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Anticonvulsant Effect of *Leucas cephalotes* (Roxb.) Flowers in Albino Mice

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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, the flowers of plant were used for the study. The flowers of *Leucas cephalotes* (Roxb.) were shade dried and extracted by soxhlet extraction method, petroleum ether and hydro-alcoholic extracts were used. After the toxicity studies, the 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 PTZ (pentylenetetrazole) and MES (Maximal Electro Shock) induced convulsion.

INTRODUCTION

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.[1] Epilepsy is most common neurological disorders affecting people across all nationalities.[2] 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.[3] 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.[4] 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.[5] 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 results 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.[1] It may be idiopathic (primary / genetic) or symptomatic (secondary / cryptogenic / reactive) epilepsy. In spite of intensive investigations, the pathophysiology is still poorly understood. 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.[7] And also, there are various other factors which cause seizures, such as oxidative stress developed by the free radical generation.[8]Epilepsy by itself means "idiopathic" in contrast to the commonly used but incorrect meaning of cause unknown.[9]

Even the current antiepileptic drugs such as oxcarbazepine, gabapentin, tiagabine, topiramate, levotiracetam, 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 are parent prodrug of phenytoin that is more tolerable than parenteral phenytoin[10.] Therefore 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 the epilepsy and to determine the effects of the chemicals for the same. This 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.[11] 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.[12]

By keeping in mind, we studied the properties of the drugs which were reported but not scientifically proven so we chose plant *Leucas cephalotes*. It was reported for various CNS activities. *Leucas cephalotes* (Roxb.) spreng. (syn- phlomicepholotes)is commonly known as "dronpushpi" in Sanskrit and peddatumni in Telugu.[13] 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.[14] Flowers are stimulant, emmenagogue, diaphoretic and expectorant and syrup sometimes mixed with honey are useful in diagnostic remedies of cough and cold. [15]Leaves are used to treat bleeding and itching in piles if smoked 1:3 ratio. It also shows activity in fever, urinary discharge. [16]Pharmacologicaly plant is reported for multiple activity including antifilarial[17], antibacterial[18], antidiabetic[19], antiinflamattory[20], antioxidant, analgesic, antithelmintic[21], antimicrobial and antioxidant[22]. By keeping in mind, we go for the

anticonvulsant activity of the plant as it has strong antioxidant with the aim of treating an epileptic is not only to abolish the occurrence of seizure but also to lead a self sustain life.

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 (ELC)) and petroleum ether (PLC) below 24^{o}C temperature. After filtration, dark green coloured solution obtained from the *Leucas cephalotes* was evaporated at 50^{0}C .[23]

Animal Selection:

Albino mice weighting (18g-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 maintained at the animal house of the institution and on standard pellet diet with 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

Pentylenetetrazole (Sigma, St.Louis, USA), Diazepam (Ranbaxy), Phenytoin sodium (M.J. Pharmaceuticals, Gujrat.)

Statistical Analysis and Calculations:

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

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

The acute toxicity study of *Leucas cephalotes* ethanolic and petroleum ether extract ELC and 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.[24]

A. Antiepileptic activity of ELC and PLC on MES induced convulsions[25]

The method used was an described by Dandiya & Sakina, 1999. Mice of either sex (18-20gm) were divided into eight groups of six mice each. The drugs and chemicals were prepared fresh; the concentration, dose and the duration before induction of convulsion were as follows:

Table No. 1 Dose and use for the administration.

Groups	Drugs	Dose and route of administration	Time of administration prior to maximal electroshock
I	Saline	1ml/mice, p.o.	30 minutes
II	Phenytoin	25mg/kg, i.p.	30 minutes
III	PLC	100 mg/kg	2 hours
IV	PLC	200 mg/kg	2 hours
V	PLC	400 mg/kg	2 hours
VI	ELC	100 mg/kg	2 hours
VII	ELC	200mg/kg	2 hours
VIII	ELC	400 mg/kg	2 hours

B. Antiepileptic activity of ELC and PLC on PTZ induced convulsions.[26,27]

In this kind of model, PTZ is administered as inducing agent for convulsion, Mice of either sex (18 to 22gm) were divided into 8 groups of 6 mice. Each animal is placed into an individual plastic cage for observation lasting 1 h. Seizures and tonic-clonic convulsions were recorded. Onset of seizures and recovery time of the animals were recorded.

Table No. 2 Drugs and their concentrations given for the PTZ.

Groups	Drugs	Dose and route of administration	Time of administration prior to PTZ
I	PTZ	80mg/kg, i.p.	30 minutes
II	Diazepam	4mg/kg, i.p.	30 minutes
III	PLC	100 mg/kg	1 hrs
IV	PLC	200 mg/kg	1 hrs
V	PLC	400 mg/kg	1 hrs
VI	ELC	100 mg/kg	1 hrs
VII	ELC	200mg/kg	1 hrs
VIII	ELC	400 mg/kg	1 hrs

The severity of convulsions was assessed by the duration of tonic extensor, and recovery phase for each animal. The duration of phase for each animal (in second) was measured by using Stopwatch. The starting time for each phase was noted and then converted to duration of each phase by deducting starting time of one phase from the starting time of the previous phase.

RESULTS AND DISCUSSION

The effect of *Leucas cephalotes* ethanolic extract and petroleum extract on various animal models was observed by monitoring different parameters during the study.

A. 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.

B. Antiepileptic effect of PLC and ELC on MES induced convulsions

Leucas cephalotes extract at 200mg/kg and 400mg/kg b.w. p.o. produced a more significant ($p<0.001$) effect in the phase of hind limb extensor, recovery found significant, as compared to control and standard. The extensor phase was significantly decreased as that of standard. It also didn't cause a decrease in the phase of flexion, which was just statistically significant ($p<0.01$) in comparison to control seconds. The MES model has served to identify antiepileptic drugs that are functionally similar to phenytoin and most of these compounds display, in common, the ability to inactivate voltage dependent Na+ channels in a dose dependent fashion, such compounds suppress sustained repetitive firing in cultured neurons. Hence, *Leucas cephalotes* may be expected to have a similar type of mechanism

Leucas cephalotes at the above mentioned dose, administered acutely, might be effective against partial and secondary generalized seizure as depicted by the protection by ELC in this model.[28]

Table No. 3 Effect of PLC and ELC on MES induced convulsions.

Groups	Treatment	Dose	Flexon	Extensor	Clonus	Recovery %
I	Control (3%PEG) MES	10ml p.o. +MES	9.63±0.905	14.73±0.2234	14.80±0.223	80
II	Phenytoin	25mg i.p.+MES	48.50±1.464	0.0±0.00	55.70±0.981	100
III	ELC+MES	100mg p.o. +MES	34.88±0.895	29.90±0.900	35.87±0.890	50
IV	ELC+MES	200mg p.o. +MES	46.25±1.098	40.00±1.201**	45.28±1.20	70
V	ELC+MES	400mg p.o.+MES	55.38±1.792	50.33±1.790***	54.32±1.70	90
VI	PLC+MES	100mg p.o. +MES	29.88±0.742^{ns}	30.90±0.740	32.23±0.745	0
VII	PLC+MES	200mg p.o. +MES	29.63±0.905^{ns}	30.90±0.740	31.90±0.740	0
VIII	PLCE +MES	400mg p.o.+MES	30.88±0.971 ^{ns}	32.99±0.973	33.90±0.740	0

C. Antiepileptic effect of PLC and ELC on PTZ induced convulsions

In PTZ induced convulsions, the parameter monitored was onset of convulsions (as indicated by Jerks, Clonus and Extensor). PTZ (70mg/kg s.c.) was used for inducing convulsions in all three groups. In ELC 200mg/kg and 400mg/kg dose groups, onset of time (seconds) to show convulsions were found as delayed respectively. The animal was 200mg/kg and 400mg/kg treated group showed a significant difference in delaying the onset of convulsions.

Table no. 4 Effect of PLC and ELC on PTZ induced convulsions

Groups	Treatment	Dose mg/kg	Onset of convulsion (in sec)	% Protection
I	3% PEG+ PTZ	10 ml p.o. +80 mg	29.75±0.2500	0%
II	Diazepam +PTZ	5 mg p.o..+ 80 mg i.p.	167.1±5.360	100
III	ELC+PTZ	100 mg p.o. +80mg i.p.	38.38±1.499	50
IV	ELC+PTZ	200 mg p.o. +80mg. i.p.	48.25±2.776**	60
V	ELC+PTZ	400 mg p.o.+80 mg i.p.	63.13±2.979***	75
VI	PLC+PTZ	100 mg p.o. +80mg i.p.	29.63 ± 0.3750^{ns}	0
VII	PLC+PTZ	200 mg p.o. +80mg. i.p.	30.13 ± 0.4407^{ns}	0
VII	PLC+PTZ	400 mg p.o.+80 mg i.p.	30.38 ± 0.3750^{ns}	0

Currently, available drugs are efficient to control the epileptic sign about 50% of the patients, another 25% may show improvement whereas the reminder does not benefit furthermore considering the side effects from the drugs often render the treatment very difficult so that the demand of new antiepileptics increases. One of the approaches for the finding of the anticonvulsant drugs is to investigate through naturally occurring compound, which may belong to new structural classes.[29] MES is commonly used models for preliminary testing of anticonvulsant drugs that produces generalized tonic-clonic seizure i.e. hind limb tonic extensor, tonic flexion and clonic convulsion.

In MES induced convulsions ELC at 200mg/kg and 400mg/kg b.w. p.o. produced a more significant ($p<0.001$) effect in the phase of hind limb extensor. It also didn't cause a decrease in the phase of flexion, which was just statistically significant ($p<0.01$) in comparison to control seconds. It may act with same mechanism of phenytoin.

PTZ induced convulsions may be prevented by reducing the T type current or by facilitating the GABA-A receptor mediated inhibitory neurotransmitter[30]

PTZ (70mg/kg s.c.) was used for inducing convulsions in all three groups. In ELC flower extract 200mg/kg and 400mg/kg dose groups, onset of time (seconds) to show convulsions were found delayed respectively. The animal is 200mg/kg and 400mg/kg treated group showed a significant difference in delaying the onset of convulsions.

CONCLUSION

As per all above study suggest, the drugs follow both the mechanism in the different dosing patterns and the stimulant or inducing agent drug may act as phenytoin and diazepam which are used as the antiepileptics. *Leucas cephalotes* flowers may have anti-epileptic activity as that of the established drugs and do not have any side effects.

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