Antiepileptic activity of leaves of *Leucas aspera*.
Ramalingam Raman1, Bindu Madhavi Boddupalli1, Ramya Miryala1, Ravinder Nath Anisetti2, Nagulu Malothu1, Deepthi Balla3

**Abstract**

*Leucas aspera* (LA) is a plant that has been used in folk medicine to treat asthma, fever, skin diseases and has several pharmacological activities. The antiepileptic property of LA was not yet been studied. Aim: The present study was aimed to investigate the antiepileptic activity of ethanolic extract obtained from leaves of LA on pentylenetetrazole (PTZ) kindling seizures in mice. Method: The ethanolic extract 200mg/kg and 400mg/kg were evaluated for the antiepileptic activity against PTZ (40mg/kg IP) induced seizure method. Diazepam was used as the standard drug. Antiepileptic activity was evaluated by observing seizure intensity, motor coordination, depression and oxidative stress by malondialdehyde (MDA) content. Results: Treatment with extract was found to be in dose dependant manner with significant prolonged onset time, decreased duration and intensity of seizure when compared with vehicle treated group. Extract has even protected the animal from loss of motor coordination and depression. Conclusion: From the results of present study it can be concluded that, the ethanolic extract of leaves of LA protected the animals from seizure effect induced by PTZ and attenuated the oxidative stress induced by PTZ without producing loss of motor coordination and depression.

**Keywords:** *Leucas aspera*, Malondialdehyde, Pentylenetetrazole

**Introduction**

Epilepsy is the most common central nervous system disorder affecting more than 50 million people world wide [1]. It is characterized by recurrent spontaneous seizures and caused by sudden abnormal and recurrent electrical discharge from the affected brain cells. There are so many drugs available currently in the market to treat epilepsy, but none of them are free from side effects such as depression, ischienia, impaired cognition and motor disability [2]. In recent years many research activities has been focused on screening of extracts obtained from herbs against epilepsy disorder. It was found that, great number of screened herbs are active against epilepsy with less side effects [3]. Therefore the present study was aimed to evaluate the protective ability of LA against epilepsy.

LA belonging to the family Lamiaceae is commonly known as Thumblai in Telugu. It is widely distributed throughout India. The plant is an annual erect, non aromatic herb. Stems are much branched, leaves are serrate, blunt tipped and the margins scalloped [4]. Traditionally, the decoction of whole plant and individual parts were used to treat asthma, fever, skin diseases, head ache, arthritic pain and snake bites [5]. The extract obtained from LA display a wide range of pharmacological activities such as antimicrobial [6], antinociceptive, antioxidant and cytotoxic activities [7]. Alcoholic extract of LA leaves showed significant free radical scavenging activity [8] and DNA protecting effect [9]. From this plant a large number of secondary metabolites such as aliphatic ketones, flavonoids, glycosides and terpenes have been isolated [5]. Since the LA has various secondary metabolites and pharmacological activities, the present investigation was focused to evaluate the protective effect of ethanolic extract of leaves of LA against PTZ induced convulsions in mice.

**Materials and Methods**

Pentylenetetrazol (PTZ), 2-Thiobarbituric acid and 1, 1, 3, 3-Tetramethoxy propane were purchased from Sigma Aldrich, Saint Louis, MO, United States. All other reagents and solvents used were of analytical grade and were obtained from various other commercial sources.

**Plant material and extraction**

Leaves from LA were collected from in and around agricultural lands surrounding Nalgonda district, Telangana, India. The leaves were shade dried and ground into fine powder. The powder was first defatted with petroleum ether at room temperature for 48H and then extracted with 70% ethanolic solution at room temperature for another 72H. The resultant ethanolic extract was concentrated under reduced pressure at room temperature using rotary vacuum evaporator.

**Animals**
Male Swiss albino mice weighing 25-30g were used in this study. All the animals were housed under 12H light and 12H dark cycle and allowed for free access to standard pellet food and ad libitum except during experiments. All experiments were performed in accordance with ethical guidelines for care and use of laboratory animals approved by institutional animal ethical committee of Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda, Telangana, India. (Ref No. SRTIPS/FM/1468/PO/a/11/CPCSEA/103/2013).

**Experimental procedure**

Extract was suspended in 0.2% carboxymethyl cellulose (CMC) suspension and given orally 1 hour before the administration of PTZ. Diazepam was used as standard and administered via IP route 30 minutes before the administration of PTZ. PTZ was dissolved in sterile saline solution and administered via IP route. Mice were randomly divided into 4 groups (n=6). All the animals in the group were treated as described below:

- **Group 1**: 0.2% CMC followed by PTZ (40mg/kg)
- **Group 2**: Extract 200mg/kg followed by PTZ (40mg/kg)
- **Group 3**: Extract 400mg/kg followed by PTZ (40mg/kg)
- **Group 4**: Diazepam 2mg/kg followed by PTZ (40mg/kg)

Kindling was induced by a total of 6 treatments with PTZ on every 5 days (1, 5, 10, 15, 20 and 25). Vehicle, Extract and Diazepam were administered daily to the animals. On 25th day mice were observed for seizure activity and subjected to rotarod test and forced swimming test [10].

**Seizure observation**

The antiepileptic activity was assessed by observing onset of seizures, duration of seizure and intensity of seizure. Intensity of seizure was evaluated using the following score [10]:

- 0: No response
- 1: Ear and facial twitching
- 2: Convulsive waves axially through the body
- 3: Myoclonic body jerks
- 4: Generalized clonic convulsions and turn over into side position
- 5: Generalized convulsions with tonic extension episode and status epilepticus
- 6: Mortality

**Motor coordination test**

This was performed after observing seizure intensity using rotarod. All the animals were previously given the training on rotating rotarod with a speed of 10rpm for 5 minutes before commencement of treatment. The animals were placed on the rotating rotarod and latency to fall in seconds from the rotarod was noticed [11].

**Forced swimming test**

After motor coordination test, the animals were subjected for forced swimming test to assess the depressive behaviour. In this test the animals were placed individually in glass cylinder (25 X 12 X 25cm) containing water at room temperature up to a level of 15cm for 5 min and total immobility period in seconds was noted. The animals were judged to be immobilised when they stopped struggling and remained floating motionless in water making only those movements necessary to keep them head above water [12].

**Malondialdehyde determination**

The animals were sacrificed by decapitation at the end of experiments. The brains were homogenized with 10% w/v 0.1M phosphate buffer (pH 7.4). The homogenized tissue was mixed with 2 volumes of cold 10% w/v trichloroacetic acid to precipitate proteins. The precipitate was centrifuged, pelleted and an aliquot of supernatant was mixed with 0.67% w/v of thio barbituric acid for 15 min in boiling water bath. After cooling, the absorbance was measured at 532 nm. The results were expressed as nM/gm of protein in brain tissues based on standard graph which was plotted by using serial dilutions of 1, 1, 3, 3 tetramethoxy propane [10].

**Statistical analysis**

Results were expressed as mean ± SEM and the data was analysed using a one way analysis of variance (ANOVA) by using the software Graph Pad Prism version 6.03. In all the treated groups were compared with vehicle treated group.

**Results and Discussion**

Repeated measures one way ANOVA test was conducted in order to test significance of results compared to control. The results of seizure activities were shown on table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Onset of seizure (sec)</th>
<th>Duration of seizure (sec)</th>
<th>Intensity of seizure (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>20.83±3.962</td>
<td>147.0±12.45</td>
<td>4.66±0.210</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>46.33±5.69**</td>
<td>123.3±8.89**</td>
<td>4.00±0.258**</td>
</tr>
<tr>
<td>3</td>
<td>400</td>
<td>87.83±8.408***</td>
<td>77.00±6.923***</td>
<td>3.00±0.258***</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>170.0±17.70****</td>
<td>19.17±2.926****</td>
<td>1±0.0****</td>
</tr>
</tbody>
</table>

All the values are represented as mean ± SEM where n=6, Symbols represent statistical significance as ns P>0.05, * P<0.05, ** P<0.01, *** P<0.001 and **** P<0.0001 vs control group.
Group Rotarod test Forced swim test MDA content

<table>
<thead>
<tr>
<th>Group</th>
<th>Rotarod test (sec)</th>
<th>Forced swim test(sec)</th>
<th>MDA content (nM/mg protein) (n= 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>11.00±1.826</td>
<td>220.3±13.79</td>
<td>0.8380±0.05</td>
</tr>
<tr>
<td>Group 2</td>
<td>49.50±6.286**</td>
<td>91.17±7.547****</td>
<td>0.8163±0.072ns</td>
</tr>
<tr>
<td>Group 3</td>
<td>95.50±13.97****</td>
<td>49.83±8.28****</td>
<td>0.6070±0.061</td>
</tr>
<tr>
<td>Group 4</td>
<td>22.50±2.432ns**</td>
<td>197.8±8.719ns**</td>
<td>0.415±0.024*</td>
</tr>
</tbody>
</table>

All the values are represented as mean ± SEM where n=6, Symbols represent statistical significance as ns P>0.05, * P <0.05, ** P <0.01, *** P<0.001 and **** P<0.0001 vs control group

Table No 2: Effects of ethanolic extract of Leucas aspera and Diazepam on rotarod performance, forced swim test and MDA content

The present study demonstrated that ethanolic extract of leaves of LA significantly suppressed seizures induced by PTZ without producing depression. The protective effect of extract may be due to its antioxidant activity which is evident by the decreased MDA content. However further research is required to identify and isolate the responsible compounds for its antiepileptic activity.

**Conclusion**

The present study demonstrated that ethanolic extract of leaves of LA significantly suppressed seizures induced by PTZ without producing depression. The protective effect of extract may be due to its antioxidant activity which is evident by the decreased MDA content. However further research is required to identify and isolate the responsible compounds for its antiepileptic activity.

**Author’s contribution**

Ramalingam Ramani conceived the present study, carried out interpretation of statistical data, drafted the manuscript and assisted in pharmacological screening.

Bindu Madhavi Boddupalli has carried out the extraction process, assisted in data collection and drafting the manuscript.

Ramya Miryala carried out the pharmacological screenings.
Ravinder Nath Anisetti revised the manuscript for its intellectual content and given final approval.
Nagulu Malotu supervised and guided the animal’s experiments.

Deepthi Balla collected the plant material and helped in statistical treatment of results.

References


[7]. Rahman MS, Sadhu SK, Hasan CM. Preliminary antinociceptive, antioxidant and cytotoxic activities of Leucas aspera root. 2007; 78: 552-555.


