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EVALUATION OF ANTIEPILEPTIC ACTIVITY OF ETHANOLIC EXTRACT OF ACORUS CALAMUS ROOT POWDER IN ALBINO WISTAR RATS

K. R. Pisal*, V. J. Chaware, A. T. Thorat and V. K. Redasani

Department of Pharmacology, YSPM's, Yashoda Technical Campus, Satara, Maharashtra, India.

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***Corresponding Author**
K. R. Pisal
Department of Pharmacology, YSPM's, Yashoda Technical Campus, Satara, Maharashtra, India.

ABSTRACT

Background – Epilepsy is socially handicapping neurological disease characterised by recurrent spontaneous seizures due to hyperexcitability and hypersynchrony of brain neurons. Maximal electroshock induced seizures induced via supramaximal stimulations at 150 mA current for 0.2 sec. Pentylenetetrazole is a GABA – A receptor antagonist. An intraperitoneal injection of PTZ (80 mg/kg) into animal induce an acute, severe seizures. Acute toxicity study of ethanolic extract of *Acorus calamus* was studied. The present study investgated that the anticonvulsant activity of ethanol extract of *Acorus calamus* (Acoraceae) in rats in order to evaluate the traditional use of this plant. **Methods –** The study was carried out on different epilepsy models such as maximal electroshock induced seizures, pentylenetetrazole induced seizures. Animals of these model were treated with ethanolic extract of *Acorus calamus* root powder (50, 100 and 200 mg/kg p.o.). **Results –** These results indicates that ethanolic extract of *Acorus calamus* root powder possess antiepileptic effect against electroshock and pentylenetetrazole induced seizures in rats. **Conclusions –** The finding from present study indicated that the use of ethanolic extract of *Acorus calamus* may be beneficial as it showed neuroprotective activity.

KEYWORDS: MES, PTZ, phenytoin, diazepam, *Acorus calamus*, Anticonvulsant activity.

INTRODUCTION

A neurological condition that severely impairs social interaction, epilepsy is typified by recurring spontaneous seizures brought on by the hyperexcitability and hypersynchrony of

brain neurons. A common brain condition known as epilepsy is characterized by frequent, spontaneous seizures. It affects people of any age, gender, or race and is one of the most prevalent CNS disorders. In affluent nations, 3-4% of individuals will experience this illness at some point in their lives. The proportion for those living in developing nations is larger. The effects of epilepsy are detrimental to the economy, society, and, in particular, the physical and mental health of those affected.[1]

About 1% of the world's population suffers from epilepsy, whose quality of life decreases due to loss of consciousness and motor skills. Unfortunately, even with optimal antiepileptic drug (AED) therapy, approximately one third of patients have poor seizure control and become drug resistant. Even worse, side effects and drug resistance have become the main reasons for treatment failure with current AEDs. Despite the improved efficacy of surgical procedures, epilepsy surgery is still adequate, as more than half of operated patients achieve long-term seizure freedom. For a small subset of drug-resistant patients.[2]

Chronic epilepsy is a long-term condition characterized by recurrent seizures brought on by aberrant electrical signals generated by damaged brain cells. Seizures are brought on by an uncontrollably high spike in electrical activity within brain cells. A seizure may cause modifications to consciousness, sensations, emotions, behaviour, and motor control All parts of the body are communicated with and received by the brain's cells. A constant electrical impulse that moves from cell to cell transmits these messages. This periodic electrical impulse pattern is disturbed by epilepsy. Rather, in one or more regions of your brain, there are electrical energy bursts between cells that resemble an erratic lightning storm. Changes in awareness (Including unconsciousness), sensations, emotions, and muscle actions are brought on by this electrical disruption.[3]

Despite the introduction of several new treatment options, a significant proportion of epilepsy patients still live with uncontrolled seizures. There is still an ideal need for an antiepileptic drug with properties such as broad-spectrum activity, rapid onset, minimal side effects. effects, good oral bioavailability and low cost. However, modern anticonvulsant treatment is not generally effective or always safe. These serious side effects include central nervous system depression, ataxia, megaloblastic anemia, cardiac arrhythmias, liver dysfunction, and teratogenicity.[4]

Currently available synthetic antiepileptic drugs fail to control seizures in up to 25% of patients. In addition, the combination of antiepileptic drugs (AED) has a high risk of toxicity and drug

interactions, which can complicate clinical treatment These limitations of traditional AEDs require the development of new antiepileptic drugs. Currently, researchers prefer to obtain herbal medicines because of their special healing properties and relatively few side effects. In Ayurvedic medicine, there is a rather complex classification of medicinal herbs according to the predominant pharmacological/therapeutic activity of mental functions.[5]

The literature review revealed that different parts (Latex stem, bark, roots, seeds, flower tops and leaves) of *Acorus calamus* is used in the Ayurvedic system of medicine for its various pharmacological activities. The root is used to treat cancer, diarrhoea, convulsions, leprosy, worms, bacteria and behavioral changes. Roots and bark are used in traditional medicine for all types of convulsions and paralytic diseases. The ethanol extract of the roots and leaves has been reported to have anti-inflammatory, anthelmintic hypolipidemic, immunosupressive, antispaspmodic, antioxidant, antiviral, diuretic, insecticidal, antiulcer activities.

This study was conducted to evaluate the anticonvulsant activity of ethanolic extract of *Acorus calamus* roots powder using maximal electroshock seizure (MES), pentylenetetrazole (PTZ) - induced convulsions.[6]

MATERIALS AND METHODS

Plant material

The root powder of *Acorus calamus* was collected from Vyankteshwar Herbal Distributor, Rajwada, Satara. The root powder of *Acorus calamus* was extracted with ethanol as solvent by using soxhlet apparatus. The extract obtained was evaporated by the water bath method and solvents were removed to yield dry extract. The dry extract was dissolved in water for administration to the animals.

Animals

In this study, 72 albino rats obtained from Animal house, YSPM's Yashoda Technical Campus, Satara. The animals weighed between 150 to 200gm were used for present study. Female rats were used because they can eliminate several antiepileptic drugs more slowly than males.

Animal housing

Animals were kept in polypropylene cages with unshelled bark as bedding. Animals were housed under standard laboratory conditions at 22 ± 2 °C, 50 ± 15% relative humidity, 12 h dark/12 h light cycle, and free access to pelleted diet and water. The experiments were

conducted in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The institutional animal ethics committee approved the study protocol.

Experimental design

72 albino rats were used in this study. Albino female rats weighing 150-200 g were randomly divided into 5 groups, with 6 rats per group in each study model of anticonvulsant activity, while for acute toxicity study either sex of rats divided into 4 groups with 3 rats in each group.

Chemicals and Drugs

Phenytoin were obtained (Abbot pharma Ltd.), Diazepam were obtained (Ranbaxy pharma Ltd.), Pentylenetetrazole were obtained from (Ozone International Ltd.), Sodium chloride and Ethanol were obtained (S. D. Lab chemical centre Mumbai).

Experimental procedure

Acute toxicity study

The acute toxic dose of the ethanol extract of *Acorus calamus* root powder was determined by oral administration of the extracts at increasing doses of 0.5, 1, 1.5 and 2 g/kg to healthy adults albino wistar rats of either sex. Animals were continuously monitored for 2 hours with the following profiles:

1. Behavioral profile: alertness, restlessness, irritability and fear
2. Neurological profile: spontaneous activity, reactivity, tactile response, pain response and gait
3. Autonomic profile: defecation and urination

   After 24 hours, any acute toxicity/dead animals were observed (% mortality).[6]

Assessment of anticonvulsant activity

Maximal electroshock seizure (MES) test

A total 30 female rats divided into six groups. Group 1 received 1 ml/rat of saline, group 2 received 25 mg/kg phenytoin, groups 3, 4 and 5 received 50, 100 and 200 mg/kg ethanolic extract of *Acorus calamus* root powder respectively.

The saline and standard reference drug were administered 45 min before induction of MES, whereas the test extract of *Acorus calamus* root powder was administered 1 hour before induction of MES.

To induce the convulsions in the control and drug treated animals, the maximal electroshock seizure test (Tonic hind limb extension) with supramaximal stimulations was carried out via transauricular copper electrodes (Introduced bilatterly into the ears) with the apparatus (Electrocinvulsiometer) using fixed current 150 mA in rats for 0.2 sec. The duration of each phase for each animal (In sec) was measured by using stopwatch. The tonic flexion, extensor, clonus, stupor and mrotality were recorded.[18]

Pentylenetetrazole – Induced seizure test

A total of 30 female rats were used. The rats were divided into five groups of six rats in each. Group 1 received 1 ml/rat saline, group 2 received 4 mg/kg diazepam, group 3, 4 and 5 received 50, 100 and 200 mg/kg ethanolic extract of *Acorus calamus* root powder. Pentylenetetrazole (PTZ) 80 mg/kg was administered intraperitoneally to induce convulsions in the animals of all five groups. The anticonvulsant activity of the drug in this model was assessed by its ability to delay the onset of action, protection against PTZ–induced convulsions.

The saline and standard reference drug were administered before 45 min induction of PTZ, whereas the test extract of *Acorus calamus* root powder was administered 1 hour before induction of PTZ. The animals were observed for 30 min after injection of PTZ and percentage of mortality was recorded.[19]

Statistical analysis

The results are expressed as mean ±SEM. The statistical analysis of all the results was carried out using one way ANOVA followed by Dunnett's 't' test graph pad instant 3. The level of significance was set at $p<0.05$.

RESULTS

Acute toxicity test

From the data, it was evident that the ethanolic extract of *Acorus calamus* root powder was well tolerated orally in rats up to the dose of 2 g/kg. LD50 for ethanol extract was considered as 2 g/kg body weight, and the ED50 ($1/10^{th}$ dose LD50) for these extract was taken as 200 mg/kg.

Table 1: Acute toxicity of *Acorus calamus* root extract.

Treatment	Incidence of symptoms at different doses (g/kg) 0.5	1.0	1.5	2.0	Mortality (%)
Vehicle	-	-	-	-	0
Ethanol extract	-	-	-	55	0

Maximal electroshock seizure test

From table 2 it was revealed that the ethanol extract of *Acorus calamus* root powder decreased the duration of hind limb extension by (7.00±0.45) at 200 mg/kg which is most significant ($p<0.01$) when compared to the effect produced by the control and ethanol extract of *Acorus calamus* root powder at 50 mg/kg and 100 mg/kg. The ethanol extract of *Acorus calamus* also decreased the duration of clonus and stupor phase of MES induced convulsions as compared to control.

Table 2: Effect of ethanol extract of *Acorus calamus* root powder against MES induced convulsions.

Sr. No.	Treatment	Flexion (Sec)	Extensor (Sec)	Clonus (Sec)	Stupor (Sec)	Recovery/ Death
1.	Control (Saline 1ml/rat)	2.83 ± 0.40	16.83± 1.58	3.84± 0.34	182.00± 2.66	Recovery
2.	Standard phenytoin (25 mg/kg i.p.)	0.00±0. 00***	0.00±0. 00***	0.00±0. 00***	0.00±0. 00***	Recovery
3.	Ethanol extract (50 mg/kg p.o.)	2.50± 0.40	11.17± 1.40	3.00± 0.36	151.70± 27.83	Recovery
4.	Ethanol extract (100 mg/kg p.o.)	2.17± 0.17	13.17± 0.86	3.50± 0.58	108.77± 9.68	Recovery
5.	Ethanol extract (200 mg/kg p.o.)	1.83± 0.30*	7.00±0. 45**	1.83±0. 17**	68.00±0. 44**	Recovery

*$p<0.05$ significant, **$p<0.01$ more significant, ***$p<0.001$ highly significant (compared with respective control).

Pentylenetetrazole (PTZ) induced seizures test

The effects of ethanolic extract of *Acorus calamus* root powder in PTZ induced convulsions are shown in Table 3. Statistical data obtained from the anticonvulsant activity of ethanolic extract of *Acorus calamus* root powder against PTZ induced has revealed that the extract of *Acorus calamus* root powder delayed the onset of action of seizure by 538.50±1.76 sec as compared to the effect produced by the control (18.4±4.87) and ethanol extract of *Acorus calamus* powder at 50 (114.50±4.79) and 100 mg/kg (128.30±11.20). The ethanolic extract of *Acorus calamus* root powder not only delayed the onset action of seizures but 50% of animals

in the group treated were protected against seizures induction. Moreover, all the animals were recovered and there was no incidence of mortality in the group of animals treated with ethanolic extract of *Acorus calamus.*

While in the animals treated with PTZ (control group) 100 % mortality was observed, animals treated with ethanolic *Acorus calamus* root extract of 50 and 100 mg/kg, 100% mortality was observed.

Table 3: Effect of ethanol extract of *Acorus calamus* root powder against PTZ induced convulsions.

Sr. No.	Group	Treatment	Onset of action (sec)	Duration of convulsions (sec)	% Mortality
1.	Control	Saline 1 ml/rat	118.4±4.87	540.08±4.12	100 %
2.	Standard	Diazepam 4 mg/kg + PTZ 80 mg/kg i.p.	0.00±0.00***	0.00±0.00***	0%
3.	Test I	Ethanol extract 50 mg/kg + PTZ 80 mg/kg p.o.	114.50±4.79	480.68±3028	100%
4.	Test II	Ethanol extract 100 mg/kg + PTZ 80 mg/kg p.o.	128.30±11.20	389.91±34.52	100%
5.	Test III	Ethanol extract200 mg/kg + PTZ 80 mg/kg p.o.	538.50±1.76**	178.57±4.27**	0%

*p<0.05 significant, **p<0.01 more significant, ***p<0.001 highly significant (compared with respective control).

DISCUSSION

Epilepsy in rats were induced by two experimental models i.e. maximal electroshock induced convulsions and pentylenetetrazole induced convulsions.

Maximal electroshock seizures (MES) are a model of generalized tonic-clonic seizures. They represent the post-brain scenes; with ear stimulation, even a threshold current induces this type of seizure. Mechanism behind the MES induced seizures is an experimental paradigm that induces synchronous neural firings in the brain by artificial current and is used to induce acute epileptic behavior.[18]

Pentylenetetrazole (PTZ), a GABA receptor antagonist, is used to establish a common chemically induced seizure model. Of all the animal models of seizures and epilepsy, pentylenetetrazole-induced seizures are classified as generalized seizure models (Vs. partial

or focal seizures). It produces myoclonic seizures that model absence seizures (Petit mal). As a generalized seizure model, it has properties that distinguish it from the MES seizure model (Also the generalized abduction model). Epileptogenesis resulting from GABAergic dysfunction. GABA is the counterpart of the excitatory glutamate, it could be theorized that epilepsy is associated with a reduction of GABA level in brain.[19]

The results of the present study show that the ethanol extract of *Acorus calamus* roots delayed the onset of convulsions and could shorten the duration of hind limb extension, clonus and stupor phase of MES-induced convulsions compared to the control group.

The standard drug phenytoin at a dose of 25 mg/kg of body weight provided 100% protection and also significantly shortened the duration of stupor compared to the control group. In other words, the ethanol extract at a dose of 200 mg/kg body weight is able to shorten the duration of the extension of the hind limb (Extension phase), clonus, and also the stupor phase, indicating that the ethanol extract has a strong anticonvulsant activity against generalized tonic-clonic seizure (Grand mal). Although other doses, as 50 and 100 mg/kg of ethanol extract of *Acorus calamus* root, showed no statistically significant effect in the extension phase compared to the control group.

The standard drug diazepam at a dose of 4 mg/kg of body weight provided 100% protection and also significantly reduced the duration of action of seizures as compared to the control group. Ethanol extract of *Acorus calamus* roots significantly delayed the onset of seizures in PTZ-induced seizures and protected 100 % of animals from seizures. Mortality was observed in the ethanol extract treated group at dose of 50 mg/kg and 100 mg/kg.

Anticonvulsants may be needed to reduce the risk of new seizures and are usually not prescribed for just one generalized seizure for which no cause can be found. But anti-seizure medications are essential for those who have had more than one seizure. Anticonvulsant drugs can completely prevent seizures in more than 50% of patients and significantly reduce the frequency of seizures. No single medication can control all types of seizures**.**

CONCLUSION

The present study shows that the ethanolic extract of *Acorus calamus* root powder may function in similar manner to phenytoin and diazepam, given its phenytoin and diazepam like action, although the specific receptor interactions were not evaluated. Further evaluation of *Acorus*

calamus is needed to establish it's exact mechanism of action. However, we concluded that the ethanolic extract of *Acorus calamus* root powder is a potent anticonvulsant drug.

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