

Original Research Article

Evaluation of Antiepileptic Property of Citrus Sinensis (Leaf Extract) By Pentylenetetrazol (PTZ) Induced Convulsions in Mice

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Abstract: *Citrus sinensis* common name sweet orange belong to Rutaceae family was widely cultivated all over the world. Citrus plant contains large amounts of flavonoids which are found to be beneficial to human health. Leaf extracts of *Citrus sinensis* have been used to treat neurological diseases. We in the present tried to evaluate the anti-epileptic property of hydro-ethalonic leaf extracts of *Citrus sinensis*. The method utilized was the ability of *Citrus sinensis* to inhibit Pentylenetetrazole (PTZ) induced convulsions in mice. The mice were divided into 4 groups having 6 mice each with Group I receiving distilled water, Group II receiving Sodium valproate 150mg/kg i.p. Group III receiving *Citrus sinensis* hydro-ethalonic extracts of 50mg/Kg p.o. and Group IV *Citrus sinensis* 100mg/kg p.o. 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. In group I the mean duration to onset of convulsion was 339.83 sec, the duration of convulsion was 24.33sec and time to recovery was 561.5 sec. In group II none of the mice developed convulsions. In group III the mean duration to onset of convulsion was 2568.75 sec the duration of convulsion was 19.0 sec and time to recovery was 395.0 sec. similarly for group IV the mean duration to onset of convulsion was 2848.5 Sec, the duration of convulsion was for 10.75 sec and time to recovery was 313.75 sec. Hydro-ethalonic extracts of *Citrus sinensis* possess anti-epileptic properties. However its antiepileptic properties are lesser when compared to sodium valproate. It may be used as an adjuvant therapy along with standard anticonvulsant drugs in seizure prevention.

Keywords: Citrus sinensis, Hydro-ethalonic extracts, Anti-epileptic.

INTRODUCTION

Epilepsy is the chronic disorder of central nervous system manifested by recurrent seizures. The word epilepsy is derived from Greek word "Epilambanein" meaning "to seize upon" or "taking hold of" [1]. Epilepsy is second most common neurological disorder after stroke in India. Epilepsy affects an estimated 7 million people in India [2]. 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 whereas prevalence is approximately 700 per 100,000 population [3]. The WHO estimated that approximately 80% people with epilepsy live in developing countries and most of them do not get adequate medical

treatment. Among the two distinct epileptic seizures (partial and 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 and molecular mechanisms of the epilepsies will lead to improved therapies as well as new insights into brain structure and function [4].

Nearly 90% epilepsy cases are in low income countries and in India, the total cost has been shown to

be equivalent to 0.5% of the Gross national product [5]. 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 [6]. *Citrus sinensis* common name sweet orange belong to Rutaceae family was widely cultivated all over the world. Many substances have been isolated from *C. Sinensis* leaves like glycosides, ruteosides, flavonoids, hesperidin, Diosmin, Triterpene lineman. Leaf extracts of *C.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-inflammatory, antihypertensive, diuretic, analgesic properties. With this background we tried to evaluate the anti-epileptic properties of *Citrus silences* by Pentylene tetrazol (PTZ) induced convulsions in mice.

MATERIALS AND METHODS

The study was conducted in the department of pharmacology at Calmed. Anand Rao Institute of Medical Sciences, Karimnagar. Prior approval was taken from Institutional Animal Ethical Committee (IAEC) of 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 estrous cycle and may be pregnant. Further the female mice were known to eliminate several anti-epileptic drugs less rapidly than male

mice does. 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].

Drugs

- a) *Citrus Sinensis* hydro-ethanolic Leaf Extract
- b) Sodium valproate

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].

Soxhlet extractor:

Soxhlet apparatus was used to obtain ethanolic extract of *Citrus sinensis* leaf extract. It contains a distilling pot where the solvent is placed, a distillation path through Which the solvent vapour travels, a condenser, a thimble in which the material to be extracted is kept, and a siphon through which the extracted material reaches the Solvent for another cycle.

Sodium valproate: (Encorate Inj):

The dose usually used in animal is 150 mg/kg given intraperitoneally 60 minutes before the induction of epilepsy. The drug presently used in our study is manufactured by Sun Pharmaceuticals Industries Ltd, Acme Plaza, Andheri Kurla Road Andheri (East) Mumbai-400059.



Fig-1: Dried leaves of *Citrus sinensis*



Fig- 2: Intraperitoneal injection in mice

RESULTS

In this Group I all mice developed clonic tonic convulsion when PTZ was administered. The mean time of onset of convulsion was 339.83 seconds, mean duration of convulsion 24.33 seconds, and mean time of recovery 561.50 seconds. Here all the mice experienced single episode of convulsion and no mice died. Group II: Standard (Sodium valproate 150mg/kg i.p.) When the standard drug was given, the mice remained normal and no mice developed convulsion in one hour observation after PTZ

administration . 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.) It was observed that four out of six mice developed single convulsion with mean time of onset 2568.75 seconds, mean duration of convulsion 19.0 seconds and mean time of recovery 395 seconds. It was observed as 33.33 percentage inhibition of convulsion compared to control Group IV: Test 2 (hydro-ethanolic leaf extract of *Citrus sinensis* 100mg/kg p.o.) It was observed that four out of six

mice developed single convulsion with mean time of onset of convulsion 2848 seconds, mean duration of convulsion 10.75 seconds and mean time of recovery

313.75 seconds. It was observed as 33.33 percentage inhibition of convulsion compared to control.

Table-1: Effect of Drugs on Pentylentetrazole (PTZ) Induced Convulsions in Mice

Drugs with doses	Time of onset of convulsion (sec)	Duration of convulsion (sec)	Time of recovery (sec)	Percentage inhibition of convulsion (%)
Group I Control: Distilled water (0.5ml/mice)	339.83 ± 137.04	24.33 ± 3.38	561.50 ± 133.7	0
Group II Standard: Sodium valproate (150mg/kg)	0**	0**	0**	100
Group III Test 1: <i>Citrus sinensis</i> (50mg/kg)	2568.75 ± 96.98	19.0 ± 11.8*	395.50 ± 12.90*	33.33
Group IV Test 2: <i>Citrus sinensis</i> (100mg/kg)	2848.50 ± 47.84*	10.75 ± 0.95*	313.75 ± 11.08*	33.33

Values represented as Mean ± SD, n=6 in each group *P < 0.05 – Significant, **P < 0.001 – highly Significant

From the above results, it was observed that all control group mice developed convulsions in contrast to standard group none developed convulsions. Both the test groups Group III and Group IV have shown anticonvulsant effect but the effect was less than standard group. The anticonvulsant effect of hydro-ethanolic leaf extract of *Citrus sinensis* was same in both test groups. However, 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 as seen in table 1.

DISCUSSION

The present study indicate that the hydro-ethanolic leaf extract of *Citrus sinensis* has anti-epilpetic activity against convulsions induced Pentylentetrazole. 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. The convulsions induced by Pentylentetrazole are useful in identifying drugs that are effective against absence seizures. Hydro-ethanolic leaf extract of *Citrus sinensis* has shown anticonvulsant effect in PTZ induced convulsions in mice. Hence, it may be useful in absence seizures. Two mechanisms have been put forth for PTZ induced convulsions, either by inhibiting Gamma Amino

Butyric Acid (GABA) pathway in CNS or by increasing the central Nor-adrenergic activity. GABA is widely implicated in epilepsy, inhibition of GABA-ergic neurotransmission or activity has been shown to promote and facilitate seizures, while enhancement of GABA-ergic neurotransmission is known to inhibit or attenuate seizures [9]. Moreover, some studies indicated that PTZ diminishes the GABAergic tone [10] probably by a competitive antagonist action on the Benzodiazepine receptors BZD receptors [11] 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. Other mechanisms may also be 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 [8].

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. Two important flavonoids Hesperidin (Hsd) and its aglycone, Hesperetin (Hst) from citrus have been shown to produce important biological properties. They have

been shown to particularly have antidepressant effects [12]. Citrus sinensis leaf extract had sedative activity with methanolic extract at $ED_{50} = 38.48 \pm 8.0$ mg/kg [13]. Anticonvulsive effects of hesperidin and the synergistic interaction between hesperidin and diazepam have been reported in the previous studies [14]. 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 petitmal epilepsy, however it is contraindicated in children (<3years) and pregnant woman because of its high hepatotoxic and teratogenic activities respectively. No mortality and adverse effects were recorded in mice administered with leaf extract of Citrus sinensis at a dose of 5g/kg [15]. 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 therapy. However its efficacy and adverse profile needs to be further 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.

CONCLUSION

Citrus sinensis possesses anticonvulsant activity against Pentylentetrazole induced seizures in mice. Although its antiepileptic properties are inferior to standard drugs, however the profile of antiepileptic activity of Citrus sinensis against PTZ induced seizures in mice suggests its potential utility in the management of epilepsy in human beings.

Conflict of Interest: None

Source of support: Nil

Ethical permission: Obtained

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