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A STUDY ON ANTIEPILEPTIC ACTIVITY OF EUGENOL EXCLUDED AQUEOUS EXTRACT OF EUGENIA CARYOPHYLLUS

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ABSTRACT

Epilepsy is a disorder characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness. The drug discovery process in this direction is very attractive, because of non-availability of safe and effective means for the management of this condition. The objective of the study is to evaluate the antiepileptic activity of eugenol excluded clove extract. Wister albino rats are used in this study for evaluation. In PTZ induced convulsion model, aqueous clove extract had significantly delayed the onset of clonic and tonic convulsions, indicating the anticonvulsant activity. In MES induced convulsions also, aqueous clove extract had significantly decreased the duration of hind limb tonic extensor phase which is an observational parameter confirming anticonvulsant activity. In the all models, convulsions induced by either chemicals or MES, the aqueous extract of clove buds exhibited a fairly good anticonvulsant effect. However, long term studies in different animals and epileptic subjects may further substantial our study result.

Keywords: Epilepsy, Eugenol excluded clove extract, Eugenia caryophyllus, Wister albino rats.

INTRODUCTION

Epilepsy is the second most common chronic neurological condition seen by neurologists. 3-5% of the populations have a seizure sometime in their life and 0.5-1% of the population have active epilepsy. In most countries worldwide, the prevalence of active epilepsy ranges from 4 to 10 per thousand populations. Higher prevalence rates ranging from 14 to 57 per thousand have been reported from some African and South American countries. The incidence of epilepsy ranges from 40 to 70 per 100,000 in most developed countries and from 100 to 190 per 100,000 in developing countries [1-3].

Epilepsy is often, but not always, the result of an underlying brain disease. Any type of brain disease can cause epilepsy, but not all people with the same brain disease will have epilepsy. In view of the fact that only a proportion of people who have a brain disease experience seizures as a symptom of that disease, it is suspected that those who do have such symptomatic seizures are more vulnerable due to biochemical/neurotransmitter reasons.

The diversity 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 [4,5]. The determination of seizure types can often help in the identification of the epileptic syndrome. In spite of the technologic advances that have contributed to the understanding and treatment of epilepsy, the initiation and selection of treatment relies on the observed details of the seizure phenomenology [6]. The diagnosis of epilepsy is essentially clinical, based on an eyewitness account of the seizure. Neurological examination and investigations may be normal between attacks [7]. Sometimes patients may not be aware of the nature of attacks; seizures occurring at night may go unnoticed and hence may not be reported. Moreover, the lack of access to EEG or neuroimaging facilities in most community-based studies may lead to inaccurate diagnosis of epilepsy, its type or etiology [8-10].

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METHODOLOGY

- **Preparation of extracts**

Maceration: Pieces of flower buds of clove are taken soaked in water and extract excluding Eugenol was obtained.

Note: Here care was taken to evaporate all the eugenol as the eugenol excluded extract was needed for the activity, and even water was used as solvent because eugenol is insoluble in water and hence gets separated from other extracts soluble in water.

- **Preliminary phytochemical screening [11]**

Preliminary phytochemical investigation was carried out on aqueous extracts of *Eugenia Caryophyllus* for detection of various phytochemicals by standard methods described in practical pharmacognosy C.K.Kokate and R.K.Khandelwal.

- **Determination of acute toxicity (LD_{50}) [12]**

The acute toxicity for aqueous extracts of the powders of *Eugenia Caryophyllus* was determined in albino mice of either sex, those maintained under standard conditions. The animals will be fasted for 3 hours prior to experiment. Animals will be administered with different doses of the extract by following up and down methods as per OECD (organization for economical and co-operation development) guidelines number 425. From LD_{50} dose $1/5^{th}$ dose is to be selected and will be considered.

Antiepileptic activity [13,14]

- **Maximal electro shock (MES) method**

Albino mice of either sex weighing between 22-25g were divided into four groups each group was consisting of six animals.

Group A - Normal control (2% gum acacia)
Group B - Standard (Valproic acid)
Group C - Eugenol (clove oil)
Group D - Eugenol excluded aqueous extracts of clove buds

Experimental procedure

Albino rats of either sex with a body weight between 200-210g were divided into four groups of 6 animals in each. Group A served as control and was administered with 2% gum acacia suspension, Group B with Valproic acid (200mg/kg p.o.) and served as standard, Group C with eugenol and Group D with aqueous extracts of clove buds for seven consecutive days. On the eighth day one hour after oral administration of acacia suspension/standard drug/eugenol/ aqueous extracts to respective groups, MES seizures were induced by electroconvulsometer. A 60 mA current was delivered transauricularly for 0.2sec in rats. This current intensity elicited complete tonic extension of the hind limbs in control rats. For recording various parameters, rats were placed in clear rectangular plastic cages with an open top, permitting full view of the animal's motor responses to seizure. In the pilot study various phases of convulsions, viz., tonic flexion, extension, clonus, stupor and mortality due to convulsions were selected as the parameters.

The following parameters were recorded during 1hr test session.

- Tonic flexion
- Tonic extension
- Clonus convulsions
- Percent protection

The values were expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using ANOVA followed by Dennett's- t -test to calculate the significance difference if any among the groups. $p<0.05$ was considered as statistically significant.

- **Pentylene tetrazole (PTZ) method**

Albino rats of either sex weighing between 200-210g were randomly selected and segregated in to four groups, each group consisting of six animals.
Group A - Normal control (2% gum acacia)
Group B - Standard (Valproic acid)
Group C - Eugenol (clove oil)
Group D - aqueous extracts of clove buds

Experimental procedure

Albino rats of either sex with body weights between 200-210g were divided into five groups of 6 animals in each. Group A served as normal control and was administered with 2%w/v Gum acacia suspension orally, Group B with Valproic acid and served as standard, Group C with eugenol and Group D with aqueous extracts of clove buds for seven consecutive days. On the eighth day one hour after the oral administration of either acacia suspension/standard drug/eugenol/aqueous extracts respectively to different groups, PTZ 50 mg/kg was administered subcutaneously. Each animal was then placed into individual plastic cages and were observed initially for 30min and later up to 24 hrs. The following parameters were recorded during test session of initial 30min and up to 24 hrs respectively:

► Latency (onset of clonus)
► Onset of tonic-clonic convulsions
► Status of animal after 1hr
► Status of animal after 24 hrs
► Percent protection

The values were expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using ANOVA followed by Student't test to calculate the significance difference if any among the groups. $p<0.05$ was considered as statistically significant.

RESULTS

Percentage Yield

Weight of clove buds taken =50.0g
Weight of extract obtained = 22.59 g

% Yield obtained = Weight of clove taken X 100
Weight of extract obtained

= (22.59/50) × 100
= 45.18 %

►Phytochemical investigation

- **Preliminary phytochemical testing of extracts**

The results of preliminary qualitative phytochemical examination are shown in table 1.

- **Thin Layer Chromatography (TLC)**

1. TLC plates: Silica coated Aluminium plates
2. Solvent: Toulene
3. Detecting reagent: Anisaldehyde reagent sprayed and observed under the U.V light

►LD_{50} determination

The whole study was carried out according to the OECD guidelines 425**.**

6 mice
↓
Drug administered (Clove extract)
↓
No Death
(Success of LD_{50} at 3000mg/kg body weight)

Table 1. Preliminary qualitative phytochemical examination

Phytoconstituents	Chemical test	Result
Carbohydrates	Molisch test	Present
Proteins	Ninhydrin test	Absent
Tannins	Ferric chloride test	Present
Flavonoids	Shinoda's test	Absent
Saponins	Foam test	Present
Fixed oils and Fats	Spot test	Present
Alkaloids	Mayer's test	Absent

Eugenol: Extract + alcohol + $FeCl_3$ didn't give blue colour proving absence of Eugenol

Table 2. MES induced convulsions (Onset of clonic seizure) in rats

Treatment	Onset of Clonic Seizures (seconds) No. of animals in the group						Mean ± Sem	Status of Animals Death/Recovery
	1	2	3	4	5	6		
Control (2%w/v gum acacia)	4	6	8	7	5	5	5.83±0.60	0/6
Valproic acid (200mg/kg body.wt)	13	17	15	14	16	13	14.66±0.67**	0/6
Eugenol (500mg/kg body.wt)	5	11	12	10	8	6	8.66±1.15*	0/6
Clove aqueous extract (3g/kg body.wt)	4	8	9	8	8	7	7.33±0.71*	0/6

Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed by Student't test. * =P>0.05 **=P<0.05

Table 3. MES induced convulsions (duration of tonic clonic seizure) in rats

Treatment	Avg. Wt (mg)	Avg. Vol. of dose (ml)	Onset of Seizure (Sec) Mean ± SEM	Duration of Tonic Clonic Seizure (Sec) Mean ± SEM	Status of Animal (No. of animals Alive)	% Protection (1hr)
Control (2% Gum acacia)	200	1.0	5.83±0.60	161 ± 17.56	0/6	100
Valproic acid (200mg/kg body.wt)	210	0.8	14.66±0.67	95.66 ± 7.14**	0/6	100
Eugenol (500mg/kg body.wt)	200	1.0	8.66±1.15	121.16 ± 9.66*	0/6	100
Clove extract (3g/kg body.wt)	210	2.4	7.33±0.71	135.66 ± 10.75*	0/6	100

Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed by Student't test. * =P>0.05 **=P<0.05

Table 4. PTZ induced convulsions (Onset of clonic seizure) in rats

Treatment	Onset of Clonic Seizures (seconds) No. of animals in the group						Mean ± Sem	Status of Animals Death/Recovery
	1	2	3	4	5	6		
Control (2%w/v gum acacia)	5	8	10	8	5	6	7 ± 0.82	0/6
Valproic acid (200mg/kg body.wt)	16	18	19	22	14	20	18.16 ± 1.16**	0/6
Eugenol (500mg/kg body.wt)	10	13	13	18	12	14	13.33 ± 1.08**	0/6
Clove extract (3g/kg body.wt)	8	10	11	16	10	12	11.16 ± 1.12**	0/6

Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed by Student't test. * =P>0.05 **=P<0.05

Table 5. PTZ induced convulsions (duration of tonic clonic seizure) in rats

Treatment	Avg. Wt (mg)	Avg. Vol. of Dose (ml)	Onset of Seizure (Sec) Mean ± SEM	Duration of Tonic Clonic Seizure (Sec) Mean ± SEM	Status of Animal (No. of animals Alive)	% Protection (1hr)
Control (2% Gum acacia)	200	1.0	7±0.82	174 ± 16.14	0/6	100
Valproic acid (200mg/kg body.wt)	220	0.9	18.16±1.16	99.83 ± 5.78**	0/6	100
Eugenol (500mg/kg body.wt)	210	0.9	13.33±1.08	146.33 ± 8.64*	0/6	100
Clove extract (3g/kg body.wt)	200	2.4	11.16±1.12	163.16 ± 13.11*	0/6	100

Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed by Student't test. * =P>0.05 **=P<0.05

Fig 1. Thin Layer Chromatography

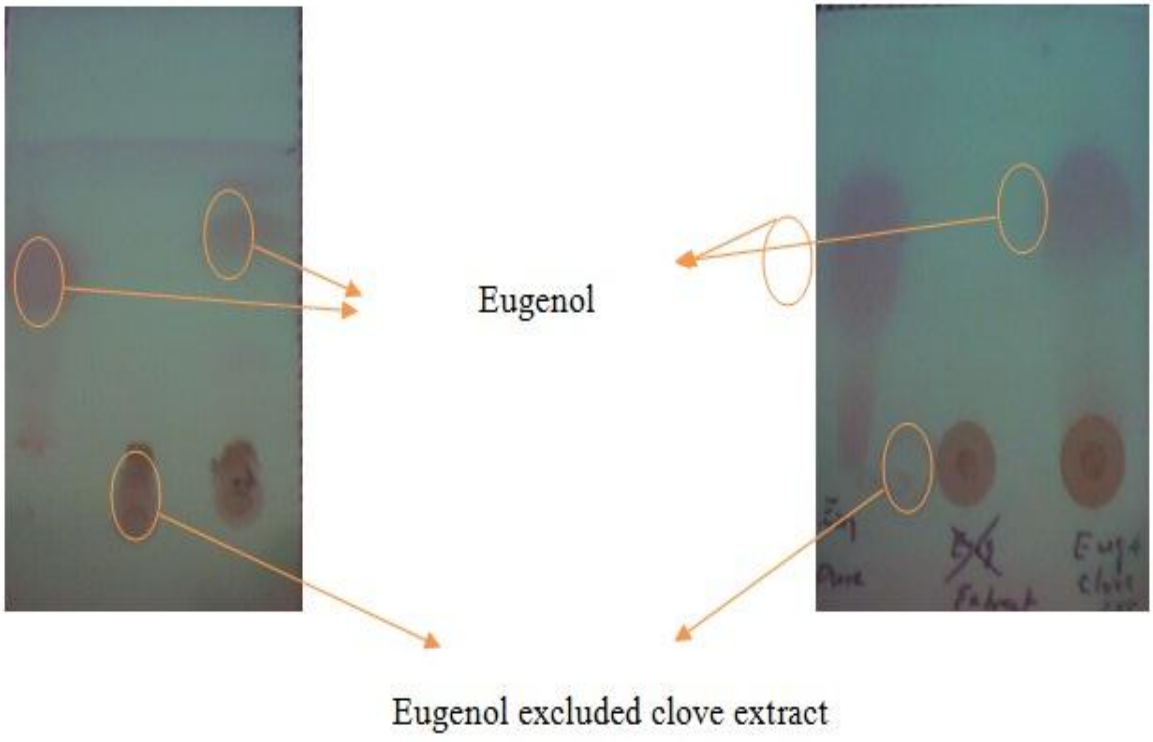


Fig 2. MES induced convulsions (Onset of clonic seizure) in rats

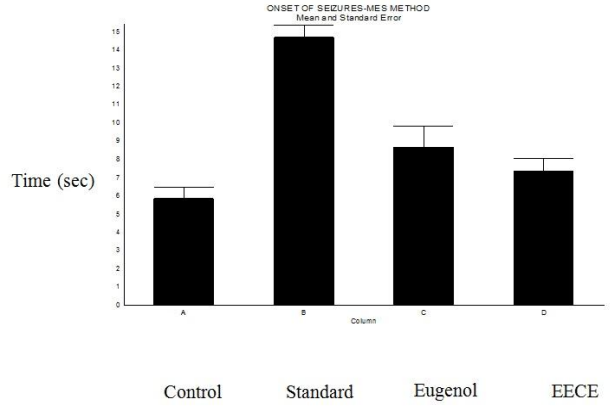


Fig 3. MES induced convulsions (duration of tonic clonic seizure) in rats

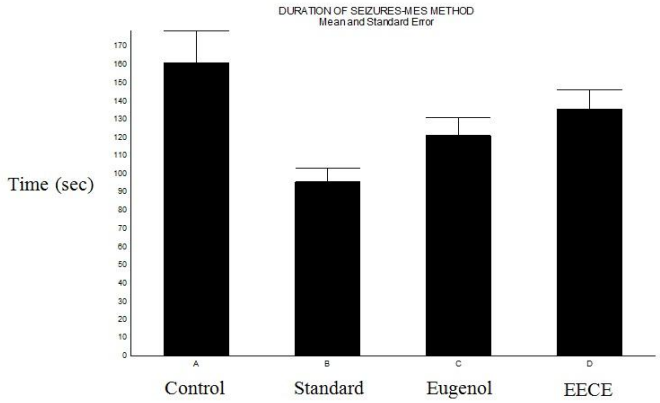


Fig 4. PTZ induced convulsions (Onset of clonic seizure) in rats

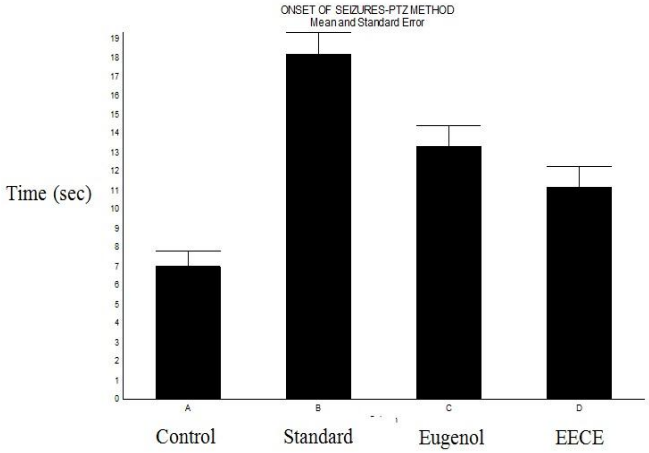
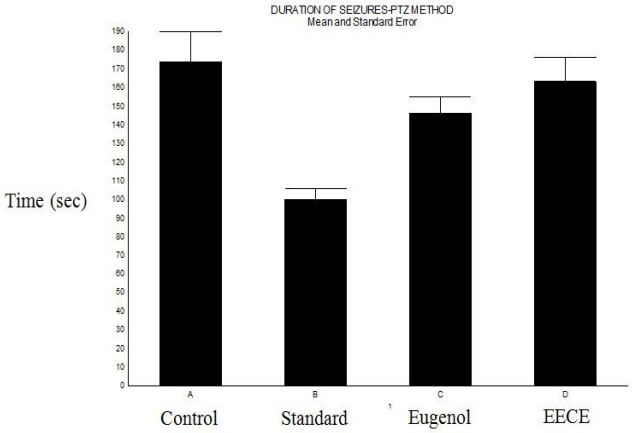


Fig 5. PTZ induced convulsions (duration of tonic clonic seizure) in rats



DISCUSSION

Anticonvulsant Activity

There are a number of synthetic anticonvulsant drugs currently available for use in the management, control and treatment of individuals with epilepsy. However, most of the synthetic drugs are not only inaccessible and unaffordable, but also possess many toxic adverse effects. Therefore, there is a great need for the development of cheap, effective and safe anticonvulsant agents from plants and other sources. The anticonvulosent activity of eugenol has already been established. Based on this, the anticonvulsant activity of eugenol excluded clove extract was studied in different experimental models employing rats. In most common forms of epileptic seizures, effective drugs appear to work either by promoting the inactivated state of voltage activated Na^+ channels or enhance GABA mediated synaptic inhibition.

Prevention of PTZ induced seizures in laboratory animals is the most commonly used preliminary screening test for characterizing potential anticonvulsant drugs. The test is assumed to identify anticonvulsant drugs effective against generalized clonic seizures, as PTZ produces clonic and tonic convulsions. It has been demonstrated that, a neural pathway sub serving clonic PTZ convulsions is located in the forebrain while the brain stem is involved in the network of tonic PTZ induced convulsions. The antiepileptic drug should abolish or increase the threshold for clonic and tonic convulsions. The mechanism by which PTZ exert its convulsant action is by acting as an antagonist at the GABA receptor complex. Drugs offer protections against tonic–clonic seizures induced by PTZ are considered to be useful to control myoclonic and absence seizures in humans. Various factors like age, sex, species, diet, water, day/light cycle, temperature, preparation dose and route of administration are known to affect the response of the animal to PTZ induced seizures.

Aqueous clove extract had significantly increased the threshold for clonic and tonic convulsions. Standard drug Valproic acid had abolished the clonic and tonic seizures with injection of PTZ (50mg/kg s.c.) and offered 100% protection.

Valproic acid acts through the activation of GABAA receptors and facilitate the GABA mediated opening of chloride channels. A dose depended activity has

seen i.e., increase in the latency (onset) of convulsion as well as decrease in duration with tonic-clonic seizure threshold.

Increase in the threshold for clonic and tonic convulsions by eugenol excluded clove extract after PTZ induced seizure suggest that, the extract might have affecting GABA-ergic neurotransmission as PTZ has been shown to interact with the GABA neurotransmitter. MES is also one of the commonly used models for preliminary testing of anticonvulsant drugs that produces generalized tonic-clonic seizures i.e. hind limb tonic extensor (HLTE) and clonic convulsions. In untreated animals a single MES produced an immediate tonic hind limb extension for 5-10 sec duration followed by clonic seizures. Previous studies have reported that immediate to MES transmitters *in vivo* increase were over the 20-30 min postictal period.

It has often been stated that antiepileptic drugs that block MES induced tonic extension act by blocking seizure spread, moreover MES induced tonic extension can be prevented either by drugs that inhibit voltage dependant Na^+ channels (phenytoin, valproate) or by drugs that block glutaminergic excitation mediated by the N-methyl- D-aspartate (NMDA) receptor.

Aqueous clove extract had significantly increased the duration of tonic extensor phase and onset of clonus as compared to control and thus exhibited anticonvulsant effect and the percent protection was 100%. Standard drug (Valproic acid) had abolished the tonic extensor phase and showed 100% anticonvulsant effect by preventing seizure spread. Hence, the anticonvulsant activity of aqueous clove extract against MES induced convulsions involve blockade of seizure spread, which perhaps occurred by inhibiting Voltage dependant Na^+ channels. Hence it is concluded that, the eugenol excluded clove extract possesses significant anticonvulsant activity against pentylenetetrazole and MES induced convulsions.

CONCLUSION

In the present study we have selected a plant *Eugenia caryophyllus* and aqueous extract was prepared from the clove buds and tested for its anticonvulsant activity in validated animal models. Preliminary phytochemical investigation of aqueous extract of flower buds of *Eugenia caryophyllus* revealed the presence of amino acids, fixed oils, alkaloids, glycosides. LD_{50} studies of the extract reveal that, extracts are safe up to the dose level of 3000mg/kg (success of LD_{50}). In PTZ induced convulsion model, aqueous clove extract had significantly delayed the onset of clonic and tonic convulsions, indicating the anticonvulsant activity. In MES induced convulsions also, aqueous clove extract had significantly decreased the duration of hind limb tonic extensor phase which is an observational parameter confirming anticonvulsant activity. In the all models, convulsions induced by either chemicals or MES, the aqueous extract of clove buds exhibited a fairly good anticonvulsant effect. However, long term studies in different animals and epileptic subjects may further substantial our study result.

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