

Research Article

Evaluation of Anti-Convulsant Activity of Aqueous Leaf Extract of *Centella asiatica* in Wistar Albino Rats.

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ABSTRACT

Objective: Evaluation of anti-convulsant activity of aqueous leaf extract of *Centella asiatica* in Maximal Electroshock induced (MES) Seizures and Pentylentetrazole (PTZ) induced seizures in rats. Materials and methods: The aqueous leaf extract of *Centella asiatica* was evaluated for its anticonvulsant activity in MES Seizure and PTZ induced seizures in rats. Phenytoin 25mg/kg, Diazepam 4mg/kg were used as the standard drugs for MES seizures and PTZ induced seizures respectively in this study. Results: In MES induced seizures Aqueous Leaf Extract of *Centella asiatica* (ALECA) significantly increased the time of onset of Tonic Hind Limb Extension (THLE) in all the doses (200mg/kg & 400mg/kg) used, and decreased the duration of THLE significantly with 400mg/kg. The percentage of protection of MES seizures with 400mg/kg is 67%. In PTZ induced convulsion ALECA significantly increased the mean latency period in all the doses used. The percentage of protection of PTZ induced seizures with 200mg/kg of ALECA is 33% and 400mg/kg is 67%. Standard drugs like phenytoin in MES induced seizures and diazepam in PTZ induced seizures possesses 100% seizure protection. Conclusion: The aqueous leaf extract of *Centella asiatica* suppresses the seizures induced by maximal electro shock and Pentylentetrazole

Keywords: Anti-convulsant activity, *Centella asiatica*, Maximal electroshock seizures, Pentylentetrazole.

INTRODUCTION

'Seizure' is a paroxysmal event due to abnormal excessive or synchronous neuronal activity in the brain. Depending on the distribution of discharges, this abnormal brain activity can have various manifestations, ranging from dramatic convulsive activity to experiential phenomena not readily discernible by an observer¹. 5–10% of the population will have at least one seizure, with the highest incidence occurring in early and late adulthood. The term seizure needs to be distinguished from that of epilepsy¹. Epilepsy describes a condition in which a person has recurrent seizures due to a chronic, underlying process. Epilepsy refers to a clinical phenomenon rather than a single disease entity¹. Using the definition of epilepsy as two or more unprovoked seizures, the incidence of epilepsy is 0.3–0.5% in different populations throughout the world, and the prevalence of epilepsy has been estimated at 5–10 persons per 1000¹. The drugs used in epilepsy has many adverse effects and *teratogenic* effects that leads to the

search for newer drugs which has less adverse drug reaction and teratogenic effect from the indigenous plant materials².

Centella asiatica L. has been used as a medicinal herb for thousands of years in India, China, Srilanka, Nepal and Madagascar. *Centella asiatica* is one of the precious herbs for revitalizing the nerves and brain cells, hence primarily known as a "Brain food" in India³. In India, *Centella asiatica* is valued as an ethnomedicine as well as in Ayurveda and Unani, the traditional Indian medicinal systems for thousands of years for different ailments like asthma, skin disorders, ulcers and body aches⁴⁻⁸, in treatment of dropsy, elephantiasis, gastric catarrh, kidney troubles, leprosy, leucorrhoea and urethritis⁹, in maternal health care¹⁰, in treatment of stomach disorders and also as a vegetable¹¹.

Centella asiatica (L.) is a prostrate, faintly aromatic, stoloniferous, perennial, creeper herb, attains height up to 15cm (6inches). Stem is glabrous, striated, rooting at the

Table 1: Maximal Electro Shock (MES) Induced Convulsion:

S.No	Group	Drug	Onset of THLE (Sec)	Duration of THLE (Sec)	Number of animals convulsed	Percentage (%) of protection
1	Group I	Normal saline 0.5 ml	2.9 ± 0.18	8.25 ± 0.7	6/6	0%
2	Group II	Phenytoin 25 mg/kg b.w.	0***	0 ***	0/6	100%
3	Group III	ALECA 200mg/Kg b.w.	5.17 ± 0.2***	7.14 ± 0.53	6/6	0%
4	Group IV	ALECA 400mg/Kg b.w.	9.26 ± 1.96***	3.98 ± 0.31***	2/6	67%

*p<0.05, **p<0.01, ***p<0.001

nodes. *Centella asiatica* flourishes extensively in shady, marshy, damp and wet places such as paddy fields, river banks forming a dense green carpet¹² and rather than clayey soil, the sandy loam (60% sand) is found to be the most fertile soil for its regeneration¹³. It contains the phytochemicals like triterpenoids, volatile and fatty acids, alkaloids, glycosides, flavonoids, and other vitamins like B, C and some amino acids³.

MATERIALS AND METHODS

Animals

Five Swiss Albino mice of either sex weighing 28–38 g were used for Acute Oral Toxicity study. 48 Wistar Albino rats of either sex weighing 200–225 g were used to evaluate the anticonvulsant activity. Animals were housed into groups of at a temperature of 28±2°C, relative humidity of 55–65% and dark: Light cycle of 12:12h. Animals are allowed for free access to food, water and ad libitum. All the experiments were started after getting the Institutional Animal Ethical Committee approval (KFMSR/IAEC/2013/007).

Pregnant animals, animals with infection, disease, injuries, and deformities were excluded from the study. All the animals were divided into 8 groups of 6 animals each. All the experiments were conducted in dim light and quiet place to avoid the external stimuli. Animals were handled minimally with care to minimize the stress and suffering.

Preparation of extract

The leaves of *Centella asiatica* was collected from Coimbatore district in the month of July. The fresh leaves were air dried and powdered in the food processor. About 500 g of powdered sample was boiled in hot water for 30min, allowed to cool and filtered using a piece of white cotton gauze. The filtrate was evaporated to dry at room temperature after transferring the material to small petridishes producing a greenish yellow colour solid residue which yielded 10% w/w of dried solid residue. The solid residues were stored in the air tight container and preserved in refrigerator at 4° C. From this stock, fresh preparations were obtained when ever required¹⁴.

Drugs

Phenytoin (Abbott, India), Pentylenetetrazole (Sigma, USA), and Diazepam (Ranbaxy, India) were used in the study. PTZ was given intraperitoneally to induce convulsion. Phenytoin and diazepam were used orally as standard drugs. All the drug solutions were prepared

freshly before the experiment by dissolving in the distilled water.

Acute toxicity study

Acute Oral toxicity study was carried out according to the OECD 425 guidelines. Five Swiss Albino mice of either sex were fasted prior to the dosing for 3–4 h. Extract of 2,000 mg/Kg was administered orally to one animal and observed at least once during the first 30 minutes after dosing, periodically during the first 24 hours with special attention given during the first 4 hours, and daily thereafter, for a total period of 14 days. The importance was given for its changes in skin, fur, eyes and mucous membranes, and also for its respiratory, circulatory, autonomic functions, somatomotor activity and behavioural pattern. After 24hrs, the remaining four animals were given the test compound in the same dose and observed for 14 days. The dose of ALECA used in this study was calculated from the acute oral toxicity study.¹⁵

Assessment of anticonvulsant activity

Maximal electroshock induced seizures (MES)

This is the model for Grandmal epilepsy and the end point is considered as Tonic Hind Limb Extension (THLE) which was evoked by electrical stimuli. The agents screened through this model are considered as an antiepileptic agent if it abolishes or suppresses THLE in rats. In this study the MES seizures are induced with the intensity of 150mA, 50Hz for 0.2sec duration through ear electrodes. 24 Wistar albino rats of either sex were divided into 4 groups of 6 animals each, after 12 hr of overnight fasting Group I received 0.5 ml of Normal saline, Group II received Phenytoin sodium 25 mg/kg b.w.¹⁶, Group III and Group IV received 200mg/kg b.w. and 400mg/kg b.w. of Aqueous Leaf Extract of *Centella asiatica* (ALECA) respectively. The standard and test drugs were given orally 60 min before the experiment. The time taken for the onset of Tonic Hind Limb Extension, duration of Tonic Hind Limb Extension and number of rats convulsed were noted.¹⁷

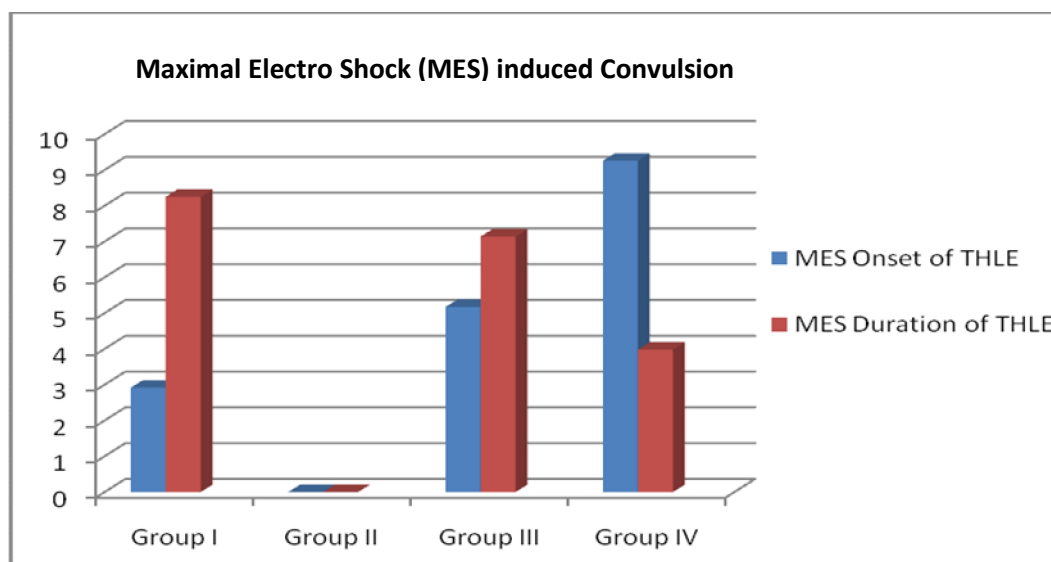
Pentylenetetrazole (PTZ) induced seizure

Pentylenetetrazole induced seizures is the model for absence seizures. This model is regarded as the good chemical model¹⁸. The standard and test drugs were administered orally 60 min before the intraperitoneal injection of PTZ (50 mg/kg). All the animals were observed for 30 minutes after PTZ injection for latency to the first forelimb clonus, and number of animals

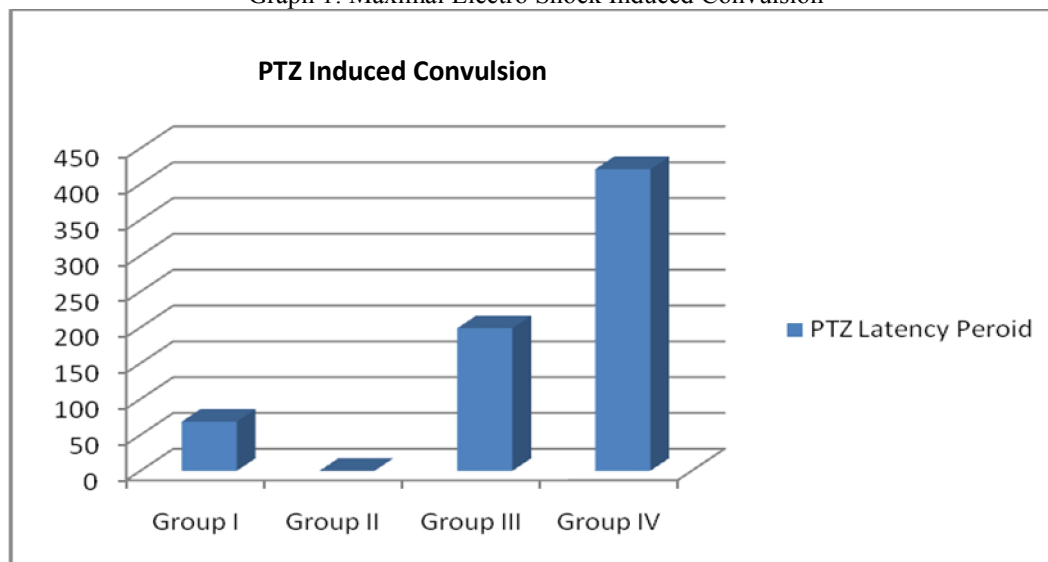
Table 2: Pentylenetetrazole induced convulsion:

S. No	Group	Drugs	Latency period (Sec)	Number of animals convulsed	Percentage (%) of protection
1	Group I	Normal saline	68.7 ± 3.4	6/6	0%
2	Group II	Diazepam(4mg/Kg)	0***	0/6	100%
3	Group III	ALECA (200mg/Kg)	199.08± 3.1**	4/6	33%
4	Group IV	ALECA (400mg/Kg)	420.96± 1.06***	2/6	67%

*p<0.05, **p<0.01, ***p<0.001



Graph 1: Maximal Electro Shock Induced Convulsion



Graph 2: Pentylenetetrazole induced convulsion

convulsed¹⁹. After 12 hr of fasting Group I received 0.5 ml of normal saline, Group II received Diazepam (4mg/kg)¹⁸, Group III and IV received 200mg/kg, and 400mg/kg b.w. of ALECA.

Statistical analysis

Results were presented as Mean ± SEM. The data was analyzed by one way ANOVA and p <0.05 was considered significant.

RESULTS

Acute toxicity study

No adverse effect and mortality was detected with the dose of 2000mg/kg of b.w. of aqueous leaf extract of *Centella asiatica* in all the five mice. The animals were alive, healthy and active for all the observational period. There was no significant weight change during the period of all the 14 days. So the LD 50 was considered as > 2000mg/kg.

*Anticonvulsant activity**Maximal electroshock induced seizures*

Table 1 shows the anticonvulsant activity of aqueous leaf extract of *Centella asiatica* in Maximal Electro Shock (MES) induced convulsion in Wistar albino rats. Phenytoin blocked the MES induced seizures in the dose of 25 mg/Kg b.w. in all the Wistar albino rats. There was significant increase in the time taken for the onset of Tonic Hind Limb Extension (THLE) in the dose dependent manner with all the doses of aqueous extract of *Centella asiatica* leaf and there was significant reduction in the duration of Tonic Hind Limb Extension in the dose of 400 mg/Kg of ALECA. The mean time taken for the onset of THLE in Group I is 2.9 ± 0.18 seconds, Group III (200mg/Kg of ALECA) is 5.17 ± 0.2 seconds ($p < 0.001$), and Group IV (400mg/Kg of ALECA) is 9.26 ± 1.96 seconds ($p < 0.001$). The mean duration of THLE of Group I is 8.25 ± 0.7 seconds, Group IV is 3.98 ± 0.31 ($p < 0.001$). In the dose of 400 mg/Kg of ALECA blocked the MES induced convulsion in 4 animals (percentage of protection is 67%).

Table 2 shows the anticonvulsant activity of aqueous leaf extract of *Centella asiatica* in pentylenetetrazole (50mg/Kg b.w.) induced convulsion in Wistar Albino rats. Diazepam (4mg/Kg b.w.) blocked the PTZ induced convulsion in all the six rats. Mean latency period of convulsed animals in control rats is 68.7 ± 3.4 seconds, low dose of ALECA is 199.08 ± 3.1 seconds ($p < 0.01$) and high dose of ALECA is 420.96 ± 1.06 seconds ($p < 0.001$). ALECA in the dose of 400 mg/kg showed the highly significant increase in the latency period than 200mg/kg of ALECA. The percentage of protection of convulsion with the dose of 200mg/Kg of ALECA is 33% (number of animals convulsed is 4/6) and with 400mg/Kg of ALECA is 67% (number of animals convulsed is 2/6).

DISCUSSION

Seizure is a characteristic feature in epilepsy and rhythmic high frequency discharge of impulses by a group of neurons in the brain.²⁰ In the present study aqueous extract of leaves of *Centella asiatica* was evaluated for the anticonvulsant activity against seizure induced by maximal electro shock and Pentylenetetrazole. The MES test predicts activity against generalized tonic-clonic seizures and the PTZ test predicts against absence seizure.²¹

MES induced seizures are abolished by the drugs that blocks voltage gated sodium channels like Phenytoin and Carbamazepine and by the drugs that block NMDA receptors like Felbamate. Whereas the drugs that block T-type Ca^{2+} current in thalamus like Sodium valproate, Ethosuximide prevents PTZ induced convulsions and the drugs like Diazepam, Clonazepam, which increases the duration of opening of GABA-Chloride channels prevents MES and PTZ induced convulsions.²²

ALECA increased the time of onset of MES induced seizures and also decreased the duration of THLE, so it increases the threshold of MES induced seizures. ALECA showed 67% of protection at the dose of 400mg/kg. The abolition of MES induced THLE by the aqueous extract

of leaves of *C asiatica* may be due to voltage gated sodium channel blockade or NMDA antagonistic activity. PTZ diminishes brain GABA (Gama Amino Butyric acid) level at sub-convulsive dose of 50mg/Kg.²³ The aqueous extract of leaves of *C asiatica* increased the threshold of PTZ-induced convulsion in rats. It showed 33% of protection at 200mg/kg and 67% protection at 400mg/kg. Many plants having anticonvulsant activity are known to inhibit GABA transaminase activity thereby increasing the GABA level in the brain.²⁴ The anticonvulsant activity of *Centella asiatica* in PTZ induced convulsion may be due to increase in the the GABA level by inhibiting GABA transaminase or increases the frequency of opening of GABA-Chloride channel.

MES induced seizure and PTZ induced seizures are also associated with oxidative stress to the brain.²⁵ *Centella asiatica* is proved to have antioxidant property²⁶ which may also contributes to its anticonvulsant activity against MES and PTZ induced convulsions. Long term administration of *Centella asiatica* prevents scopolamine-induced cognitive impairment and associated oxidative stress²⁷. Schulz et al., found out that there is increase in free radicals and decreased glutathione level during the process of combating oxidative stress. YK Gupta et al., found out that aqueous extract of whole plant of *Centella asiatica* induces malondialdehyde levels which is the marker of lipidperoxidation and increases the glutathione level which may also be the reason for antiepileptic activity.^{28,29}

Praveen et al., found out that aqueous extract of whole plant of *C. asiatica* at the dose of 100mg/kg suppressed THLE in 3 out of 6 mice and all the 6 animals at the dose of 300mg/kg in MES seizures. The convulsion induced by PTZ 80mg/kg was suppressed at the dose of 300mg/kg but not at 100mg/kg.³⁰

S. Sudha et al., used crude drug and methanolic extract of whole plant of *C. asiatica*. They concluded that both at the dose of 1000mg/kg showed 50% reduction in THLE. In PTZ (70mg/kg) induced convulsion both does not afford any protection, 100% mortality was seen in rats and mice. De Lucia et al., used the leaves of *C asiatica* & showed the crude extract has moderate activity.^{31,32}

In our study *Centella asiatica* suppressed the PTZ induced seizures even in low dose (200mg/kg) compared to MES induced seizures which was suppressed only in high dose (400mg/kg) which may be reasoned by the use of sub-convulsive dose of PTZ.

CONCLUSION

In MES induced seizures aqueous leaf extract of *Centella asiatica* increased the time of onset and decreased the duration of Tonic Hind Limb Extension. ALECA also increases the latency period of PTZ induced seizures. So the aqueous extract showed the anticonvulsant effect in both the MES & PTZ induced seizures. This concludes that the aqueous leaf extract of *Centella asiatica* can increase the threshold of generalized tonic clonic seizures and absence seizures in humans.

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