National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

Evaluation of anticonvulsant activity of ethanolic extract of leaves of *Ocimum sanctum* (tulsi) in albino rats

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Received: March 06, 2017; **Accepted:** March 22, 2017

ABSTRACT

Background: Epilepsy is a chronic neurological disorder. *Ocimum sanctum* (OS) (tulsi) has analgesic, anticancer, antiasthmatic, antidiabetic, antifertility, hepatoprotective, hypotensive, hypolipidemic, anti-inflammatory, antioxidant, immune modulatory, and antistress properties. **Aims and Objectives:** The objective was to evaluate the anticonvulsant effect of ethanolic extract of leaves of OS and to compare the anticonvulsant effect with the standard sodium valproate in electrically and chemically induced epileptic animal models. **Materials and Methods:** A total of 60 albino rats (150-200 g) of male sex were randomly selected. They were divided into five groups (per model) of six rats each. Control group received normal saline (0.35 ml/100 g), standard group sodium valproate (300 mg/kg), and the test groups were given OS at three different doses (1.75, 4.25, and 8.5 mg/kg). The anticonvulsant activity was screened using maximal electroshock seizure (MES) model and pentylenetetrazole (PTZ) model. Results were analyzed by ANOVA followed by *post-hoc* turkey's test. **Results:** The ethanolic extract of OS leaves at doses of 4.25 and 8.5 mg/kg has shown significant anticonvulsant by decreasing the duration of tonic hind limb extension activity in MES model and by prolonging the duration of seizure latency in PTZ model. **Conclusion:** The ethanolic extract of OS leaves possess anticonvulsant activity which can be comparable with the standard sodium valproate.

KEY WORDS: *Ocimum sanctum*; Anticonvulsant; Maximal Electroshock Seizure Model; Pentylenetetrazole Model; Sodium Valproate

INTRODUCTION

Epilepsy is a heterogeneous symptom complex, a chronic disorder characterized by recurrent seizures affecting approximately 1% of the world's population and second most common neurological disorder after stroke.[1] Seizure is defined as abnormal, disordered discharges of brain nerve cells resulting in a temporary disturbance of sensory, motor or mental function.[2] The incidence rate is estimated from 40 to 60/1,000,000 population/year.[3] Approximately 5% of general population experience at least one seizure, excluding febrile seizures.[4]

The conventional antiepileptic drugs (AED) have contributed significantly in the management of epilepsy. About 60-70% of patients with epilepsy achieve control of their seizures with the use of AEDs. However, in nearly one-third of epileptic patients, the seizure control is not achieved even with continued use of AEDs.[5] Moreover, these AEDs are associated with dose-related side effects, chronic toxicity as well as teratogenicity.[6] As such, there is an increased need to discover drugs which are effective in refractory

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2017.7.0308122032017	

epilepsy having lesser adverse effects. Medicinal plants offer important sources of new chemical substances with therapeutic benefits besides being safe, easily available, effective, and economical.[7]

Ocimum sanctum (OS) commonly known as holy basil or tulsi means "one that is comparable" belongs to family *Lamiaceae*. It is regarded in Ayurveda as a kind of "elixir of life" and believed to promote longevity.[8] It enhances general health and well-being, having positive overall effects on the body and mind.[7] The plant has analgesic, anticancer, antiasthmatic, antidiabetic, antifertility, hepatoprotective, hypotensive, hypolipidemic, anti-inflammatory, antioxidant, immune modulatory, and antistress properties.[9] Here in this study, an effort was made to investigate the anticonvulsant effect of ethanolic extract of leaves of OS and to compare the anticonvulsant effect with the standard sodium valproate in electrically and chemically induced epileptic animal models as it will be worth evaluating the alternative forms of medicines which can be used for its treatment.

MATERIALS AND METHODS

Drugs and Chemicals

Test drug (OS, ethanolic extract, used at doses of 1.75, 4.25, and 8.5 mg/kg)[10] procured from Himalaya Health Centre, Bengaluru. Sodium valproate at 300 mg/kg[11] dose was obtained from Sun Pharma Laboratories Ltd., Mumbai. Pentylenetetrazole (PTZ) at 80 mg/kg[12] dose was obtained from HiMedia Laboratory, Mumbai. Normal saline at 0.35 ml/100 g dose used as control and as a vehicle, to suspend sodium valproate and OS extract. Drugs and vehicles were administered by intraperitoneal (IP) route.

Animals

Healthy Swiss albino male rats weighing 150-200 g were included in the study. Pregnant and diseased animals and animals used in other experiments were excluded from this study. The experiments were conducted in research hall, Department of Pharmacology, AIMS, B G Nagar, between 9:00 am and 4:00 pm A total of 60 animals ($N = 60$) were used. They were divided into five groups (per model) of 6 animals each (Table 1). The experimental study was approved by the Institutional Animal Ethical Committee.

Methods

Maximal electroshock seizure (MES) model

Swiss albino rats weighing 150-200 g were used. Seizures were evoked by supramaximal electroshock stimulation of 150 mA, 50 Hz for 0.2 s using electroconvulsiometer through transauricular electrodes. Seizures pass through phases of tonic flexion and extension of limbs, clonus period. The abolition of tonic hind limb extension (THLE) is taken as an index of anticonvulsant activity.[13] OS along with control and standard drugs were administered to respective groups of rats 30 min before application of electroshock. The duration of THLEs was noted.

Table 1: The animals were divided into five groups with six animals in each group for both models

Groups (N=6)	Treatment	
I	Control - normal saline	0.35 ml/100 g
II	Sodium valproate	300 mg/kg
III	OS	1.75 mg/kg
IV	OS	4.25 mg/kg
V	OS	8.50 mg/kg

N=Number of animals in each group. OS: *Ocimum sanctum*

PTZ model

PTZ is a CNS stimulant. PTZ (80 mg/kg) dissolved in normal saline was given 30 min after injecting the control, standard and test drug for a respective group of rats IP, which produces excitement, myoclonic jerks, and clonic seizures. The onset of convulsions was observed until 30 min after administering PTZ. Prolongation of the duration of seizure latency was taken as an index of protection indicating the anticonvulsant activity of the test compound.[14]

Statistical Analysis

The effect of OS in different doses in both MES and PTZ seizure induction models was expressed as mean ± standard error mean. Data were analyzed using ANOVA followed by *post-hoc* turkey's test. $P < 0.05$ was considered significant.

RESULTS

MES Model (Table 2)

The THLE phase was abolished in the standard group. When control group is compared with OS-1.75 mg/kg, the difference between the mean of THLE is not significant statistically, but when compared with OS (4.25 and 8.5 mg/kg), the difference between the mean of THLE is significant statistically ($P < 0.05$). This shows that the OS extract at doses 4.25 and 8.5 mg/kg has significantly reduced the mean duration of THLE when compared to control group indicating that it possesses anticonvulsant property.

PTZ Model (Table 3)

When control group is compared with standard, there was significant prolongation in mean duration of seizure latency. When control group is compared with a test drug, there was significant prolongation in mean duration of seizure latency ($P < 0.05$) at two doses of OS (4.25 and 8.5 mg/kg) but not at

Table 2: Effect of ethanolic extract of *Ocimum sanctum* leaves on MES-induced seizures in albino rats

Groups	Treatment	THLF	THLE	Clonus	Stupor	Postictal depression
I	Control (NS- 0.35 ml/100 g)	3.93±0.17	7.13±0.61	10.36±0.51	272.50±39.01	245.33±28.33
II	Standard (sodium valproate - 300 mg/kg)	8.23±0.4**	0.00±0.00**	12.68±0.40**	290.87±28.73	289.16±26.24**
III	OS (1.75 mg/kg)	4.26±0.25	6.86±0.80	19.20±0.48**	125.0±16.36**	239.83±36.86
IV	OS (4.25 mg/kg)	4.50±0.21	5.98±0.6**	16.61±0.21**	103.5±24.64**	192.0±27.67**
V	OS (8.50 mg/kg)	5.28±0.34*	3.96±0.3**	12.05±0.41**	85.16±12.60**	115.5±25.11**

All values are mean±SEM, statistical analysis by one-way ANOVA followed by Turkey's *post-hoc* test, *P<0.05, **P<0.01. SEM: Standard error mean, THLF: Total hind limb flexion, MES: Maximal electroshock seizure, THLE: Total hind limb extension

Table 3: Effect of ethanolic extract of *Ocimum sanctum* leaves on PTZ induces seizures in albino rats

Group	Treatment	Seizure latency or onset (seconds)	Duration of myoclonic jerks (seconds)
I	Control (NS - 0.35 ml/100 g)	212.16±15.10	703.3±20.24
II	Standard (sodium valproate - 300 mg/kg)	324.83±29.44**	442.83±28.56**
III	OS (1.75 mg/kg)	220.0±27.80	666.16±34.73
IV	OS (4.25 mg/kg)	275.16±25.19**	503.66±31.65**
V	OS (8.50 mg/kg)	299.33±25.84**	425.0±29.26**

All values are mean±SEM, statistical analysis by one-way ANOVA followed by Turkey's *post-hoc* test, **P<0.01. SEM: Standard error mean, PTZ: Pentylenetetrazole, OS: *Ocimum sanctum*

the dose of 1.75 mg/kg. This shows that OS at doses of 4.25 and 8.5 mg/kg, prolong the mean duration of seizure latency significantly indication that it possesses anticonvulsant property.

DISCUSSION

Epilepsy is a very common disorder affecting 1% of the world's population. The incidence in India is around 20-50 cases/lakh population.[15] The anticonvulsants available is neither effective universally nor invariably safe. Due to long-term therapy with unwanted effects of many drugs the compliance with medication is very minimal. This study is to evaluate the anticonvulsant activity of ethanolic extract of leaves of OS in MES and PTZ seizure-induced rats.

MES and PTZ tests are the best-validated method for assessment of AED in human generalized tonic–clonic seizures and absence seizures, respectively, among the tests used for evaluation of anticonvulsant activity.[16]

In our study, it was found that treatment with OS extracts (4.25 and 8.5 mg/kg) in rats significantly reduced THLE in MES-induced seizure model. MES-induced seizures are abolished by the drugs that block voltage-gated Na^+ channels such as phenytoin and carbamazepine or by the drugs that block N-methyl-D-aspartate receptors like felbamate.[17] Protection of OS extract against THLE indicates that the drug possesses the ability to inhibit or abolish the spread of seizures within the brain suggesting the presence of an anticonvulsant compound in the extract. Similarly, it was found that treatment with OS extracts (4.25 and 8.5 mg/kg) significantly prolong the mean duration of seizure latency in PTZ seizure model. PTZ induced convulsions are prevented by the drugs that block T-type Ca^{2+} current in thalamus like sodium valproate or the drugs which possess gamma-aminobutyric acid ($GABA_A$) agonistic like diazepam.[17] Protection of OS extract against PTZ induced seizure suggests a possible interaction with GABA-ergic neurotransmission indicating the presence of an anticonvulsant compound in the extract. Sakina et al.[18] a similar study states that OS prolongs the phenobarbitone induced sleeping time and also has antiepileptic activity against MES and PTZ induced seizures.

CONCLUSION

In conclusion, the results suggest that the ethanolic extract of leaves of OS possesses anticonvulsant activity which can be compared with the standard sodium valproate in electrically and chemically induced epileptic animal models. Further studies are required to elucidate the exact mechanism by which this plant acts as an anticonvulsant agent.

ACKNOWLEDGMENTS

The authors are grateful to Himalaya Health Care, Bengaluru, for providing the ethanolic extract of leaves of OS.

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How to cite this article: Manu G, Padmanabha ST, Chandrakantha T, Ravishankar M. Evaluation of anticonvulsant activity of ethanolic extract of leaves of *Ocimum sanctum* (tulsi) in albino rats. Natl J Physiol Pharm Pharmacol 2017;7(7):762-765.

Source of Support: Nil, **Conflict of Interest:** None declared.