THE ANTICONVULSANT EFFECT OF EUPATORIUM BIRMANICUM DC LEAVE EXTRACT ALONE AND IN COMBINATION WITH LAMOTRIGINE AGAINST MES SEIZURE IN ALBINO MICE.

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ABSTRACT: OBJECTIVE: To evaluate the anticonvulsant activity of aqueous extract of *Eupatorium birmanicum* DC leave (EB) alone and in combination with lamotrigine against MES seizure in albino mice. **METHOD:** Aqueous extract of EB was prepared using Soxhlet apparatus. The anticonvulsant and effect of its combination extract was tested on prescreened albino mice at 3 doses (200, 400 & 800 mg/kg). After 1 hr of oral administration of EB the animals were subjected to MES seizures by convulsimeter with a current of 45 mA for 0.2 sec via trans auricular electrodes and the duration of the THLE was recorded. Sub-anticonvulsant dose of lamotrigine was also determined and the effect of their combination with the most effective dose of EB tested. **RESULTS:** EB aqueous extract exhibited significant anticonvulsant activity in the MES model at doses 400 mg/kg ($p < 0.02$) & 800 mg/kg ($p < 0.001$). The THLE was combined with the sub anticonvulsant totally abolished ($p < 0.001$) when EB extract at 800mg/kg was combined with sub anticonvulsant dose of lamotrigine (5 mg/kg). **CONCLUSION:** The aqueous extract of *E. birmanicum* leaves showed significant anticonvulsant activity in MES seizure model in albino mice and the THLE was totally abolished when EB extract at 800 mg/kg was combined with sub anticonvulsant dose of lamotrigine in the same animal model. **KEY WORDS:** MES, Lamotrigine, Anticonvulsant, *Eupatorium birmanicum* DC.

INTRODUCTION: Convulsion, as a term, refers to an intense paroxysm of involuntary contraction, which is one of the many manifestations of seizure, depending on the distribution of discharge.¹ Seizure (from the latin sacire, "to take possession of"), on the other hand, is a paroxysmal event due to abnormal, excessive, hyper synchronous discharges from an aggregate of central nervous system neurons. Although a variety of factors influence the incidence and prevalence of seizures, approximately 5-10% of the population will have at least one seizure, with the highest incidence occurring in early childhood and late adulthood. Epilepsy describes a condition in which a person has recurrent seizures due to a chronic, underlying process.² The underlying neuronal abnormality in epilepsy is poorly understood but it may be associated with enhanced excitatory amino acid transmission, impaired inhibitory transmission, or abnormal electrical properties of the affected cells.³

Eupatorium birmanicum DC (Manipuri: langthrei) belonging to the family Asteraceae, is a widely abundant pubescent under shrub with serrated leaves and white flowers. There are about 1200 species of *Eupatorium* across America, Europe, Asia and Africa, of which 7 species are found in India. It grows abundantly in Manipur. ⁴ Various species of *Eupatorium* have been studied for a wide range of activity: *E. squalidum* for antimalarial activity,⁵ *E. odoratum* on wound healing,⁶ *E. birmanicum* for antifungal activity,⁷ *E. glutinosum*⁸ and *E. salvia*⁹ for antimicrobial properties,

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E. laevigatum, *E. arnottianum*, *E. subhastatum*¹⁰ and *E. buniifolium*¹¹ for antinociceptive effect. Various species of this plant have been widely used traditionally not only in India but in various parts of Europe, America and Asia as an emetic, diuretic, emmenagogue, purgative, stimulant, diaphoretic, tonic and is even applied to foul ulcers and used as a remedy for snake bite. The present study was planned to find out the anticonvulsant effect. Further study was done to see its effect on combining with standard drug like lamotrigine.

MATERIAL AND METHOD:

Preparation of the plant extract: The leaves of *E. birmanicum* DC was collected during the month of August and authenticated by botanist at the Life Science Department, Manipur University and the Life Sciences Manipur University Herbarium Code of *E. birmanicum* DC is COLL No. 003421. It was cleaned with water and air dried in shade for several days and then powdered using a grinder. Extraction was done using the method described by Verma SCL.¹² The powdered material (47.83 gm) was then soxhleted with roughly ten times its volume of distilled water. A deep brown residue (17.76 gm) was obtained and was stored at 4°C for further use. The yield was 37.13%.

Animal: Healthy albino mice of either sex weighing 20-30 gm were obtained from the Animal House, RIMS and then housed in groups of 5-10 per cage, maintained in natural light-dark cycle with free access to food and water. They were divided into groups of 6 mice each as follows:

GROUPS	TREATMENT
C (control)	2% gum acacia suspension, p.o.
T ₁ (test)	<i>E. birmanicum</i> extract (200mg/kg), p.o.
T ₂	<i>E. birmanicum</i> extract (400mg/kg), p.o.
T ₃	<i>E. birmanicum</i> extract (800mg/kg), p.o.
L	Lamotrigine (sub anticonvulsant dose)
L + T _{1/2/3}	Lamotrigine (sub anticonvulsant dose)
	+ <i>E. birmanicum</i> extract (most effective dose), p.o.

The study protocol was approved by Institutional Ethics Committee of RIMS.

Drugs: The standard drug, lamotrigine (Torrent, India) and the extract were suspended in 2% gum acacia and administered in a volume of 25 ml/kg p.o. The control group received 2% gum acacia suspension prepared in distilled water.

Maximal Electroshock Seizure test: Evaluation by this method was done as described by Swinyard EA et al.¹³ Mice were prescreened with a current of 45 mA for 0.2 sec via trans auricular electrodes using a Techno convulsimeter. Only those showing tonic hindlimb extension (THLE) were taken for the study. A recovery period of 3-4 days was given before repeating the experiment. The drugs & 2% gum acacia were administered 1 hour prior to the electroshock given in the same manner as in the screening. The duration of THLE was recorded in seconds. Later, a sub anticonvulsant dose of lamotrigine was determined and its combination with the most effective dose of EB was seen in the same model.

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Acute Toxicity study: The aqueous extract of *E. birmanicum* was administered in doses of 400, 800, 1600 and 3000 mg/kg, p.o. to 4 groups of mice, each consisting of 10 mice and mortality was observed after 24 hrs.

Statistical analysis: The results obtained from the study were analyzed by the 'Analysis of Variance' (ANOVA) followed by Dunnet's 't' test. A level of 5% was considered significant.

RESULT:

Acute Toxicity study: The aqueous extract of *Eupatorium birmanicum* DC was found to be safe in the doses used and there was no mortality upto a dose of 3 mg/kg, p.o. after 24 hrs.

Maximal Electroshock Seizure (MES) test: The duration of THLE in each group of mice is tabulated in the table.

Group	Duration of THLE in secs (mean $\pm$ SEM)
C	13.50 $\pm$ 0.34
T ₁	13.20 $\pm$ 0.37
T ₂	12.25 $\pm$ 0.62 *
T ₃	11.50 $\pm$ 0.50 #
L	5.66 $\pm$ 0.32 #
L+ T ₃	Absent ^{#α}
ONE WAY	F
ANOVA	d.f
	P
	197.14
	7,23
	< 0.01

Table : Effect of aqueous extract of *Eupatorium birmanicum* leaves on MES seizures in albino mice, alone and in combination with lamotrigine.

* p < 0.02, # p < 0.001 compared to control, and ^α p < 0.001 compared to T₃ and L

The duration of THLE in mice treated with vehicle was 13.5 $\pm$ 0.34 secs. EB extract at 200 mg/kg did not significantly reduce the duration of THLE. The duration of THLE at 400 and 800 mg/kg were 12.25 $\pm$ 0.62 secs (p<0.02) and 11.50 $\pm$ 0.50 secs (p<0.001), respectively. The duration of THLE did not decrease with further increase in the dose of the extract. The dose of 800 mg/kg produced maximum decrease in the duration of THLE and the incidence of convulsion in this group was 33.33%. There was 33.33% mortality rate in the control group but there was no mortality in any group treated with the extract or the standard drug.

Lamotrigine at 10 mg/kg abolished the THLE stage completely. The duration of THLE at sub-anticonvulsant doses of lamotrigine (5 mg/kg) was 5.66 $\pm$ 0.32 secs and was significant when compared to control (p<0.001). There was no mortality in these groups.

The combination of lamotrigine (5 mg/kg) with the most effective dose of the aqueous extract of EB (800 mg/kg) resulted in the complete abolition of the THLE phase (p<0.001).

DISCUSSION: Efficacy in this model was determined by decrease in the duration of tonic hindlimb extension (THLE). Inhibition of the MES induced seizure predicts activity against generalized tonic

clonic and cortical focal seizures and it is said that this model evaluates the capacity to prevent seizure spread. Calcium ion influx appears to be involved in the origin of seizure.¹⁴ In the present study, the aqueous extract of EB produced significant decreases in the duration of THLE at doses of 400 mg/kg and 800 mg/kg, p.o. ($p < 0.01$ and $p < 0.001$, respectively) compared to control. The duration of THLE in control group was 13.50 ± 0.34 secs and was consistent with the findings of Sahadevan P and Rema MN.¹⁵ These findings showed that aqueous extract of EB possesses significant anticonvulsant activity in MES model in albino mice.

The present study also confirms the antiepileptic efficacy of lamotrigine and it is in consonance with the fact that it is effective against generalized tonic clonic seizure and is used for grandmal epilepsy. Lamotrigine, like phenytoin blocks sustained repetitive firing of the neurons and delays the recovery from inactivation of recombinant Na⁺ channel. However, lamotrigine is effective against a broader spectrum of seizure than phenytoin suggesting additional mechanism of action, one of which is the inhibition of synaptic release.¹⁶ The protection with lamotrigine in the model is consistent with the finding of Goel R et al.¹⁷

The daily maintenance dose of lamotrigine is 100-200 mg¹⁸ and on extrapolation of 100 mg of human dose to albino mice according to body surface area¹⁹ it came roughly to 13 mg/kg body weight. But for convenience, 10 mg/kg was taken for the study and it was found to completely abolish THLE stage in MES model. So, a sub-anticonvulsant dose of lamotrigine (5 mg/kg) was taken to assess the effect of its combination with the extract. The duration of THLE with sub-anticonvulsant dose of lamotrigine (5 mg/kg) was 5.66 ± 0.32 secs ($p < 0.001$ compared to control). But on combining the same dose of lamotrigine with 800 mg/kg of aqueous extract of *Eupatorium birmanicum*, the THLE was completely abolished.

Thus, combination of lamotrigine with the most effective dose of aqueous extract of EB elicited significant increase in the protection against MES induced seizures. It may be due to the aqueous extract acting by a different mechanism from lamotrigine or it may be due to the drug interaction, which may be either pharmacokinetic or pharmacodynamic and it needs further elucidation. Phytochemical studies of EB have revealed the presence of various compounds like coumarin, β -sitosterol, cerebroside, ceramide and quercetin 3-o-rutinoside.²⁰ The anticonvulsant property of the aqueous extract of EB may be due the presence of one or more of these compounds and confirmation of this remains to be elucidated.

CONCLUSION: The aqueous extract of the *Eupatorium birmanicum* DC leaves was tested for anticonvulsant activities in maximal electroshock seizure models in albino mice. It showed significant anticonvulsant activity in this model. Moreover, it significantly increased the anticonvulsant effects of lamotrigine when given in combination.

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