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Anticonvulsant activity of the ethanolic extract of *Platycladus orientalis* (Cupressaceae), leaves in rats

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Abstract

The main aim of this study was to determine the anticonvulsant activity of ethanolic extract of *Platycladus orientalis* leaves in albino rats. *P. orientalis* has an effective natural origin that has a tremendous future for research as the novelty and applicability are still hidden. Thuja is used traditionally for treatment of bronchial catarrh, enuresis, cystitis, psoriasis, epilepsy, ulcer, uterine carcinomas, amenorrhea, rheumatism, asthma, skin infections, mumps, bacterial dysentery, arthritic pains and premature blandness. The present study was therefore carried out to evaluate the anticonvulsant activity of ethanol leaf extract of *P. orientalis* in rats. The anticonvulsant activity of ethanolic extract of leaves of *Platycladus orientalis* (100mg/kg, 200 mg/kg and 400 mg/kg) was assessed in rats using maximum electroshock seizure (MES) test and pentylenetetrazole (PTZ) induced seizure test. The extract of *Platycladus orientalis* leaves significantly ($p<0.01$) reduced the hind limb tonic extension in the MES test in a dose dependent manner. In the PTZ model also, the extract signicantly ($p<0.01$) reduced the duration of clonic convulsions as well as delay the onset of seizures in a dose dependent manner.

Keywords: Anti-convulsant activity, *Platycladus orientalis*, Rat, Traditional, MES, PTZ

Introduction

Epilepsy is the second most common neurological disorder after stroke, affecting approximately 1% of the world's population.[1] Epilepsy is the term used for a group of disorders characterized by recurrent spontaneous seizures that apparently result from complex processes involving several neurotransmitter systems such the glutamatergic, cholinergic, and gabaergic system.[2] Seizure is defined as a paroxysmal event which occurs due to abnormal, excessive hyper-synchronous discharges from aggregates of central neurons.[3] According to the WHO, about 450 million people in the entire world have suffered mental, neurological, or behavioral problems at some time in their life and the prevalence rate for epilepsy are 1–2% of the world population.[4] The essential feature of the epilepsies is the appearance of behavioral changes, termed seizures. Such seizures are thought to occur via an alteration in the behavior of neuronal networks in the brain that induce the spontaneous expression of periods of synchronized burst firing interspersed by periods of normal electrical activity.[5]

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Glutamate and ɣ-amino butyric acid (GABA) are quantitatively the most important excitatory and inhibitory neurotransmitters, respectively, in the mammalian brain.[6] Thus, receptors for these two neurotransmitters are regarded as important targets for antiepileptic drugs. Despite the state-of-the-art medical treatment, drug-resistance remains a major clinical problem for one in three epileptic patients. Approximately 30% of patients with partial epilepsy and 25% of patients with generalized epilepsy are not well controlled on medications.[7] These patients often receive multiple medical treatments to control their seizures. Thus, there is an unmet need for new anti epileptic drugs. Herbal medicine could be a source for new therapeutics.[8, 9, 10]

Platycladus orientalis, also called morpankhi is a species of plant from the Cupressaceae family that was one of the original sources of thusa. Platycladus orientalis (L.) is a monoecious, multipurpose, evergreen plant which has been used anciently for its medicinal importance and association with long life and vitality in China and India. [11, 12] Platycladus orientalis is small, slow-growing, reaching 15–20 m (49–66 ft) and 0.5 m (1 ft 8 in) trunk diameter. Biochemical studies reveal that fresh plant contains essential oil, reducing sugar, watersoluble polysaccharides, water-soluble minerals, free acid, tannic agents1, flavonoids, saponins, glycosides and alkaloids2**.** The frequently occurring active constituents of the leaves were labdane-type and pimarane-type deterpenes, such as isopimaric acid, sandaracopimaric acid, pinusolide and 15-methoxypinusolidic acid (15-MPA), and flavonoids, such as quercetin, quercitrin and amentoflavone. The essential oil of the fresh leaves (related to the monoterpene fraction) composed of 65% **thujone**, 8% **isothujone**, 8% fenchone, 5% sabines and 2% -pinene as the main **monoterpenes.** A new bicyclic sesquiterpene, l-borneol, bornyl acetate, α-thujone and camphor, and a new sesquirterpenenic alcohol also isolated from plant.[13-17]

It is widely distributed in China and India used traditionally to treat peptic ulcer disease, cancer, Fever, **Convulsion**, Pain, Inflammation. [25-33] Thuja is also occasionally used for treating diseases of skin, blood, gastrointestinal tract, kidney, brain, warty excrescences, spongy, hepato-protective and as antioxidant.[18-24] Also used as Antimicrobial, Antioxidant, Anti-Inflammatory, Antidiabetic, antihelminthic etc. Hepato-protective activities and antioxidant activity of Thuja occidentalis also reported. Anti-proliferative and apoptosis- inducing properties of Thuja occidentalis has been also evaluated.[34-38]

Present study was aimed to investigate the pharmacological effect of *Platycladus orientalis* against **convulsion** by using **Maximum Electro Shock (MES)** and **Pentylenetetrazole** (**PTZ**) methods in Wistar rats.

Nimesulide is chemically N-(4-nitro-2-phenoxyphenyl) methane sulfonamide. It is Odorless yellow crystalline powder.

Material and Methods

Collection and preparation of plant extract:

The fresh leaves of ***Platycladus orientalis*** (L) Franco. (Cupressaceae) were collected from local area Alwar Rajasthan, during the month of October-November. Taxonomic identification was established by ethno botanist. Two herbarium were prepared one was sent to Department of Botany for proper authentication and a voucher specimen was submitted at Institute's herbarium department for future reference no. SRU 128/11

Extraction

The plant material was dried under shade at room temperature for about 5 days. The dried plant samples were coarsely powdered by mechanical grinder and sieved to give particle size 100 to 150 mm. The course powder of leaves was extracted with ethanol solvent in Soxhlet apparatus (Hot continuous percolation) at a temperature not exceeding 60 °C as plant contain mainly volatile oil. The extracts were concentrated under reduced pressure in a rotary evaporator to yield a crude semi-solid mass. It was then dried and used.

A portion of residue from each extract was subjected to phyto-chemical analysis to test the presence of carbohydrates, glycosides, alkaloids, flavonoids, tannins, sterols and tri- terpenoids in the leaves extracts. The preliminary phyto-chemical screening was performed with the standard procedures and the nature of the phyto-constituents was identified. [15, 16, 18, 23]

Drugs

Phenytoin was obtained from Zydus Cadila Healthcare Limited, Diazepam obtained from Ranbaxy Laboratories, New Delhi and

Pentylenetetrazol was obtained from Sigma Aldrich India, Bangalore

Preliminary phytochemical screening:

The extracts of *P. orientalis* leaves was tested for the presence of various phytoconstituents such as carbohydrate, alkaloids, glycoside, phenolic compound and tannins, saponins, flavonoids, fixed oils and fat test. All phytochemical tests were done as per the procedure given in the standard book.[21, 22, 23] Physicochemical values such as the foreign organic matter, moisture content, ash value as well as extractive value were determine as per the official methods (Anonymous,1996) as well as per WHO guidelines of quality control method for medicinal plant materials. (WHO, 1998; WHO, 1992)

Experimental animals:

Albino rats (Rattus norvegicus) of 12–16 weeks old, weighing 150-200 mg each of either sex were used in the experiments. The animals were housed in standard cages and maintained under standard conditions (12 hours light/dark cycle; 25 ± 3ºC). The animals were given standard diet of Bengal gram, maize, wheat and fasted overnight before the day of experiment. Water was given ad libitum. The animals were well acclimatized to laboratory conditions before commencement of experiments. The experiment protocol was duly approved by the Institutional Animal Ethics Committee of department of Pharmacology, Ponda Education Society's Rajaram and Tarabai Bandekar College of Pharmacy.

Table 1: Grouping and dose of drug

Model of Stress	Drug and Doses
MES/PTZ induced convulsion	
Group I	Control (Received D/W)
Group II	P. orientalis 100mg (i.p.) and inducing materials
Group III	P. orientalis 200mg (i.p.) and inducing materials
Group IV	P. orientalis 400mg (i.p.) and inducing materials
Group V	Phenytoin 25mg/kg/ Dizepam 4mg/kg (ip) and inducing mat

Acute oral toxicity studies:

Acute oral toxicity study was done as per OECD guideline 423. A group of three Wistar rats of either sex selected randomly and were used for acute toxicity study. The extracts were administered orally at the dose level of 50 mg/kg body weight to the animals and observed for 14 days. Since no mortality was observed, the procedure was repeated for further higher doses of 200, 500 and 2000 mg/ kg body weight. The extract showed no mortality at doses upto 2000mg/kg.

Evaluation of antiepileptic activity:

Maximum electro shock (MES) induced seizure model:

Albino rats were taken and divided into five groups containing 6 animals each. Group I served as control and received 2% gum acacia solution (10 ml/kg, p.o). Rats in groups II III and IV received ethanolic extract of *Platycladus orientalis* orally at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight respectively. Group V received the standard drug phenytoin at a dose of 25mg/kg intraperitoneally. All drugs were administered 1 hour prior to induction of seizures by MES. After 1 hour, electric current of 150 mA for 0.2 seconds was administered through ear electrodes to induce convulsions in all the experimental animals with the help of a convulsiometer. The different phases of convulsions were noted down along with the duration of each phase. Abolition or reduction in the duration of hind limb tonic extensor (HLTE) phase was taken as a measure of protection against MES induced seizures.

Pentylenetetrazole (PTZ) induced seizure model:

Thirty albino rats were taken and divided into five groups containing 6 animals each. Group I served as control and received 2% gum acacia solution (10 ml/kg, p.o). Rats in groupsI, II and III received ethanolic extract of *Platycladus orientalis* orally at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight respectively. Group V received the standard drug diazepam at a dose of 4 mg/kg intra-peritoneally. All drugs were administered 1 hour prior to induction of seizures by PTZ. After 1 hour, all the animals received convulsive doses of pentylenetetrazole (80 mg/kg) intraperitoneally. The animals were observed for 30 minutes after the administration of PTZ. The different parameters noted were the onset and

duration of clonic convulsions. The anticonvulsant property was assessed by the ability to reduce the duration of clonic convulsions and increase the latency of seizures.

Statistical analysis:

All the results were expressed as Mean ± SD. The statistical significance was analysed by performing one-way ANOVA followed by post hoc Dunnett's test. The difference was taken to be statistically significant at p value < 0.05.

Results and Discussion

Phytochemical screening:

The preliminary phytochemical screening of the methanolic extract of *Platycladus orientalis* revealed the presence of volatile oil, carbohydrates, phytosterols, glycosides, alkaloids, saponins and tannins.

Table 2 Preliminary phytochemical test of extract of *Platycladus orientalis*

S.No.	Phyto Constituents	Phytochemical tests	Results
1	Carbohydrate	Fehling's test	Positive
2	Alkaloids	Dragendroff's, Mayer's, Wagner's, Hager's test	Positive
3	Glycoside	Keller-Killani test With con. H_2So_4	Positive
4	Tannins	With $FeSO_4$, lead acetate test and ferric chloride	Negative
5	Saponins	Foam test	Positive
6	Flavonoids	With NaOH, with lead acetate test, H_2So_4	Positive
7	Fixed oils, fat test	Spot test	Positive
8	Volatile Oil	Sudan III, Tincture Alkane	Positive

Effect of *P.orientalis* on MES induced seizures:

The *P.orientalis* at dose 100mg/kg did not show significant result while at doses of 200mg/kg and 400mg/kg did not completely abolish the hind limb tonic extensor (HLTE) phase as seen with phenytoin, however there was a significant ($p<0.01$) reduction in the duration of HLTE phase in a dose dependent manner. Phenytoin treated animals showed 100% protection against MES induced seizures whereas *P.orientalis* extract at dose of 200 mg/kg and 400 mg/kg showed 47% and 69.24% protection respectively. Death percentages also considerable compare to standard drug phenytoin in case of 400 mg/kg dose. (Table 3 and Figure 1)

Table 3: Effect of ethanol extract (EEPO) in Maximal Electroshock induced convulsion

Group	Treatment (Dose of Drug/Extract in mg/kg, & Route	Number of Animals (n)	Duration of Convulsion (Time in Sec)			Death %age
			Flexion	Extensor	Stupor	
Group-I	Control (0.9% Normal saline orally	6	26 ± 0.34	48 ± 0.07	192 ± 0.50	4/6 (66.66)
Group-II	Standard (Phenytoin 25, i.p)	6	12 ± 0.43**	_	_	0/6 (0.00)
Group-III	EEPO (100, orally)	6	21 ± 0.32	29 ± 0.64	134 ± 0.32	2/6 (33.33)
Group-IV	EEPO (200, orally)	6	16 ± 0.65	24 ± 0.76	119 ± 0.50	1/6 (16.66)
Group-V	EEPO (400, orally)	6	14 ± 0.12*	18 ± 0.07*	102 ± 0.30*	1/6 (16.66)

Effect of *P.orientalis* on PTZ induced seizures:

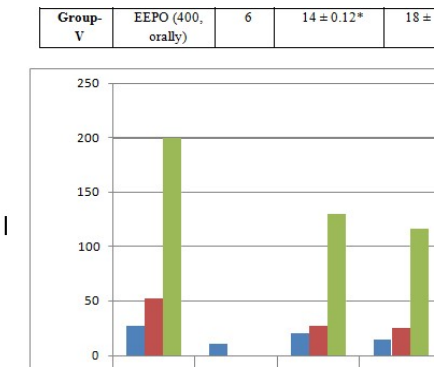
In present study EEPO (Ethanol Extract of *Platycladus orientalis*) of platycladus orientalis at the dose 400mg/kg shows significant activity against PTZ induced convulsions. In this model, 200 and 400mg/kg of *P. orientalis* ethanolic extract delayed the onset of clonic seizure especially at the dose of 400mg/kg, significantly ($P < 0.01$). Similarly, the *Platycladus orientalis* extract deferred the induction of tonic seizure particularly at the 400mg / kg dose. Standard drug diazepam inhibits onset of tonic seizure and death caused by convulsion. Death rate and tonic seizure were reduced by *P. orientalis* at dose of 400mg/kg, refer Table 4 and Figure 2 shows the graphical presentation of results of EEPO on PTZ model including comparative study of different groups taken to death %age caused by them.

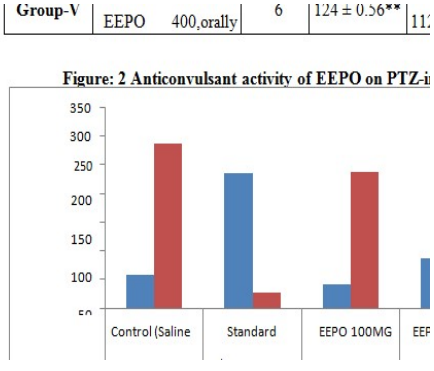
Table: 4 Anticonvulsant activity of EEPO on PTZ-induced seizures

Group	Treatment (Dose of Drug/Extract in mg/kg, Route of Admin.	Number of Animals	Onset of convulsion (sec)	Duration of Convulsion (sec)	Death %age
Group-I	Control (Saline 0.9%, orally)	6	55±0.21	270±0.1	5/6 (83.33)
Group-II	Standard (diazepam 4, i.p)	6	212 ± 0.24**	32 ± 0.2**	0/6 (0.00)
Group-III	EEPO 100,orally	6	46 ± 0.21	214 ± 0.43	3/6 (50.00)
Group-IV	EEPO 200,orally	6	92 ± 0.23*	160 ± 0.12*	2/6 (33.33)
Group-V	EEPO 400,orally	6	124 ± 0.56**	112±0.24**	1/6 (16.66)

This study was carried out to evaluate the anti-convulsant effect of leaf extract of PO on PTZ and MES induced convulsion animal models. The MES test identifies compounds/extracts which prevent seizure spread. In this model, all the extracts of Platycladus orientalis significantly and dose dependently increased the onset time of THLE (tonic hind limb extension) and decreased the duration of THLE (tonic hind limb extension). But ethanol extract at dose of 400mg/kg exhibited maximum significant antiepileptic activity (69.24%). All the extracts of Platycladus orientalis might prevent the seizure spread and contribute to the activity. But the maximum antiepileptic activity of the ethanol extract may be due to the presence of phytoconstituents such as terpenoids and flavonoids.

PTZ test identifies compounds/extracts which primarily raise seizure threshold. In this model, all the extracts of Platycladus orientalis significantly increased the onset time of convulsions and decreased the duration of convulsions in a dose-dependent manner. But ethanol extract at 400 mg/kg showed maximum antiepileptic activity (70.82%).





Conclusion

The findings in this study confirm the absence of oral acute toxicity at the doses employed, and presence of anti-convulsant pharmacologic activity of PO. Its efficacy is comparable to the standard drugs.

Overall the present study demonstrated that the ethanol extract of aerial parts of Platycladus orientalis exhibited maximum antiepileptic activity in all tested models. Ethanol extract may act at seizure focus, prevent spread of the seizure and suppresses THLE (tonic hind limb extension) induced by MES. In PTZ model, the extract might raised the seizure threshold or act as GABA agonist and enhanced GABAergic neurotransmission by increasing GABA levels in brain by facilitating the opening of GABA-activated chloride channels at GABAA receptors. Thus, the present work validates the use of PO for convulsion in the Indian and chinese folk medicine.

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