Antiepileptic activity of Murraya Koenigii Leaf Extracts

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ABSTRACT
The aim of the present study was to investigate antiepileptic effect of the aqueous extract of the leaves of Murraya koenigii L. Spreng (AEMK) on electrically and chemically induced seizures. The aqueous extract of the leaves of M. koenigii (200 and 300 mg/kg) were studied for its antiepileptic effect on maximal electroshock induced seizures and pentylenetetrazole induced seizures in mice. AEMK (200 and 300 mg/kg) significantly reduced the duration of seizures induced by maximal electroshock (MES) as well as protected animals from pentylenetetrazole induced tonic seizures. The results suggest that the aqueous extract of the leaves of M. koenigii may produce its antiepileptic effects via non-specific mechanisms since it reduced the duration of seizures produced by maximal electroshock as well as delayed the latency of seizures produced by pentylenetetrazole.

Keywords: Murraya koenigii, Antiepileptic activity, Seizures, Pentylenetetrazole.

INTRODUCTION
Traditional medicine occupies an important place in the healthcare systems of developing countries. The people in developing countries depend on traditional medicine, because it is cheaper and more accessible than orthodox medicine.[1,2] Herbal medicine is currently enjoying a revival in popularity in the west and in fact it is the primary form of medicine in many parts of the world.[3] Epilepsy is a progressive disorder comprising of many seizure types and syndromes. Despite the introduction of many new antiepileptic drugs (AEDs) but a significant percentage of patients with epilepsy continue to experience seizures. Hence, there continues to be an unmet clinical need for more effective and less toxic anti-epileptic drugs.[4]

Murraya koenigii (L.) family rutaceae is an aromatic more or less deciduous shrub or a small tree up to 6m. in height found throughout India and is commonly known as Meethi neem and karry tree, is used traditionally as antiemetic, antidiarrhoeal, febrifuge and blood purifier.[5] The whole plant is considered to be a tonic and stomachic. The leaves are used extensively as a flavoring agent in curries and chutneys. Phytochemical screening of M. koenigii revealed the presence of some vitamins, carbazole alkaloid, terpenoids, phenolic compounds and mineral content such as calcium, iron, zinc and vanadium etc.[6]

The aim of the present study was to evaluate the antiepileptic potential of the aqueous extract of the leaves of M. koenigii in experimental animal models to validate the use of the plant’s leaf in the management of convulsions and epilepsy.

MATERIALS AND METHODS
1. Plant material/ Preparation of Extract
The leaves of Murraya koenigii were collected from the local area of Anand, (Gujarat) in the month of December in the year 2010 and it was authenticated by the Directorate of Medicinal and Aromatic Plants Research, Boriavi (Anand). The fresh leaves were oven dried at 45 °C for 3 days, ground into power and 20g soaked in 300ml of distilled water overnight at room temperature.[7] The filtrate obtained was evaporated to constant weight in a hot-air oven at 45 °C for 4 days and the yield of the extract was approximately 60%. The extract was weighed (2g) and reconstituted in appropriate volume of distilled water before administration to the animals.

2. Phytochemical analysis

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AEMK were subjected to identify the presence of various phytoconstituents viz. alkaloids (Dragendorffs test), steroids and terpenoids (Leibermann Burchard test), tannin and phenolic compounds (Ferric chloride test), flavonoids (Shinoda test), amino acids (Ninhydrin test), etc. by usual methods prescribed in standard texts.\(^8\)

3. Animals
Swiss albino mice of either sex were used for the study of the crude extract. Institution Animal Ethics Committee has approved the project (CPCSEA/IAEC/ARCP/11-12/06). The animals were kept at 27 ± 2\(^\circ\)C, relative humidity 44-56% and light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. Animals were provided with water ad libitum and standard diet.

4. Maximal electroshock induced seizures (MES):
The seizure was induced by maximal electroshock in mice with the help of electroconvulsiometer by passing current of 120 mA for 0.2 second using ear clip electrodes. The animals were divided into four groups each containing 6 animals (n = 6). The test samples were given 1 hour prior to induction of convulsions.

- **Group I (Control):** Received normal saline (1 ml/kg, po).
- **Group II (Standard):** Received Phenytoin (25 mg/kg, i.p).
- **Group III:** Received AEMK (200 mg/kg, po).
- **Group IV:** Received AEMK (300 mg/kg, po).

The animals were observed for the extensor phase as well as its duration. The abolition of extensor (tonic phase) in groups treated with extract and Phenytoin were considered as criteria for antiepileptic activity when compared with the control group.

5. Pentylenetetrazole induced seizures (PTZ):
The mice were divided into four groups each containing 6 animals (n = 6).
- **Group I (Control):** Received normal saline (1 ml/kg, po).
- **Group II (Standard):** Received Diazepam (2mg/kg, i.p).
- **Group III:** Received AEMK (200 mg/kg, po).
- **Group IV:** Received AEMK (300 mg/kg, po).

After 30 minutes of the dosing all the groups were injected with the convulsing agent pentylenetetrazole (50 mg/Kg) and animals were kept in individual plastic cages to observe convulsions for 1 h.

6. Statistical analysis
The results of the study were expressed as mean ± SEM, n = 6. ANOVA was used to analyze and compare the data, followed by Tukey's multiple comparisons.

### RESULTS
In the case of MES, it was observed that the AEMK 200 and 300 mg/kg were showed 67.75% and 81.56% inhibition of convulsion, respectively. The Phenytoin inhibited 91.25% of convulsion (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Duration of HLTE (sec)</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal saline</td>
<td>10 ml p.o</td>
<td>15.14± 0.10</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Phenytoin</td>
<td>25 mg i.p</td>
<td>3.41±0.12</td>
<td>100</td>
</tr>
<tr>
<td>III</td>
<td>AEMK</td>
<td>200 p.o</td>
<td>8.46± 0.09*</td>
<td>67.75</td>
</tr>
<tr>
<td>IV</td>
<td>AEMK</td>
<td>300 p.o</td>
<td>13.43±0.13**</td>
<td>81.56</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 6). Statistical significance was determined by ANOVA, followed by Tukey's Multiple Comparison Test (n=6); ***P < 0.001, **P < 0.01, *P < 0.05 when compared against control * P < 0.001, when compared against standard |

HLTE: Hind limb Tonic Extension

In the model of PTZ induced seizures, in mice treated with vehicle, clonic convulsion appeared for 192.3± 0.76 seconds after PTZ and all mice died after seizures. The AEMK at doses of 200 mg/kg and 300 mg/kg significantly delayed the onset of clonic convulsions for 296.4 ± 0.65 (p<0.001) and 354.2 ± 0.86 (p<0.001) seconds respectively in dose dependent manner. Whereas, the standard drug diazepam (2 mg/kg, i.p) delayed the onset
of clonic convulsions. Diazepam treated animals have shown 100% protection against PTZ induced seizures where as AEMK 200 mg/kg and 300 mg/kg have shown 70.33% and 82.88% protection of convulsion and 83.33% and 100% protection of mortality respectively (Table-2).

Table 2: Effect of AEMK on PTZ induced convulsions in mice

<table>
<thead>
<tr>
<th>Group No</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Onset of convulsion (sec)</th>
<th>Duration of convulsion (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal saline</td>
<td>10 ml p.o</td>
<td>192.3 ± 0.76</td>
<td>8.092 ± 0.2303</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam</td>
<td>2 mg i.p</td>
<td>No clonus</td>
<td>No tonic</td>
</tr>
<tr>
<td>III</td>
<td>AEMK</td>
<td>200 p.o</td>
<td>296.4 ± 0.65**</td>
<td>2.738 ± 0.05**</td>
</tr>
<tr>
<td>IV</td>
<td>AEMK</td>
<td>300 p.o</td>
<td>354.2 ± 0.86**</td>
<td>2.827 ± 0.00**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 6). Statistical significance was determined by ANOVA, followed by Tukey’s Multiple Comparison Test (n=6); ***P < 0.001, **P < 0.01, *P < 0.05 when compared against control * P < 0.001, when compared against standard

DISCUSSION AND CONCLUSION

Epilepsy is one of the most common brain diseases in human. About 1% of the population diagnosed with the disease. Several different types of human epilepsies have been characterized based on the classification of International League against Epilepsy (ILAE). According to this classification, epilepsy has been divided into partial epilepsy (simple and complex), generalized symptomatic epilepsy and unclassified epilepsy. An imbalance between the excitatory and inhibitory neurotransmitters is responsible for seizures. At neuronal level, seizures activity often occurs when glutamatergic excitatory neurotransmitters overrides gamma aminobutyric acid (GABA) mediated inhibition. In the assessment of antiepileptic study, several models have been developed. Many drugs that increase the brain contents of GABA have exhibited the antiepileptic against seizures induced by MES induced seizures and PTZ induced seizures. The MES is probably the best validated method for assessment of antiepileptic drugs in generalized tonic-clonic seizures. At the highest tested dose (300 mg/ Kg), aqueous extract was found to significantly decrease the duration of the hind limb tonic extensor phase whereas the lower dose (200 mg/ Kg) shown less effect against seizures. The extracts of leaves of M. koenigii exhibited anticonvulsant activity by delaying the onset of PTZ induced seizures and protecting treated mice from mortality induced by seizures. Drugs protecting against tonic-clonic seizures induced by PTZ are considered as useful in controlling myoclonic and absence seizures in humans. The phytochemical study of extracts revealed the presence of alkaloids, tannins, triterpene and steroids. The phytochemicals such as carbazole, triterpene and steroids were reported as active substances for anticonvulsant activity. Hence, these phytochemicals might be contributing to the anticonvulsant activity of AEMK . Further study is necessary to determine the mechanism of action and isolation of active principle(s) from aqueous extract of leaves of Murraya koenigii for antiepileptic activity.

REFERENCES

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