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Original Research Article

Effect of ethanolic extract of *Centella asiatica* on pentylene tetrazol induced seizure in albino mice

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ABSTRACT

Background: Various preparations of the plant *Centella asiatica* were used in traditional medicine for treating memory disorders, insanity, epilepsy, headache, fever and dysentery. Hence this study was conducted to test the effect of *Centella asiatica* on classical animal model of epilepsy.

Methods: First the ethanolic extract of *Centella asiatica* was subjected to acute toxicity study. Intraperitoneal injection of pentylene tetrazol in albino mice produces seizure. Plant extract was evaluated for protection from these experimental seizures. Four groups of animals were given the extract orally at 100mg, 200mg, 500mg and 1000mg per kilogram body weight. One group received phenobarbitone as standard drug and another group received distilled water as control. Time of onset of clonus is the study parameter. Statistical analysis of data was done using analysis of variance followed by Dunnet's test.

Results: Various doses of plant extract ranging from 100mg to 1000mg/kg exhibited protection from experimentally induced seizures. Compared to control, the plant extract showed statistically significant delay in time of onset of clonus.

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

Keywords: *Centella asiatica*, Epilepsy, Pentylene tetrazol

INTRODUCTION

Epilepsy is a major disorder of central nervous system. The main abnormality is excessive electrical discharge of certain neurons in the brain.¹ Modern medicine uses many drugs to treat epilepsy and it is only partially successful. About 80% of patients are completely controlled of seizures and still suffer adverse effects.² Search for an ideal drug with lesser adverse effect and useful in different types of epilepsy is still going on. India has rich flora due to extreme variation in geography and climate. Many of these plants are potential remedy for human diseases and are un explored even now.³ In this study *Centella asiatica* is subjected to experiments and

find out whether it has anti-convulsant effect. *Centella asiatica* is a small trailing herb distributed throughout the world in tropical and subtropical regions. It grows in wet places like banks of pond and stream. It has slender stem and round sessile leaves. In north India this plant is called Brahmi whereas south Indian region calls *Bacopa Monnieri* as Brahmi.⁴ Several uses of this plant in folk medicine have been recorded in literature. Sushruta has mentioned its use for improving duration of human life and memory.⁵ Polyherbal unani medicine Dimagheen containing *Centella asiatica*, *Emblica officinalis* and *Rosa damascena* reduced anticholinesterase activity in brain and improved cognitive functions in experimental models of rat and mice.⁶ Indian physicians used *Centella*

asiatica for various diseases like epilepsy, insanity, memory boosting, rejuvenation therapy and leprosy.⁷ Ayurvedic polyherbal preparation containing *Centella asiatica* and *Nardostachys jatamansi* controlled generalized anxiety in twelve patients.⁸ Powdered leaves of *Centella asiatica* was taken with milk to boost memory.⁹ Leaves of this plant were used to treat delay in starting of speech in children.¹⁰

METHODS

Preparing the plant extract

Centella asiatica whole plant was collected locally from Trivandrum district of Kerala in January month. It was pharmacognostically identified by Government Ayurveda College, Trivandrum. Plant was thoroughly cleaned to remove soil and shade dried, cut into smaller bits and used for preparing ethanolic extract. 50% ethanolic extract was prepared by method of Rosenthaler using Soxhlet apparatus.¹¹ Solvent was evaporated and a thick greenish black tarry mass obtained. This was stored in desiccator and taken out as and when required. Pharmacognosy tests were conducted to detect major phytochemical constituents.¹²

Experimental animals

Adult swiss albino mice weighing 25-30 g were used. It was maintained under identical conditions at the animal house of Govt. medical college, Trivandrum. They were provided commercial pellet diet and water ad libitum. Acclimatization to laboratory environment was done and maintained natural light dark cycle. Experimental protocol was approved by the institutional animal ethics committee of Govt. medical college Trivandrum.

Drugs and chemicals

Pentylene tetrazol as dry powder was procured from Himedia Laboratories, Mumbai. It was freshly diluted in sterile water for injection. Phenobarbitone tablets were obtained from Abbott Pharmaceuticals, Chennai.

Statistical analysis

Time at which clonic seizure start is noticed for each animal. Mean value of this time measured in seconds is tabulated. Analysis of data was done using graphpad prism-8 Software. One-way analysis of variance (ANOVA) performed to compare means of each group with the mean of the control group. Dunnett test was done for post hoc analysis after ANOVA. p-value<0.05 was considered to be statistically significant.

Acute toxicity study

Acute toxicity studies were conducted according to the organization for economic co-operation and development

423 guidelines.¹³ All the treated animals were observed for any abnormal or toxic manifestations and mortality. After overnight fasting, five groups of mice with two mice in each group were taken. First group of animals received distilled water as control. *Centella asiatica* extract was orally administered in doses of 100mg, 200mg, 500mg and 1000mg/kg body weight to the remaining group of animals. They were observed for one hour, two hours, four hours, twenty-four hours, forty-eight hours and seventy-two hours.¹⁴

Anticonvulsant effect evaluation

Anticonvulsant effect was investigated against pentylene tetrazol induced seizures as per the method described by Fischer.¹⁵ Six groups with six animals in each group were used. Seizure was induced by single injection of 80mg/kg bodyweight of pentylene tetrazol subcutaneously. *Centella asiatica* extract was administered orally one hour prior to the injection at dose of 100mg, 200mg, 500mg and 1000mg/kg bodyweight. Distilled water was used to dilute plant extract. One group of mice received standard drug oral phenobarbitone at dose of 50mg/kg bodyweight. Last group received distilled water orally and serves as control. Experimental convulsion begins with transient intermittent myoclonic jerks without loss of posture and the tail gets erect. Subsequently the convulsion become generalised, posture is lost and clonus sets in.¹⁶ Mice was observed for 30 minutes for the onset of clonus. Time of onset of clonus is the measured data and tabulated. Delay in this parameter indicates its clinical advantage as drug for petitmal epilepsy.¹⁷

RESULTS

Acute toxicity study did not show any mortality or signs of toxicity at doses of 100mg, 200mg, 500mg and 1000mg/kg body weight. On chemical analysis of this extract, there was presence of alkaloids, flavanoids, triterpenes, steroids, tannins and carbohydrates.

Pentylene tetrazol induced seizure was seen in all animals used for this study. Ethanolic extract of *Centella asiatica* has protected the animals from seizure. There was dose dependant delay in the time of onset of clonic convulsions at all given incremental doses. Onset of clonus is delayed by 14.00 ± 0.7380 seconds when standard drug phenobarbitone is given. 1000mg of plant extract gives a very close value of 12.00 ± 0.5825 . Corresponding values for other test groups and the control group are shown (Table 1).

Doses of 200mg, 500mg and 1000mg have produced statistically significant delay in clonus onset as learned from p-value<0.0001. This was comparable to the effect produced by standard drug phenobarbitone. However, the effect was not superior to standard drug (Figure 1).

Table 1: Effect of *Centella asiatica* on time of clonus onset following pentylene tetrazol injection in albino mice.

| Treatment given | Dose (mg/kg) body weight | Number of mice | Time of clonus onset in seconds Mean±SEM | Incidence of seizure (%) |
|-----------------|--------------------------|----------------|--|--------------------------|
| Distilled water | - | 6 | 3.333±0.1406 | 100 |
| Plant extract | 100 | 6 | 5.317±0.5695 | 100 |
| Plant extract | 200 | 6 | *7.000±0.3679 | 100 |
| Plant extract | 500 | 6 | **10.30±0.4844 | 100 |
| Plant extract | 1000 | 6 | **12.00±0.5825 | 100 |
| Phenobarbitone | 50 | 6 | 14.00±0.7380 | 100 |

*p-value<0.001, **p-value <0.0001 versus control ANOVA post hoc Dunnett test.

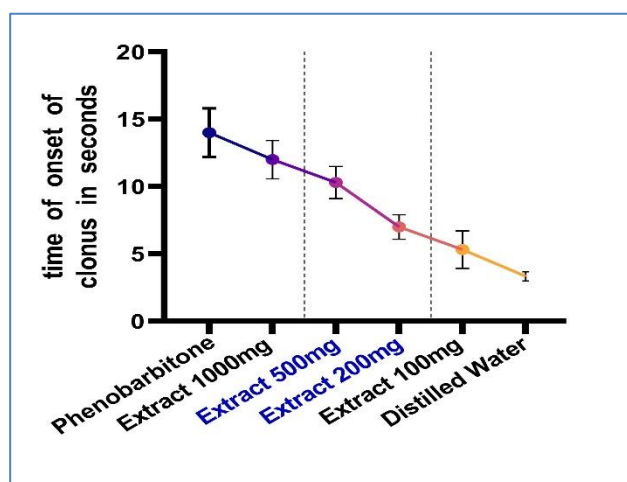


Figure 1: Mean and standard deviation of time of clonus onset in each group.

DISCUSSION

About 1% of world population has epilepsy and drug therapy is the most widely effective mode of treatment. Following the introduction of phenytoin in 1930, modern medicine has developed numerous drugs for epilepsy.¹⁸ This was mainly possible due to specific animal models of epilepsy and knowledge of neurotransmitters.¹⁹ Pentylene tetrazol induced convulsion is a classical experiment for evaluation of anticonvulsant drugs.²⁰ Drugs for controlling absence seizure is identified by this experimental model. Findings of this study clearly indicate that the oral administration of ethanolic extract of *Centella asiatica* exhibit significant anticonvulsant effect in rodents. This finding is in support of its traditional use for epilepsy.

Acute toxicity study also did not show any symptoms of toxicity. There are many earlier studies which observed effect of this plant in various body systems. One research has confirmed the anti-inflammatory activity of this plant.²¹ There was positive effect on local application of *Centella asiatica* extract over striae gravidarum.²² Stress induced gastric ulcer shows healing in experimental rodents.²³ Antioxidant property was demonstrated by the elevated activity of superoxide dismutase enzyme.²⁴

Centella asiatica also has antimicrobial activity as demonstrated by the inhibition of pseudomonas in culture.²⁵ Albino rats showed stimulation of immunity following seven days of this plant extract administration.²⁶

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