



PHYTOCHEMICAL SCREENING AND ANTICONVULSANT STUDIES OF
ETHANOLIC EXTRACT OF *CELASTRUS PANICULATUS* WILLD ON LABORATORY
ANIMALS

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ABSTRACT

Bio-efficacy of plants and their derivatives have been reemphasized in recent times. *Celastrus paniculatus* Willd (*C. paniculatus*, Celastraceae), commonly known as Malkangni or Jyotishmati was used to treat brain related disorders. *C. paniculatus* seeds and seed oil have been used in Ayurvedic medicine for stimulating intellect and sharpening the memory. *Celastrus* seeds have been reported to possess hypolipidemic and antiatherosclerotic, antispermatogenic, antioxidant, anxiolytic, antistress and nootropic activities. The objective of the present study was to investigate the phytochemical properties and the anticonvulsant potential of the ethanolic leaf extract of *C. paniculatus*. The phytochemical screening was carried out using standard protocol while the anticonvulsant activity was studied using isoniazid-induced (300mg/kg, i.p) seizure in Swiss albino mice. The preliminary phytochemical screening carried out on the crude ethanol extract revealed the presence of alkaloids, terpenoids, carbohydrates, phenols, flavonoids, steroid and tannins. Epileptic seizure challenged animals treated with ethanolic leaves extract of *C. paniculatus* at doses 100mg/kg and 300mg/kg showed reduction of Isoniazid (INH)-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 ethanolic leaves extract of *C. paniculatus* significantly delayed the onset of epileptic seizure induced by INH. Thus the overall results suggest that the ethanolic leaves extract of *C. paniculatus* contains some active principles which may possess significant antiepileptic activity.

Keywords: *Celastrus paniculatus* Willd, Antiepileptic seizure, Phytochemical, Isoniazid.

INTRODUCTION

Epilepsy is a common and frequently devastating disorder affecting an estimated 7 million people in India and 50 million people worldwide. Approximately 40% of them are women. The estimated incidence rate ranges from 40 to 60 per 1,000,000 population/year. The WHO estimated that approximately 80% patients with epilepsy live in developing countries and most of them do not get

adequate medical treatment. Unfortunately, currently used antiepileptic drugs cause side effects which vary in severity from minimal impairment of the central nervous system to aplastic anemia or hepatic failure. Moreover, approximately 30% of people with epilepsy have intractable seizures that do not respond to even the best available treatment (Reddy, 2005). Approximately 30% of the patients continue to have seizures with current

antiepileptic therapy (Mattson, 1995). This fact demands new safer antiepileptic drugs for better and effective control of epilepsy. Since plants have provided many drugs in the past and they remain a rich source of novel compounds based on nature's combinatorial natural products chemistry during million of years of evolution, they should be continuously investigated as a source of novel therapeutic agents (Phillipson, 2003). The Ayurvedic system of medicine has a quite sophisticated classification of medicinal plants as per the dominant pharmacological/therapeutic activity of mental functions (Vaidhya, 1997). Ethnopharmacological approaches have provided leads to identify potential new drugs from plant sources, including targets for neuronal disorders (Howes and Houghton, 2003). Numerous studies point to medicinal plants as an interesting source of novel antiepileptic drugs (Schachter, 2009; Nsour *et al.*, 2000). One interesting example in this regard is losigamone derived from the kava kava plant and originally used by traditional healers in the South Pacific as an anxiolytic, which is now in early clinical development as novel antiepileptic drugs (Malawska, 2005; Willmore *et al.*, 2001). Likewise, *C. paniculatus* 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 (Nadkarni, 1976). Ayurveda, the ancient Indian traditional system of medicine has used the plant seed for prevention of various diseases (Vaidyaratnam, 1994). The seed oil is bitter, thermogenic and intellect promoting and is useful in abdominal disorders, beri-beri and sores (Sastry *et al.*, 2008). 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, anti-arthritic activity, antifertility activity, wound healing activity, antimalarial activity, antibacterial activity, cardiovascular activity, antioxidant activity, hypolipidemic activity etc (Atigari *et al.*, 2012). Though the oil obtained from the seeds of the plant had been studied to possess sedative and anticonvulsant activity (Gaitonde *et al.*, 1957) in the past, no one had previously studied the antiepileptic activity of the leaves of plant. Since there is dearth of scientific data proving the antiepileptic activity of the leaves of plant, the present study was carried out to investigate the antiepileptic activity of the ethanolic extract of *C. paniculatus* leaves against Isoniazid induced epileptic seizures.

MATERIALS AND METHODS

Plant materials

Leaves of *C. paniculatus* were collected from local area of Sager (M.P.).

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

Extraction Procedure

Defatting of plant material

Powdered Plant material (leaves) *C. paniculatus* was shade dried at room temperature. The shade dried material was coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a soxhlet

apparatus. The extraction was continued till the defatting of the material had taken place.

Extraction

200gm of dried plant material were exhaustively extracted by refluxing in ethanol at room temperature. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts and preserved in a refrigerated at 4°C for the experiments.

Phytochemical screening

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures (Khandelwal, 2005; Kokate, 1994). After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

Animals

Mice of either sex (18 to 22 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Mice received standard rodent chow and water *ad libitum*. Animals were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of mice was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity study

Acute oral toxicity was conducted according to the method of Organization for Economic

Co-operation and Development (OECD) (OECD, 1996). Ethanolic extract of *C. paniculatus* (5, 50, 300, and 2000 mg/kg) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-convulsion activity.

Antiepileptic Activity

Isoniazid-induced epileptic seizure model

Isoniazid can precipitate convulsions in patients with seizure disorders. The compound is regarded as a GABA-synthesis inhibitor. Clonic tonic seizures are elicited in mice which are antagonized by anxiolytic drugs. Ten mice of either sex with a weight of 18 to 22 g are treated with the test compound or the standard (e.g. diazepam 10 mg/kg i.p.) by oral or intraperitoneal administration. Controls receive the vehicle only. 30 min after i.p. or 60 min after p.o. treatment the animals are injected with a subcutaneous dose of 300 mg/kg Isoniazid (isonicotinic acid hydrazide). During the next 120 min the occurrence of clonic seizures, tonic seizures and death is recorded (Arka *et al.*, 2016; Vogel and Vogel, 2002).

Body weights of the animals were recorded and they were randomly divided into 5 groups of 6 animals each as follows:

Group I served as normal

Group II served as induced Isoniazid 300mg/kg s.c.

Group III animals were administered with standard Diazepam+ INH 5mg/kg i.p. +300mg/kg s.c

Group IV animals were administered treatment (at low dose), ethanolic extract of *C. paniculatus* + INH 100 mg/ kg i.p. + 300 mg/kg s.c.

Group V animals were administered with treatment (at high dose), ethanolic extract of *C. paniculatus* + INH 300mg/ kg i.p. +300mg/kg s.c.

The percentage of seizures or death occurring in the control group is taken as 100%. The suppression of these effects in the treated groups is calculated as percentage of controls. ED50-values are calculated.

RESULTS AND DISCUSSION

The crude extracts so obtained after extraction process was concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of ethanolic extracts was found to be 9.2%. The results of qualitative phytochemical analysis of the crude powder of leaves of *C.*

paniculatus were shown in Table 1. Ethanolic extracts of *C. paniculatus* showed the presence of alkaloids, terpenoids, carbohydrates, phenols, flavonoids, steroid and tannins. The antiepileptic activity of the ethanolic extract of *C. paniculatus* leaves 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 71.05±0.75sec and the extract treated groups at doses 100 mg/kg, and 300mg/kg significantly delayed the onset of epileptic seizure. The standard antiepileptic drug, diazepam 5mg/kg i.p abolished the effects of INH induced epileptic seizures in mice.

Table 1: Result of phytochemical screening of extracts of *C. paniculatus*

Phytochemicals	Result
Glycosides	-
Terpenoids	+++
Proteins	-
Amino acids	-
Alkaloids	++
Carbohydrates	++
Phenols	+++
Flavonoids	+++
Saponins	-
Steroid	++
Tannins	++

Here, +++: excellent, ++: good, -: not present

Table 2: Effect of ethanolic extract of *C. paniculatus* leaves on INH-induced epileptic seizure in Mice

Groups	Treatment	Dose	Convulsions		Status of animals after 30mins (no. of animals alive)	Status of animals after 24hrs (no. of animals alive)
			Onset	Nature and severity		
Control	Normal saline	10ml/kg	71.05±0.75	No effect	6	6
Induced	Isoniazid	300mg/kg s.c.	72.15±0.65	Clonic seizure	4	2
Standard	Diazepam+ INH	5mg/kg i.p. +300mg/kg s.c	69.25±0.79	Stiffening of body	6	4
Treatment (at low dose)	Ethanolic extract of <i>C. paniculatus</i> + INH	100mg/kg i.p. +300mg/kg s.c.	64.33±49	Continuous staring	3	1
Treatment (at high dose)	Ethanolic extract of <i>C. paniculatus</i> +INH	300mg/kg i.p. +300mg/kg s.c.	67.5±0.79	Jerking of the body	4	3

Values are expressed as Mean ± SEM (standard error mean); each groups contains 6 animals, n=6 : i.p. = intraperitoneal, s.c.= subcutaneous route of administration:
INH = Isonicotinic acid hydrazide (Isoniazid)

CONCLUSION

The results of the present study revealed significant antiepileptic potential of the ethanolic leaves extract of *C. paniculatus*. 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.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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