VITEX TRIFOLIA LINN. (VERBANEACEAE): EVALUATION OF ANTICONVULSANT ACTIVITY IN EXPERIMENTAL ANIMALS

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ABSTRACT
The present study was carried out to evaluate the anti-convulsant activity of Vitex trifolia Linn. (Verbaneaceae) leaves extracts. The anti-convulsant activity of plant extracts was evaluated by using two models Maximal Electroshock (MES) induced convulsions and Pentylenetetrazole (PTZ) induced convulsions. The convulsions in experimental animals were induced with Maximal electroshock (Inco Electroconvulsimeter model# 100-3) of 150 mA current for 0.2 sec and PTZ (80 mg/kg, sc) after administration of normal saline, standard drug and leaves extracts at respective doses. Both the extracts exhibited a significant (P<0.05) reduction in various phases of epileptic seizure. The alcoholic and aqueous extracts treated groups showed significant decrease in duration of hind limb extension and decreased the duration of clonus and stupor phase of MES induced convulsions. In PTZ induced convulsions, both the extracts delayed onset of clonus with no mortality. The Vitex trifolia Linn possessed significant anticonvulsant activity against MES and PTZ induced convulsions.

Key words: MES, PTZ, Phenytoin, Diazepam, Vitex trifolia, Verbaneaceae.

INTRODUCTION
Epilepsy is the common chronic central nervous system disorder characterized by transient signs and/or symptoms of abnormal, excessive or synchronous neuronal activity in the brain\(^1\). The prevalence rate for epilepsy are 1–2% of the world population\(^2\). Epilepsy is not cured but usually controlled with drug treatment. However, even with the currently available antiepileptic drugs treatment, over 30% of epilepsy patients do not have seizure control and more than 50% of epilepsy patients have experienced unwanted side effects and chronic toxicity\(^3,4\). The conventional antiepileptic agents like phenytoin, carbamazepine and sodium valporate carry with them several serious side effects notably neurotoxicity\(^5\).

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects\(^6\). The present study covers scientific evaluation of herbal plant Vitex trifolia Linn for anticonvulsant activity. The plant Vitex trifolia Linn (Verbaneaceae) is well known in Hindi as ‘Pani-ki-Sanbhalu’. It is stout aromatic shrub or a small tree, found from the foot of Himalayas southwards throughout greater part of
India, western ghat and in Andamans. Leaves contain aucubin, agnuside, casticin, orientin, Iso-orintin, and α-D-glucoside of tetrahydroxy-monomethyl flavone. Leaves are used traditionally, for rheumatic pain, inflammation, analgesic, anticonvulsant and sedative, hypnotic etc. Leaves also possess insecticidal, cytotoxic, fungicidal properties. The roots are antiemetic, expectorant, tonic and beneficial in thirst. However no scientific data regarding anti-convulsant activity of said plant is available.

MATERIALS AND METHODS

Plant material:
The plant Vitex trifolia Linn. was collected from the local areas of Belgaum, and authenticated from Dr. R.S. Gaudar, Botanist & Head, Department of Botany, R.L.S. Institute, Belgaum. The leaves were dried, powdered and used for the extraction process. The ethanolic extract and aqueous extract of Vitex trifolia Linn. obtained were used as the test drug for the evaluation of anticonvulsant activity.

Preparation of plant extracts:
After authentication, the leaves were dried at room temperature until they were free from the moisture. The coarsely powdered form was then successively extracted for 48 h with 90% ethanol in a Soxhlet apparatus. The extract was filtered and concentrated in a rotatory evaporator, at 30-400°C, to obtain semi-solid material. The viscous residue thus obtained was kept in a vacuum desiccator to obtain a completely dry solid mass. The ethanolic extract yield was 12 %. Aqueous extract was prepared by maceration process (Yield 8 %). For screening purpose, the extracts were weighed and triturated with tween 80 (1%) and then was suspended in distilled water quantity sufficient to produce a suspension of suitable strength. The extracts were administered at doses 200mg/kg b.w.p.o.

Animals:
The Swiss albino mice (22-25gm) and the Albino Wistar strain rats (150-250gm) of either sex were housed in a group of five per cage and were maintained under natural day and night cycle at 25±2°C ambient temperature, 45-55% relative humidity. They were allowed to acclimatize one week before the experiment. A 12:12, light: dark cycle was following during the experiment. The animals were allowed with free access to standard pellet and water ad libitum.
Drugs, reagents and chemicals:
Pentylenetetrazole (Sigma, USA), Phenytoin injection (Ranbaxy), Diazepam injection (Ranbaxy). PTZ was dissolved in water for injection and all the drugs were administered intraperitoneally.

Acute toxicity study:
There was no mortality amongst the graded dose groups of mice up to a dose of 2000 mg/kg for duration of 72 h. This finding probably suggests that the plant extract is relatively safe or non-toxic in mice at the doses used for this study.

Assessment of Anticonvulsant Activity:

Maximum electroshock (MES) induced seizures:
The rats were divided into four groups of 5 animals each. Group I received Normal saline (p.o.), Group II received 25 mg/kg of Phenytoin (i.p.). Group III and Group IV received 200 mg/kg of p.o. Ethanolic extract and Aqueous extract respectively. Maximal electroshock (Inco Electroconvulsimeter model# 100-3) of 150 mA current for 0.2 sec was administered through ear electrodes to induce convulsions in the control and various drugs treated animals. The duration of tonic extension of hind limb was used as end point for the evaluation of activity.

Pentylenetetrazole (PTZ) induced seizures:
The mice were divided into four groups of 5 animals each. Group I received Normal saline (p.o.), group II received 5 mg/kg of Diazepam (i.p.). Group III and Group IV received 200 mg/kg of p.o. Ethanolic extract and Aqueous extract of Vitex trifolia Linn. respectively. PTZ was administered (80 mg/kg, sc) 45 min after administration of Normal saline, Standard drug and Ethanolic and Aqueous extracts. Animals were observed for 30 min after injection of PTZ. The ability to delay the onset of myoclonic spasm and clonic convulsions and mortality were used as end points.

Statistical Analysis:
The data were expressed as MEAN ± SEM. The data were analyzed by One way analysis of variance (ANOVA) followed by Dunnet’s Multiple Comparison test. P<0.05 were considered significant.

RESULTS

MES induced convulsions:
The anticonvulsant activity against MES induced convulsions is shown in Table 01. The Standard drug Phenytoin, both the extracts exhibited a significant (P<0.05) reduction in various phases of epileptic seizure. The alcoholic and aqueous extracts treated groups showed significant decrease in duration of hind limb extension (9.720 ±0.3652 sec and 11.56 ±0.3530 sec respectively) as compared to control group (13.50±0.6058 sec). The alcoholic and aqueous extracts also decreased the duration of clonus (15.94±0.4622 sec and 17.00±0.3975 sec respectively) and stupor (86.66±2.185 sec and 89.34±0.9490 sec respectively) phase of MES induced convulsions as compared to control (78.08±1.345 sec clonus and 97.22±1.730 sec stupor).

Table No. 01: Effect of Vitex trifolia Linn. against MES Induced convulsions

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Drug Treatment</th>
<th>Mean Time (in sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Flexon</td>
</tr>
<tr>
<td>01</td>
<td>Control</td>
<td>3.740±0.4622</td>
</tr>
<tr>
<td>02</td>
<td>Standard Drug (Phenytoin) treated</td>
<td>1.400±0.1871***</td>
</tr>
<tr>
<td>03</td>
<td>Alcoholic Extract treated</td>
<td>2.020±0.1772**</td>
</tr>
<tr>
<td>04</td>
<td>Aqueous Extract treated</td>
<td>2.260±0.1077**</td>
</tr>
</tbody>
</table>

Values are expressed as MEAN±SEM. One way Anova followed by Dunnets ‘t’ test. n=5 in each group. *P<0.05, **P<0.01, ***P<0.001 Vs Control

PTZ Induced convulsions:

The anticonvulsant activity against PTZ induced convulsions is shown in Table 02. In PTZ induced convulsions, Ethanolic and Aqueous extracts showed delayed onset of clonus (87.14 ±2.051sec and 83.98±1.828sec respectively) and extensor (308.7±4.980sec and 303.8 ± 4.967 sec respectively), resulting in significant anticonvulsant activity as compared to control (Clonus 78.08±1.345 sec and extensor 284.5 ±4.216). Both the
extracts and standard drug Diazepam protected all the animals in the group as no mortality was observed.

**Table No. 02: Effect of *Vitex trifolia* Linn. against PTZ Induced Convulsions**

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Drug Treatment</th>
<th>Mean Onset time (in sec)</th>
<th>Recovery/Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Control</td>
<td>46.66 ±3.546</td>
<td>78.08 ±1.345</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>284.5 ±4.216</td>
</tr>
<tr>
<td>02</td>
<td>Standard Drug (Diazepam) treated</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>03</td>
<td>Alcoholic Extract treated</td>
<td>62.74 ±2.990**</td>
<td>87.14 ±2.051**</td>
</tr>
<tr>
<td>04</td>
<td>Aqueous Extract treated</td>
<td>59.40 ±3.239*</td>
<td>83.98 ±1.828*</td>
</tr>
</tbody>
</table>

Values are expressed as MEAN±SEM. One way Anova followed by Dunnets ‘t’ test. n=5 in each group. *P<0.05, **P<0.01 Vs Control

**DISCUSSION**

The plant extracts of *Vitex trifolia* Linn. were studied against MES and PTZ induced convulsions in experimental animals.

The MES is the best validated method for assessment of antiepileptic drugs in generalized tonic clonic seizures\(^{13, 14}\). The PTZ induced seizures are similar to the symptoms observed in the absence seizures and drugs useful in treatment of absence seizures suppresses PTZ induced seizures.

Phenytoin is used as a standard drug because it is the most commonly used antiepileptic drug that abolishes tonic phase of MES seizures and limits the spread of seizure activity. Diazepam is a drug of choice for emergency control of convulsions but is not used for long term treatment because of its sedative action.

Ethanolic extract and aqueous extract have shown a significant anticonvulsant activity against MES and PTZ seizures models indicating usefulness in holding generalized tonic-clonic seizures by regulating GABA mediated synaptic inhibition and absence seizures by increasing the brain content of GABA respectively.
The effects of extracts may be attributed due to presence of flavonoid contents which are revealed in preliminary phytochemical investigation. Further an extensive work is desirable.

CONCLUSION

In the present study, the preliminary work carried out in lab indicate that Vitex trifolia Linn possessed significant anticonvulsant activity against MES and PTZ induced convulsions which is further supported by decrease in duration of hind limb extension in MES induced seizure model and delayed onset of clonus & extensor phase in PTZ induced seizures model.

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REFERENCES


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