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ANTICONVULSANT ACTIVITY OF AQUEOUS EXTRACT OF *EUPATORIUM BIRMANICUM* DC. LEAVES ON SEIZURE INDUCED BY PTZ IN ALBINO MICE, ALONE AND IN COMBINATION WITH ETHOSUXIMIDE

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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 ethosuximide against seizure induced by PTZ in albino mice. **Method:** Aqueous extract of EB leaves was prepared using Soxhlet apparatus. The albino mice were fed with EB at 3 doses (200, 400 & 800 mg/kg). One hour after feeding, pentylenetetrazole (PTZ) was given at a dose of 60 mg/kg, s.c. after dissolving in distilled water, at the nape of the neck at a volume of 5 ml/kg to produce convulsion. The animals were observed for upto 30 mins after the PTZ challenge for onset of myoclonic spasm and clonic convulsion. The time of onset of myoclonic spasm and clonic convulsion were noted in seconds. Sub-anticonvulsant dose of ethosuximide was also determined and the effect of its combination with the most effective dose of EB tested. **Results:** The aqueous extract of *Eupatorium birmanicum* at doses 200mg/kg, 400mg/kg and 800 mg/kg significantly ($p<0.001$) increased the latency of onset of myoclonic spasm and clonic convulsion compared to the control. The combination of 800 mg/kg of the extract and the sub anticonvulsant dose of ethosuximide also significantly ($p<0.001$) increased the latency of onset of myoclonic spasm and clonic convulsion compared to the control. **Conclusion:** The aqueous extract of *E. birmanicum* leaves showed significant anticonvulsant activity in PTZ model in albino mice.

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.

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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. perfoliatum* for anti-inflammatory effects and antiplasmodial effect against *Plasmodium falciparum* [5], *E. odoratum* on wound healing [6], *E. birmanicum* for antifungal activity [7], *E. glutinosum* [8] and *E. lindleyanum* [9] for antimicrobial properties, *E. laevigatium, 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 ethosuximide.

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 Azwanida NN [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^{o}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.

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

Anticonvulsant testing: Anticonvulsant activity of aqueous extract of *E. birmanicum* in this model was tested following the method as described by Showraki A *et al.,* [13]. No pretesting was done. The drugs were given at a uniform volume of 25 ml/kg, p.o. They were divided into groups of 6 mice each as follows:

TABLE 1: GROUPS OF SIX MICE

S. no.	Group	Treatment
1	C (control)	2% gum acacia suspension, p.o.
2	T_1 (test)	*E. birmanicum* extract (200mg/kg), p.o.
3	T_2 (test)	*E. birmanicum* extract (400mg/kg), p.o.
4	T_3 (test)	*E. birmanicum* extract (800mg/kg), p.o.
5	E	Ethosuximide (200mg/kg), p.o.
6	T_3 + E	*E. birmanicum* extract (800mg/kg) + Ethosuximide (200mg/kg), p.o.

One hour after feeding, pentylenetetrazole (PTZ) was given at a dose of 60 mg/kg, s.c. after dissolving in distilled water, at the nape of the neck at a volume of 5 ml/kg to produce convulsion. The animals were observed for upto 30 mins after the PTZ challenge for onset of myoclonic spasm and clonic convulsion. The time of onset of myoclonic spasm and clonic convulsion were noted in seconds.

Later, a sub-anticonvulsant dose of ethosuximide was determined and then given along with the most effective dose of the extract together to see its effect. The Ethics Committee, RIMS approved the protocol of the study.

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.

RESULTS:

Acute toxicity: 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.

The anticonvulsant activity of the aqueous extract of *Eupatorium birmanicum* in the chemoshock convulsion test induced by pentylenetetrazole (PTZ) was evaluated by the method described by Showraki A *et al.,* [13]. The results of the test are tabulated below in **Table 2**.

TABLE 2: EFFECT OF AQUEOUS EXTRACT OF *EUPATORIUM BIRMANICUM* ON SEIZURE INDUCED BY PTZ IN MICE, ALONE AND IN COMBINATION WITH ETHOSUXIMIDE

Groups		Onset of myoclonic spasm in secs (mean ± SEM)	Onset of clonic convulsion in secs (mean ± SEM)
C		211.33 ± 3.48	361.83 ± 11.11
T_1		407.83 ± 11.94 *	801.50 ± 53.58*
T_2		512.83 ± 33.59*	892.66 ± 10.75*
T_3		523.80± 21.03*	922.50 ± 17.51*
E		497.75 ± 18.30*	846.66 ± 34.53*
T_3 + E		544.66 ± 32.35*$^{\#}$	914.33 ± 35.88*$^{\alpha\beta}$
One Way ANOVA	F	33.30	36.80
	df	5,24	5,17
	p	< 0.001	< 0.001

*p < 0.001 compared to control, $^{\#}$p> 0.1 compared to both T_3 and E, $^{\alpha}$p > 0.5 compared to T_3, $^{\beta}$p > 0.1 compared to E

The latencies of onset of myoclonic spasm and clonic convulsion in the mice treated with vehicle were 211.33 ± 3.48 secs and 361.83 ± 11.11 secs, respectively. The latencies of onset of myoclonic spasm in the groups treated with the aqueous extract *Eupatorium birmanicum* at 200, 400 and 800 mg/kg were 407.83 ± 11.94 secs, 512.83 ± 33.59 secs and 523.80 ± 21.03 secs, respectively. This prolongation in the latency of myoclonic spasm was found to be significant (p< 0.001) compared to the control. The latencies of onset of clonic convulsion in the groups treated with 200, 400 and 800 mg/kg of the aqueous extract were 801.50 ± 53.58, 892.66 ± 10.75 and 922.5 ± 17.51 secs, respectively and were statistically significant (p<0.001) compared to the control.

Ethosuximide at 350 mg/kg completely abolished the clonic convulsion phase in mice. The latencies of onset of myoclonic spasm and clonic convulsion at a sub-anticonvulsant dose of 200 mg/kg of ethosuximide were 497.75 ± 18.30 secs and 846.66 ± 34.53 secs, respectively and these were significant (p<0.001) compared to the control.

On treating the mice with a combination of 800 mg/kg of the extract and 200 mg/kg of ethosuximide, the latency of onset of myoclonic spasm was significantly (p<0.001) increased to 544.66 ± 32.35 secs compared to the control. This was not found to be significant (p > 0.1) compared individually to the groups treated with the extract (800 mg/kg) and ethosuximide (200 mg/kg) alone.

In the same group, the latency of clonic convulsion was significantly (p < 0.001) increased to 914.33 ± 35.88 secs compared to the control but was not significant compared to the groups treated with the extract at 800 mg/kg (p > 0.5) and ethosuximide at 200 mg/kg (p>0.1) alone.

DISCUSSION: The PTZ induced seizure test was done by the method described by Showraki A *et al.,* [13]. Efficacy in this model was determined by increase in latency of myoclonic spasm and clonic convulsion. Efficacy in this model predicts effectiveness against absence seizures [14]. The clonic convulsions are paralleled by spike wave complexes in the EEG [15]. The mechanism of convulsant action of PTZ seems to be related to the inhibition of the inhibitory function of the GABA neurotransmitter. PTZ have affinity for the chloride ionophore of the post synaptic GABA receptor complex and antagonizes GABAergic function [16].

In the PTZ model, the aqueous extract of *Eupatorium birmanicum* at doses 200mg/kg, 400mg/kg and 800 mg/kg significantly (p<0.001) increased the latency of onset of myoclonic spasm and clonic convulsion.

In human, the daily maintainance dose of ethosuximide is 1-1.5 gm and 1.5 gm [17] in man roughly extrapolates to 195 mg/kg in albino mice. But for convenience 200 mg/kg was taken for the study in PTZ model. But it didn't give complete protection against clonic convulsion and on increasing the dose it gives complete protection

against clonic convulsion at 350 mg/kg. So, the sub-anticonvulsant dose of 200 mg/kg was taken for the combination study.

Ethosuximide at sub anticonvulsant dose of 200 mg/kg significantly ($p<0.001$) increased the latency of onset of both myoclonic spasm and clonic convulsion to 497.75 ± 18.30 secs and 846.66 ± 34.53 secs, compared to the control. The result obtained confirms the efficacy of ethosuximide in the PTZ model and it is in accordance with the findings of Melissa A R *et al.,* [18]. Ethosuximide reduces low threshold Ca^{2+} current (T current) in thalamic neurons at clinically relevant concentration, without modifying the voltage dependence of steady state inactivation or the time course of recovery from inactivation It does not inhibit sustained repetitive firing or enhance GABA response at clinically relevant concentrations [19]. On combining this sub-anticonvulsant dose of ethosuximide with 800 mg/kg of aqueous extract of *Eupatorium birmanicum*, the increase in latency of onset of myoclonic spasm (544.66 ± 32.35 secs) and clonic convulsion (914.33±35.88 secs) was significant ($p<0.001$)compared to control but not significant ($P>0.01$) compared to either groups alone, indicating no increased protection when combined.

This study shows the anticonvulsant effect of the aqueous extract of *Eupatorium birmanicum* in the PTZ model. But the combination of ethosuximide and the most effective dose of the aqueous extract of *Eupatorium birmanicum* did not significantly offer increased protection against PTZ induced seizure.

Phytochemical studies of *Eupatorium birmanicum* have revealed the presence of various compounds like coumarin, β-sitosterol, cerebroside, ceramide and quercetin 3-o-rutinsoside [20]. Other species of *Eupatorium* are reported to contain different phytochemical compounds namely hexadecanoic acid, 2,6,10-trimethyl,14-ethylene-14-pentadecne, Bicyclo[4.1.0]heptane, 7-butyl- , Decanoic acid, 8-methyl-, methyl ester, 1-undecanol, 1-hexyl-1-nitrocyclohexane, 1,14-tetradecanediol, Octadecan oic acid, 2-hydroxy-1,3-propanediyl ester and 2-hydroxy-3-[(9E) -9-octadecenoyloxy] propyl(9E)-9-octadecenoate [21]. The anticonvulsant property of the aqueous extract of *Eupatorium birmanicum* may be due the presence of one or more of these compounds and confirmation of this remains to be elucidated.

CONCLUSION: In the pentylenetetrazole induced seizure model, aqueous extract of *Eupatorium birmanicum* showed significant anticonvulsant activity. However combining it with sub-anticonvulsant dose of ethosuximide did not significantly increase the latencies of myoclonic spasm and clonic convulsion.

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CONFLICT OF INTEREST: There is no conflict of interest for the research.

REFERENCES:

1. Raymond DA, Victor M and Ropper AH: Epilepsy and other seizure disorders, Principle of Neurology. McGraw Hill, New York, 6th Edn., 1997:313-343.
2. Daniel HL: Seizures and epilepsy, Harrison's Principles of Internal Medicine. Mc Graw Hill, New York, 19th Edn., 2015: 2: 2542-2559.
3. Rang HP, Dale MM, Ritter JM and Flower RJ: Antiepileptic drugs, Rang and Dale's Pharmacology. Churchill Livingstone, London, 8th Edn., 2016: 546- 558.
4. Deb DB: Compositae (Asteraceae), The flora of Tripura state. Today and tomorrow's printers and publishers, New Delhi, 1st Edn., 1983: 2: 119-231.
5. Hensel A, Maas M, Sendker J, Lechtenberg M, Petereit F, Deters A, Schmidt T and Stark T: *Eupatorium perfoliatum* L.: Phytochemistry, traditional use and current applications. J Ethnopharmacol. 2011; 138(3):641-51.
6. Phan TT, Hughes MA, Cherry GW, Lee TT and Pham HM: An aqueous extract of the leaves of *Chromolaena odorata* (formerly *Eupatorium odoratum*) (Eupolin) inhibits hydrated collagen lattice contraction by normal human dermal fibroblasts. J. Altern. Complement Med. 1996; 2: 335-343.
7. Damyanti M, Susheela K and Sharma GH: Effect of plant extracts and systemic fungicide on the pineapple fruit rotting fungus, *Ceratocystis paradoxa*. Cytobios 1996; 86: 155-165.
8. El Sheedi HR, Ohara T, Sata N and Nishiyama S: Antimicrobial diterpenoids from *Eupatorium glutinosum* (Asteraceae). J. Ethnopharmacol 2002; 81: 293-396.
9. Li- Lian, Yu-Ming Luo and Gui-Longyan: Studies on the antimicrobial activities of extracts from *Eupatorium lindleyanum* DC. against food spoilage and food-borne pathogens. Food Control. October 2008; 19(10): 995-1001
10. Clavin ML, Gorzalczany S, Mino J, Kadarian C, Martino V, Ferraro G and Acevedo C : Antinociceptive effect of some Argentine medicinal species of Eupatorium. Phytother. Res. 2000; 14: 275-277.
11. Miao J, Muschietti L, Ferraro G, Martino V and Acevedo C: Antinociceptive activity of *Eupatorium bunifolium* aqueous extract. Fitoterapia 2005; 76: 100-103.

12. Azwanida NN: A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med Aromat Plants 2015; 4:196.
13. Showraki A, Emamghoreishi M and Oftadegan S: Anticonvulsant Effect of the Aqueous Extract and Essential Oil of *Carum Carvi* L. Seeds in a Pentylenetetrazol Model of Seizure in Mice. Iran J Med Sci. 2016; 41(3): 200-8.
14. Phulen S and Anusuya B: Models of epilepsy used in antiepileptic drug discovery: a review. Int J Pharm Pharm Sci. 2014; Vol 6(11): 1-7.
15. Ludmyla Kandratavicius, Priscila Alves Balista, Cleiton Lopes-Aguiar, Rafael Naime Ruggiero, Eduardo Henrique Umeoka, Norberto Garcia-Cairasco, Lezio Soares Bueno-Junior, and Joao Pereira Leite: Animal models of epilepsy: use and limitations. Neuropsychiatr Dis Treat. 2014; 10: 1693–1705.
16. Huang RQ, Bell-Horner CL, Dibas MI, Covey DF, Drewe JA and Dillon GH: Pentylenetetrazole-induced inhibition of recombinant gamma-aminobutyric acid type A (GABA(A)) receptors: mechanism and site of action. J Pharmacol Exp Ther. 2001 Sep; 298(3):986-95.
17. Sweetman SC, Blake PS, McGlashan JM, Neathercoat GC and Parsone AV: Antiepileptics, Martindale: The Complete Drug Reference. RPS Publishing, London, 37th Edn. 2011:422-464.
18. Melissa A. Riegle, Melissa L. Masicampo, Hong Qu Shan, Victoria Xu, and Dwayne W. Godwin: Ethosuximide Reduces Mortality and Seizure Severity in Response to Pentylenetetrazole Treatment During Ethanol Withdrawal. Alcohol Alcohol 2015 Sep; 50(5): 501–508.
19. Mc Namara JO: Pharmacotherapy of the epilepsies, The Goodman and Gillman's Pharmacological Basis of Therapeutics. McGraw Hill, New York, 12th Edn. 2011: 555-584.
20. Devi LR, Singh TS and Singh LW: Antifungal and phytochemical studies of *Eupatorium birmanicum* DC. Indian J. of Chemistry 2007; 46: 1868-1872.
21. Christy Selvamangai, Anusha Bhaskar: GC-MS Analysis of Phytocomponents in the Methanolic Extract of *Eupatorium triplinerve*. Int. J. Drug Dev. & Res. 2012; 4(4): 148-153.

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