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Research Article

The study of antiepileptic activity of clove oil in chemical induced convulsions in mice

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ABSTRACT

Background: The objective was to evaluate and compare the effect of an extract of essential oil of clove with the standard sodium valproate on pentylenetetrazole (PTZ) induced seizures in animal models.
Methods: A total of 30 mice were taken, they were given a chemo shock at the concentration of 60 mg/kg using PTZ. 30 mice were divided into 5 groups of 6 animals each the control group received distilled water 5 ml/kg i.p., standard received injection sodium valproate 200 mg/kg i.p. another group received sesame oil – 10 ml/kg i.p. (control), test groups received Clove oil - 0.075 ml/kg i.p., clove oil - 0.1 ml/kg i.p., respectively. All the injections were given 30 mins before the administration of PTZ.
Results: Clove oil produced a significant antiepileptic effect at all the doses.
Conclusion: Clove oil has shown significant antiepileptic activity in mice.

Keywords: Epilepsy, Clove oil, Albino mice

INTRODUCTION

Epilepsy is one of the most common neurological disorders. It is estimated to affect 0.5-1% of the global population, which translates to approximately 50 million people worldwide.[1] Epilepsy is a chronic disorder characterized by recurrent seizures. Seizures are episodes of brain dysfunction resulting from the abnormal discharge of cerebral neurons. The causes of seizures may include the full range of neurologic diseases from infection to neoplasm and head injury.[2] The variety of symptoms that can result from an epileptic seizure arises from the differing brain regions that when deprived of their function, give rise to the particular features of an individual seizure.[3,4] Currently available antiepileptic drugs (AEDs) fail to prevent seizures in approximately 30% of people with epilepsy. These agents are associated with significant dose-related adverse effects, occasional reactions and do not appear to influence the development or progression of epilepsy. There is no evidence that the new AEDs introduced over the past two decades has improved the progression of seizure disorders and little to suggest that compounds currently in developmental will do any change. Many number of patients demonstrates progressively increasing seizure frequency. New therapeutic strategies to address the significant clinical burden of drug-resistant epilepsy are

required. Patients with uncontrolled seizures experience significant morbidity and mortality and discrimination also.[5] Medicinal herbs constitute the cornerstone of traditional medicinal practice worldwide. The majority of the population in developing countries remains dependent on them for health care.[6] Various herbal extracts can be used as antiepileptic remedies. Clove oil, the test drug of our experiment is one such herbal medicinal plant.[7] Clove oil is an important aromatic spice, which belongs to the family Myrtaceae. Clove is cultivated in India, Madagascar, Sri Lanka and Malaysia.[8,9] From the phytochemical analysis it has been reported that eugenol is the main component of clove oil.[10,11] It also contains beta-caryophyllene, alfa humulene as well as carvacrol. Few studies have shown the anticonvulsant and anti-stress properties of eugenol, carvacrol, and humulene.[7]

METHODS

Source of data

The study was carried out at the Department of Pharmacology, JJM Medical College, Davangere, Karnataka, India, after the approval from Institutional Animal Ethics Committee.

Experimental animals

Male albino mice, weighing 20-30 g that were bred in the central animal house of J. J. M. Medical College, Davangere, Karnataka, India were used for the study.

Chemicals and drugs

- Injections pentylenetetrazole (PTZ) - 60 mg/kg
- Injections Sodium valproate - 200 mg/kg
- Intraperitoneal injection of extract of sesame oil - 10 ml/kg
- Intraperitoneal injection of extract of clove oil - 0.075, 0.1 ml/kg.

Dried buds of clove were obtained from the local market, and it was authenticated by Department of Pharmacognosy (Bapuji College of Pharmacy). Clove oil was prepared using dried buds of clove in Clevenger apparatus with 5% yield.

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.

Method of collection of data (including sampling procedure, if any)

A total of 30 mice were taken. Were given a chemo shock at the concentration of 60 mg/kg using PTZ. Only those animals showing convulsive activity were selected for study.

Toxicity assessment

The animals were divided into four groups with six animals in each group. Doses 0.2, 0.4, 0.8, and1.0 ml/kg of clove oil were administered i.p., in different group and incidence of mortality was noted up to 48 hrs.

Grouping of animals

Thirty mice were divided into 5 groups of 6 animals each, which were given PTZ at the dose of 60 mg/kg i.p.

- Group A: Distilled water 5 ml/kg i.p. as a control.
- Group B: Injections sodium valproate 200 mg/kg i.p. as standard
- Group C: Injections sesame oil - 10 ml/kg i.p.
- Group D: Injections clove oil - 0.075 ml/kg i.p.
- Group E: Injections clove oil - 0.1 ml/kg i.p.

All the injections were given 30 mins before the administration of PTZ.

Parameters observed for PTZ model

1. Abolition of clonic seizures is taken as an index of anticonvulsant activity
2. Time duration between injection of PTZ and onset of seizures.

Evaluation

Abolition of clonic seizures is taken as an index of anticonvulsant activity. Increased time duration between injection of PTZ and onset of seizures, i.e., latency of clonic seizures shows that anticonvulsant is more effective.

Statistical analysis

Protection in PTZ induced seizures were recorded as percentage and compared using Chi-square test. Other values expressed as a mean ± standard error and statistical significance was calculated by ANOVA and *post-hoc* Tukey's test for intergroup comparison. $p<0.05$ was taken as significant.

RESULTS

Table 1 shows there is increase in the duration of onset of clonic seizures in the test group compared to that of the control group. Table 2 shows Group wise comparison of the abolition of clonic seizures in PTZ model. (percentage of protection), Table 3 shows Tukey's *post-hoc* multiple comparison test in PTZ model showing difference between groups in respect of duration of onset of clonic seizures (seconds). Figure 1 shows percentage of animals showing abolition of clonic seizures in PTZ Model. Figure 2 shows

Table 1: Response of different group of animals.

Groups	Parameters	Animals						Mean	SD
		1	2	3	4	5	6		
Group A (distilled water-5 ml/kg)	Clonic seizures	+	+	+	+	+	+		
	Duration of onset of clonic seizures (sec)	118	132	168	180	90	120	134.67	33.65
Group B (sodium valproate-200 mg/kg)	Clonic seizures	-	-	-	-	-	-		
	Duration of onset of clonic seizures (sec)	0	0	0	0	0	0	-	-
Group C (sesame oil-10 ml/kg)	Clonic seizures	+	+	+	+	+	+		
	Duration of onset of clonic seizures (sec)	119	168	120	180	132	96	135.83	32
Group D (clove oil-0.075 ml/kg)	Clonic seizures	+	+	+	+	+	+		
	Duration of onset of clonic seizures (sec)	284	302	115	164	180	201	207.7	72.15
Group E (clove oil-0.1 ml/kg)	Clonic seizures	+	+	+	+	+	+		
	Duration of onset of clonic seizures (sec)	482	660	409	428	398	495	478.67	97.04

SD: Standard deviation

Table 2: Clonic seizures in PTZ model.

Drugs given	% Protection
Group A (distilled water - 5 ml/kg)	0 (0)
Group B (sodium valproate - 200 mg/kg)	6 (100)
Group C (sesame oil - 10 ml/kg)	0 (0)
Group D (clove oil - 0.075 ml/kg)	0 (0)
Group E (clove oil - 0.1 ml/kg)	0 (0)

PTZ: Pentylenetetrazole

Table 3: Tukey's *post-hoc* multiple comparison test in PTZ model.

Groups	Mean difference	Significance ($p<0.05$)
Groups A and C	1.16	0.999 not significant
Groups A and D	73.00	0.218 not significant
Groups A and E	344.00	$p<0.001$ significant
Groups C and D	71.83	0.232 not significant
Groups C and E	342.83	$p<0.001$ significant
Groups D and E	271.00	$p<0.001$ significant

Group A, C, D, E are compared with each other. Group E showed significant increase in duration of onset of clonic seizures when compared with control. ($p<0.001$), PTZ: Pentylenetetrazole

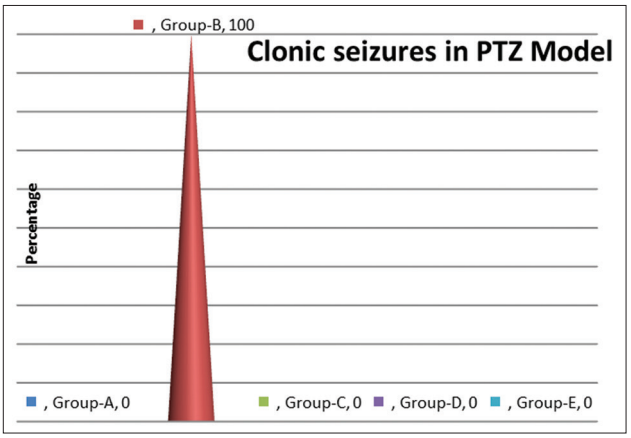

Figure 1: Clonic seizures in pentylenetetrazole model.

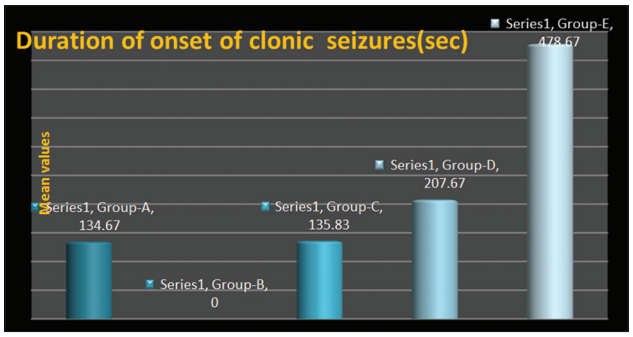

Figure 2: Duration of onset of clonic seizure in pentylenetetrazole model.

comparison of duration of onset of clonic seizure in PTZ model.

DISCUSSION

Clove oil is an important aromatic spice, which belongs to the family Myrtaceae. Clove is cultivated in India, Madagascar, Sri Lanka and Malaysia. It has many biological activities such as antibacterial, antifungal, insecticidal and anti-oxidant properties besides it has antiphlogistic and anti-vomiting. Its analgesic effect has been reported by many researchers. It also has cytotoxic and anti cancerogenic effects. From phytochemical analysis, it has been reported that eugenol is the main component of clove oil. It also contains beta-caryophyllene, alfa humulene as well as carvacrol. Few studies have shown the anticonvulsant and anti-stress properties of eugenol, carvacrol, and humulene.

There are a number of models that could potentially be used to screen for anticonvulsant activity. In the present study, PTZ models were used to evaluating the anticonvulsant effect of clove oil and compare with the respective standards.

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter and enhancement of its neurotransmission leads to attenuation of convulsions. PTZ is believed to be an antagonist at GABAa receptor complex and produces seizures by inhibiting the GABA pathway in the central nervous system resulting in an imbalance between the ionic concentrations of the membrane.

An increase in latency and decrease in duration of seizures or total abolition of seizures is considered suggestive of protection against PTZ convulsions. 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.

Group D animals that received 0.075 ml/kg of clove oil showed clonic seizures indicating 0% protection. The mean duration of latency of clonic seizure was 207.7±72.15. The mean duration of this parameter was significantly raised when compared to control group; this implies that clove oil at 0.075 ml/kg has significant anticonvulsant activity in PTZ model.

Group E animals that received 0.1 ml/kg of clove oil showed clonic seizures indicating 0% protection. The mean duration of latency of clonic seizure was 478.67±97.04. The mean duration of this parameter was significantly raised when compared to control group; this implies that clove oil at 0.1 ml/kg has statistically significant anticonvulsant activity in PTZ model.

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

CONCLUSION

The following conclusions can be drawn after observing the results of the present study. Clove oil has shown significant anticonvulsant action in PTZ models; it increases the threshold of clonic seizures induced by infusion of PTZ. It seems like clove oil contains certain active compounds that elevates the threshold of clonic seizures. In view of promising results of Clove oil in increasing the duration of latency of clonic seizure, further screening and evaluation in other species may prove beneficial to the development of novel therapeutic approach in the treatment of epilepsy.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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