in Picrotoxin induced convulsions in mice.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>Latency (Onset of Tonic seizure) mean±SEM</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal Control (0.5% Sodium CMC, 10 ml/kg, p.o)</td>
<td>138.8 ± 5.683</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>Standard (diazepam, 2 mg/kg, i.p.)</td>
<td>NO</td>
<td>100</td>
</tr>
<tr>
<td>3.</td>
<td>PEEAC (200 mg/kg, p.o)</td>
<td>312.2 ± 8.479***</td>
<td>83.33</td>
</tr>
<tr>
<td>4.</td>
<td>MEAC (200 mg/kg, p.o)</td>
<td>847.8 ± 9.505***</td>
<td>100</td>
</tr>
<tr>
<td>5.</td>
<td>AEAC (200 mg/kg, P.o)</td>
<td>No</td>
<td>100</td>
</tr>
</tbody>
</table>
Values are expressed as mean ± SEM, n=6, ***p<0.001 as compared to control, No stand for Abolished convulsions using One way ANOVA followed by Dunnet’s multiple test for comparision.

Fig.: 01, Showing onset of tonic seizures in picrotoxin induced convulsion in mice.

Assessment of Anxiolytic activity

Open Field test

Diazepam has long been reported for anxiolytic activity in mice with the OFT. In our study also, a significant anxiolytic effect was recorded with diazepam as increased number of crossing in periphery square, centre square, the number of rearing and decrease the immobility and grooming time when compared to control. Different three extracts i.e. PEEAC, MEAC and AEAC (200 mg/kg) were subjected for anxiolytic activity using open-field test. These dose were subjected 30 min prior to study. After 30 min of 0.5% Sodium
CMC, all extracts and diazepam administration, the animal were placed into Open-Field apparatus one by one for 5 min, the significant increase in number of crossing in both square and also significantly increased rearing time was observed with administration of all three extracts. Also significantly decrease in grooming time were observed with all three extracts. Except PEEAC, two extracts i.e. MEAC and AEAC significantly decreases the immobility time, when compared with control. Hence, all three extracts possess significant anxiolytic activity (Table No. 02, fig.: 03, 04).

**Table no.: 02, Showing effects of PEEAC, MEAC and AEAC in Open-Field Test in mice.**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment</th>
<th>No. of crossing in periphery square mean±SEM</th>
<th>No. of crossing in centre square mean±SEM</th>
<th>Rearing Frequency mean±SEM</th>
<th>Immobility time (sec) mean±SEM</th>
<th>Grooming time (sec) mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal Control (0.5% Sod. CMC, 10 ml/kg, P.o.)</td>
<td>43.50 ± 3.085</td>
<td>14.00 ± 2.033</td>
<td>5.667 ± 0.8819</td>
<td>32.33 ± 2.472</td>
<td>31.67 ± 2.552</td>
</tr>
<tr>
<td>2.</td>
<td>Standard diazepam (2 mg/kg, P.o.)</td>
<td>105.8 ± 4.159***</td>
<td>42.67 ± 2.044***</td>
<td>14.33 ± 0.8819***</td>
<td>15.17 ± 1.905***</td>
<td>15.67 ± 1.202***</td>
</tr>
<tr>
<td>3.</td>
<td>PEEAC (200 mg/kg, P.o.)</td>
<td>66.83 ± 2.926**</td>
<td>27.83 ± 1.939***</td>
<td>10.17 ± 1.078 *</td>
<td>25.83 ± 1.797**</td>
<td>24.17 ± 1.537*</td>
</tr>
<tr>
<td>4.</td>
<td>MEAC (200 mg/kg, P.o.)</td>
<td>101.2 ± 4.686***</td>
<td>38.00 ± 2.176***</td>
<td>12.33 ± 1.202***</td>
<td>19.67 ± 2.362***</td>
<td>18.83 ± 1.014***</td>
</tr>
<tr>
<td>5.</td>
<td>AEAC (200 mg/kg, P.o.)</td>
<td>90.17 ± 4.651***</td>
<td>35.17 ± 1.869***</td>
<td>11.50 ± 1.258**</td>
<td>23.00 ± 1.713*</td>
<td>22.83 ± 1.641**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=6, *P<0.05, **P<0.01, ***P<0.001, and ns stand for not significant as compared to control using One way ANOVA followed by Dunnet’s multiple test for comparison.

**Fig.-03, Showing number crossing of animal in periphery square, Centre square and number of rearing in Open-Field test in mice.**
Fig.: 04, Showing immobility time and grooming time in open field test in mice.

DISCUSSION
Convulsion are one of the most dangerous disease in the world, so research in the field of anticonvulsant are most useful and unique. Due to excessive electrical discharge or abnormality in dopaminergic or GABAergic balance or head injury causes convulsions. In our study convulsions were induced by chemical i.e. picrotoxin were used.

There are a number of synthetic anticonvulsant drugs available for use in the management, control and/or treatment of individuals with epilepsy. However, most of the drugs are not only inaccessible and unaffordable, but also possess much toxic effects. Therefore, a must need of the development of cheap, effective and safe anticonvulsant agents from natural sources.

Acorus Calamus Linn. used in Asia since last 2000 years for a number of beneficial and medicinal effects. From beginning, the administration through nasal route is salutary in headache, haviness, epilepsy and hysteria\textsuperscript{[14]}. Apart from these many more literature showed that the drug having neuroprotective\textsuperscript{[15]} and anticonvulsant activities\textsuperscript{[16]}. Based on the above data three different extracts of A. calamus Linn. i.e. PEEAC, MEAC and AEAC were selected for anticonvulsant effect in picrotoxin induced convulsions.

In most common forms of epileptic seizures, effective drugs appear to work either by promoting the inactivated state of voltage activated Na\textsuperscript{+} channels or enhance GABA mediated synaptic inhibition\textsuperscript{[17]}. 
Common model used for the screen of anticonvulsant drug is picrotoxin induced convulsion model in mice. Post synaptic GABA$_A$ – receptors are functionally linked to BDZ, barbiturate receptors and chloride-ion channels to form GABA- chloride ionophore complex, which is intimately involved in the modulation of GABAergic neurotransmission$^{[18]}$. Picrotoxin a GABA-receptor antagonist produces seizure by blocking the chloride ion channel linked to GABA$_A$ – receptors, thus preventing the entry of chloride ions in to the brain. This process will in turn inhibit GABA neurotransmission and activity of brain. Phenobarbitone and diazepam are believed to enhance GABAergic neurotransmission by increasing chloride ion flux through chloride ion channel at GABA-receptor sites. This hypothesis may explain the observed protective effects and/or antagonistic actions, of phenobarbitone and diazepam against picrotoxin (PCT)-induced seizure in mice.

PEEAC, and MEAC (200 mg/kg) were showed anticonvulsant action by significantly increased in the onset of tonic seizure and offered high percent of protection i.e. 83.33%, and 100% respectively, when compared to control. Where as, AEAC abolished the tonic seizure and offered 100% of protection, hence, the extracts having very good anticonvulsant activity. Standard drug, diazepam (2 mg/kg) exhibited significant anticonvulsant activity by abolishing the tonic convulsion and offering 100% protection of animals, when compared with control. Picrotoxin produces convulsion by blocking the chloride channel linked to GABA$_A$-receptors, thus preventing the entry of chloride ions in to the brain. This process will in turn inhibit GABA neurotransmission and activity of the brain, so anticonvulsant effect produced by PEEAC, MEAC and AEAC might be through blocking of the chloride ion channel linked to GABA$_A$-receptors. In the Open-Field Test, the confrontation with the situation induces anxiety behavior in rodents is triggered by two factors i.e. individual testing as the animal were separated from its social group and agoraphobia, as the arena is very large, relative to the animals breeding or the natural environment. In such situations rodents show thigmotaxic behavior identified by spontaneous preference to the periphery of the apparatus and reduced ambulation. Rodents demonstrate anxiety, fear and curiosity when placed in a new environment, and an overall assessment of behavior could be determined through the observation of number of crossing in periphery square, number of crossing of centre square, rearing frequency, immobility time and grooming time. The PEEAC, MEAC and AEAC (200 mg/kg) possessed significant anxiolytic activity by increase in number of crossing in centre square, and also significantly increased rearing frequency and decreased in immobility time and grooming time when compared with control. Similar to the effects observed after
administration of the standard anxiolytic drug diazepam. Anxiolytics are known to exert pharmacological action by causing an increase in GABA content in the cerebral hemisphere. GABA receptors are involved in anxiety and their direct activation would have an anxiolytic effects.

CONCLUSION
From the experimental study it can be concluded that PEEAC, MEAC and AEAC had exhibited significant anxiolytic and anticonvulsant effects in mice. Phytoconstituents like flavonoids and saponins were reported for their anxiolytic and anticonvulsant activity and those two were present in all three extracts. These active principle can be accounted for both anxiolytic and anticonvulsant activity. AEAC showed much anticonvulsant effect than MEAC and PEEAC. Where as, in anxiolytic model, MEAC showed much significant activity than AEAC and PEEAC. PEEAC showed lesser anticonvulsant and anxiolytic activity than both AEAC and MEAC.

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ABREVIATIONS USED

REFERENCES