EVALUATION OF THE ANTICONVULSANT EFFECT OF AQUEOUS EXTRACT OF CENTELLA ASIATICA IN ALBINO MICE

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ABSTRACT

Objective: To evaluate the antiepileptic activity of aqueous extract of Centella asiatica in maximal electroshock (MES) and pentylenetetrazole (PTZ) induced convulsions.

Methods: The anticonvulsant activity of leaves of Centella asiatica (200 mg/kg and 400 mg/kg) in mice was assessed using MES and PTZ induced seizure models. Abolition of tonic hind limb extension (MES and PTZ) and increase in seizure latency (PTZ) when compared to control group, were taken as a measure of protection. Statistical analysis was done using one-way ANOVA followed by Tukey-Kramer multiple comparisons test. The test was considered to be significant at p<0.05.

Results: The aqueous extract of Centella asiatica at a dose of 200 mg/kg has abolished tonic hind limb extension in 1 out of 6 animals in MES while there was no anticonvulsant action in PTZ convulsions. At a dose of 400 mg/kg body weight, the aqueous extract of Centella asiatica has shown a significant anticonvulsant effect against both MES and PTZ convulsions, where it has abolished tonic hind limb extension in 4 mice in MES method and in all 6 mice in PTZ method.

Conclusion: The aqueous extract of Centella asiatica showed efficacy in both MES and PTZ convulsions in mice at a dose of 400 mg/kg. Since the clinical correlates of MES seizures are tonic-clonic convulsions and correlates of PTZ seizures are absence seizures, the aqueous extract of Centella asiatica is likely to be useful in the treatment of tonic-clonic and absence seizures.

Keywords: Anticonvulsant activity, Centella asiatica, Epilepsy, MES, PTZ model

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The design of the study is as follows:

The plant was collected from the vicinity of basic science building (SMCH). It was authenticated by Dr. Ashis Nath (Associate professor, Department of Botany, G. C. College, Silchar [No/GCC/ SIL/2014/198]) It was cleaned with water and air dried in the shade. It was then powdered using a mixture grinder. 30 g of the powder was soaked in 200 ml of cold water for ~18 hr at room temperature. The extract was first filtered through Whatman no. 1 filter paper to clarify and then through a 0.45 μm membrane filter[14]. The filtrate was evaporated to dryness at room temperature in a steady air current and the yield recorded as a percentage of the quantity of initial plant material used. The filtrate was evaporated to dryness at room temperature in a steady air current and the yield recorded as a percentage of the quantity of initial plant material used, and it was 33%. The test solution of Centella asiatica was prepared by dissolving 2 g of an aqueous extract of Centella asiatica in 100 ml of distilled water at room temperature. This solution had a concentration of 20 mg/ml.

The mice were subjected to maximal electroshock (MES) convulsions using electro-convulsiometer (INCO, Ambala, India) by applying a current of 50 mA for 0.2 seconds via ear electrodes. The electrodes were moistened with saline solution before application. The resultant seizure passes through various phases: phase of tonic limb flexion, tonic limb extension, clonus, and post-ictal depression followed by recovery or death [15]. The mouse was considered as protected if the drug prevented the appearance of hind limb tonic extensor component of the seizure.

In the PTZ method, the mice received 80 mg/kg of PTZ subcutaneously [15]. Each mice was pretreated with drugs one hour before giving PTZ. Only those animals that exhibited a convulsive response in the form of clonus, tonic fore and hind limb flexion, tonic limb extension, post-ictal depression followed by recovery or death were used for the experiment. In this method abolition of tonic, hind limb extension phase was considered as protection conferred by the drug. The results of this study are expressed as mean±standard error of mean (mean±SE). Results are analysed by ANOVA and post hoc test was done by Tukey-Kramer multiple comparisons test. The significance is established when probability value (p-value) is less than 0.05. P values are denoted as *P<0.05 as significant, **P<0.01 as highly significant and ***P<0.001 as very highly significant.

The Institutional Ethics Committee, Silchar Medical College, Silchar approved the protocol of the study (SMCH/IEC/SIL/2013/12-067).

The mean duration of tonic hind limb flexion, tonic hind limb extension, clonus, post-ictal depression and seizure latency (in case of PTZ method) are recorded for different test dose of Centella Asiatica (T1 200 mg/kg and T2 400 mg/kg for MES method and T3 200 mg/kg and T4 400 mg/kg for PTZ method) and findings are compared with the mean duration of above-mentioned parameters recorded for the control group used for both PTZ and MES method.

### Table 1: It shows comparison of mean duration (in seconds) with control group of different parameters in MES method

| Parameters duration in second | Group C1 | Group S1 | Group T1 | Group T2 |
|-------------------------------|----------|----------|----------|----------|
| Tonic hind limb flexion       | 2.67±0.4216 | 0***      | 0.75±0.359 | 0.33±0.333*** |
| Tonic hind limb extension     | 12.33±0.6146 | 0***      | 11.33±2.30ns | 1.83±1.47*** |
| Clonus                        | 22.8±2.149 | 11.2±0.7491*** | 11.5±2.46*** | 13.3±1.542** |
| Post ictal depression         | 17±4.819 | 0***      | 25.7±5.256*** | 12.5±6.021*** |

Data are expressed as MEAN±SE. *p<0.05, **p<0.01, ***p<0.001 compared with control, N=6, ns=not significant. One way ANOVA followed by Tukey-Kramer multiple comparisons test.

### Table 2: Shows Comparison of mean duration (in seconds) with control group of different parameters in PTZ method

| Parameters duration in second | Group C2 | Group S2 | Group T3 | Group T4 |
|-------------------------------|----------|----------|----------|----------|
| Seizure latency               | 32.5±5.141 | 0***      | 43.5±4.11*** | 0***     |
| Tonic hind limb flexion       | 1.7±0.21 | 0***      | 1.75±0.21 | 0***     |
| Tonic hind limb extension     | 9.8±0.31 | 0***      | 9.8±0.6 | 0***     |
| Clonus                        | 4.8±0.61 | 0***      | 3.67±0.56** | 0***     |
| Post ictal depression         | 33±5.38 | 0***      | 27.9±5.33±1.51*** | 0***     |

Data are expressed as MEAN±SE. *p<0.05, **p<0.01, ***p<0.001 compared with control, N=6, ns=not significant. One way ANOVA followed by Tukey-Kramer Multiple Comparisons test.
From table 1 it was evident that there was a decrease in the mean duration of tonic hind limb extension for both the test dose (T1 200 mg/kg and T2 400 mg/kg) and it was highly significant compared with control for T2, it was statistically not significant for T1 with compared with control.

From table 2 it was observed that the decrease in mean duration of tonic hind limb extension was only statistically significant compared with control T4 (400 mg/kg of Centella asiatica).

In this study, for the screening of aqueous extract of Centella asiatica for anticonvulsant activity, two standard methods namely MES and PTZ methods had been used [16]. The parameters observed were the duration of tonic hind limb flexion, tonic hind limb extension, clonus, post-ictal depression and incidence of recovery and death. In both MES and PTZ methods, the mouse was considered protected if the drug abolished the tonic hind limb extension [14].

In MES method, comparison of mean duration of tonic hind limb extension of control group (12.33±0.6146) with test groups indicate that there is a decrease in mean duration of tonic hind limb extension in both groups T1 (11.33±2.305) and T2 (1.83±1.47) and it is statistically significant (p<0.001) only in group T2. In group S1, there is the complete abolition of tonic hind limb extension which is statistically significant (p<0.001). A comparison of test groups T1 (11.33±2.305) and T2 (1.83±1.47) with group S1 (±0.00), indicate that there is a significant difference between S1 and T1 (p<0.001), while no significant difference between S1 and T2. Since abolition of tonic hind limb extension is considered suggestive of protection against MES convulsions [15] and standard antiepileptic drugs such as phenytoin, valproate and lamotrigine, which are clinically proven to be competent in the treatment of generalized tonic-clonic and partial seizures, all abolish the hind limb tonic extension in the MES model [17, 18], the aqueous extract of Centella asiatica has anticonvulsant effect against MES convulsions at a dose of 400 mg/kg. This effect is comparable to that of phenytoin in this study.

In PTZ method, comparison of mean duration of tonic hind limb extension of control group (9.83±0.907) with test groups and standard indicates that there is no significant difference between group C2 (9.83±0.307) and group T3 (9.83±0.601), while in groups S2 and T4, there is abolition of tonic extensor phase which is statistically significant (p<0.001) compared to control group. Comparison of mean duration of tonic hind limb extension of standard group S2 (0±0.00) with test groups T3 (9.83±0.601) and T4 (±0.00) indicates that there is a significant difference between group S2 and group T3 (p<0.001), while no significant difference between group S2 and group T4.

The abolition of tonic hind limb extension has occurred in all mice in group T4 while in group T3 there is no abolition of tonic hind limb extension. Since the abolition of tonic hind limb extension is considered suggestive of protection against MES and PTZ convulsions [14, 15], the aqueous extract of Centella asiatica has anticonvulsant effect against PTZ convulsions at a dose of 400 mg/kg. This effect is comparable to that of sodium valproate in this study.

From our study, we can conclude that aqueous extract of Centella asiatica has shown efficacy in both MES and PTZ convulsions in mice at a dose of 400 mg/kg. Further studies are required to estimate the exact mechanisms, active principles and safety of the plant as a medicinal remedy for epilepsy.

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CONFLICT OF INTERESTS
Declared none

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