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Original Research Article

Experimental Evaluation of Anticonvulsant Property of *Citrus sinenses* (Leaf Extract) in Mice

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Abstract: Leaf extracts of *Citrus sinensis* have been used in local folk medicine to treat neurological diseases. Its methanolic extracts have sedative activity. Diosmin and Hesperidine have been shown to posses' anti-inflammatory and analgesic actions. We in the present study tried to evaluate the anticonvulsant property of hydro-ethanolic leaf extracts of *Citrus sinensis*. It was evaluated in albino mice by Maximal Electric Shock (MES) induced convulsions in mice. The mice were closely observed for 30 minutes and the following parameters were recorded, Onset of convulsion, Duration of convulsion and Time of recovery Percentage of inhibition of seizures compared with standard drug Sodium valproate. With *Citrus sinensis* 50mg/Kg MES produces single convulsion in 50% of mice with mean onset of convulsion 11.33 sec, mean duration of convulsion 5.0 sec and mean time of recovery 5 sec. With *Citrus sinensis* 100 mg/kg 33.3% developed convulsion with mean onset of 15.5 sec, mean duration 2 sec and Mean time of recovery 2.5 sec. No mice developed convulsion with standard drug Sodium Valproate. With the increase in dose 50mg/kg and 100mg/kg compared with the control group have shown the time of onset of convulsion prolonged, duration reduced and time of recovery decreased and the values were statistically highly significant $P < 0.001$. The present study demonstrated that *Citrus sinensis* possesses anticonvulsant activity against Maximal Electric Shock induced seizures in mice. The anticonvulsant activity was better with dose of 100mg/Kg. However its anticonvulsant properties were inferior to the standard drug sodium valproate. It may act as an adjuvant therapy along with standard anticonvulsant drugs in seizure prevention.
Keywords: *Citrus sinensis*, Anticonvulsant, Sodium Valproate.

INTRODUCTION

Epilepsy is second most common neurological disorder after stroke in India. Epilepsy affects an estimated 7 million people in India[1]. Worldwide there is an estimated population of at least 65 million living with epilepsy. The annual incidence of epilepsy is nearly 50 per 100,000 population where as prevalence is approximately 700 per 100,000 population[2]. The WHO estimated that approximately 80% people with epilepsy live in developing countries & most of them do not get adequate medical treatment[1]. Among the two distinct epileptic seizures (partial & generalized) the bulk of cases in India belong to generalized seizures. Current therapy is symptomatic. Available drugs reduce seizure frequency in majority of patients, but only 40% are free of seizures despite optimal treatment. Neither an effective prophylaxis nor a cure of any of these disorders is available except neurosurgical resection of epileptic tissue in selected instances. Understanding the cellular & molecular mechanisms of the epilepsies will lead to improved therapies as well as new insights into brain structure & function[3].

Nearly 90% epilepsy cases are in low income countries including India[4]. Epilepsy is a neurological disorder characterized by paroxysmal dysrhythmia, seizure with or without bodily convulsions & sensory or psychiatric phenomenon. Antiepileptic drugs were used to prevent or interrupt seizures. Although ten new antiepileptic drugs were made available since the late 1980's refractoriness to treatment is still an important issue in epilepsy care. Only two – thirds of patients are seizure free under pharmacological treatment. The current therapy of epilepsy with modern antiepileptic drugs is associated with dose related side effects, chronic toxicity, as well as teratogenic effects.

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Several plants used for the treatment of epilepsy in different systems of traditional medicine have shown activity when tested in modern bioassays for the detection of anticonvulsant activity and many such plants are yet to be scientifically investigated[5]. *Citrus sinensis* common name sweet orange belong to Rutaceae family was widely cultivated all over the world. *Citrus sinensis* is a spreading evergreen tree with slender blunt spines which grows about 1.5 m. Its leaves are narrow and rounded at the base, 2 cm long, ovate oblong, pointed at the tip, green on the upper surface and deep green on the lower surface. Petioles are short with very narrow wings. Its inflorescence is small and white. It bears sweet smelling rounded fruits which are deep yellow to orange in colour [6]. Many substances have been isolated from *Citrus sinensis* leaves like glycosides, ruteosides, flavonoids, hesperidin, Diosmin, Triterpene, Linomin. Leaf extracts of *Citrus sinensis* have been used in Nigerian local folk medicine to treat neurological diseases. It also had sedative activity with methanolic extract. Diosmin and Hesperidin have been shown to have anti-inflamatory, antihypertensive, diuretic, analgesic properties. With these properties of *Citrus sinensis* we tried to evaluate its anticonvulsant activity in albino rats and compared it standard Drug Sodium valproate.

MATERIALS AND METHODS

The study was conducted in the Department of pharmacology at Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar. Prior approval was taken from Institutional Animal Ethical Committee (IAEC) of our institute before the study was undertaken. Twenty Four albino mice weighing between 21 to 36 grams were selected from the central animal house of our institution for the study. Male mice were chosen rather than female mice because the female mice have different response with changing hormonal status due to Oestrous cycle and may be pregnant. Mice were kept in groups of 3 to 4 per cage and were fed regularly in the animal house. Animals were maintained in the standard environmental condition at room temperature with 12 hr light and 12 hr dark cycles and with proper feed and water ad libitum as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines [7].

***Citrus sinensis* Hydro-ethanolic Leaf Extract:** Healthy leaves of *Citrus sinensis* were collected in the Warangal town during the winter season. The plant was identified by the department of botony of S.R.R College, Karimnagar. The leaves were spread out and dried in laboratory at room temperature until they broke easily by hand. Air dried plant material was ground with motor and pestle and extracted with ethanol. A crude hydro-ethanolic extract was obtained after grounded leaves were packed in Soxhlet apparatus. A green colour extract was obtained. The extract was concentrated in a rotary evaporator[8].

Sandard Drug Sodium valproate: (Encorate Inj) The dose usually used in animal is 150 mg/kg given Intraperitoneally 60 minutes before the induction of epilepsy[9]. The drug presently used in our study is manufactured by Sun Pharmaceuticals Industries Ltd, Acme Plaza, Andheri Kurla Road Andheri (East) Mumbai-400059.

The anticonvulsant property of hydro-ethanolic leaf extract was evaluated in albino mice by the following method. Maximal Electric Shock (MES) induced convulsions in mice.

Maximal electric shock (MES) induced convulsions in mice: Twenty four male albino mice with body weight between 21 and 36g were divided into 4 groups with six mice each. Each group is treated as follows:

- **Group I**: **CONTROL**; received distilled water 0.5ml p.o.
- **Group II**: **STANDARD**; Sodium valproate in a dose of 150mg/kg i.p.
- **Group III**: **TEST 1**; *Citrus sinensis* (hydro-ethanolic leaf extract) 50mg/kg p.o.
- **Group IV**: **TEST 2**; *Citrus sinensis* (hydro-ethanolic leaf extract) 100mg/kg p.o.

The albino mice of all groups were subjected to MES 60 minutes after the above treatment. Electroconvulsive meter with ear electrodes was used to deliver the stimuli. The electrical stimulus of 50mA, Voltage of 220v and 50Hz was delivered for 0.2 sec duration in the mice through ear-clip electrodes after ears were moistened with normal saline [10].

The mice were closely observed for 30 minutes and the following parameters were recorded:

1. Onset of convulsion
2. Duration of convulsion
3. Time of recovery

Percentage of inhibition of seizures with standard drug and test drug was calculated compared with control group. Statistical analysis was done using SPSS software for windows. Descriptive parameters were expressed as Mean, Standard deviation and Standard error. Student‘t’ test was done to evaluate statistical significance.

RESULTS

In the Group I Control (Distilled water 0.5ml/mice p.o.) It was observed that all the mice developed convulsion after MES with mean onset of convulsion 6.0 seconds, mean duration of convulsion 14.50 seconds and mean time of recovery 31 seconds. All the animals had a single episode of convulsion. These observations were tabulated in table – 1.

Table 1: Effect of Drugs on Maximal Electroshock (MES) Induced Convulsions in Mice

Drugs with doses	Time of onset of convulsion (sec)	Duration of convulsion (sec)	Time of recovery (sec)
Group I Control: Distilled water (0.5ml/mice)	6.0 ± 2.09	14.50 ± 3.01	31 ± 7.5
Group II Standard: Sodium valproate (150mg/kg)	0**	0**	0**
Group III Test 1: *Citrus sinensis* (50mg/kg)	11.33 ± 4.04*	5.0 ± 0.0*	5.0 ± 2.0*
Group IV Test 2: *Citrus sinensis* (100mg/kg)	15.50 ± 0.70**	2.0 ± 0.0**	2.5 ± 0.57**

Values represented as Mean ± S.D (n = 6) in each group * <0.05 Significant ** <0.001Highly Significant.

Group II Standard (Sodium valproate 150mg/kg i.p.) In this group when standard drug Sodium Valproate was given no mice developed convulsion in half an hour observation after MES. It was observed as 100 Percent inhibition of convulsion in this group. Group III: Test 1 (hydro-ethanolic leaf extract of *Citrus sinensis* 50mg/kg p.o.) When *Citrus sinensis* was administered prior to MES only three mice (50%) developed single convulsion with mean onset of convulsion 11.33 seconds, mean duration of convulsion 5.0 seconds and mean time of recovery 5 seconds. In this group 50 percentage of inhibition of convulsion was observed. The above findings were recorded in table – 1.

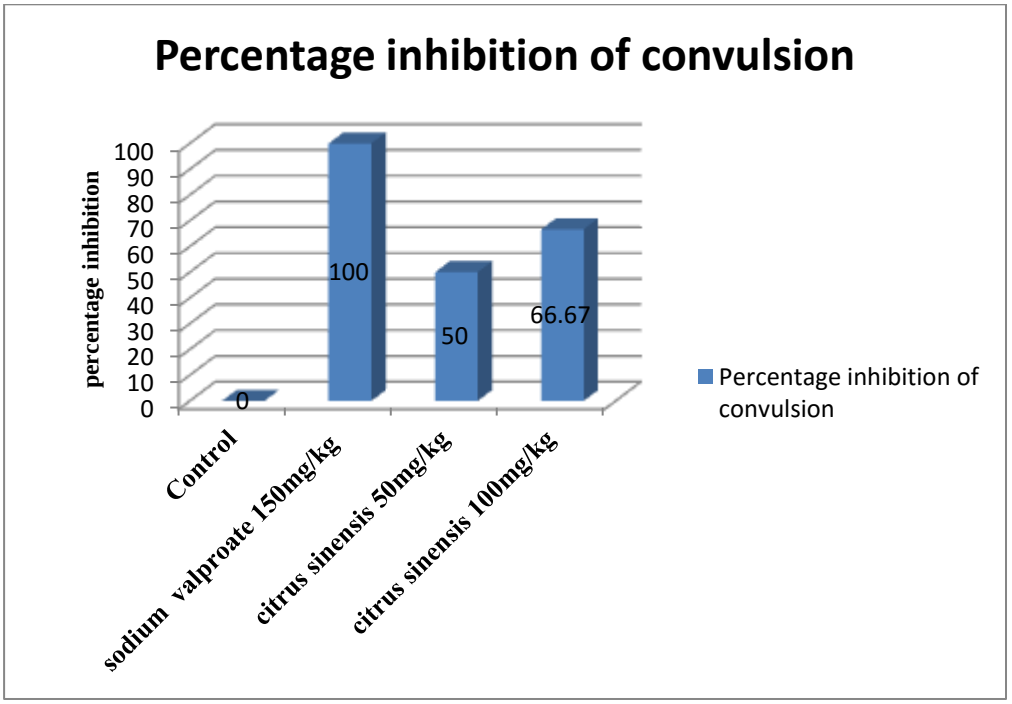

Fig-1: Effect of Drugs on Percentage Inhibition on Maximal Electro Shock (MES) Induced Convulsions in Mice

Group IV: Test 2 (hydro-ethanolic leaf extract of *Citrus sinensis* 100mg/kg p.o.) It was observed that two mice developed convulsion after MES with mean onset of convulsion 15.50 seconds, mean duration of convulsion 2 seconds and mean time of recovery 2.5 seconds From the above values, it was observed that all control group mice developed convulsions in contrast to standard group none developed the convulsions. Both test groups 'T1' and 'T2' have shown anticonvulsant effect but the effect was less than standard group. The anticonvulsant effect in test group II was higher than test group I. When the test group 'T1' and 'T2', were compared with control group, mean time of onset of convulsion was prolonged, mean duration of convulsion and mean time of recovery were decreased and the values were statistically highly significant $P < 0.001$ as seen in table 1.

DISCUSSION

The observations from the present study indicate that the hydro-ethanolic leaf extract of *Citrus*

sinensis has anticonvulsant activity against convulsions induced by Maximal Electric Shock. The anticonvulsant effect of hydro-ethanolic leaf extract of *Citrus sinensis* was more when it was given in the dose 100mg/kg than 50mg/kg. Therefore it is likely that hydro-ethanolic leaf extract of *Citrus sinensis* might possibly be producing anticonvulsant action by increasing level of (GABA), an inhibitory neurotransmitter in the central nervous system. However, several mechanisms are involved in anticonvulsant activity; it is very early at this stage of the study to say that anticonvulsant action appears to be due to increased levels of GABA. This could be a possible hypothesis, but certainly needs further investigation in future. It is well known fact that drugs which provide protection against seizures induced by Maximal Electric Shock are generally predicted to be effective against generalized tonic clonic seizures and cortical focal seizures. MES induced convulsions can be prevented effectively by drugs that inhibit voltage dependent Na channels, such as Phenytoin, Valproate [8]. Hydroethanolic leaf extract of *Citrus sinensis* has shown dose dependent anticonvulsant effect against MES induced convulsion. It may be useful in grandmal epilepsy also.

The multiplicity of mechanism of actions and the broad spectrum of anticonvulsant activity of hydro-ethanolic leaf extract of *Citrus sinensis* might be due to the presence of different active components. According to the previous study, phytochemical screening of the hydro-ethanolic leaf extract showed presence of glycosides (Apigenin and Diosmetin), Ruteosides (luteolin), Hesperidin and Diosmin[9]. *Citrus sinensis* leaf extract had sedative activity with methonolic extract at ED50 =38.48 ± 8.0 mg/kg[11]. Anticonvulsive effects of Hesperidin and the synergistic interaction between Hespiridin and Diazepam have been reported in the previous studies[12]. In conclusion, these findings suggest that hydro-ethanolic leaf extract of *Citrus sinensis* possesses anticonvulsant activity against both MES and PTZ induced seizures in mice. It is well known that very few drugs available like valproic acid which is effective against grandmal and petitimal epilepsy, however it is contraindicated in children (<3years) and pregnant woman because of its high hepatotoxic and tetratogenic activities respectively. No mortality and adverse effects were recorded in mice administered with leaf extract of *Citrus sinensis* at a dose of 5g/kg[6]. Hence it is prudent to know that at this stage hydro-ethanolic leaf extract of *Citrus sinensis* having sedative, anticonvulsant properties could be another substitute to the standard therapy. However its efficacy and adverse profile has to be screened thoroughly. This could explain the basis for the use of hydro-ethanolic leaf extract of *Citrus sinensis* in traditional medicine for management of epilepsy. Further research is required to isolate the active principles and elucidate the actual mechanisms involved in the anticonvulsant activity of this plant.

CONCLUSIONS

The present study demonstrated that *Citrus sinensis* possesses anticonvulsant activity against Maximal Electric Shock induced seizures in mice. The anticonvulsant activity was better with dose of 100mg/Kg as compared to 50mg/Kg. However its anticonvulsant properties were inferior to the standard drug Sodium valproate. It may only act as an adjuvant therapy along with standard anticonvulsant drugs in seizure prevention.

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