Evaluation of anticonvulsant activity of stem bark extract of *Tephrosia purpurea*

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**ABSTRACT**

The anticonvulsant potential of Ethanol extract of stem bark extract of *Tephrosia purpurea* (200 mg/kg and 400 mg/kg), was studied for their anticonvulsant effect on maximal electroshock induced seizures (MES) and pentylenetetrazole (PTZ) induced seizures in mice. The duration of hind limb tonic extension in case maximal electroshock induced seizures and onset of convulsions in case of pentylenetetrazole induced seizures were noted. The percentage mortality was calculated. *Tephrosia purpurea* significantly reduced the duration of seizures by MES. The effect of *Tephrosia purpurea* PTZ induced convulsions was not marked. The data suggest that the ethanolic extract of *Tephrosia purpurea* will produce its anticonvulsive effect by acting on voltage dependent sodium ion channels since it reduced the duration of seizures produced by maximal electroshock.

**1. INTRODUCTION**

Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures. These seizures are transient signs and/or symptoms of abnormal, excessive or synchronous neuronal activity in the brain. About 50 million people worldwide have epilepsy, with almost 90% of these people being in developing countries. Epilepsy is more likely to occur in young children or people over the age of 65 years; however it can occur at any time.

A large number of agents called antiepileptic drugs are available to treat various types of seizures with the objective to reduce seizure frequency and severity with in a framework of acceptable level of side effects. The ideal antiseizure drug would suppress all seizures without causing any unwanted effect. Unfortunately drugs used currently not only fail to control seizures activity in some patients but they frequently cause side effects. In addition safety, tolerability, efficiency, expenses especially in long term therapy, serum drug monitoring etc. are other limitations with synthetic antiepileptic drugs. Further a large number of drug interactions seen with almost all current antiepileptic drugs make it more difficult to control seizures easily.

Traditional systems of medicine are popular in developing countries and up to 80% of the population relies on traditional medicines or folk remedies for their primary health care need. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Several plants such as *Withania somnifera*, *Bacopa monnieri*, *Croton zambesicus*, *Hypericum perforatum*, *Ficus religiosa* used for the treatment of epilepsy in different systems of traditional medicine have shown activity when tested in modern bioassays for the detection of anticonvulsant activity and many such plants are yet to be scientifically investigated. So the present study is to evaluate the antiepileptic activity ethanolic extract stem bark extract of *Tephrosia purpurea*.

**2. MATERIALS AND METHODS**

**Plant Material Collection:** The plant material of *Tephrosia purpurea* (TP) was collected from Ranga Reddy dist, Telangana State in the month of August and was identified and authenticated by Dr. K. Madhav Chetty, Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupathi, and Andhra Pradesh, India. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

**Extraction procedure:** Fresh plants material of *Tephrosia purpurea* (TP) were collected and dried under shade. The extracts used were prepared by using soxhelet apparatus by taking containing 500 ml of ethyl
alcohol equivalent to two portions. Boiled up to 50-60°C for 4-5 hours, the filtrate was boiled until the concentrated residue is formed. Powdered Drug is extracted with ethyl alcohol yielding a crude extract. To separate two portion of ethyl alcohol extraction of TP one portion used pharmacological screening second one used fractionated with chloroform and ethyl acetate extract.

Preparation of Ethanolic extract: The dried marc from the above process was extracted successively with Ethanol to get Ethanolic extract. The extract was collected and obtained dried extract used for further investigation.

Preliminary qualitative phytochemical analysis: The Ethanolic extract stem bark of *Tephrosia purperia* was subjected to qualitative examination for different phytoconstituents like Alkaloids, Carbohydrates, Flavonoids, Proteins, Lipids, and Reducing sugar, Phenol, Tannins, Saponins, Terpenoids and Steroids by using standard methods.

Acute Toxicity Study: The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD Guidelines 425). The animals were fasted overnight prior to the experiment. The first group was treated with oral dose of 1000 mg/kg body weight with the Ethanolic extract of the stem bark of *Tephrosia purperia*. The extracts was given in two different groups and the animals were observed continuously for 4-5 hours for general, behavioral, neurological, autonomic profiles and finally death after 24 hours. If there was no mortality and no sign of toxicity and the extract was found to be safe at that dose level, then a higher dose of 2000mg/kg body weight of the ethanolic extract was administered in another 2 groups. If no mortality was observed, the maximum tolerated dose level was taken as 2000 mg/kg body weight. The doses for pharmacological studies were taken as 400, 200, 100 mg/kg body weight i.e. 1/5th, 1/10th, 1/20th of the maximum tolerated dose (i.e. 2000mg/kg).

Anticonvulsant activity of *Tephrosia purperia*:

Electrically induced seizures: In the electrically-induced seizure experiment, the maximal electroshock (MES) method will be employed. In brief, tonic convulsions of the hind extremities of the mice was induced by passing alternating electrical current of 50 Hz and 150 mA for 0.2 sec through corneal electrodes. The animals were divided into four groups containing six animals each group.

Group I : Control and administered with Normal saline
Group II : *T. Purperia* stem bark ethanolic extract (200 mg/kg)
Group III : *T. Purperia* stem bark ethanolic extract (400 mg/kg)
Group IV : Reference standard Phenyltine (25mg/kg, i.p)

The above treatment was continued for 15 days prior to the induction of convulsion. The number of animals protected from hind limb tonic extension seizure (HLTE) and the time spent in this position were determined for each dose group.

Pentylenetetrazole induced seizures: The animals were randomly divided into four groups containing six animals each.

Group I : Control and administered with Normal saline
Group II : *T. Purperia* stem bark ethanolic extract (200 mg/kg)
Group III : *T. Purperia* stem bark ethanolic extract (400 mg/kg)
Group IV : Reference standard Diazepam (0.5mg/kg, i.p)

Seizures were induced in mice with standard convulsing agents, pentylenetetrazole (60 mg/kg., s.c), after 30 min of drug treatment and the animals were observed for one hour for tonic convulsion episode. Hind limb extension was taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period was noted. The ability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity.

3. RESULTS AND DISCUSSION

Preliminary Qualitative Phytochemical Screening: The ethanolic extract of *T. Purperia* showed the presence of alkaloids, carbohydrates, flavonoids, proteins, amino acids, steroids, fats and oils, and saponins, triterpenoids and glycosides.

Acute toxicity study: The ethanolic extract of stem bark extract of *Tephrosia purperia* was found to be safe at the maximum dose of 2000 mg/kg body weight by oral route. After 24 hours animals were found well tolerated. There was no mortality and no signs of toxicity. General behaviors, neurological, autonomic profiles were found to be normal and the both the extracts were found to be safe.

MES produced hind limb tonic extension seizures in all the animals used. The control mice showed tonic limb extension for the duration of 18.4±0.29 sec. Ethanolic extract of *T. Purperia* 400mg/kg protected 83.33 % of mice and alter the incidence of seizures elicited by MES to a significant extent. Ethanolic extract of *T. Purperia* 200mg/kg protected 66.67% of mice and considerably decreased the duration of HLTE produced by MES. The standard antiepileptic drug, phenytoin (25mg/kg) also protected all the animals and significantly reduced the duration of HLTE.
PTZ produced tonic seizures in all the animals used. A dose of 50mg/kg of Ethanolic extract of *Tephrosia purperia* 400 mg/kg protected 66.67% of animals against PTZ induced seizures and Ethanolic extract of *Tephrosia purperia* dose of 200 mg/kg protected only 33.33% of mice delayed the latency of seizures produced by PTZ, and protected all the mice against the seizures. The standard antiepileptic drug, diazepam (0.5mg/kg) profoundly antagonized the seizures produced by PTZ.

Table 1. Effect of *Tephrosia purperia* on maximal electroshock induced seizures in mice (Anticonvulsant activity of *Tephrosia purperia*)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of HLTE (sec)</th>
<th>Quantal protection</th>
<th>Percentage protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>18.43±0.29</td>
<td>4/6</td>
<td>66.67</td>
</tr>
<tr>
<td>Group II</td>
<td>12.34 ± 0.38**</td>
<td>5/6</td>
<td>88.33</td>
</tr>
<tr>
<td>Group III</td>
<td>14.89 ± 0.49**</td>
<td>4/6</td>
<td>66.67</td>
</tr>
<tr>
<td>Group IV</td>
<td>07.71±0.31***</td>
<td>6/6</td>
<td>100</td>
</tr>
</tbody>
</table>

The values are shown in mean +SEM. Data was analyzed by Student T-test. Values of * = p < 0.05, ** = p < 0.01, *** = p < 0.001 were considered as a significant. Mean percentage ±SEM; n = 6 animals in each group.

Table 2. Effect of *Tephrosia purperia* on pentylenetetrazole induced seizures in mice (Anticonvulsant activity of *Tephrosia purperia*)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg),i.p</th>
<th>Onset of seizures(min)</th>
<th>Quantal protection</th>
<th>Percentage protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>-</td>
<td>3.04 ± 0.24</td>
<td>1/6</td>
<td>16.67</td>
</tr>
<tr>
<td>Group II</td>
<td>400</td>
<td>6.48 ± 0.25**</td>
<td>4/6</td>
<td>66.67</td>
</tr>
<tr>
<td>Group III</td>
<td>200</td>
<td>3.55 ± 0.33**</td>
<td>2/6</td>
<td>33.33</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.5</td>
<td>11.59 ± 0.42***</td>
<td>6/6</td>
<td>100</td>
</tr>
</tbody>
</table>

The values are shown in mean +SEM. Data was analyzed by Student T-test. Values of * = p < 0.05, ** = p < 0.01, *** = p < 0.001 ns = not significant were considered as a significant. Mean percentage ±SEM; n = 6 animals in each group.

Discussion: The results of the present study indicate that ethanolic extract of stem bark extract of the roots of *Tephrosia purpurea* possesses anticonvulsant activity in mice. Gama amino butyric acid (GABA) is the chief inhibitory neurotransmitter in the brain while glutamic acid is an excitatory neurotransmitter in the brain. The inhibition of Gama amino butyric acid neurotransmitter and the enhancement of the action of glutamic acid have been shown to be the vital factors in epilepsy. Our study shows that the ethanolic extract of the stem bark extract of *Tephrosia purpurea* confined some of the animals against seizures induced by maximal electroshock, pentylenetetrazol and picrotoxin delayed the latency of the seizures.

In the present study maximal electroshock produced convulsions in all the animals used antiepileptic drugs that inhibits the MES-induced tonic extension are known to act by inhibiting the seizure spread. Moreover, drugs that block voltage-dependent sodium channels, such as phenytoin can put off MES-induced tonic extension. However, phenobarbitone is as effective against electric shock-induced convulsion as it is against pentylenetetrazol induced convulsions in mice and phenobarbitone is known to decrease the electrical activity of neurons within a chemically-induced epileptic focus in the cortex, while diazepam does not suppress the focal activity but prevents it from spreading.

Diazepam had anticonvulsant effect on both PTZ-induced seizures and MES induced seizures, in which diazepam effect on the earlier (100% protection) is better than the latter (50% protection). This is consistent with the report that benzodiazepine (BDZ) agonists such as diazepam and clonazepam, etc., are more potent in the prevention of PTZ-induced seizures than in that of MES-induced tonic seizures.

The result of present study showed that the ethanolic extract of *T.Purperia* decreased the duration of tonic hind leg extension in MES- induced seizures. So the ethanolic extract of *T.Purpurea* seems to act on the voltage dependent sodium ion channels thereby stopping the repetitive firing of action potential and thus produce their anticonvulsant effect.

Pentylenetetrazole (PTZ) induced convulsions in all the mice used. Pentylenetetrazol may elicit seizures by inhibiting gabaergic mechanisms. Standard antiepileptic drugs, diazepam and phenobarbitone, are supposed to produce their effects by enhancing GABA-mediated inhibition in the brain. It is, therefore, possible that the anticonvulsant effects shown in this study by the drugs against seizures produced by PTZ may be due to the activation of GABA neurotransmission. Since the extract similarly antagonized seizures elicited by pentylenetetrazole in mice, it is likely, therefore, that it may also be exerting its anticonvulsant effects by affecting gabaergic mechanisms.
PTZ produces its anticonvulsant effect by inhibiting the activity of GABA at GABA$_A$ receptors thereby inhibiting the neuronal responsiveness and activity by increasing the chloride ion conductance through opening of the chloride-ion channel. Ethanollic extract of *T.Purperia* increased the latency of convulsion. Therefore, *T.Purperia* decrease the seizure threshold by acting on the GABAergic system.

### 4. CONCLUSION

Ethanolic extract of stem bark extract of *Tephrosia purpurea* decreased the duration of tonic hind leg extension in maximal electroshock-induced seizures probably by acting on voltage gated sodium ion channels and increased the latency of convulsion and decreased the seizure threshold by acting on the GABAergic system.

From the results of the present study it may be concluded that ethanolic extract of stem bark extract of *Tephrosia purpurea* produces anticonvulsant effect by acting on sodium ion channels, inhibition of prostaglandin synthesis and monoamine oxidase enzyme as well as by the decreasing the influx of calcium ions acting on the glycnergic transmission. So the present study indicates the ethanolic extract of stem bark extract of *Tephrosia purpurea* possess the anti-epileptic activity by different mechanisms. However, the exact mechanism and the active principle by which these extracts exert their action remain unclear. Further studies are required to study the individual mechanism of actions.

### REFERENCES

2. Indian medicinal plants, a comendium of 500 Species, orient longmann, orient longmann pvt.Ltd, 5, 2006, 249.