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Original Article

EVALUATION OF ANTICONVULSANT ACTIVITY OF LEAF EXTRACTS OF *HOLOPTELEA INTEGRIFOLIA* (ROXB.) PLANCH IN EXPERIMENTAL ANIMALS

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ABSTRACT

Objective: *Holoptelea Integrifolia* (Roxb.) Planch has been used from long time in traditional medicine. The main objective of the work was to evaluate the anticonvulsant activity of *Holoptelea Integrifolia* (Roxb.) Planch.

Methods: The anticonvulsant activity of petroleum ether and methanolic extract of

Holoptelea Integrifolia leaves was evaluated using *Pentylenetetrazole* (PTZ) induced convulsions in mice and maximal electro shock (MES) induced C onvulsions and *lithium-pilocarpine* induced status epilepticus in rats.

Results: Preliminary Phytochemical investigation of the Petroleum ether extract of Holoptelea Integrifolia leaves reveals the presence of steroids, terpenoids, alkaloids, glycosides, flavonoids, proteins, tannins, and carbohydrate While methanolic extract of Holoptelea integrifolia showed the presence of steroids, alkaloids, flavonoids, proteins and carbohydrates. The petroleum ether extract (100 and 300 mg/kg) and methanolic extract (300 mg/kg) delayed onset of PTZ- induced convulsions and also prolonged the onset of tonic convulsions in mice. Both the extracts failed to protect the rats from MES induced convulsions. The extracts also protected rats against seizures induced by *lithium-pilocarpine*. In *Lithium-pilocarpine* model the petroleum ether extract (100 and 300 mg/kg) and methanolic extract (300 mg/kg) delayed the latency to rearing with forelimb clonus significantly.

Conclusion: The results indicate thatpetroleum ether and methanol extracts contained such phytochemial compounds which are active in case of *Pe ntylenetetrazole* (PTZ) and *lithium pilocarpine* induced status epilepticus, which support the ethnomedicinal application of the plant as an anticonvulsant agent.

Keywords: Anticonvulsant, *Pentylenetetrazole*, Maximal electroshock, *Lithium-pilocarpine*, *Holoptelea Integrifoila*

INTRODUCTION

Epilepsy is a common neurological disorder that demands immediate medical attention and, often,long-term therapy [1]. In developed countries,annual new cases are between 40 and 70 per 100,000 people in the general population. This figure is often close to twice as high due to the higher risk of experiencing conditions that can lead to permanent brain damage. At the present day, six antiepileptic drugs, *gabapentin,lamotrigine, tiagabine, topiramate, vigabatrin* and *zonisamide*, have been used for the treatment of epilepsy. They have all been shown to be effective in short-term add-on clinical trials in patients with uncontrolled epilepsy. Synthetic antiepileptic drugs are associated with side-effects, including teratogenicity, chronic toxicity and adverse effects, on cognition and behavior [1,2].

An ideal antiepileptic drug should suppress all seizures without causing any untoward effect.Unfortunately, the drugs available in the modern medicine not only fail to control the seizure activity in some patients, but quite frequently cause unwanted effects that range in severity from minimal impairment of the CNS to death from aplastic anemia or hepatic failure [3]. Rich floral biodiversity of India has provided herbal health practitioners and other traditional healers in the country with an impressive pool of "natural pharmacy" from which plants are selected as ingredients to prepare herbal remedies and medicines (phytomedicine) for the treatment [4], management and control of a variety of human ailments. In traditional system of medicine, bark and leaves of *Holoptelea Integrifoila* used as bitter, astringent, acrid, thermogenic, anti-inflammatory, digestive, carminative,laxative,anthelmintic,depurativ e,repulsive,urinary astringent and in rheumatism[5,6]. The plant *Holoptelea integrifolia* is used traditionally for the treatment of inflammation, gastritis, dyspepsia, colic, intestinal worms, vomiting, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhoea and rheumatism [7]. But, its anticonvulsant activity is not yet validated scientifically as on date. Hence in the current dissertation the anticonvulsant activity of petroleum ether and methanol extract of leaf of *Holoptelea Integrifolia* in experimental animals is evaluated.

MATERIALS AND METHODS

Plant Introduction

Holoptelea Integrifoila belongs to the family ulmaceae commonly called as Indian Elm and frequently used in India by the tribal people for it's medicinal properties. The mucilaginous bark is boiled and the juice squeezed out and applied to rheumaticswellings[8].Leaves of *Holoptelea Integrifoila* were collected in the Month of August from the agricultural fields of Tirunelveli district, Tamilnadu. The plant w as identified and leaves of *Holoptelea Integrifolia* were authenticated and confirmed from Dr.V.Chelladurai, Research Officer,Botany, C.C.R .A.S. (Retired), Govt. OfIndia by compairing morphological features (l eaf and stem arrangement, flower /inflorescence arrangement,fruit and seed morphology etc.). The collected plant material was shade d ried to retain its vital phytoconstituents and then subjected to size reduction for further extraction process.

Preparation of Different Extracts: Successive extraction of leaves of *Holoptelea Integrifolia* was prepared on the basis of solvent polarity

Preparation of Petroleum ether and methanol extract: The powder of *Holoptelea Integrifoila* leaves was charged in to the thimble of a Soxhlet apparatus and extracted using petroleum ether. Appearance of colourless solvent in the siphon tube was the

indication of exhaustive extraction and based on that the further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50 ◦ C to get petroleum ether extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated. The perfectly dried extract was then stored in an air tight container in a refrigerator below 10°C. After obtaining the petroleum ether extract the marc was pressed and it is air dried and again it was extracted using methanol. Appearance of colourless solvent in the siphon tube was the indication of exhaustive extraction and based on that the further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50◦ C to get semi solid mass of methanol extract. The extract was stored in an airtight container in a refrigerator below 10°C.

The Petroleum ether and Methanol extracts of *H.Integrifolia* leaves were subjected to the following investigations,

1. Preliminary phytochemical screening.

2. Pharmacological activities

a. Determination of acute toxicity (LD_{50})

b. Anticonvulsant activity

Animals

Albino mice of either sex weighing between 20-30g And albino rats of either sex weighing between (180-220) gm were procured from Central Animal House, Rajah Muthiah Medical College & Hospital, Faculty of Medicine, Annamalai University, Annamalai Nagar- 608002, Tamilnadu for experimental purpose. The animals were acclimatized to laboratory conditions for 7 days. The animals were supplied with commercially available standard diet. Water was allowed ad libitum under hygienic conditions. All animal studies were performed in accordance to guideline of CPCSEA and Institutional Animal Ethical Committee (IAEC) of Central Animal House, Rajah Muthiah Medical College & Hospital, Annamalai University, Tamilnadu (CPCSEA registration number- 160/1999 /IAEC/ CPCSEA).

Drugs

Phenytoin (Shreeji Pharma International, Vadodara, India), *Pentylenetetrazole* (Sigma,USA), *Diazepam* and *Clonazepam* (Campose injection, Ranbaxy, India), *Lithium carbonate* (Glenmark Pharmaceuticals, India), and *pilocarpine* (FDC Limited, India) were used in the study. All other chemicals were of analytical grade. *PTZ, Phenytoin, Diazepam inj., Lithium carbonate, pilocarpine nitrate* were dissolved in distilled water just before administration.The extracts were suspended in CMC (0.5 %). A gastric catheter was used for oral drug administration. The extracts did not show any sign of toxicity till the oral dose of 2000 mg/kg hence the extracts were used in the range of 100–300 mg/kg orally assuming that LD50 dose is 2000 mg/kg.

Preliminary phytochemical screening of extracts

The extracts were subjected to following chemical tests to detect the chemical constituents present in them. 0.5 gm of extract was dissolved in 5 ml of distilled water and filtered. The filtrate was used to determine the presence of various phytoconstituents [9]

Determination of LD_{50} of Leaf Extract of *Holoptelea Integrifolia*

The acute toxicity of leaf extracts of *H. Integrifolia* was determined by using albino mice of either sex weight between (20-25 g), maintained under standard conditions. The animals were fasted for 3 hr prior to the experiments. Animals were administered with single dose of either Petroleum ether or Methanol leaf extract of *H. Integrifolia* and observed for its mortality up to 48 hr study period (short term toxicity). Based on the short-term toxicity profile,the next dose was decided as per OECD guidelines No 425. Since no mortality was observed upto dose 2000mg/kg From the LD50 dose, 100 mg/kg and 300 mg/kg doses were selected and considered as low and high doses respectively.

Assessment of anticonvulsant activity

Treatment schedule

Albino rats and mice were used to evaluate anticonvulsant activity. Rats were used in the Maximal electroshock induced seizures and *Lithium pilocarpine* induced status-epilepticus and mice were used in *pentylenetetrazole*-induced convulsions. Animals were divided in six groups. One group received vehicle, two groups received PEHI (100 & 300 mg/kg), two groups received MHI (100 & 300 mg/kg) and the sixth group received the reference standard.

***Pentylenetetrazole(PTZ)* induced seizures**

60 min after above mentioned drug treatment Clonic seizures were induced in mice by subcutaneous injection of 80mg/kg *Pentylenetetrazole*. The latency to the onset of clonic convulsions in non-protected mice and lethality during the following 24 hour was recorded and compared with those of vehicle treated control mice to assess the anticonvulsant activity[10, 11, 12]. One group received *clonazepam* 0.1 mg/kg - i.p. as a reference standard 30 min before *PTZ.* The animals were observed for onset of convulsion up to 30 min after *PTZ.* Each animal was then placed into individual plastic cages and were observed initially for 30 min and later up to 24 hrs. The following parameters were recorded during test session of initial 30 min and up to 24 hrs respectively: Latency (onset of clonus), Onset of tonic-clonic convulsions, Status of animal after 1hr Status of animal after 24 hrs, Percent protection

Maximal Electro Shock Induced seizures (MES)

One group received *phenytoin* (20 mg/kg- p.o.) as a as a reference standard.Tonic clonic convulsions were induced by giving maximal electroshock seizures (MES) (150 mA for 0.2sec) using an electroconvulsometer (INCO, Ambala, India) via crocodile ear clip, 60 minutes after administration of either vehicle or test drug dosese and 90 minutes after *Phenytoin* (20mg/kg-p.o.). The number of animals protected from tonic hind limb extension seizure (i.e. abolition of tonic hind limb extension within 10 sec after delivery of the electroshock was considered as protected rat) and duration of observed tonic hind limb extension seizure (HLTE) was recorded in each dose group[10,13]. For recording various parameters, rats were placed in clear rectangular plastic cages with an open top, permitting full view of the animal's motor responses to seizure. In the pilot study various phases of convulsions, viz., tonic flexion, extension, clonus, stupor and mortality due to convulsions were selected as the parameters.

***Lithium pilocarpine* induced status-epilepticus**

Status epilepticus was induced by administration of *pilocarpine* (30 mg/kg i.p) 24 h after *lithium carbonate* (3 mEq/kg i.p). The effect of PEHI & MHI (each 100 & 300 mg/kg, p.o) was studied on the rearing with forelimb clonus by administering both extracts 30 min. before injection of pilocarpine [14]. *Diazepam* was used as a reference standard in a dose of 1 mg/kg i.p.

RESULTS

Phytochemical Examination of Extracts.

Preliminary phytochemical analysis of petroleum ether extract of *Holoptelea integrifolia* showed the presence of steroids, terpenoids, alkaloids, glycosides, flavonoids, proteins, tannins and carbohydrates. Methanolic extract of *Holoptelea integrifolia* showed the presence of steroids, alkaloids, flavonoids, proteins and carbohydrates

Acute toxicity study

Both the petroleum ether and methanol extracts did not produced any sign of toxicity.

Assessment of Anticonvulsant Activity of *H.Integrifolia* Leaves.

***PTZ* - Induced seizures**

H.Integrifolia leaves were screened for anticonvulsant activity using *PTZ* induced convulsion model in mice. Study was conducted using low, and high doses of PEHI & MHI (100 & 300 mg/kg respectively).

The above mentioned doses were administered as mentioned earlier. It was observed that both low and high doses of PEHI while only high dose of MHI exhibited a significant anticonvulsant exhibited a significant anticonvulsant effect. The petroleum ether extract was found to be more effective than methanol extract. The standard drug *clonazepam* (0.1 mg/kg-i.p.) exhibited a significant anticonvulsant activity and offered 100% protection. The observations are given in Table 1.

MES Induced seizures

H.Integrifolia leaves were screened for anticonvulsant activity using MES induced convulsion model in rat. Study was conducted using low and high doses of PEHI & MHI (100 & 300mg/kg) respectively.

The above mentioned doses were administered as mentioned earlier. It was observed that both the low dose and high doses of PEHI & MHI failed to protect the rats and to produce anticonvulsant effect as compared to control by reducing the duration of tonic extensor phase and tonic-clonic seizures.

The standard drug *phenytoin* (20 mg/kg- p.o.) exhibited a significant anticonvulsant activity and offered 100% protection. The observations are given in Table 2.

Table 1: Effect of petroleum ether and methanol extracts of *holoptelea integrifolia* Leaves on PTZ (80mg/kg-s.c.) Induced Convulsions in mice

Treatment(mg/kg)	Onset of firstclonus(second)	No. of animals survived/used	Percent mortality (%)
Vehicle Control	211.14 ± 07.57	0/6	100 %
PEHI - 100	237.27 ± 04.77*	2/6	66.66 %
PEHI-300	273.49 ± 06.27**	3/6	50 %
MHI-100	200.46 ± 02.92 NS	1/6	83.33 %
MHI-300	234.23 ± 05.48*	1/6	83.33 %
Clonazepam - 0.1	Nil	6/6	0.00%

Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed by Dunnetts test. Where, *P<0.05, **P<0.01,***P<0.001, PEHI: Petroleum ether extract of *Holoptelea Integrifolia* leaves, MHI: methanol extract of *Holoptelea Integrifolia* leaves, PTZ: *Pentylenetetrazole.*

Table 2: Effect of petroleum ether and methanol extracts of *holoptelea integrifolia* Leaves on MES Induced Convulsions in rats

Treatment (mg/kg)	Duration of hind limb extension (second)	Rats convulsed/Rats used
Vehicle Control	28.26± 02.01	6/6
PEHI-100	26.38 ± 02.20	6/6
PEHI-300	27.41 ± 01.35	6/6
MHI-100	25.11 ± 01.50	6/6
MHI-300	26.92 ± 01.65	6/6
Phenytoin-20	02.31 ± 0.45**	0/6

Values are mean ± SEM; n=6, One way analysis of variance (ANOVA)followed by Dunnettstest,Where, *P<0.05, **P<0.01,***P<0.001, PEHI: Petroleu m ether extract of *Holoptelea Integrifolia* leaves, MHI: methanol extract of *Holoptelea Integrifolia* leaves, MES: Maximal electro shock.

Table 3: Effect of petroleum ether and methanol extracts of *holoptelea integrifolia* Leaves on *Lithium pilocarpine* induced status-epilepticus

Treatment (mg/kg)	Latency to rearing with forelimb clonus (min)
Vehicle Control	18.17± 0.7491
PEHI-100	37.17± 1.922*
PEHI-300	69.17± 1.167**
MHI-100	20± 0.5774 NS
MHI-300	39± 0.9661*
Diazepam - 1	76.67± 0.882**

Values are mean ± SEM; n=6, NS non significant, One way analysis of variance (ANOVA) followed by Dunnetts test, Where, *P<0.05, **P<0.01,***P<0.001, PEHI: Petroleum ether extract of *Holoptelea Integrifolia* leaves, MHI: methanol extract of *Holoptelea Integrifolia* leaves.

***Lithium pilocarpine* induced status-epilepticus**

H.Integrifolia leaves were screened for anticonvulsant activity using *Lithium pilocarpine* induced status epilepticus model in rat. In vehicle treated group latency to forelimb clonus was observed at 18.17±0.7491 min after *pilocarpine.* Study was conducted using low, and high doses of PEHI & MHI (100 & 300 mg/kg-p.o. respectively). The above mentioned doses were administered as mentioned earlier. It was observed that both low and high doses of PEHI while only high dose of MHI exhibited a significant anticonvulsant effect by showing significant delay in latency to rearing with forelimb clonus when compared to control group. The petroleum ether extract was found more effective than methanol extract. The standard drug *diazepam* (1mg/kg-i.p.) exhibited a significant anticonvulsant activity. The animals were normal in behaviour after 180 min. The observations are given in Table 3.

DISCUSSION

There are a number of synthetic anticonvulsant drugs currently available for use in the management, control and treatment of individuals with epilepsy. However, most of the synthetic drugs are not only inaccessible and unaffordable, but also possess many toxic adverse effects. Therefore, there is a great need for the development of cheap, effective and safe anticonvulsant agents from plants and other sources.

GABA is the primary inhibitory neurotransmitter in the central nervous system (CNS).

Diminution of brain GABA level has been reported after PTZ. Diminution of brain GABA level has been reported after subconvulsive dose of PTZ [15]. Many plants having anticonvulsant activity are known to inhibit GABA transaminase activity thereby increasing brain contents of GABA.The MES test predicts activity against generalized tonic clonic and cortical focal seizures and the PTZ test against absence seizure, while Lithium-pilocarpine was found useful in status epilepticus[14].

Pretreatment of *lithium* initiates limbic seizures after administration of subconvulsant doses of *pilocarpine* and other cholinergic agonist; Still *lithium* does not have proconvulsant activities[16]. If *lithium*

and *pilocarpine* administred concurrently it results in an accumulation of inositol monophosphate and reduction in cortical inositol that are about 10 times greater than the effects obtained with either drugs alone[16,17]. *Lithium-pilocarpine* induced convulsion have used to study the effect of *fluoxetine* on post- status epilepticus induced depression in rats.The study has shown that depression in epilepsy may have specific mechanisms and not only altering serotonergic pathways. serotonergic or cholinergic mechanisms may be responsible for inhibition of *lithium-pilocarpine*-induced convulsion[18]. *Phenobarbitone, sodium valproate*, *diazepam* and *trimethadione* prevent the limbic seizures induced in rats by *pilocarpine,* however, *phenytoin* and *carbamazepine* are ineffective[19]. *Lithium-pilocarpine*-induced seizures were inhibited by blocking of serotonergic transmission and inhibition of post-synaptic 5-HT receptors[20]. On observation and reference to reported data from Phytochemical tests, it was clear that, both the extracts Petroleum ether and Methanol of *H.Integrifolia* leaves showed the presence of flavonoids, steroids, triterpenoids. Flavonoids, sterols and terpenoidshave been implicated in various pharmacological actions on central nervous sy stem including anticonvulsant and anxiolytic activity[21,22]. Flavon oids and sterols have been involved in central inhibitory and neuromodulatory effects[22, 23]. The anticonvulsant activity may be due to the presence of flavonoids and sterols in the extracts. From t he above data it is concluded that *H.Integrifolia* leaves possesses sign ificant anticonvulsant activity against *pentylenetetrazole, Lithium-pilocarpine* and not against MES induced convulsions.

CONCLUSION

The petroleum ether extract of *holoptelea integrifolia* leaves is more potent for showing the anticonvulsant activity than methanol extract.The extracts showed dose dependent effect.Further studies are required to find and isolate active principles and determine the mechanism of their anticonvulsant action, also our study suggests th e application of *holoptelea integrifolia* leaves in the treatment of convulsive disorders as a need of modern health science.

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