The study of antiepileptic activity of clove oil by MES model in mice

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Abstract

Background: The objective was to evaluate and compare the effect of extract of essential oil of clove with the standard sodium valproate on MES model.

Methods: A total of thirty mice were taken, they were given an electroconvulsive shock. Thirty mice were divided into five groups of six animals each, the control group received distilled water 5ml/kg i.p, standard received Inj. Sodium valproate 200 mg/kg i.p. another group received Sesame oil – 10ml/kg i.p(control), test groups received Clove oil- 0.075 ml/kg i.p., Clove oil- 0.1ml/kg i.p respectively. All the injections were given 30 minutes before the test.

Results: Clove oil produced significant antiepileptic effect at all the doses.

Conclusion: Clove oil has shown significant antiepileptic activity in mice.

Keywords: Epilepsy, Clove oil, Albino mice, MES model.

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Introduction

Epilepsy is regarded as a symptom of pathological excitatory processes in the brain, rather than a disease in itself, and is commonly defined as a group of chronic neurological disorders characterized by recurrent and unprovoked seizures.[1] The word "epilepsy" is derived from the Greek prefix *epi*, meaning "upon", and the Greek verb *labein*, meaning "to take", "to grasp" or "to seize".[2]

Epilepsy is one of the commonest neurological disorders estimated to affect 0.5-1% of the global population.[2] It is estimated that there are more than ten million people suffering from epilepsy in India.[3] This being higher in rural (1.9%) population as compared with the urban population (0.6%).[4,5]

The first genuine anticonvulsant, potassium bromide, was serendipitously discovered in 1857. Consequently, the inorganic bromides became the primary antiepileptic therapy for the next 55 years, despite their tendency to cause dermatitis and psychosis. Due to their high toxicity, the bromides are generally not used today.[6]

Valproic acid, Carbamazepine, benzodiazepines and the cyclic ureides which are collectively referred to as the "first generation" drugs are used nowadays.[7] Indeed, the most commonly prescribed AED worldwide is phenobarbital and phenytoin is the most frequently used AED in the United States.[8] The discovery and study of the first-generation anticonvulsants greatly improved our understanding of epilepsy at the molecular level, and ultimately led to the modern and more strategic approach of rational antiepileptic drug design.

Even then only 50-60% of these individuals are able to control their seizures with available medications[9]. Currently available drugs for epilepsy has many side effects like nystagmus, gingival hyperplasia, hirsuitism, nausea, vomiting, carbamazepine has diplopia and ataxia.[10]

Clove oil derived from the dried flower buds of the clove tree was used for medicinal properties in the earliest Chinese medicines.

M H Pourgholami et al. have concluded in his study that clove oil possesses anticonvulsant activity against MES induced tonic seizures.[11]

The above mentioned study prompted us to undertake this topic to evaluate and confirm the effect of extract of essential oil of clove on Maximal electro shock induced seizures in animal models.

Aims and Objectives

1. To evaluate the effect of extract of essential oil of clove by Maximal electro shock induced seizures in animal models.
2. To compare the effect of extract of essential oil of clove with standard dose of sodium valproate on Maximal electro shock induced seizures in animal models.

Materials and Methods

The study was carried out in the Department of Pharmacology, JJM Medical College, Davanagere, after the approval from institutional Animal Ethics committee. (IAEC). Male albino mice, weighing 20 to

30g which were bred in central animal house of J.J.M. Medical College, Davangere were used for the study.

Chemicals and drugs:

- Inj. Sodium valproate- 200 mg/kg.
- Intra peritoneal injection of extract of sesame oil- 10ml/kg[11]
- Intra peritoneal injection of extract of clove oil – 0.075, 0.1 ml/kg[11]

Sesame oil was mixed with clove oil to achieve the desired volume. To exclude any unknown anticonvulsant activity of Sesame oil, it was used in a separate group.

A total of 30 male mice were taken and were given maximal electroshock stimulation through trans-auricular electrodes priorly covered with saline moistened cotton wool with a current of 12mA, 50Hz for 0.2 seconds using convulsiometer.[12] Only those animals showing convulsive activity were selected for study.

Animals weighing 20-30g male albino mice with healthy with normal behavior were included in the study. Animals weighing more than 30g and less than 20g and animals used for any other experimental procedure in the recent past were excluded.

Five groups of six animals each were given maximal electro shock.

Group A- Distilled water 5ml/kg i.p. (intraperitoneal) as control.

Group B- Inj. Sodium valproate 200 mg/kg i.p. as standard.

Group C- Inj. Sesame oil – 10ml/kg i.p.

Group D- Inj. Clove oil- 0.075 ml/kg i.p

Group E- Inj. Clove oil-0.1ml/kg i.p.

All the injections were given 30 minutes before the electroshock.

Parameters observed in maximal electro shock:

1. Abolition of Hind limb tonic extension (HLTE) is taken as index for anticonvulsant activity.
2. Duration of HLTE.
3. Duration of time to regain righting reflex (from the end of HLTE till the animal can stand on 4 legs).

Evaluation: Abolition of hind limb tonic extension is taken as an index for anticonvulsant activity. Duration of HLTE, time taken to regain righting reflex provides information regarding the recovery time after a seizure attack. An effective anticonvulsant shortens the time to regain righting reflex.

Statistical analysis

Protection in MES induced seizures were recorded as percentage and compared using chi square test. Other values expressed as mean+/-standard error and statistical significance was calculated by ANOVA and Post –hoc Tukey's test for inter group comparison. P<0.05 was taken as significant.

Results

Clove oil has anticonvulsant effect against MES convulsions at both 0.075ml/Kg and 0.1ml/Kg.

Group D that received 0.075ml/kg of clove oil showed that mean duration of HLTE is 8.33±6.59, mean duration to regain righting reflex is 25.17±19.63. The mean duration of these parameters were significantly reduced when compared to control group, this implies that clove oil at 0.075ml/kg has some protection in MES induced seizures.

Group E that received 0.1ml/kg of clove oil showed that mean duration of HLTE is 6.78±5.54, mean duration to regain righting reflex is 20.5±15.97. The mean duration of these parameters were significantly reduced when compared to control group, this implies that clove oil at 0.1ml/kg also has some protection in MES induced seizures.

So, we conclude that clove oil at 0.075ml/kg and 0.1ml/kg has statistically significant anticonvulsant activity in MES model.

Table 1: Group wise comparison of abolition of HLTE in MES model (percentage of protection)

Drugs given	% Protection
Group A (Distilled water-5ml/kg.)	0 (0%)
Group B (Sodium Valproate-200mg/kg.)	6 (100%)
Group C (Sesame oil-10ml/kg.)	0 (0%)
Group D (Clove oil-0.075 ml/ kg.)	2 (33.33%)
Group E (Clove oil-0.1 ml/kg.)	4 (66.66%)

Table 2: Comparison of groups using ANOVA

Groups	Statistical Analysis		
	Mean	SD Deviation	ANOVA
Group A (Distilled water-5ml/kg.)	27	7.64	F =24.25 df= 4,25 P<0.000
Group B (Sodium Valproate-200mg/kg.)	0	0	
Group C (Sesame oil-10ml/kg.)	26.83	7.63	
Group D (Clove oil-0.075 ml/ kg.)	8.33	6.59	
Group E (Clove oil-0.1 ml/kg.)	6.78	5.54	

Table 3: Tukey's post-hoc Multiple Comparison test showing difference between groups on duration of HLTE (Sec) in MES Model

Difference between groups		
Groups	Mean Difference	Significance (P<0.05)
Group A & C	0.16	1.000 Not Significant
Group A & D	18.66	P<0.000 Significant
Group A & E	20.21	P<0.000 Significant
Group C & D	18.5	P<0.000 Significant
Group C & E	20.05	P<0.000 Significant
Group D & E	1.55	.992 Not Significant

Table 4: Showing comparison of groups in respect to time to regain righting reflex in MES Model

Groups	Statistical Analysis		
	Mean	SD	ANOVA
Group A (Distilled water-5ml/kg.)	84.83	12.94	F=29.48, df=4, 25, P<0.000
Group B (Sodium Valproate-200mg/kg.)	0	0	
Group C (Sesame oil-10ml/kg.)	75	23.94	
Group D (Clove oil-0.075 ml/ kg.)	25.17	19.63	
Group E (Clove oil-0.1 ml/kg.)	20.5	15.97	

Table 5: Tukey's post-hoc multiple comparison test in MES Model showing difference between groups in respect of time to regain righting reflex

Difference between groups		
Groups	Mean Difference	Significance (P<0.05)
Group A & C	9.83	0.84 Not Significant
Group A & D	59.67	P<0.000 Significant
Group A & E	64.33	P<0.000 Significant
Group C & D	49.83	P<0.000 Significant
Group C & E	54.50	P<0.000 Significant
Group D & E	4.67	0.987 Not Significant

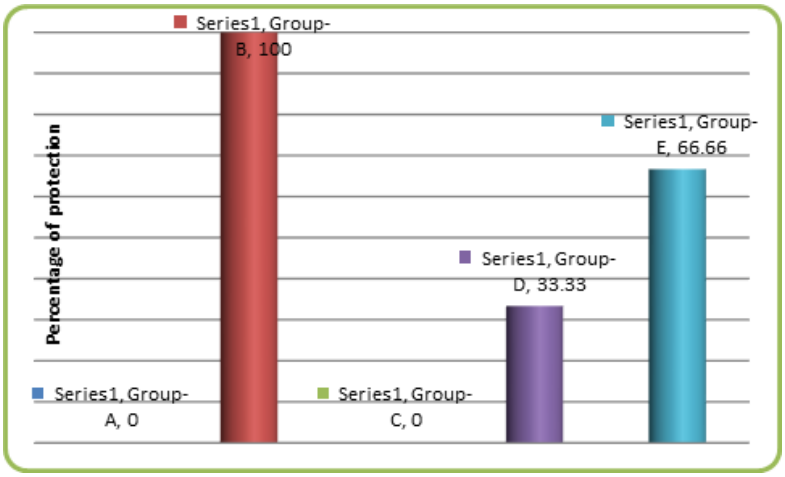

Fig. 1: Bar diagram depicting percentage of animals showing abolition of HLTE in MES Model

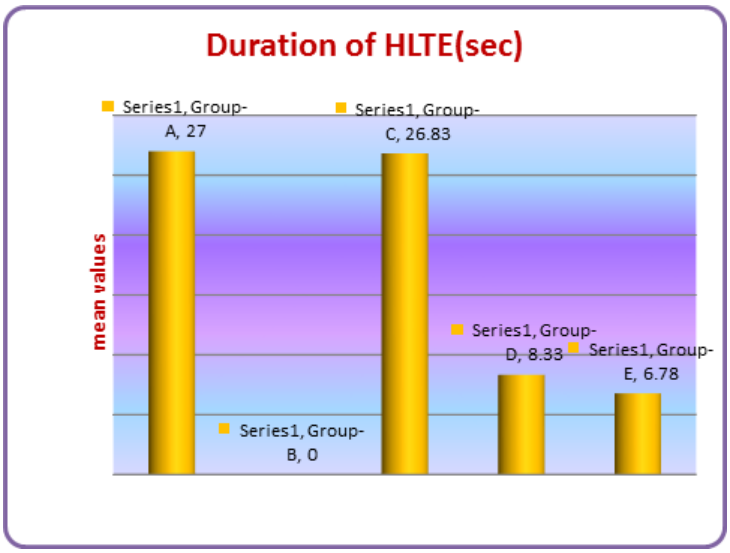

Fig. 2: Bar diagram depicting comparison of mean duration of HLTE between different groups in MES Model

Discussion

There are number of models that could potentially be used to screen for anticonvulsant activity. In the present study, MES model was used to evaluate the anticonvulsant effect of clove oil and compare with the respective standards. MES model is the most validated experimental method for evaluation of antiepileptic drug effective in generalized tonic-clonic seizures.

Anticonvulsant drugs like valproate and phenytoin act by modulation of ion channels. Hence valproate was chosen as the standard drug in this study. Several studies have shown that NMDA receptor antagonists are also effective in antagonizing MES induced seizures. Since decrease in duration of tonic hind limb extension is considered suggestive of protection against MES convulsions.[13]

GABA is a major inhibitory neurotransmitter and enhancement of its neurotransmission leads to attenuation of convulsions.[14]

In the present study, clove oil has shown results comparable to that of valproate in terms of seizure latency i.e., clove oil has delayed the seizure onset.

Therefore, we conclude that clove oil at 0.1ml/kg has statistically significant anticonvulsant activity in MES model.

Similar study conducted previously by M H Pourgholami et. al.[11] showed that, clove oil blocked tonic seizures induced by MES at different doses. This is comparable to our study.

Conclusion

- Clove oil has shown significant anticonvulsant action in both MES model.
- In view of promising results of Clove oil in generalised tonic clonic seizures, the further screening and evaluation in other species may prove beneficial to the development of novel therapeutic approach in treatment of epilepsy.

References

1. Fisher, R. S. Epileptic Seizures and Epilepsy: Definitions Proposed by the International League against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia 2005;46:470.
2. Nybakken, O. Greek and Latin in Scientific Terminology. Iowa State University Press 1962;1:23.
3. Sridharan R, Murthy BN. Prevalence and pattern of epilepsy in India. Epilepsia 1999;40:631–6.
4. Leonardi M, Ustun TB. The global burden of epilepsy. Epilepsia 2002;43(Suppl. 6):21–5.
5. Pahl K, de Boer HM. Atlas: epilepsy care in the world. Geneva: WHO; 2005. Epilepsy and rights; pp. 72–3.
6. Porter R J, Rogawski M A. New Antiepileptic Drugs: From Serendipity to Rational Discovery. Epilepsia 1992;33: S1.
7. Loscher W. Current Status and Future Directions in the Pharmacotherapy of Epilepsy. Trends Pharmacol. Sci. 2002;23:113.
8. Shorvon S D, Farmer P J. Epilepsy in Developing Countries: A Review of Epidemiological, Sociocultural, and Treatment Aspects. Epilepsia 1988;29:S36.
9. Jacobs M. P, Leblanc G G, Brooks-Kayal A, Jensen F E, Lowenstein D H, Noebels J L, et al. Curing Epilepsy: Progress and Future Directions. Epilepsy Behav 2009;14:438-445.
10. Roger PJ, Brian MS. Antiseizure drugs. In: Katzung BG, Masters SB, Trevor AJ, Editors. Basic and clinical pharmacology. 12th ed. Newyork: Tata McGraw-Hill; 2012. p. 403-27.
11. Pourgholami MH, Kamalinejad M, Javadi M. Evaluation of the anticonvulsant activity of the essential oil of Eugenia caryophyllata in male mice. Journal of Ethnopharmacology 1999;64:167-171.
12. Vogel WH, Huger FP. Psychotropic and neurotropic activity. In: Vogel HG Editor Drug discovery and evaluation, Pharmacological assays. 2nd ed. New York: Springer;2002.p.422-87.

13. Inan S, Buyukafsar K. Antiepileptic effects of two Rho-kinase inhibitors, Y-27632 and fasudil, in mice. Br J Pharmacol. 2008;155:44–51.
14. Nagakannan P, Shivsharan BD, Boreddy S Thippeswamy. Sedative and antiepileptic effects of Anthocephalus cadamba Roxb. Indian J Pharmacol. 2011;43(6):699–702.