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ANTICONVULSANT ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF *LEPISANTHES TETRAPHYLLA (SAPINDACEAE)*

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KEYWORDS:

Anticonvulsant, *Lepisanthes tetraphylla,* Sapindaceae, MES & PTZ method.

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ABSTRACT

Lepisanthes tetraphylla (Sapindaceae) traditionally used for the treatment of Elephantiasis, skin disease, fever and claimed for its anti-convulscent property. The present study was aimed to evaluate the preliminary phytochemical analysis and anti-convulscent activity by MES (Maximal electroshock induced convulsion) and PTZ (Pentalinetetrazole induced convulsion) method. The result showed highly significant activity similar to the standard drug Phenytoin. Thus the study proof scientifically the folklore claim and traditional uses of the plant *Lepisanthes tetraphylla*.

INTRODUCTION:

Epilepsy is a brain disorder in which a person has repeated seizures (convulsions) over time. Seizures are episodes of disturbed brain activity that cause changes in attention or behavior. A measurement of electrical activity in the brain and brain scans such as magnetic resonance imaging or computed tomography are common diagnostic tests for epilepsy [3]. The current therapeutic treatment of epilepsy with modern antiepileptic drugs (AEDs) is associated with side-effects, dose related and chronic toxicity. Approximately 30% of the patients continue to have seizures with current therapy. Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of AEDs with novel structures and better safety and efficacy.

Lepisanthes tetraphylla

In the traditional the leaves are one of the ingredient in Ayurvedic shampoos and cleansers. The plant is being used as a nontoxic laundry detergent. Traditionally the plant is used for the treatment on Elephantiasis and Skin diseases [4]. The juice of the leaves is used in fever, cough. The plant is used as an anti-convulscent, mucolytic agent and surfactant. The plant is used in Ayurvedic medicine in the treatment of eczema, psoriasis and for removing frechlets. There is however no scientific studies on anticonvulsant activity of *Lepisanthes tetraphylla* have been done. Hence the present study was aimed to carry out the presence of phytoconstituents and anticonvulscent activity.

MATERIALS AND METHODS

Collection and authentication of plant material

The whole plant of *Lepisanthes tetraphylla* (Vahl) Radlk. were collected from Madhakadipattu, Puducherry, India in the month of November, 2012 and the plant was authenticated by the taxonomist of Department of Botany, French Institute, Pondicherry. For a future reference Herbarium No: HIFP.026652.

Preparation of plant powder:

The leaves of *Lepisanthes tetraphylla* (Vahl) Radlk. were collected by hand plucking and then it was washed thoroughly to remove the foreign matters and dried in shade. Then the dried plant material was powdered and passed through the sieve (mesh size #40). Finally the powder was stored in air tight container at room temperature away from the sunlight.

Extraction

The leaves was dried under shade, reduced to moderately coarse powder, loaded into soxhlet extractor and was subjected to successive extraction with Petroleum Ether, Chloroform, Ethanol and Water to get different extracts.

Preliminary phytochemical screening

The preliminary phytochemical screening of Ethanolic extract for the leaves of *Lepisanthes tetraphylla* (Sapindaceae) was carried out as per Pulok K Mukherjee.

Experimental animals [5-6]

Albino Wister rats (150-200g) of either sex will be used. The animals were maintained under standard laboratory conditions (12 hr light and 12 hr dark) in a room with controlled temperature (24 ± 3^0 C) during experimental period. Food of normal rat chow and water was provided *ad libitum*. Rats shall be randomly assigned to four groups six animals in each group.

Acute toxicity study

Acute toxicity study was performed according to OECD guidelines 423. The methods use defined doses at 100, 200, 400, 2000mg/kg body weight. The starting dose of ethanolic extract 2000mg/kg body weight was administered. They were continuously observed for 3 days to detect changes in the skin, fur, eyes, mucous membrane, respiratory, circulatory, autonomic central nervous system, somoto motor activity and behavior pattern. A group of animals treated with the vehicle 0.1ml of 0.5%w/v carboxy methyl cellulose served as control. Based on the results of preliminary toxicity testing, the dose of 200mg and 400mg/kg body weight of *Lepisanthes tetraphylla* were chosen for further experiments.

The screening of anti-convulscent activity was done by MES and PTZ induced convulsion

a)Maximal electroshock seizures (MES) test

Group I - Control (which received no treatment)

Group II - Standard drug (Phenytoin 25mg/kg, i.p).

Group III - Test drug (200 mg/kg, p.o)

Group IV- Test drug (400 mg/kg, p.o)

After one hour the drug treatment - seizures was induced to all the animals by using Electroconvulsiometer (150 mA current for 0.2 sec). The duration of various phases of convulsion was observed by giving importance to hindleg tonic extension.

b)PTZ-induced seizures [7-8]

Group I - Control (which received 0.5% w/v carboxy methyl cellulose)

Group II - Standard drug (Diazepam 4 mg/kg, p.o).

Group III- Test drug (EELT 200 mg/kg, p.o)

Group IV- Test drug (EELT 400 mg/kg, p.o)

After one hour, PTZ (Pentalinetetrazole 90 mg/kg b.w) was administered to all the groups to induce clonic convulsion. Animals were observed for a period of 30 mins after PTZ administration. Ability to delay the onset and duration of clonic convulsion was noted percentage protection of animals for mortality was recorded.

Statistical Analysis [9]

Data obtained were statistically analyzed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison post hoc tests to compare the level of significance between control and experimental groups. All statistical analysis was evaluated using SPSS version 17.0 software. The values of $p < 0.05$ were considered as significant.

Results: Preliminary Phytochemical Screening of *Lepisanthes tetraphylla* (Sapindaceae)

Table.1 Preliminary Phytochemical Screening of *Lepisanthes tetraphylla* (Sapindaceae)

S.No.	Test for the Phytoconstituent	Experiment	Inference (Ethanol)
1.	Carbohydrates	1. Molisch's reagent	+
		2. Fehling's solution on A&B	_
		3. Benedict's reagent	+
		4. Barfoed's reagent	_
2.	Protein and Amino acids	1. Biuret test	+
		2. Millon's reagent	+
3.	Alkaloids	1. Dragendroff's reagent	_
		2. Mayer's reagent	_
		3. Hager's reagent	_
		4. Wager's reagent	_
4.	Steroids	1. Libermann's burchard reagent	+
		2. Salkowski test	+
5.	Phenols	1. Ferric chloride	+
		2. 10% sodium chloride	+
6.	Tannins	1. 10% sodium chloride	+
		2. Aqueous bromine solution	_
7.	Flavonoids	1. Shinoda's test	_
		2. Zinc hydrochloride test	+
8.	Gylcosides	1. Keller-killiani's test	_
		2. Borntrager's test	_
		3. Legal's test	_
9.	Saponins	1. Foam test	+
		2. Heamolysis test	+

The screening of anticonvulsant activity was done by MES and PTZ induced convulsion

a)Maximal electroshock seizures (MES) test:

Table.2 Anticonvulsant effect of leaf extract of *Lepisanthes tetraphylla*

Drug treatment	Various phase of convulsion (sec)			
	Flexion	Extension	Stupor	Recovery
Control	8.167 ± 0.4773	23.67 ± 0.6667	36.33 ± 0.4216	150.3 ± 0.7149
Standard	5.833 ± 0.6009*	0.00 ± 0.00	17.50 ± 0.5627*	86.50 ± 0.4282*
EELT (200 mg/kg)	6.667 ± 0.3333	8.500 ± 0.4282	26.67 ± 0.5578	116.0 ± 1.065
EELT (400 mg/kg)	5.500 ± 0.4282*	4.167 ± 0.4773*	17.83 ± 0.4773*	87.17 0.7923*

**P<0.001 when compared with control.*

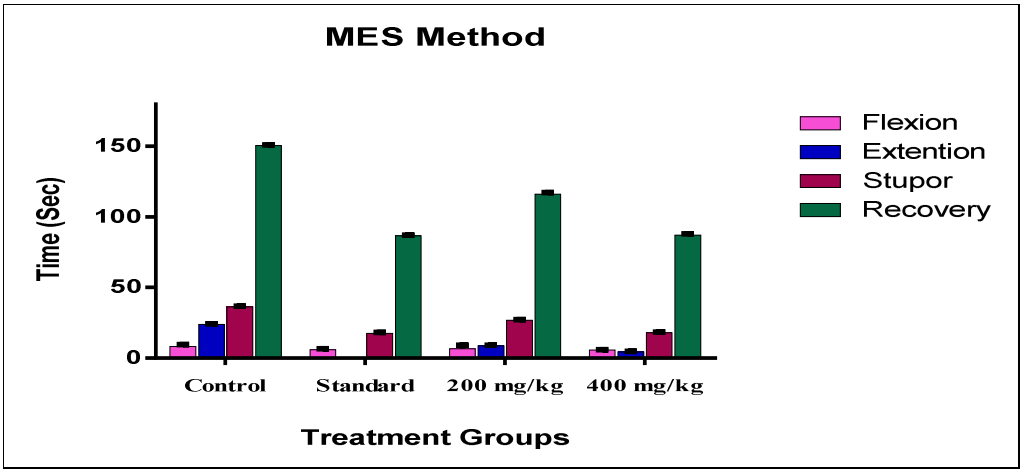

Fig. 1 Anticonvulsant effect of leaf extract of *Lepisanthes tetraphylla*

b)PTZ-induced convulsion:

Table.3 Anticonvulsant effect of leaf extract of *Lepisanthes tetraphylla*

Drug treatment	Onset of convulsion	Duration of convulsion	Nature & severity
Control	54.75 ± 4.100	601.3 ± 4.53	Jerkey movement straub tail clonic convulsion
Standard	0.00 ± 0.00*	0.00 ± 0.00*	Absence
EELT (200 mg/ml)	177.5 ± 4.173	251.3 ± 8.518	Jerkey movement clonic convulsion
EELT (400 mg/ml)	0.00 ± 0.00*	0.00 ± 0.00*	Absence

**P<0.001 when compared with control.*

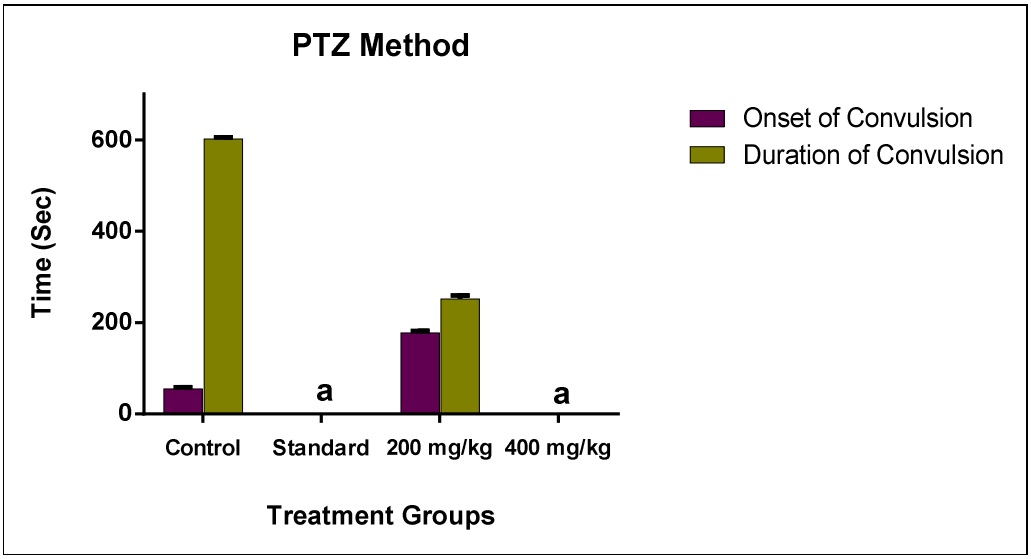

Fig. 2 Anticonvulsant effect of leaf extract of *Lepisanthes tetraphylla*

DISCUSSION:

It was found from the above observations that Ethanolic extract of *Lepisanthes tetraphylla* shows anticonvulsant activity against seizures induced by MES and PTZ at a dose 200 and 400 mg/kg. Preliminary phytochemical analysis showed the presence of carbohydrates, protein, steroids, tannins, phenols and saponins.

CONCLUSION:

In the traditional system of medicine the plant *Lepisanthes tetraphylla* (Sapindaceae) is being used in the treatment of epilepsy, elephantiasis, fever and skin disease. From the above study it was concluded that the ethanolic leaf extract of *Lepisanthes tetraphylla* exhibits highly significant activity equivalent to that of the standard drug Phenytoin and Diazepam.

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