

Original Research Article

Effect of ethanolic extract of *Centella asiatica* on maximal electroshock seizure in albino mice

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ABSTRACT

Background: Animal models had always served as integral part in introducing newer drugs for epilepsy. India has long tradition of using herbal medicines. *Centella asiatica* is an ingredient of various ayurvedic preparations to treat brain related disorders like insanity, memory loss and epilepsy. This research is done to find out the usefulness of *Centella asiatica* in epilepsy.

Methods: Ethanolic extract of the plant is prepared using Soxhlet apparatus. Experimental seizure is induced in albino mice by maximal electroshock method. Resulting seizure goes through different phases. Duration of the phase of hind limb extension was the measured data. Clinically used anti epileptic drugs can abolish hind limb extension. Effect is compared to that of standard drug phenytoin. Four different doses of the plant extract; 100mg, 200mg, 500mg and 1000mg per kilogram bodyweight was given orally. Statistical analysis of data was done by one way ANOVA and Dunnett test.

Results: Ethanolic extract of *Centella asiatica* exhibited statistically significant protection from maximal electroshock seizures. All given doses of the extract had $p < 0.05$ when compared to control.

Conclusions: *Centella asiatica* is potential source of anti-epileptic drug. Detailed phytochemical studies and animal experiments are recommended.

Keywords: *Centella asiatica*, Epilepsy, Maximal electroshock seizures

INTRODUCTION

British neurologist Jackson explained epilepsy as sudden excessive and disorderly discharge of cerebral neurons. Intense paroxysm of involuntary muscle contractions are called convulsions and it is common symptom of epilepsy. The drugs for epilepsy are also called anticonvulsants.¹ Epilepsy has been recorded throughout history and humans had tried to find remedies using herbal preparations. Judicious selection of drugs from traditional medicine attained new dimension by scientific methods of research. As the science of pharmacology flourished, there was sufficient and accurate methods to experiment antiepileptic drugs in animals.² Two

experimental models of seizure are widely used. In the first model, seizure is induced in rodents by pentylene tetrazol injection and the second is by electric current. Drugs which reduce seizure following electric stimulation are clinically useful in tonic clonic convulsion or major epilepsy.³

This research is done to evaluate effect of ethanolic extract of *Centella asiatica* on electroshock seizures using albino mice. *Centella asiatica* is a small trailing herb from Umbelliferae family. It is seen worldwide and is called Indian pennywort. The plant has slender stem with nodes and internodes. Round sessile leaves, flowers and roots emerge from this nodes.⁴ Literature mentions

the use of this plant for various diseases related to nervous system. Indian physicians used *Centella asiatica* for various diseases like epilepsy, insanity, memory boosting, rejuvenation therapy and leprosy.⁵ Experiments in thirty mentally retarded children showed significant improvement in performance IQ social quotient, immediate memory span and reaction time.⁶ Leaves of this plant were used to treat delay in starting of speech in children.⁷ Ayurvedic polyherbal preparation MENTAT containing *Centella asiatica* significantly prevented anxiety and depression in albino rats.⁸ Sushruta has mentioned its use for improving duration of human life and memory.⁹ Powdered leaves of *Centella asiatica* were taken along with milk for improving memory.¹⁰

METHODS

Plant extract preparation

Locally available whole *Centella asiatica* plants were collected from rural areas of Trivandrum district in Kerala. Government Ayurveda College, Thiruvananthapuram confirmed the authenticity of the specimen. Plants were cut into small bits after drying under shade. Using the Soxhlet apparatus 50% ethanolic extract was prepared.¹¹ Final thick and tarry extract was analyzed for chemical constituents and found to have carbohydrates, steroids, tannins, alkaloids, flavonoids, triterpenes.¹² This extract was freshly reconstituted with distilled water for administering to animals.

Experimental animals

Swiss albino mice of either sex weighing 25-30 grams were used for the experiments. Mice were obtained from the animal house of Government medical college, Trivandrum. It was allowed free access to rodent food pellets and sufficient water. Natural dark and light cycle was maintained and acclimatized for the laboratory conditions before the experiments. Institutional animal ethics committee of Government medical college Trivandrum approved the study protocol.

Chemicals

Phenytoin supplied by Zydus Cadila Healthcare Ltd., Mumbai. Electro-convulsimeter purchased from Labtech equipments, Ambala, Haryana.

Acute toxicity study

Acute toxicity study of plant extract was done as per the organisation for economic co-operation and development guidelines. Total ten mice were used as five groups containing two in each. *Centella asiatica* extract was orally administered in doses of 100 mg/kg, 200 mg/kg, 500 mg/kg and 1000 mg/kg body weight. Another group was given distilled water as control. At one hour, two hours, four hours, twenty four hours, forty eight hours

and seventy two hours the animals were observed to find toxic effects.

Maximal electroshock seizure

Animals are fasted overnight for 12 hours. Six groups with six mice in each group are used. The mice are subjected to 30mA current for 0.2 seconds through corneal electrodes.¹³ Resulting convulsion has various phases. At the beginning there is tonic flexion of all limbs followed by tonic extension of hind limbs. Finally whole body goes into clonic convulsion and post ictal depression. Lowering of the duration of the hind limb extension is considered as a measure of protection from convulsions.¹⁴ Extract of *Centella asiatica* is given at doses of 100 mg/kg, 200 mg/kg, 500 mg/kg and 1000 mg/kg orally one hour before applying electric shock. An initial screening for their ability to develop full seizure was performed and only those which responded well were used in actual study.

Statistical analysis

Duration of hind limb extension is the compared parameter. It is tabulated as mean value in seconds. Comparison of mean value for statistical significance performed using one way analysis of variance and post hoc Dunnett test. Graphpad prism 8 software was used for the statistical evaluations. $P < 0.05$ was considered to be statistically significant.

RESULTS

Ethanolic extract of *Centella asiatica* has protective action against maximal electroshock seizures in albino mice. It did not show any signs of acute toxicity at doses of 100 mg/kg, 200 mg/kg, 500 mg/kg and 1000 mg/kg body weight. Plant extract reduced duration of hind limb extension of mice stimulated by maximal electroshock (Figure 1).

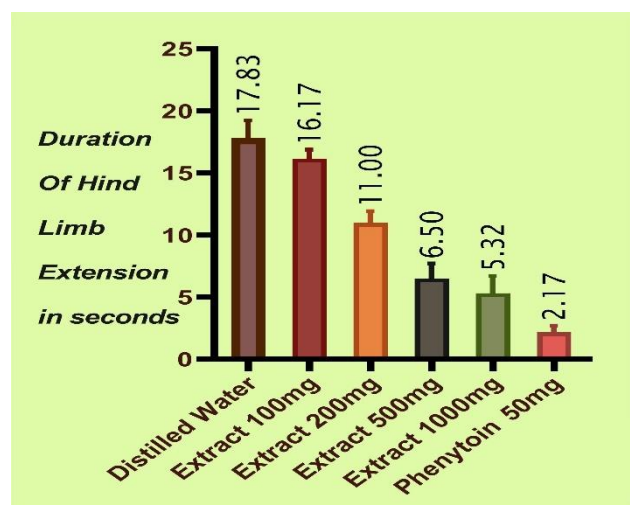


Figure 1: Mean and standard deviation value of experimental groups.

Table 1: Effect of *Centella asiatica* on maximal electroshock seizure in albino mice.

Treatment groups, N=6	Dose (mg/kg)	Duration of hind limb extension (seconds), Mean±SEM	Incidence of seizure (%)
Distilled water	--	17.83±0.5719	100
Plant extract	100	16.17±0.2906*	100
Plant extract	200	11.00±0.3768**	100
Plant extract	500	6.50±0.4906**	100
Plant extract	1000	5.317±0.5695**	100
Phenytoin	50	2.167±0.2201**	66.66

*p-value<0.05, **p-value<0.0001v/s control, SEM: standard error of mean.

The mean value of this duration for each experimental group is tabulated. Distilled water given to control group showed hind limb extension duration for 17.83 seconds. This time duration is just 2.17 seconds with standard drug phenytoin. Response with dose of 200 mg/kg, 500 mg/kg and 1000 mg/kg was significant statistically with $p < 0.0001$ (Table 1).

DISCUSSION

Epilepsy is major disease of central nervous system where drugs are main facet of treatment.¹⁵ Available drugs are either enhancing inhibitory neurotransmitter or suppressing excitatory neurotransmitters. Few drugs also modify ionic conduction of neurons.¹⁶ Still the drugs are effective only in 80% of patients. Patient acceptance is not satisfactory due to adverse effect and long duration of treatment. Hence there is clear need for research to develop more specific and effective antiepileptic drugs.¹⁷ Grand mal epilepsy characterized by generalized tonic clonic convulsion is the most common type in epilepsy. Drugs to treat this type of epilepsy is seen to abolish hind limb extension of rodent following maximal electro shock induced seizures.¹⁸ In this study ethanolic extract of *Centella asiatica* had shown statistically significant result by reducing hind limb extension phase.

The plant extract is also found to be safe at dose used for this study. *Centella asiatica* had undergone several animal studies in the past. It is clear that the plant has variety of phytochemicals acting in various body systems. In vitro study has demonstrated its ability to inhibit angiotensin converting enzyme.¹⁹ Research in guinea pigs has shown better wound healing effect of this plant.²⁰ Plant extract has antioxidant property and ability to reduce stress as demonstrated in albino mice.²¹ Studies in rodents have shown analgesic effect also.²² Research has proved its ability to inhibit proliferation of tumor cell lines in laboratory.²³ *Centella asiatica* has also benefitted canines infested with *Dirofilaria immitis*.²⁴

CONCLUSION

This study arrives at a conclusion that *Centella asiatica* inhibits experimental seizure in animals. Use of *Centella asiatica* in folk medicine to treat epilepsy is substantiated

scientifically by this study. Hence detailed study by extracting active phytochemicals may provide novel anti-epileptic drugs.

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