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Antiepileptic Activity of Ethanolic and Petroleum Ether Extracts of *Leucas cephalotes* (Roxb.) in Isoniazid and Strychnine Induced Convulsions

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ABSTRACT

The present study was carried out to evaluate the flowers of *Leucas cephalotes* (Roxb.) for their anticonvulsant effect as it was extensively used traditionally for the treatment of the various ailments in Ayurveda with very least side effects. Whole plant has been used in edema, inflammation, asthma, dyspepsia, paralysis and snake bite. As it has some neurological effect, we planned the study. Flowers of *Leucas cephalotes* (Roxb.) were shade dried and extracted by soxhlet extraction method, petroleum ether and ethanolic extracts were used. After the toxicity studies, extracts are divided in 100, 200, 400mg/kg and implemented in various animal models for the evaluation of anticonvulsant effect. Studies carried out for both the extracts and the results strongly point towards the hydroalcoholic extracts doses of 200 and 400mg/kg were shown to be significant in the models like isoniazid and strychnine induced convulsion.

INTRODUCTION

Epilepsy is most common neurological disorders affecting people across all nationalities.[1] The word epilepsy in derived from the Greek verb **epilamvanein** (to be seized", "to be taken hold off", or "to be attacked" indicating that the person having a seizure is 'possessed' or at least out of control[2]. Epilepsy includes a group of heterogeneous and diverse conditions. The terms epilepsy and seizure are not synonymous and the distinction must be made clear. 'A seizure is an abnormal behavior (with symptoms or signs) resulting from abnormal discharges of cortical neurons and it is an observable phenomenon that is finite in time. Epilepsy refers to chronic conditions characterized by recurrent seizures[3]. Epilepsy is one of the most common neurological disorders characterized by sudden, transient alterations of brain function usually with motor, sensory autonomic or psychic symptoms often accompanied by loss of, or altered consciousness[4]. Coincidental pronounced alteration in the electroencephalogram (EEG) might be detected during these episodes. Epilepsy occurs due to abnormal activity of brain tissue. A convulsion (seizure or fit) is the abnormal event that result from the sudden change in the electrical function of the cell in the brain. Seizures can vary widely in their clinical presentations, depending on the sites extent and mode of propagation of the paroxysmal discharge and hence now looked at the as spectrum of clinically different varieties than the single disease[5]. Epilepsy is becoming the most serious brain disorder and affects about 40 million people and about 100 million will be affected same tie in their life. Overall it accounts for 1% of World's burden of disease and the prevalence rate is reported at 2%. In addition, the incidence rate for the primary generalized tonic-clonic and absence seizures are highest in infants and children[5]. It may be idiopathic (primary / genetic) or symptomatic (secondary / cryptogenic / reactive) epilepsy. In spite of intensive investigations, the pathophysiology is still poorly understood[5](find ref.). Several biochemical hypotheses suggest the involvement of decreased activity of inhibitory GABAergic system or increased activity of excitatory amino acids (glutamate and aspartate system) in epilepsy [6]. And also, there are various other factors which cause seizures, such as oxidative stress developed by the free radical generation[7]. Epilepsy by itself means "idiopathic" in contrast to the commonly used but incorrect meaning of cause unknown.[8]

Even the current antiepileptic drugs such as oxcarbazepine, gabapentin, tiagabine, topiramate, levetiracetam, lamotrigine, felbamate and fosphenytoin have the drawbacks like limited spectrum

or drug interactions with oral contraceptives. Three drugs of these gabapentine, lamotrigine and topiramate are approved for use in adults with partial seizure or without generalization. It is felbamate and lamotrigine have potential of significant side effects. fosphenytoin and lamotrigine is parent pro-drug of phenytoin that is more tolerable than parenteral phenytoin[20]. Therefore, this is not surprising that the currently used antiepileptic drugs fail to provide satisfactory seizure control and toxicities associated with these drugs can further compromise quality of life while drug-drug interactions may complicate clinical management.

Keeping these complications in mind, various herbal medicines have been tried in the past for their potent anticonvulsant properties. There are various models for epilepsy and to determine the effects of the chemicals for the same. May due to application, physical and chemicals models are used for the experimental evaluation of the same, chemicals like PTZ, picrotoxin, strychnine and INH-isoniazid are reproducible laboratory animal models for preclinical evaluation of the potential drug for epilepsy[21]. Hence, we turn to Ayurveda. Ayurveda is the knowledge of healthy living and not merely confined to the treatment of diseases or disorders. It is an ancient and holistic system of diagnosis and treatment involving nutrition, hygiene and rejuvenation originating in India more than 5000 years ago.[22]

By keeping in mind, we studied the properties of the drugs which were reported but not scientifically proved so we chosen plant *Leucas cephalotes*. It was reported for various CNS activities. *Leucas cepholotes* (Roxb.) spreng. (syn- phlomicepholotes) is commonly known as "dronpushpi" in Sanskrit and peddatumni in Telugu.[23] It is rainy season weed belonging to family labitatae/Lamiaceae and grows in all parts of India alone the roadside and waste lands. This plant is used in homeopathic drug indigenous system of medicine. It has been used for the diagnosis of disease like edema, diaphoretic, inflammatory and obstinate urinary tubules. Plant is valuable drug in snake bite[24]. Flowers are stimulant, emmenagogue, diaphoretic and expectorant and syrup sometimes mixed with honey are useful in diagnostic remedies of cough and cold.[25] Leaves are used to treat bleeding and itching in piles if smoked 1:3 ratio[26]. It also shows activity in fever, urinary discharge.[27] Pharmacologicaly plant is reported for multiple activity including antifilarial[28], antibacterial[29], antidiabetic[30], antiinflamattory[31], antioxidant, analgesic, antithelmintic[32], antimicrobial and antioxident.[33] By keeping in mind we go for the anticonvulsant activity of the plant as it has strong antioxidant activity.

MATERIALS AND METHODS

Preparation of Extracts:

Whole plant of *Leucas cephalotes* were collected from Southern Ghats region drug was authenticated by Dr. K.Madhawa Chetty, Shri. Venkateswara University, Tirupati. The flowers from collected drugs were shade dried and powdered. The powder of *Leucas cephalotes* flowers was passed through sieve no 40 and extracted by soxhlet using 70% ethanol (100gm in 500ml) and petroleum ether below 24^{o}C temperature. After filtration, dark green coloured solution obtained from the *Leucas cephalotes* was evaporated at 50^{0}C .[33]

Animal Selection:

Male albino rats (150-200g) and albino mice (18-25g) of either sex procured from M/s. National Toxicological Center, approved by FDA Maharashtra state, LIC. NO. P-D-T-L-7ISO 9OO1:2OO8 certified laboratory (Regd. No. IPU{152.07) were used with the approval of the Institute Animal Ethics committee. Animals were reared and maintained at the animal house of the institution and were on standard pellet diet and water *ad libitum*. They were initially acclimatized to the laboratory environment for one week prior to their use. Each group of animals was housed separately, with a distinct identity throughout the study.

Drugs and chemicals

Pentylene tetrazole (Sigma, St.Louis, USA), Diazepam (Ranbaxy), Phenytoin sodium (M.J. Pharmaceuticals, Gujrat.), Isoniazid (INH), Strychnine (STR)

Statistical Analysis and Calculations:

Data was statistically analyzed by one way analysis of variance (ANOVA) followed by Tukey karamar using graph pad prism.

Methods Used

Determination of acute toxicity study (LD $_{50}$)

The acute toxicity study of *Leucas cephalotes* ethanolic (ELC) and petroleum ether extract (PLC) respectively, was determined by albino mice of either sex (18-22gm). The animals were

fasted 3 hours prior to the experiment, Acute toxic class method (OECD guideline no 423) of ELC and PLC was adopted for the toxicity study. Animals were administered with the single dose of extract. The dose for the next animal was determined as per as OECD guideline No-423.[34]

Strychnine induced convulsions in mice:(33-35)

Mice of either sex (18-25gm) were divided into 8 groups 6 mice in each was fasted overnight prior to the test but water was supplied *ad libitum*. It receives the doses as mentioned in the table.

Table No. 1 Drugs and their concentrations given for the strychnine.

Groups	Drugs	Dose and route of administration	Time of administration before convulsive stimuli
I	Strychnine	2mg/kg, i.p.	30 minutes
II	Diazepam	5mg/kg, i.p.	30 minutes
III	ELC	20mg/kg, po	1 hours
IV	ELC	40mg/kg., po	1hours
V	ELC	60mg/kg,p.o.	1 hours
VI	PLC	20mg/ml,p.o.	1 hours
VII	PLC	40mg/kg,p.o.	1 hours
VIII	PLC	60mg/kg,p.o.	1 hours

The extracts were administered once daily for 7 days. On 7th day, 60 min after respective treatment and extract administered. The parameters were recorded during treatment session of initial, 30min, status of animal after 24h and percentage protection.

Isoniazid induced seizures-(33,37)

Albino mice (18-22gms) of either sex were divided into 8 groups. 6 mice each was fasted overnight prior to the test but water was provided *ad libitum*. The drugs and their concentrations as well as dosing is done as follows

Table No. 2 Drugs and their concentrations given for the Isoniazid (INH).

Groups	Drugs	Dose and route of administration	Time of administration
I	Isoniazid	2mg/kg, i.p.	30 minutes
II	Diazepam	5mg/kg, i.p.	30 minutes
III	ELC	20mg/kg, po	1 hours
IV	ELC	40mg/kg., po	1 hours
V	ELC	60mg/kg,p.o.	1 hours
VI	PLC	20mg/ml,p.o.	1 hours
VII	PLC	40mg/kg,p.o.	1 hours
VIII	PLC	60mg/kg,p.o.	1 hours

RESULTS AND DISCUSSION

Pharmacological investigations

Acute toxicity study

An acute toxicity study of ELC and PLC was determined in mice as per OECD guideline no. 423. The extract was administered orally to different groups of mice at the different dose levels and extracts produced no mortality up to 2000mg/kg. Hence, $1/5^{th}$, $1/10^{th}$, $1/20^{th}$ of LD50 doses were selected for the present study.

Effect of ELC and PLC extract on isoniazid induced convulsions

The effect of *Leucas cephalotes* ethanolic extract and petroleum ether extract on various animal models were observed by monitoring different parameters during the study. In isoniazid induced convulsions, the parameter monitored was onset of convulsions. P-values <0.01 were considered as significant. *Leucas cephalotes* extract (ELC) at 200mg/kg and 400mg/kg b.w. p.o. produced a more significant ($p<0.001$) doses in isoniazid induced convulsions recovery was not found significant, as compared to control and standard. Hence, *Leucas cephalotes* may be expected to

have a similar type of mechanism as diazepam in case of standard, it shows 100% protection and any sign of convulsion.

INH is used widely for the treatment and chemoprophylaxis of tuberculosis, but can have serious effects on the CNS causing seizures and comas. The INH is thought to be inhibition of GABA synthesis in the CNS.[31] So diazepam treated group was showed up to 100% of protection of the animals. But the PLC not showed significant protection of the animal, it was ineffective. At the same time, we found the ELC more effective than PLC. The extract might be having either by stimulation of L-glutamate or prevention of GABA degradation by GABA transaminase.

Table no. 3 Effect of ELC and PLC on isoniazid induced convulsions

Groups	Treatment	Dose	Onset latency (in sec.) (Mean ±SEM)	Time of occurrence of tonic convulsions (in sec)	% Protecti on
I	Control(GA) INH	10 ml p.o.+300mg i.p.	33.1±1.04	34.1±1.45	0
II	Diazepam+ INH	5mg i.p.+ 300mg i.p.	168±3.44	Nil***	100
III	ELC+INH	100 mg p.o. +300mg i.p.	41.4±1.97	42.5±1.98	30
IV	ELC+INH	200 mg p.o. +300mg. i.p.	49.0±2.01	52.0±3.01**	40
V	ELC+INH	400 mg p.o.+300 mg i.p.	60.1±1.55	65.2±1.57***	60
VI	PLC +INH	100mg P.O+300 mg i.p	33.6±0.944	34.65±0.968	0
VII	PLC +INH	200mgP.O+300mgi.p	33.5±0.945	34.75±0.968	0
VIII	PLC +INH	400mgP.O.+300mg i.p	34.3±0.977	36.7±0.987	0

Effects of PLC and ELC on STR induced convulsions

STR (2mg/kg s.c.) was used for inducing convulsions in all three groups. ELC and PLC 200mg/kg and 400mg/kg groups, onset of time (seconds) to show convulsions were found as respectively. The animal is 200mg/kg and 400mg/kg treated group showed significant difference in delaying the onset of convulsions. Where in PLC there was no any significant change.

Table no. 4 Effect of PLC and ELC on strychnine induced convulsion

Groups	Treatment	Dose	Latency (on set of convulsion in sec.)	% Protection
I	Control+ Str	10 ml p.o. +2 mg i.p.	82.50±1.282	0 %
II	Diazepam + Str	5 mg i.p. + 2 mg i.p.	120.00±0.00	100%
III	ELC+ Str	100 mg p.o.+ 2 mg i.p	53.88±4.090	50%
IV	ELC+ Str	200 mg p.o.+ 2 mg i.p	90.00±2.752	55%
V	ELC+ Str	400 mg p.o.+ 2 mg i.p	106.9±5.177***	60%
VI	PLC+ Str	100 mg p.o.+ 2 mg i.p	82.50±1.126 ns	0%
VII	PLC+ Str	200 mg p.o.+ 2 mg i.p	82.88 ±1.407 ns	0 %
VIII	PLC+ Str	400 mg p.o.+ 2 mg i.p	82.88± 1.202 ns	0%

In the strychnine-induced seizure model, it is known that strychnine a potent spinal cord convulsant, blocks glycine receptor selectively to induce excitatory response in the CNS.[31]

Glycine is an inhibitory neurotransmitter in the CNS and strychnine is competitive antagonist of glycine receptor.[32] The PLC did not show any significant inhibition against STR induced convulsions, but ELC shows the good results. It might not interfere with glycine transmission. The suppression of seizure by diazepam was indirectly enhancing glycine inhibitory mechanisms[31]. Here, we found that ELC may act as that of the diazepam.

CONCLUSION

In both the models INH and STR, *Leucas cephalotes* (Roxb.) flowers ethanolic extract was found to be the significant protector and delayer of the action

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