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(Research Article)

Evaluation of Antiepileptic Activity of Methanolic Extract of *Celastrus paniculatus* Willd Whole Plant in Rodents

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ABSTRACT

The objective of the present study was to evaluate the antiepileptic activity of the methanolic extract of Celastrus paniculatus Willd. whole plant(MECP) using different in vivo experimental models. Epileptic seizure challenged animals treated with methanolic extract of Celastrus paniculatus Willd whole plant (MECP) at doses 200 mg/kg,400 mg/kg and 600 mg/kg showed reduction of Isoniazid (INH)- and Pentylenetetrazole (PTZ)- induced epileptic seizure. Onset of seizure was found to increase and the extension phase of seizure was found to be lowered in the extract treated animals as compared to control group. The methanolic extract of Celastrus paniculatus Willd. whole plant(MECP) significantly delayed the onset of epileptic seizure induced by INH and significantly reduced the duration(in sec) of tonic hind limb extension phase of PTZ-induced epileptic seizure. Thus the overall results suggest that the methanolic extract of Celastrus paniculatus Willd. whole plant(MECP) contains some active principles which may possess significant antiepileptic activity.

Key Words: *Celastrus paniculatus* Willd., Antiepileptic seizure, Isoniazid, Pentylenetetrazole

INTRODUCTION

Epilepsy is one of the most common affliction of human beings with a prevalence rate of approximately 1% of the total population[1]. Epilepsy (Greek- to seize) is a common chronic neurological disorder[2]. It is characterized predominantly by recurrent and unpredictable interruptions of normal brain function, called epileptic seizures. It is a variety of disorders reflecting underlying brain dysfunction that may result from different causes[3]. The current therapeutic treatment of epilepsy with modern antiepileptic drugs is associated with side-effects, dose-related and chronic toxicity, teratogenic effects and approximately 30% of the patients continue to have seizures with current antiepileptic drug therapy[4,5]. Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be alternative source for the discovery of antiepileptic drugs with novel structures and better safety and efficacy profiles[6]. Now, various phytochemical and pharmacological studies have been carried out on these antiepileptic plants[7]. Thus, it is necessary to investigate for an antiepileptic agent that is highly efficacious as well as safe in terms of drug related toxicity. The aim of treating an antiepileptic drug is not only to abolish the occurrence of seizures but also to lead a self sustained life. Hence, search should continue to develop newer, more effective, and safer neuroprotective agents for treatment of epilepsy.

In the present study, we selected a plant namely *Celastrus paniculatus* Willd (Celastraceae), which is a large, woody, climbing shrub, distributed almost all over India upto an altitude of 1800m and is known for its ability to improve memory [8]. Ayurveda, the ancient Indian traditional system of medicine has used the plant seed for prevention of various diseases[9]. The seed oil is bitter, thermogenic, and intellect promoting and is useful in abdominal disorders , beri-beri and sores[10]. The bark is abortifacient, depurative and a brain tonic. The leaves are emmenagogue and the leaf sap is a good antidote for opium poisoning. Earlier the plant has been pharmacologically studied for its analgesic and anti-inflammatory activity[11,12], anti-arthritic activity[13], antifertility activity[14], wound healing activity[15], antimalarial activity[16], antibacterial activity[17], cardiovascular activity[18], antioxidant activity[19,20], hypolipidemic activity[21] etc. Though the oil obtained from the seeds of the plant had been studied to possess sedative and anticonvulsant activity [22] in the past, no one had previously studied the antiepileptic activity of the whole plant. Since there is dearth of scientific data proving the antiepileptic activity of the whole plant, the present study was carried out to investigate the antiepileptic activity of the methanolic extract of *Celastrus paniculatus* Willd. whole plant(MECP) against Isoniazid-

induced epileptic seizures and Pentylenetetrazole-induced epileptic seizures.

MATERIALS AND METHODS

Collection of Plant Materials

The whole plant of *Celastrus paniculatus* Willd was collected from Tirumala hills, Tirupathi, Andhra Pradesh, India. The plant was identified and authenticated by Dr. K.Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh,India and voucher specimen has been deposited in the departmental herbaria.

Preparation of Extract

The whole plant species was collected and then dried under shade for a period of four weeks. The dried plant material (500g) was milled to a fine powder using commercial laboratory blender. The dried powder (300g) was extracted in a Soxhlet extractor with Methanol. The extraction was continued until the solvent in the thimble became clear. After complete extraction, the extract was filtered and the solvent was distilled off. Then it was concentrated at 40^0 C under reduced pressure using Buchi R-153 Rotavapour to obtain the dry residue. The yield of the crude methanol extract was 30g. The extract was the stored in desiccators until use.

Drugs and Chemicals

Drugs and chemicals used in the study were obtained commercially and were of analytical grade. Phenytoin and Pentylenetertrazole (Sigma, USA), Isoniazid(Novartis India ltd., Hyderabad, India), DMSO and methanol (Hi-pure fine chem Industries, Hyderabad, India).

Animals

For the screening of antiepileptic activity, studies were carried out using Swiss albino mice (18-22g) and Wistar albino rats (150-180 g) of either sex. All the animals were procured from Sainath Agencies, Hyderabad, India for experimental purpose. After procuring, all the animals were acclimatized for 7 days and housed in groups of six under standard laboratory conditions, like room temperature 26 ± 2^0C, relative humidity 45-55% and light/dark cycle of 12h. All the animals were provided with synthetic standard diet and water was provided *ad libitum* under strict hygienic conditions. Animal experimentation protocols are approved by Institutional Animal Ethical Committee (IAEC) of GSN Pharmaceuticals Pvt. Ltd., Kukatpally, Hyderabad, India.

Preliminary Phytochemical Screening

The preliminary phytochemical investigations were carried out with the methanolic extract of *Celastrus paniculatus* Willd. whole plant for qualitative identification of phytochemical constituents using standard conventional protocol. All the chemicals and reagents used were of analytical grade [23].

Acute Toxicity Study

The acute toxicity of the methanolic extract of Celastrus panicualtus Willd. whole plant was determined as per the OECD guideline no. 423 (Organization for Economic Co-operation and Development). It was observed that the test extract was not mortal even at a dose of 2000 mg/kg body weight. Hence, 200 mg/kg, 400 mg/kg and 600 mg/kg doses were selected for further study.

Antiepileptic Activity

Isoniazid-induced epileptic seizure model

Swiss albino mice(18-22 g) of either sex were divided into V groups of six animals each. Group I served as control and was administered 10% (w/v) DMSO (5 ml/kg, p.o). Group II was administered phenytoin (5 mg/kg, i.p) on the first day alone and served as standard group. Groups III, IV and V were treated with different doses of methanolic extract of *Celastrus paniculatus* Willd. whole plant (200 mg/kg, 400 mg/kg and 600 mg/kg, p.o) respectively once daily for seven days. On the seventh day 60 min after control, standard and extract administration into respective groups, Isoniazid (300 mg/kg s.c) was administered. The following parameters were recorded during test session of initial, 30 min and upto 24 h.The animals were observed for latency(onset of epileptic seizure), status of animal after 30 min, status of animal after 24 h period and the percentage [24].

Pentylenetetrazole-induced epileptic seizure model

The seizure was induced by administration of pentylenetetrazole (80 mg/kg i.p) to Wistar albino rats of either sex. The rats showing response were divided into IV groups of six animals each. Group I was administered 10%(w/v) DMSO (5 ml/kg, p.o) which served as control. Group II was allotted for standard drug where animals were treated with Phenytoin (25 ml/kg, i.p) [25]. Group III and IV were treated with different doses of methanolic extract of *Celastrus paniculatus* Willd whole plant (200mg/kg and 400 mg/kg ,i.p) respectively. The doses were given for seven days as multiple dose studies. Animals were fasted overnight prior to the test but water was supplied *ad libitum*. Drug pretreatment was given 1 hr prior to the administration of pentylenetetrazole, then animals were placed in plastic cages individually and were observed for the duration of tonic flexion, tonic extension, clonus, death or recovery and percentage protection of the animal, initially for 30 min and later upto 24 hr period.

Statistical Analysis

The results were expressed as mean ± SEM and statistically analyzed by one way ANOVA followed by Tukey- krama test. The results obtained were compared with the control group. p values < 0.05 were considered to be statistically significant (p denotes probability).

RESULTS

Phytochemical screening

The qualitative analysis of MECP showed the presence of various phytoconstituents such as alkaloids, steroids, tannins, flavonoids, glycosides, sesquiterpenes, proteins and amino acids (Table 1).

Table1: Results of Phytochemical Screening of the methanolic extractof *Celastrus paniculatus* Willd. whole plant (MECP)

Phytoconstituents	MECP
Reducing sugars	-
Alkaloids	+
Tannins	+
Flavonoids	+
Glycosides	+
Phytosterols	+
Triterpenoids	+
Proteins and Amino acids	+

+ : Positive result ; - : Negative result

Antiepileptic Activity

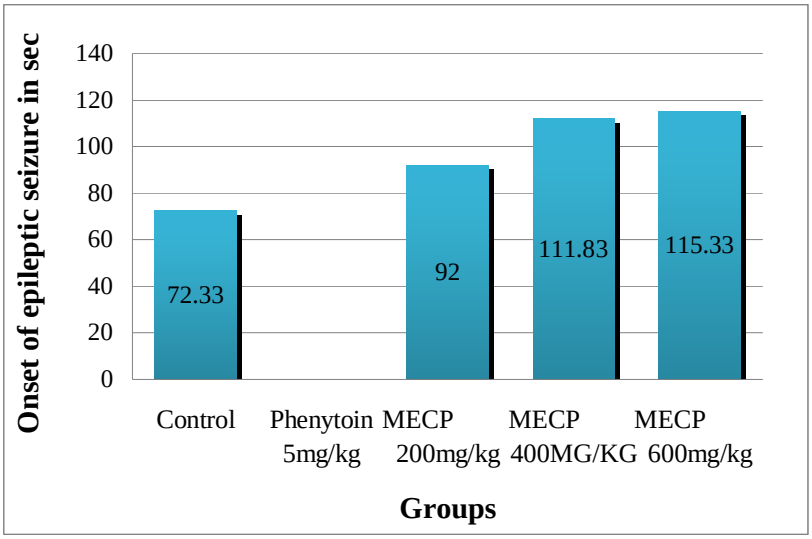
INH- induced epileptic seizure in mice

The antiepileptic activity of the methanolic extract of *Celastrus paniculatus* Willd. whole plant using INH-induced epileptic seizure in mice is expressed in (Table 2). In this test the onset of epileptic seizure in the control group occurred at 72.33±0.75 sec and the extract treated groups at doses 200 mg/kg, 400 mg/kg and 600 mg/kg significantly ($p< 0.00$) delayed the onset of epileptic seizure time to 92.0±0.57 sec, 111.83±0.79 sec and 115.33±0.49 sec respectively (Graph 1). The standard antiepileptic drug, phenytoin 5mg/kg i.p totally abolished the effects of INH-induced epileptic seizures in mice with 100% protection, whereas the various doses of MECP at 200mg/kg, 400mg/kg and 600 mg/kg showed the percentage protection of 50%, 33% and 50% respectively.

Table 2: Effect of methanolic extract of *Celastrus paniculatus* Willd. whole plant on INH-induced epileptic seizure in Mice

Groups	Treatment	Dose (kg^{-1})	Latency(onset of epileptic seizure in sec)	Status of animal after 30 min(no.of animals alive)	Status of animal after 24 hr (no.of animals alive)	Percentage protection
I	Control(10% w/v DMSO) *p.o*+INH *s.c*	5ml+300mg	72.33±0.75	4	1	16
II	Phenytoin *i.p* + INH *s.c*	5mg+300 mg	NIL**	6	6	100
III	MECP *p.o*+INH *s.c*	200mg+300mg	92.0±0.57	4	3	50
IV	MECP *p.o*+INH *s.c*	400mg+300mg	111.83±0.79*	3	2	33
V	MECP *p.o*+INH *s.c*	600kg+300mg	115.33±0.49*	3	3	50

*Values are expressed as Mean±SEM(Standard Error Mean); Values are calculated as compared to control using one way-ANOVA followed by Tukey-kramer test, *indicates p<0.05,**indicates p<0.01vs. control;n=6; p.o.:per oral ; s.c.:subcutaneous ; i.p.: intraperitoneal route of administration.*



Graph-1: Effect of MECP on Onset of epileptic seizure in INH-induced epileptic seizue in Mice

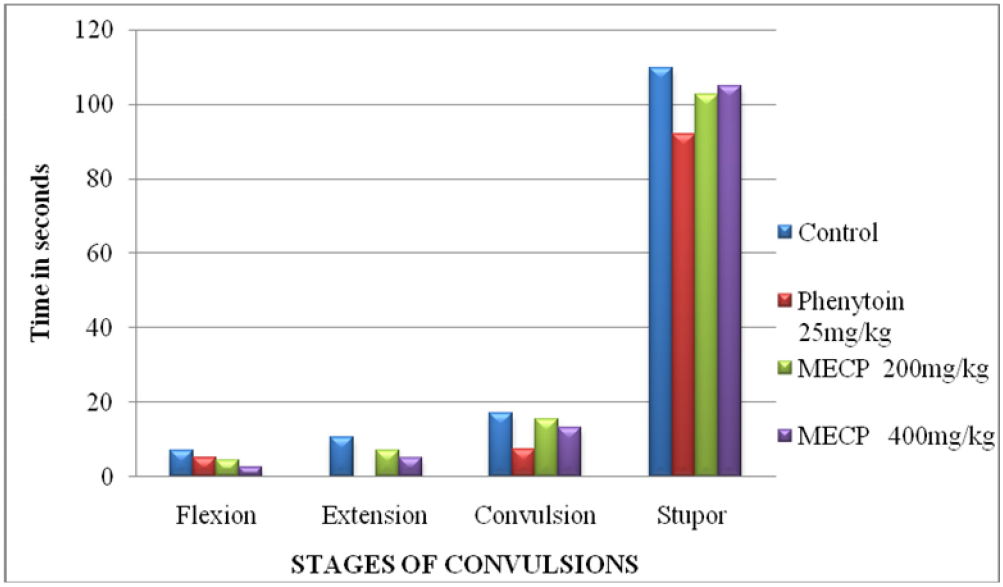
PTZ-induced epileptic seizure in rats

Table 3 showed the antiepileptic activity of the methanolic extract of *Celastrus paniculatus* Willd. whole plant using PTZ-induced epileptic seizure in rats. MECP exhibited significant antiepileptic activity by lowering the duration of extension phase when compared with the control group. The duration of tonic and hind limb extension in rats with the extract treated groups at doses 200 mg/kg and 400 mg/kg was 7.16 ± 0.67 and 5.0 ± 0.99 respectively. The methanolic extract of *Celastrus paniculatus* Willd. whole plant at different doses was comparable ($p<0.00$)with that produced by standard drug Phenytoin 25 mg/kg. The different stages of epileptic seizure *vs* Time in seconds is shown in Graph 2.

Table 3: Effect of methanolic extract of Celastrus paniculatus Willd. whole plant on PTZ- induced epileptic seizure in Rats

Groups	Treatment	Dose (kg^{-1})	Time in seconds of various phases of convulsions				Recovery	% protection
			Flexion	Extension	Convulsion	Stupor		
I	Control(10% DMSO) *p.o*+ PTZ *i.p*	5ml+80 mg	7.16±0.47	10.6±0.34	17.1±0.30	109.6±0.494	192.24	0
II	Phenytoin *i.p*+ PTZ *i.p*	25mg+80mg	5.16±0.36	0**	7.5±0.76**	91.8±0.401**	93.5	100
III	MECP *i.p*+PTZ *i.p*	200mg+80 mg	4.33±0.66*	7.16±0.67*	15.33±0.42	102.5±0.428*	156.79	70.93
IV	MECP *i.p* +PTZ *i.p*	400mg+80 mg	2.66±0.49**	5.0±0.99**	13.16±0.47*	104.8±0.421	134.51	83.04

*Values are expressed as Mean±SEM (Standard Error Mean); Values are calculated as compared to control using one way-ANOVA followed by Tukey-kramer test, *indicates p<0.05, **indicates p<0.01 vs. control ;n=6; p.o.:per oral ; i.p.: intraperitoneal route of administration.*



Graph 2: Effect Of MECP on stages of convulsions in PTZ-induced epileptic seizure in Rats

DISCUSSION

Currently available antiepileptic drugs are able to efficiently control epileptic seizures in about 50% of the patients, another 25% may show improvement, whereas the remaining 25% of antiepileptic drugs do not benefit significantly. Furthermore, undesirable side effects from the drugs used clinically often render treatment difficult so that a demand for new types of antiepileptics exists. One of the approaches to search for new antiepileptic drugs is to investigate the naturally occurring compounds, which may belong to new structural classes [26].

Isoniazid is used widely for the treatment and chemoprophylaxis of Tuberculosis, but can have serious effects on the central nervous system causing seizures and comas [27]. The factor responsible for INH-induced epileptic seizure is the decrease of GABA below a critical level in some neurons. Perhaps the decrease in the amount of GABA stored presynaptically causes a reduction in the amount of GABA released by nerve impulses. Hence, the GABA receptors are regulated at the level of maximal sensitivity in order to maximize the action of GABA. Phenytoin treated group showed 100% protection of the animals. INH-induced epileptic seizure in mice significantly delayed the onset of seizures. The test drug treated groups showed protection of the animals suggesting that methanolic extract of *Celastrus paniculatus* Willd. whole plant has antiepileptic activity.

The extract also exhibited pronounced delay in the duration of extension phase against PTZ-induced epileptic seizure. Pentylenetetrazole(PTZ) is a selective blocker of the chloride ionophore complex to the GABA-A receptor, and after repeated or single dose administration leads to the decrease in GABAergic function and to the stimulation and modification of density or sensitivity of different glutamate receptor subtype in many brain regions. PTZ may also trigger a variety of biochemical processes including the activation of the membrane phospholipase, protease and nucleases. Alterations in the membrane phospholipid metabolism cause the liberation of free fatty acids, diacylglycerols, eicosanoids, lipid peroxidase and free radicals. The tonic extensor phase is selectively abolished by the drugs effective in generalized tonic clonic seizure [28]. Repeated and high doses of PTZ reliably produces tonic clonic epileptic seizures in rats and mice [29,30]. MECP at dose 200 mg/kg and 400 mg/kg significantly reduced the duration of the extension phase compared to the control group. The standard drug Phenytoin at a dose of 25 mg/kg body weight provided 100% protection and the extract treated groups at dose of 200 mg/kg and 400 mg/kg showed 70.93% and 83.04% protection respectively. The findings of the present study suggest that MECP shows the antiepileptic activity against PTZ-induced epileptic seizure either by enhancing, or in some ways interfering with GABAergic neurotransmission.

One of the approaches to search for the new antiepileptic drugs is the investigation of naturally occurring compounds, which may belong to new classes. Herbal medicines are often considered to be gentle and safe alternative to synthetic drugs. More than half of the medicinally important pharmaceutical drugs are either natural products or derivatives of the natural products [31,32].

In the present study the antiepileptic action of the methanolic extract of *Celastrus paniculatus* Willd. whole plant was evaluated in rodents against INH-induced epileptic seizure and PTZ-induced epileptic seizure.

CONCLUSION

In conclusion the results of the present study revealed significant antiepileptic potential of the methanolic extract of *Celastrus paniculatus* Willd. whole plant. It is therefore possible that the antiepileptic activity of the plant may be exerted by the various phytoconstituents present in the plant *viz*., alkaloids, flavonoids, steroids, tannins, glycosides, sesquiterpenes, proteins and amino acids and justify its use as a traditional folk remedy for central nervous system related activities. However, further studies are necessary to ascertain its clinical effectiveness and the exact mechanism(s) of action of the extract and its active compounds.

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