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Research Article

ANTICONVULSANT ACTIVITY OF *CITRUS MAXIMUS* LEAVES IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

Objective: To assess the anticonvulsant activity of ethanolic extract of *Citrus maximus* (EECM) leaves of maximal electroshock seizure (MES) and pentylenetetrazol (PTZ)-induced seizure models on albino (Wistar strain) rats and mice.

Methods: Anticonvulsant activity was carried out by MES model and PTZ-induced clonic convulsions model; in each model, albino rats (Wistar strain) of either sex were taken and divided into five groups, each consisting of 6 rats. One group was used as control (3% w/v gum acacia), one as standard (phenytoin), and three groups for the test drug of EECM leaves (doses of 50, 100, and 200 mg/kg) treatment. The reduction in time or abolition of tonic extensor phase of MES-convulsions was recorded for all the animals. In PTZ model, either delay or complete abolition of convulsions in rats treated with diazepam and EECM leaves was noted for all the animals.

Result: EECM leaves reduced the extensor phase of convulsion in MES in a dose-dependent manner and decrease in the duration of convulsions in PTZ model with increasing dose. Anticonvulsant activity was seen maximum at the dose of 200 mg/kg.

Conclusions: Thus, from the above two seizure models of MES and PTZ, it can be concluded that EECM leaves have got an anticonvulsant effect in an increasing dose-dependent manner.

Keywords: Anticonvulsant, *Citrus maximus*, Maximal electroshock seizure, Pentylenetetrazol.

INTRODUCTION

Epilepsy describes a condition, in which a person has recurrent seizures due to a chronic, underlying process. This implies that a person with a single seizure, or recurrent seizures due to correctable or avoidable circumstances, does not necessarily have epilepsy. Epilepsy refers to a clinical phenomenon rather than a single disease entity. Since there are many forms and causes of epilepsy. However, among the many causes of epilepsy, there are various epilepsy syndromes, in which the clinical and pathological characteristics are distinctive and suggest a specific underlying etiology [1].

Citrus maximus is commonly known as pomelo (English), rabab-tenga (Assamese), Chinese grapefruit, pummelo, pummel jabot, shaddock, or Jeruk Bali. It is a citrus fruit which belongs to the Rutaceae family and is usually pale green to yellow when ripe, with sweet flesh and thick spongy ring [2].

C. maximus has been used in the traditional medicine in Asia for 100 of years. The decoctions of the leaves, flowers, and rind are used for their sedative effect in cases of epilepsy, chorea, and severe cough. The hot leaf decoction is applied on swellings and ulcers. The fruit juice is taken as a febrifuge. The sarcocarps are employed against coughs, dyspepsia, and lumbago. The pulp is considered an effective aid in the treatment of urinary disorders. Leaf extractions have shown antibiotic activity [3]. Traditional medicinal plants with various active principles and properties have been used since ancient times by physicians and laymen to treat a variety of diseases such as epilepsy. The anticonvulsant activity of *Citrus maximus* leaves has not been studied earlier in the north-eastern region of India. Keeping in view with the above ideas, the present study has been undertaken to evaluate the anticonvulsant activity of *C. maximus* leaves in albino rats and mice, respectively.

METHODS

Collection of plant material

C. maximus leaves were collected from the PG-Hostel campus of Assam Medical College, Dibrugarh, in the month of March and identified by Botanist, Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam, India, with identification voucher number being DU/LS/208.

Method of extraction

The required amount of leaves of *C. maximus* was collected and dried at room temperature. The dried leaves were ground into powder separately. Sufficient amount of powdered leaves was moistened with 95% ethyl alcohols and allowed to remain for 6 hrs in a percolator. When the liquid began to drop from the percolator, the orifice was closed and the content was allowed to macerate for 24 hrs. After 24 hrs, it was allowed to percolate slowly, a rate not exceeding 1 ml/minutes [4] and the solution was collected in Petri dishes. Alcohol was allowed to evaporate at room temperature. When the extract got completely dried, it was scrapped out, weighed and stored [4] and the yield at the end of extraction was found to be 16.7 g.

Acute toxicity testing

In the present study, for acute toxicity testing for both oral (not intended route) and intraperitoneal (i.p.) (intended route), routes were performed. Oral acute toxicity testing was studied in albino mice as well as in albino rats according to the OECD Guidelines for 425 [5], and no mortality was recorded up to the dose of 2000 mg/kg.

Grouping and preparation of drug doses

a. Vehicle (Group A): 3% (w/v) gum acacia suspension was prepared and used in the control group.
b. Test drugs (Group B_1, Group B_2, and Group B_3): 50, 100, and 200 mg/kg of the ethanolic extract of *C. maximus* (EECM) leaves were prepared with 3% (w/v) gum acacia as a suspending agent.
c. Standard drugs (Group C): For anticonvulsant models-phenytoin 25 mg/kg, and diazepam 4 mg/kg. All the suspensions of phenytoin and diazepam were prepared with 3% (w/v) gum acacia.
d. CNS stimulant: Pentylenetetrazol (PTZ) 40 mg/kg [6], a stock solution containing 4 mg/ml of the drug was prepared using distilled water.

EXPERIMENTAL PROCEDURES

Permission of the Institutional Animal Ethical Committee was obtained before conducting the study.

Maximal electroshock seizure (MES) model

Induction of the MES, using an electroconvulsiometer, is a commonly used model for evaluation of anticonvulsant drugs.

Healthy albino rats (Wistar strain) of either sex were taken and divided into five groups, each consisting of 6 rats. One group was used as control (3% w/v gum acacia), one as standard (phenytoin), and three groups for the test drug (EECM in the doses of 50, 100, and 200 mg/kg) treatment.

- Each animal was properly held, and earlobe electrodes were placed on the earlobes and current of 150 mA was passed for 0.2 seconds. Different stages of convulsions as mentioned earlier were noted down, along with the time (seconds) spent by the animal in each phase of the convulsions. The same procedure was repeated with other animals of the control group. The current was passed 30 minutes after i.p. injection of 3% (w/v) gum acacia.
- For the test drug groups, the same procedure as the earlier step was repeated for three different doses (50, 100, and 200 mg/kg, i.p.) of EECM.
- Phenytoin (standard) was also injected i.p. to all the 6 rats. After waiting for 30 minutes, animals were subjected to electroconvulsions as described in the earlier steps.
- The reduction in time or abolition of tonic extensor phase of MES-convulsions was recorded for all the animals [7].

PTZ-induced clonic convulsions

Healthy albino rats (Wistar strain) of either sex were taken and divided into five groups, each consisting of 6 rats. One group as a control, one as standard (diazepam), and three as tests (EECM in the doses of 50, 100, and 200 mg/kg) were taken for the study.

- 3% (w/v) gum acacia was injected intraperitoneally to the control animals, and after 30 minutes, PTZ was injected subcutaneously to these animals, and the onset of action (indicated by Straub's tail, jerky movements of the whole body and convulsions) and severity of convulsions due to the drug were noted.
- EECM in the doses of 50, 100, and 200 mg/kg were taken as test drug groups, and the same procedure as the earlier step was followed.
- Diazepam was injected intraperitoneally to a group of 6 rats, which were taken as the standard group. After waiting for 30 minutes, the animals were subjected to subcutaneous injection of PTZ as described in the earlier step.
- Either delay or complete abolition of convulsions in rats treated with diazepam and EECM was noted [7].

RESULTS

Statistical analysis

The data were statistically analyzed using one-way analysis of variance (ANOVA) test, followed by Dunnett's multiple-comparison test, using the GraphPad Prism 5 software (Tables 1 and 2).

DISCUSSION

The present study was undertaken to evaluate an anticonvulsant activity of the EECM leaves in experimental animal models.

Although the MES test predicts activity against generalized tonic-clonic and cortical focal seizures and the PTZ test against absence seizures, the underlying neuronal abnormality is not well understood. Diminution of brain GABA level has been reported after subconvulsive dose of PTZ [8].

The MES-induced convulsions for the screening of the anticonvulsant drug are the standard experimental model for evaluating a drug in experimental animals its anticonvulsant property, which represents grand mal epilepsy in human beings [7].

The anticonvulsant effect by the MES model is determined by the effect of the drug in the tonic hindlimb extensor phase of the convulsion [9]. By either completely abolishing it or by reducing its duration [7]. In the present study, it was found that the duration of the extensor phase in the control group was 12.5±0.428 seconds, which is similar to that found by Manocha *et al.* [10] as 15.34±0.93 seconds, Rewari and Prabhu [11] as

Table 1: MES model

S. No.	Groups	Pre-treatment (mg/kg, i.p.)	Rats	Onset time (mean±SEM in seconds)					No extensor seizures
				Tonic limb flexion	Tonic extensor	Clonus	Stupor	Recovery	
1	Group A (control)	3% gum acacia	6	5.5±0.428	12.5±0.4282	5.333±0.421	139±5.526	162.33±5.590	0
2	Group B_1 (test 1)	EECM 50	6	4.833±0.307	5.5±0.223[a]	4.333±0.614	146±7.479	160.67±7.269	0
3	Group B_2 (test 2)	EECM 100	6	4.5±0.223	3.833±0.307[a]	4.5±0.428	125.83±2.535	138.67±2.716[a]	0
4	Group B_3 (test 3)	EECM 200	6	4±0.258[a]	2.167±0.307[a]	4.667±0.666	124.50±4.958	135.33±5.044[a]	0
5	Group C (standard)	Phenytoin 25	6	3.5±0.223[a]	0[a]	4.667±0.494	114.33±3.169[a]	122.50±3.096[a]	6
p		F		6.625	268.49	0.506	6.176	11.658	
		df		4.25	4.25	4.25	4.25	4.25	
		P		<0.01	<0.01	>0.05	<0.01	<0.01	

N=6 in each group; all the values were expressed in mean±SEM. [a]p<0.01 is significant when compared with control (ANOVA followed by Dunnett's multiple comparison test). MES: Maximal electroshock seizure, EECM: Ethanolic extract of *Citrus maximus*, SEM: Standard error of mean, i.p.: Intraperitoneal

Table 2: PTZ-induced seizure model

S. No.	Number of animals	Pre-treatment (mg/kg, i.p.)	Latency (Seconds±SEM)	Number of convulsions	Average duration of convulsions (Seconds±SEM)	Number of animals recovered
1	6	3% gum acacia	670.33±25.43	1.677±0.210	14.583±0.374	6
2	6	EECM 50	1305.2±37.92	1	9.5±0.428	6
3	6	EECM 100	1610.5±28.40	1	7.667±0.557	6
4	6	EECM 200	2090±37.3	1	5.333±0.494	6
5	6	Diazepam 4	-	0	-	6
One-way ANOVA		F	330.07	40	70.298	
		df	3.20<0.01	4.25<0.01	3.20<0.01	
		p				

N=6 in each group; all the values were expressed in mean±SEM. P<0.01 is significant when compared with control (ANOVA followed by Dunnett's multiple comparison test). PTZ: Pentylenetetrazol, EECM: Ethanolic extract of *Citrus maximus*, SEM: Standard error of mean, i.p.: Intraperitoneal

11.6±0.40 seconds, and Achliya *et al*. [12] as 14.39±0.52 seconds in their respective control groups. The extensor phase in the three test groups, viz., Group B_1, B_2, and B_3 were found to be 5.5±0.2236, 3.833±0.3073, and 2.167± 0.3073 seconds, respectively, which shows there was a reduction in its duration.

There was complete abolition of the extensor phase in the standard group of phenytoin. Similar effects were found with different doses of phenytoin by Chattopadhyay *et al.* [13], where there was reduction in the extensor phase of MES-induced convulsion. Phenytoin sodium exerts an antiepileptic effect by stabilization of the neuronal membrane, and thus, prolongation of recovery of inactivated sodium channels. On high doses, phenytoin can also block the calcium influx during depolarization [14]. There was a 0% incidence of mortality of rats in all the groups, which correspond to the findings of Sonavane *et al*. [15].

From the above findings, it can be said that the EECM leaves reduced the extensor phase of convulsion in the dose-dependent manner [16] with maximum effect seen at the dose of 200 mg/kg.

The seizure is produced by the PTZ-induced seizure model resembles in the absence or petit mal seizure in human beings. A drug which causes either delay or complete abolition of convulsions in the PTZ-induced seizure model has reportedly got anticonvulsant activity [7].

In the present study, latency to convulsion in the control group was 670.33±25.438 seconds, and for the test groups B_1, B_2, and B_3, it was found to be 1305.2±37.926, 1610.5±28.402, and 2090±37.3 seconds, respectively, which shows an increase in the latency with increasing dose. In the standard group of diazepam (4 mg/kg), there was complete absence of convulsions within the specified time of 60 minutes of observation after subcutaneous PTZ injection [6], which corresponds to the findings of Khosla and Pandhi [17], for their standard group (diazepam, 4 mg/kg) and Sonavane *et al*. [15]. There were convulsions in all (100%) the animals in the control and test groups of EECM, which correlates with the findings of Abed [18]. The incidence of convulsions in the control group of Ambawade *et al*. [19] was also found to be 100%. The number of convulsions in all the test groups was found to be 1, whereas it was 1.677±0.2108 in the control group.

The average duration of convulsions in the control Group A and the test groups B_1, B_2, and B_3 was 14.583±0.3745, 9.5±0.4282, 7.667±0.5578, and 5.333±0.4944 seconds, respectively, which shows a decrease in the duration of convulsions with increasing dose. Animals were observed for 30 minutes to detect the onset of spasm and generalized tonic-clonic seizures and further up to 2 hr to detect mortality if any [20] animals devoid of seizures were considered as protected [21]. In this model of seizure, also it was found that there was no mortality of rats in any of the groups, and the maximum anticonvulsant activity was found with the 200 mg/kg dose.

Thus, from the above two seizure models of MES and PTZ, it can be concluded that EECM leaves have got an anticonvulsant effect in the increasing dose-dependent manner.

CONCLUSION

Thus, the present study showed that EECM leaves have got significant anticonvulsant effect, and it would be of great value to confirm these findings for different other doses and to find the exact mechanism of action with further studies on experimental animals and finally doing clinical studies to make it available for use in human beings in a commercial way. With the change in the global scenario toward the use of non-toxic herbal and ayurvedic medicines, development of more purified forms of *C. maximus* leaf extract should be emphasized for the control of seizures in the future.

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