EFFECT OF *ACORUS CALAMUS* ON ELECTRICAL AND CHEMICAL INDUCED SEIZURES IN MICE

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ABSTRACT: *Acorus calamus* Linn. (Family: Araceae) is an aromatic semi-aquatic perennial marshy herb. Experimental studies have indicated the efficacy of this plant against various types of epileptic seizures, but the results vary with the models and the type of extract used. These conflicting reports and the unavailability of the data regarding the effects of aqueous extract of *Acorus calamus* (AEAC) prompted us to evaluate the efficacy of AEAC on electrical and chemical induced seizures in albino mice. Either normal saline or sodium valproate or AEAC was given sixty minutes prior to the experiment in acute study, whereas in chronic study, they were given twice daily for ten days and the last dose was given one hour prior to the exposure of the animal either to maximal electrical shock (MES) or pentylenetetrazole (PTZ) administration. On acute administration, AEAC dose dependently reduced the duration of tonic hind limb extension in MES induced seizure which was comparable to that produced by sodium valproate. Whereas, in PTZ induced seizures, the test drug decreased the latency and increased the duration of seizures as well as mortality. On repeated administration (chronic study) the test drug significantly reduced the duration of tonic hind limb extension and also the clonus phase of MES induced seizures. However, in PTZ induced seizures, results were similar to that obtained in acute study. Results indicates that AEAC has protective effect against MES, but not against PTZ induced seizures

Key words: *Acorus calamus,* Maximal electrical shock, Epilepsy, Pentylenetetrazole

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INTRODUCTION

Epilepsy is one of the most common disorders of central nervous system. Although several drugs are available for the treatment of epilepsy, their use is associated with a number of short comings and side effects like sedation, attention deficit, cognitive impairment, etc (Emilio P, 2007). Secondly, these agents are also unable to control seizures effectively in as many as twenty five percent of patients (Homan RW, et.al., 1987). Owing to the above reasons, many attempts have been made in the past to obtain anticonvulsant from plant origin which are better tolerated than the conventional drugs and have a better efficacy profile. The most important benefit afforded by traditional drugs is that their safety has been proven by decades of use in humans.

Acorus calamus Linn. (Family: Araceae) is an aromatic semi-aquatic perennial marshy herb commonly known as Bach and Ugragranthi in Sanskrit, Baje in Kannada and sweet flag in English. The rhizome of this plant is used empirically in the treatment of wide variety human diseases (3). It is used in the treatment of insomnia, melancholia, neurosis, epilepsy and other mental disorders either alone or as a component Ayurvedic preparations (Nadakarni KM, et.al., 1989, Dandia PC, et.al., 1970). Recently it has been reported that *Acorus calamus* (AC) has antistressor activity and prevents stress induced changes in the rat brain by its antioxidant activity (Manikandan S, et.al., 2005). The essential oil, alcoholic and aqueous extract of this rhizome have been found to be pharmacologically active (Agarwal SL, et.al., 1956, Das PK, et.al., 1962). The essential oil has been shown to protect the animals against the maximal electrical shock (MES) induced seizures, but not against pentylenetetrazole (PTZ) induced seizures (Madan BR, et.al., 1962). The essential oil contains 2 active principles, viz. α – asarone and β – asarone, the β – asarone content present in aqueous extract of *Acorus calamus* not more than 0.005 mg per gram of herbal drug. This low level of beta asarone is acceptable for therapeutic use(Dandia PC, 1970). β - asarone has been shown to facilitate electroshock and PTZ induced seizures while α- asarone has slight protective action against both the type of seizures (Dandia PC & Sharma JD, 1962, Dandiya PC & Menon MK, 1963). Vohra *et al*. (Vohra SB, et.al., 1990) screened the ethanolic extract of this plant for central nervous system effects by using a battery of tests in rodents. A large number of effects observed were similar to that observed with α – asarone. Experimental studies have indicated the efficacy of this plant against various types of epileptic seizures, but the results vary with the model and the type of extract used (Madan BR, et.al., 1962, Dandiya PC & Menon MK, et.al., 1963, Martis GR & Karanth KS, et.al., 1991). These conflicting reports and the unavailability of the data regarding the effects of aqueous extract of *Acorus calamus* prompted us to initiate this study with the aim to add to the existing knowledge regarding the antiepileptic efficacy of this medicinal plant.

MATERIALS AND METHODS

Animals: The study protocol was approved by the Institutional Animal Ethics Committee, Kasturba Medical College, Mangalore. Inbred male albino mice (Swiss strain) weighing between 25-30g were used in the study. Animals were acclimatized for a period of 7 days prior to screening/experimentation. They were housed in groups of six in clean polypropylene cages with 12 hour light/dark cycle at 25±2^{0}C and 65±5% humidity. They had access to food (standard pellet diet, Hindustan Lever Ltd) and water *ad libitum* except during overnight fasting prior to acute study and during the test period in acute and chronic study. All experiments were carried out between 11 AM and 4 PM.

Drugs: Aqueous extract of AC (AEAC; Natural Remedies, Bangalore), sodium valproate (Cadila Laboratories, India) and PTZ (Sigma, USA) were dissolved in normal saline, which served as the vehicle. Animals were divided into six groups (n=6) for both, MES induced seizures and PTZ induced seizures. After overnight fasting, group I received 2ml/kg vehicle orally and served as the control. Group II and III received sodium valproate (20 and 40mg/kg orally) and Groups IV, V and VI received AEAC (125, 250 and 500mg/kg orally) 60 minutes prior to the test in acute study. Doses of AEAC and sodium valproate that were used in the study did not produce either gross changes in behaviour (Janssen PAJ, et.al., 1960) or motor in-coordination in animals (results not shown) as assessed by the rotor-rod and the traction tests (Robert AT, 1965). For chronic study drugs/vehicle were administered daily for 10 days without overnight fasting and the convulsive challenge was given 60 minutes after administration of the last dose.

MES induced seizures: Forty eight hours before the test, each animal was exposed to the shock and those animals which showed all the phases of convulsion were chosen for the study. On the day of experimentation, 60 minutes after administration of drug/vehicle, seizures were induced by delivering an electrical shock (50mA at 50Hz for 0.2 sec) by means of a convulsiometer (Techno India) through a pair of ear clip electrodes. The duration of flexor and extensor phases was noted (Gupta YK, et.al., 1999).

PTZ induced seizures: sixty minutes after administration of drug/vehicle, animals were challenged with a convulsive dose of pentylenetetrazole (80 mg/kg body weight i.p.). The time taken for the onset of convulsion (seizure latency), duration of convulsion and the mortality were noted (James JEP, et.al., 1964).

Statistical analysis. The difference between groups was analysed by one-way ANOVA followed by Dunnet's multiple comparison test. P value <0.05 was considered significant.

RESULTS

A. Acute study- single dose pre-treatment *a) MES induced seizures* (Table 1)

Table1.Effect of AEAC pre-treatment on MES induced seizures in mice- acute study.

Treatment group (n = 6)	Dose /kg	Duration (seconds)		
		Tonic flexion	Tonic hind limb extension	Clonus
Control Normal saline	10ml	2.12± 0.20	14.37±0.53	3.62 ± 0.43
Sodium valproate	20mg	2.14± 0.55	8.71± 2.65	1.00 ± 0.78**
	40mg	1.57± 0.66	1.33 ± 1.14**	0
AEAC	125mg	2.16± 0.30	13.66± 0.80	2.29 ±0.20*
	250mg	2.66± 0.96	3.00± 0.70**	1.67 ±0.55*
	500mg	2.00± 0.69	0	1.00 ± 0.44**

(Values are mean± SEM, * P< 0.05, ** P< 0.01 *versus.* control group)

Sodium valproate at a dose of 20mg/kg did not alter any of the observed parameters to any significant level. But at the higher dose tested (40mg/kg), it significantly reduced the duration of tonic hind limb extension and completely abolished clonus. However, the decrease in the duration of tonic flexion was not statistically significantly. AEAC at higher doses 250mg and 500mg/kg significantly reduced the duration of hind limb extension and clonus. The duration of tonic flexion was found to be increased in the lower two doses (125 and 250mg/kg) and reduced only in the highest dose (500mg/kg) treated group. However, the effect of AEAC on tonic flexion was not statistically significant.

b) PTZ induced seizures. (Table 2)

Sodium valproate at the dose of 20 mg/kg did not increase the time of onset of convulsion. However, it reduced the duration of seizures in the treated animals. At the dose of 40mg/kg it delayed the onset of convulsion and also decreased the duration of seizures. There were no mortalities in the sodium valproate treated groups.

On the other hand, pre-treatment with AEAC dose dependently reduced the time of onset of convulsion, prolonged the duration of seizures and increased the mortality when compared to vehicle treated group. The aggravation of PTZ induced seizures was statistically significant as compared to the control group.

Table 2. Effect of AEAC pre-treatment on PTZ induced seizures in mice- acute study.

Treatment group	Dose / kg	Latent period (minutes)	Seizure duration (seconds)	Mortality
Control Normal saline	10ml	6.83 ±0.48	13.25±1.39	3/6
Sodium valproate	20mg	8.00± 2.08	6.66±2.22*	0/6
	40mg	11.66±1.57*	2.50± 1.77**	0/6.
AEAC	125mg	4.53± 0.43*	14.33 ±1.44	5/6
	250mg	4.16± 0.93*	24.66± 2.29**	6/6
	500mg	3.55± 0.14**	25.83 ±1.17**	6/6

(Values are mean± SEM, * P< 0.05, ** P< 0.01 *versus.* control group)

B. Chronic Study

a). MES induced seizures (Table 3)

Table 3. Effect of AEAC pre-treatment on MES induced seizures in mice – chronic study

Treatment group	Dose /kg	Duration (seconds)		
		Tonic flexion	Tonic hind limb extension	Clonus
Control Normal Saline	10ml	2.00± 0.18	15.29 ± 0.84	3.25 ± 0.37
Sodium valproate	20mg	1.57± 0.70*	4.14± 1.69**	1.00 ± 0.78
	40mg	0.58± 0.79**	1.43±1.10**	0
AEAC	125mg	1.86± 0.16	16.71±0.31	2.28± 0.20
	250mg	2.00±1.07	7.14± 2.79**	1.00±0.53**
	500mg	1.00±0.53	3.29± 2.32**	0.85±0.44**

(Values are mean± SEM, * P< 0.05, ** P< 0.01 *versus.* control group)

On chronic administration sodium valproate at both the doses tested (20 & 40mg/kg) significantly reduced the duration of tonic hind limb extension and flexion as compared to the vehicle treated group. At 40mg/kg, it completely abolished the clonus phase.

AEAC at higher doses (250mg and 500mg/kg) significantly decreased the duration of tonic hind limb extension and clonus as compared to the vehicle treated group. However, the duration of tonic flexion was not significantly altered at any of the tested doses.

b) PTZ induced seizures. (Table 4)

Table 4. Effect of AEAC pretreatment on PTZ induced seizures in mice – chronic study

Treatment group	Dose / kg	Latent period (minutes)	Seizure duration (seconds)	Mortality
Normal saline	10ml	4.00 ± 0.66	14.50± 0.63	5/7
Sodium valproate	20mg	5.71± 3.03	3.29±0.81**	0/7
	40mg	9.00± 1.70*	1.29± 0.77**	0/7
AEAC	125mg	2.99± 0.85	11.00± 2.08	7/7
	250mg	3.25± 0.62	14.14± 2.29	6/7
	500mg	3.27± 0.38	13.71±0.87	4/7

(Values are mean± SEM, * P< 0.05, ** P< 0.01 *versus.* control group)

Sodium valproate at the dose of 20mg/kg significantly delayed the onset and decreased the duration of convulsions in the treated animals as compared to the control. At 40mg/kg, it showed complete protection against PTZ induced seizures. There were no mortalities in any of the sodium valproate treated groups.

On chronic administration AEAC did not produce any protection against PTZ induced seizures. At all the three doses tested it reduced the time for onset of convulsions but did not have any effect on the duration of seizures. As compared to the control animals, it increased the mortalities in the lower two dose treated groups (125 and 250mg/kg).

DISCUSSION

The rhizomes of *Acorus calamus* have traditionally been used in the treatment of insomnia, melancholia, neurosis, epilepsy and other mental disorders either alone or as a component in Ayurvedic preparations (Nadakarni KM, et.al., 1989, Dandiya PC & Chopra YM, et.al., 1970). The essential oil, alcoholic and aqueous extract of this rhizome have been found to be pharmacologically active (Agarwal SL, et.al., 1956, Das PK, et.al., 1962). Experimental studies with essential oil and alcoholic extracts of this plant in experimental seizures are ambiguous and results vary with the model used (Madan BR, et.al., 1962, Dandiya PC & Menon MK, et.al., 1963, Martis GR & Karanth KS, et.al., 1991). Therefore the present study was carried out to scientifically evaluate the antiepileptic activity of AEAC, as experimental studies with this extract is lacking.

In the present study, AEAC on single and repeated administration, dose dependently reduced /abolished the tonic hind limb extensor phase in MES induced seizures in experimental animals. On the other hand, when the convulsive challenge was made with PTZ, acute administration of AEAC decreased the latency of onset and increased the duration of seizures in the treated animals as compared to the control. Mortality was also higher in the extract treated groups. A very similar result was again obtained on repeated administration, as AEAC decreased the latency of onset and mortality in the treated animals. However, the duration of seizures was similar to that seen in the control animals.

Our results are in contradiction to previously published reports on the antiepileptic effect of this plant. Martis *et al* (Martis GRA & Karanth KS, et.al., 1991) have reported that both alcoholic and aqueous extracts of *Acorus calamus* have protective effects against pentylenetetrazole induced seizures but not against maximal electrical shock induced seizures. In our study, firstly, the aqueous extract of *Acorus calamus* was protective against maximal electroshock induced seizures on both acute and chronic administration. Secondly, the extract was shown to aggravate PTZ induced seizures on both acute and chronic administration.

These differences between studies might be due to variations in the concentration of active principles, α and β-asarone (Dandiya PC & Sharma JD, et.al., 1961, 1962). Recent evidence indicates that asarone block NMDA (N-methyl-D-aspartate) receptors and thus has neuroprotective activity against NMDA or glutamate induced excitotoxicity. In this regard α-asarone has been reported to be more potent than β-asarone (Cho J, et.al., 2002). Several studies (Lehmann J, et.al., 1988, Kulkarni SK & Ticku MK, et.al., 1989) have shown that NMDA receptor antagonists are more effective in antagonizing MES induced seizures, as compared to PTZ/ picrotoxin induced seizures. More over, the convulsive effect of PTZ is via specific GABA (gamma-amino-butyric-acid) coupled chloride channels blockade (Corda MG, et.al., 1991). Although reports regarding interaction of *Acorurs calamus* extracts and GABA receptors are lacking, the active principle of *Acorus calamus*, α-asarone has been shown not to have any interaction with GABA receptors in the brain (Liao JF, et.al., 1998)

This could be the reason why *Acorus calamus* is not effective against PTZ induced seizures and effective against maximal electro-shock induced seizures. As MES induced seizures test is the most validated experimental method for assessment of antiepileptic drug effective in generalized tonic-clonic seizures (Fisher RS, et.al., 1989, Loscher W, et.al., 1991), the profile of anticonvulsant activity of AEAC against MES induced seizures suggests its potential utility in the management of generalized tonic-clonic seizures.It has been found that drugs effective against PTZ induced convulsions in rodents are generally effective in absence seizures (McNamara JO, et.al., 1996). As the aqueous extract of *Acorus calamus* potentiated PTZ induced seizures on acute administration and had no protective effect on chronic administration, it suggests that the extract may aggravate absence seizures and should be used cautiously in patients with this condition. However, the mechanism behind aggravation of PTZ induced seizures is not clear at present and further studies are required to elucidate the same.

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