

Research Article

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Anticonvulsant activity of ethanolic extract of *Acorus calamus* rhizome in swiss albino mice

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Abstract

Acorus calamus Linn. also known as Vacha is one of the oldest herbs with creeping rhizome in Indian traditional system and has been used as laxative, diuretic, antioxidant and as an antimicrobial agent. **Objectives:** The present study was carried out to investigate the possible anticonvulsant activity of ethanolic extract *Acorus calamus* Rhizome (EEACR) by Maximal Electroshock (MES) and Pentylentetrazole (PTZ) induced Seizure tests in Swiss Albino mice. **Methods:** A total of 48 male Swiss albino mice were divided into eight groups containing six in each. First four groups of animals were evaluated for anticonvulsant activity by MES induced seizures, whereas remaining 4 groups by PTZ induced seizure model after 60 minutes of oral drug administration. First four groups of mice received Normal Saline 3ml/kg (Control), Phenytoin sodium 50mg/kg (Standard), EEACR 250 mg/kg and EEACR 500mg/kg (Test groups) respectively by oral route. Similarly, the remaining four groups received normal saline 3ml/kg (Control), valproate sodium 200mg/kg (Standard), EEACR 250 mg/kg and EEACR 500mg/kg (Test groups) respectively by oral route. **Results:** EEACR when given in a dose of 250 mg/kg and 500mg/kg significantly reduced hind limb extension and tonic flexion of forelimbs when compared to control ($p < 0.001$) in MES induced seizure model. However, EEACR (250 mg/kg and 500mg/kg) failed to prevent mortality in PTZ induced seizures model (100% mortality). **Conclusion:** These findings show that EEACR in a dose of 250 mg/kg and 500mg/kg can be effective in generalized tonic-clonic convulsions but not in petit mal epilepsy.

Keywords: *Acorus calamus*, Maximal electro shock (MES), Anti-Convulsant activity, PTZ induced Seizure, Phenytoin, Pentylentetrazole.

Introduction

Acorus calamus L. commonly known as sweet flag belongs to the family of Araceae. It is perennial herb, which is indigenous to central Asia, India, and the Himalayan region, is found commonly on the banks of streams and in damp marshy places. It has a cylindrical rhizome with a diameter of 3 to 4 cm. The plant changes from pale green to pink as it grows. The leaf scars are spongy, brown and white in colour. The plant possesses slender roots, and its leaves are few and distichously alternate.¹ The rhizome of *A. calamus* has various medicinal properties.

A. calamus herb is used for the appetite and as an aid to the digestion. It is also used in fever, stomach cramps and colic. Its rhizomes are used for toothache, gingival congestion, and also used to treat several diseases like asthma and bronchitis and as sedative.² It has the property of improving the memory power and intellect.

Acorus calamus is also helpful in the conditions like hoarseness, flatulence, dyspepsia, helminthiasis, amenorrhea, dysmenorrhea; nephropathy, calculi and stragury.³ Epilepsy, mental ailments, chronic diarrhea and dysentery are also benefited by *Acorus calamus*. It also has insecticidal, antifungal, antibacterial, antidyslipidemic, neuroprotective, antioxidant, anticholinesterase, and vascular modulating activities.^{4,5}

The various extracts of *Acorus calamus* are also traditionally used as antidiabetic, antiproliferative, immunosuppressive, mitogenic and anticarcinogenic activity towards human lymphocytes.⁶ The extract were used in the traditional Chinese prescription and its beneficial effects on memory disorders, on learning performance and anti-aging effect in senescence have been reported.⁷

The objective of the present study was to investigate the anticonvulsant effect of ethanolic extract *Acorus calamus* rhizome by Maximum Electroshock Seizure and Pentylene-tetrazole induced Seizure Tests in Swiss albino mice.

Materials and Methods

Institutional Animal Ethics Committee (IAEC) approval was obtained from Kasturba Medical College, Mangalore, Manipal University, Karnataka, India before starting the experiment. Study was conducted in Post graduate Pharmacology Laboratory. All animals were handled and taken care according to guidelines of "Principles of Laboratory Animal Care" (NIH Publication No. 85-23, Revised 1985) and CPCSEA guidelines, New Delhi, India.

Experimental Animals:

A total of 48 male Swiss albino mice weighing 25-30g were included in the present study. The mice were housed under standard conditions with food and water ad libitum, in the central animal house Kasturba Medical College, Mangalore, Manipal University. Animals were divided into eight groups containing six in each. First four groups of animals were evaluated for anticonvulsant activity by MES induced seizures, whereas remaining four groups by PTZ induced seizure model after 60 minutes of oral drug administration. First four groups of mice received Normal Saline 3ml/kg (Control), Phenytoin sodium 50mg/kg (Standard), EEACR 250 mg/kg and EEACR 500mg/kg (Test groups) respectively by oral route. Similarly, the remaining four groups received Normal Saline 3ml/kg (Control), Sodium valproate

200mg/kg (Standard), EEACR 250 mg/kg and EEACR 500mg/kg (Test groups) respectively by oral route.

Drugs and chemicals:

The standard drugs Phenytoin and valproate sodium were obtained from pharmaceutical company. *Acorus calamus* rhizome was purchased from Ayurvedic pharmacy in Mangalore, Karnataka, India and ethanolic extract of *A. calamus* (500gm) was prepared in Pharmacology laboratory, Kasturba Medical College, Mangalore by using 90% ethanol. The percentage yield was 15.6%. The doses for each drug selected for the present experiment was based on the previous studies.⁸

Maximal Electroshock Seizure (MES) Test:

This model was developed by Merritt and Putnam in 1938.⁹ This model is helpful in the screening of drugs effective against primary and secondary generalized tonic-clonic seizure.^{10,11} In this experiment electrical stimulation was applied via trans-auricular electrodes with a current of 12mA, 50Hz for 0.2 seconds after 60 minutes of oral drug administration to first four groups of mice. In mice, Maximal seizures consist of initial tonic flexion of forelimbs, tonic hind limb extension (THLE), and terminal clonus. Duration of THLE and time taken for regaining recovery were noted.

Pentylenetetrazole (PTZ) Test:

Pentylenetetrazole Test is used for screening of drugs effective in petit mal epilepsy or absence seizures.⁹ PTZ believed to act by antagonizing the inhibitory GABAergic transmission in central nervous system.¹² 80mg/kg of 1% PTZ was given subcutaneously to the next four groups of mice after 60 minutes of oral drug administration. Animals were observed for protection against myoclonic jerks, clonic seizures or death.⁹

Statistical Analysis

Results are presented as Mean±SD. One way ANOVA followed by Dunnett's multiple comparison tests were applied for comparison between groups. For all the tests a 'p' value of 0.05 or less was considered as statistical significance.

Results

Present study has shown EEACR when given in a dose of 250 mg/kg and 500mg/kg significantly reduced hind limb extension and tonic flexion of forelimbs in MES induced

seizure model (Table 1 & Figure 1) when compared to control ($p < 0.001$). However, EEACR (250 mg/kg and 500mg/kg) failed to prevent mortality in PTZ induced seizures model (100% mortality) suggesting failure of role in petit mal epilepsy (Table 2 & Figure 2).

Table 1: Showing Anti-Convulsant activity of EEACR by MES Induced Seizure in Swiss albino mice

Groups	Latency (Sec)	Tonic Flexion of fore limbs (Sec)	Tonic Extension of hind limbs (Sec)	Tonic-Clonic convulsions (Sec)	Recovery (Sec)
Control (Normal Saline) 3ml/Kg	0.02±0.007	0.021±0.004	0.106±0.029	0.343±0.088	0.198±0.088
Phenytoin Sodium 50mg/Kg	0.01±0.002***	0.01±0.006***	0.06±0.007***	0.055±0.025***	0.123±0.062*
EEACR 250mg/Kg	0.01±0.0018***	0.01±0.008***	0.11±0.012*	0.008±0.025***	0.21±0.025*
EEACR 500mg/Kg	0.01±0.001***	0±0***	0.116±0.02*	0.03±0.021***	0.206±0.039*

n=6. The observation are mean ± SD. * $p > 0.05$, ** < 0.05 , *** $p < 0.01$ as compared to control (ANOVA followed by Dunnett's multiple comparison test), EEACR - Ethanol Extract of Acorus calamus rhizomes

Table 2: Showing Anti-Convulsant activity of EEACR by PTZ induced Seizure in Swiss albino mice

Groups	Latency (Sec)	Jerky Movement (Sec)	Straub's Tail (Sec)	Clonic convulsion (Sec)	Mortality		Mortality Percentage (%)
					Animal Survived	Animals died	
Control (Normal Saline) 3ml/Kg	118±0.286	18±4	10±1.789	428±2.528	0	6	100
Sodium Valproate 200mg/Kg	180±6.812***	3±1.414***	3±1.673***	292±1.262***	2	4	66.66
EEACR 250mg/Kg	136±6.603***	12±1.789***	8±2.828*	398±2.828***	0	6	100
EEACR 500mg/Kg	140±14.142** *	8±1.265***	6±1.789***	381±0.632***	0	6	100

n=6. The observation are mean ± SD. * $p > 0.05$, ** < 0.05 , *** $p < 0.01$ as compared to control (ANOVA followed by Dunnett's multiple comparison test), EEACR - Ethanol Extract of Acorus calamus rhizomes

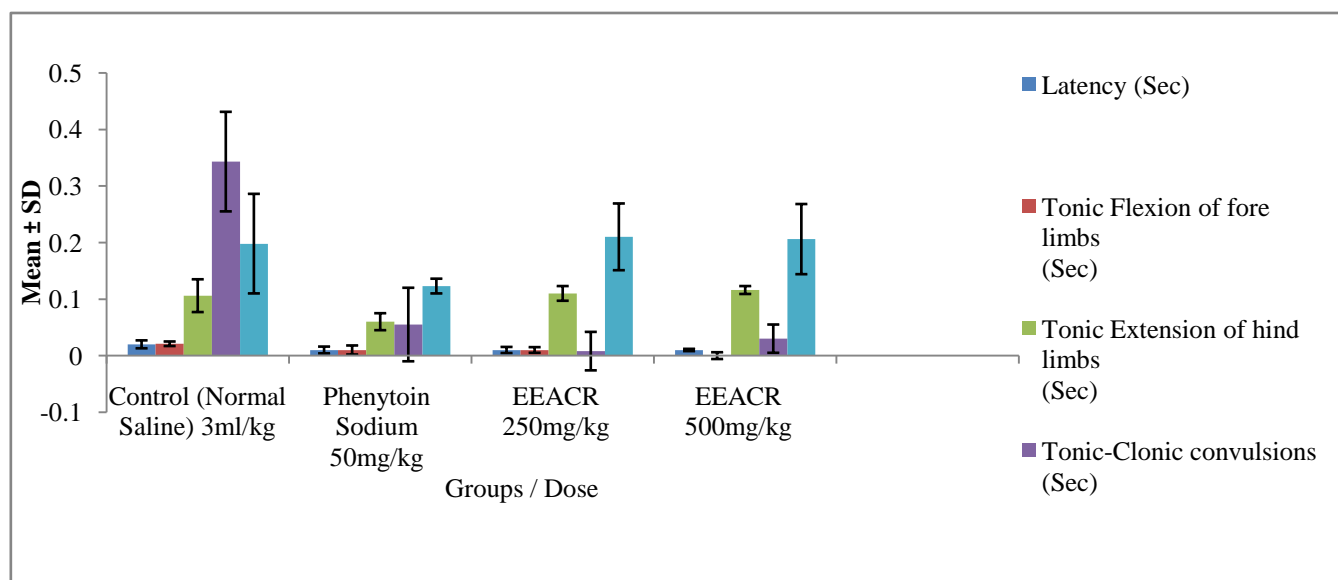


Figure 1: Showing Anti-Convulsant activity of EEACR by MES induced convulsion in Swiss albino mice

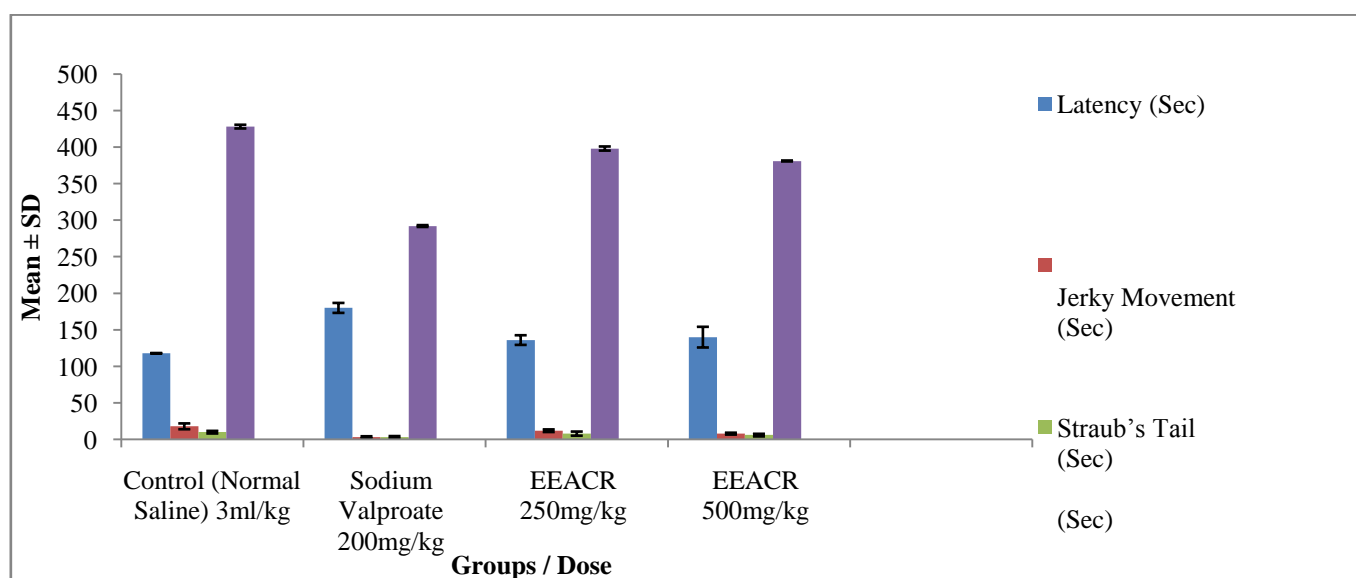


Figure 2: Showing Anti-Convulsant activity of EEACR by PTZ induced Convulsion in Swiss Albino Mice

Discussion

Epilepsy is one of the most common chronic neurological disorders characterized by recurrent and unprovoked seizures.¹³ The prevalence of epilepsy Worldwide is estimated to be around 1% and has important medical, social and psychological consequences.¹⁴ It is estimated that 6-10 million epileptics in India, accounts for nearly 1/5th of global burden.¹⁵

Even with availability of present conventional antiepileptic drugs, a significant fraction of the epileptics continue to live with uncontrolled seizures¹⁶ and also conventional

antiepileptics are associated with unusual adverse effects like central nervous system depression, ataxia, megaloblastic anemia, cardiac arrhythmias, hepatic dysfunction and teratogenicity.¹⁷ Hence, there is a need for an ideal antiepileptic drug with broad spectrum activity, rapid onset of action, least side effects, good oral bioavailability and low cost.

Imbalance between excitatory and inhibitory neurotransmission is considered to be an important mechanism for the generation of seizures.¹⁸ Recently, reactive oxygen species have also been implicated in the development of seizures under pathological conditions and

linked to seizure-induced neurodegeneration.¹⁹ Both the roots and leaves of *A. calamus* have shown antioxidant activity in previous studies.²⁰⁻²²

A. calamus found to have many phytochemical constituents namely alpha-asarone, Beta-asarone and eugenol.^{23, 24} It was found that main constituent, alpha-asarone modulates GABAergic transmission in hippocampus in experimental animal exerting its antiepileptic action.²⁵ Thus, *A. calamus* can be new drug for epilepsy in near future by its antioxidant and modulation of GABA activity in central nervous system. However, further extensive clinical and preclinical studies are essential to elucidate its exact mechanism and toxicity profile.

Conclusion

Present study has shown the anticonvulsant property of *A. calamus* in MES model of Swiss albino mice. Thus, *A. calamus* can be considered for further research in the management of generalized tonic-clonic convulsions.

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