Evaluation of anti-epileptic activity of *Cuscuta reflexa* Roxb.

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ABSTRACT

In present article the anticonvulsant activity of *Cuscuta reflexa* Roxb. was studied. Which contain flavonoids, glycosides, steroids, alkaloids, cuscutalin, cuscutin, and amarvelin as revealed by phytochemical screening and the main active chemical constituent flavonoid which responsible for depressant activity. It also investigated by studying the effects on seizures induced by pentylenetetrazole, and maximal electroshock convulsive methods in mice. *Cuscuta reflexa* Roxb. (200 mg/kg oral route 41.9 % and 400 mg/kg oral route 61.25 %) significantly reduced the duration of convulsion in tonic seizure induced by pentylenetetrazole (85 mg/kg i. p.). *Cuscuta reflexa* Roxb. (200 mg/kg oral route 16.66 % and 400 mg/kg oral route 21.44 %) significantly reduced the tonic extension convulsion induced by maximum electroshock-induced convulsions. The data obtained suggest that *Cuscuta reflexa* Roxb. have anticonvulsant property and may probably be affecting both GABA aminergic and glycine inhibitory mechanism.

Keywords: *Cuscuta Reflexa* Roxb., Maximum Electroshock Seizure (MES), Pentylenetetrazole (PTZ), Antiepileptic activity

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INTRODUCTION

Epilepsy (also called seizures) is characterized by uncontrolled excessive activity of either part or all of the central nervous system. A person who is predisposed to epilepsy has attacks when the basal level of excitability of the nervous system (or of the part is susceptible to the epileptic state) rises above a certain critical threshold [1,2].

In industrialized countries the incidence rate decreased in children but increased among the elderly during the three decades prior to 2003, for reasons not fully understood [3].

GABA (A) benzodiazepine receptors and epilepsy: GABA (A) benzodiazepine receptors and epilepsies. Gamma-Amino butyric acid (GABA) is quantitatively one of the most important neurotransmitters in the central nervous system. Since the predominant action of GABA on neurons is inhibitory, activation of GABA receptors, and especially of GABA (A) receptors, causes an anticonvulsive effect. GABA (A) receptors can be activated either directly by GABA or GABA-agonists, or indirectly by allosteric modulation of these receptors. For instance, benzodiazepines enhance the postsynaptic actions of GABA by binding to benzodiazepine receptors which are allosteric modulatory binding sites on GABA (A) receptors. Conversely, there are compounds which bind to the same benzodiazepine receptors, but reduce the postsynaptic actions of GABA. These compounds cause convulsions and are called "inverse agonists" of the benzodiazepine receptors. Recent evidence indicates the existence of several different benzodiazepine receptor subtypes. Since these receptor subtypes exhibit a different regional distribution in the central nervous system, the development of subtype-selective GABA (A) receptor agonists or benzodiazepine receptor agonists should result in anticonvulsants with less side effects [4].

Mechanism of Action

The antiseizure action of diazeapm as well as other effect occurs at non sedating doses; result in large part of their ability to enhance GABA-mediated synaptic inhibition. Molecular cloning and study of recombinant receptor have demonstrated that the benzodiazepam receptor is an integral part of the GABA receptors. At therapeutically relevant concentration, benzodiazepine act at subset of GABA (A) receptors and increases the frequency, but no duration of action of opening at GABA-activated Cl⁻ channel. At high concentration, diazepam reduced the sustained high-frequency firing neurons, similar to effect of phenytoin carbamezepine and valproate. Although these concentration correspond to concentration achieved in patient during treatment of status epilepsy with diazepam, they considerably higher than those associated with anti-seizure in ambulatory patient [5,6].
MATERIALS AND METHOD

Plant collection and Authentication

The leaves of *Cuscuta reflexa* Roxb. were collected during November from Pune Municipal Corporation, Shivaji Nagar, Dist.- Pune., and were authentify by Agharkar research institute (Maharashtra association for the cultivation of science) an autonomous grant-in-aid institute under the department of science and technology, Government of India, agarkar road, Pune- 411004.

Plant preparation and extraction

The leaves were dried in sunlight and reduced to a coarse powder. Then the powder was subjected to macerate with ethanol for 72 hours at a temperature of 50-60 °C. The extract was concentrated and the solvent was completely removed. They were freeze dried and stored in the vacuum desiccators until further use.

Maceration process

Procedure

As indicated in the Pharmacopoeia the process consists of the following:

Place the solid material with whole menstrum in close vessel and allow stand for 7 days shaking occasionally. Strain presses the mark and mix the liquid obtained. Clarify by subsidence or filtration. This process is normally used for the preparation of tincture or extract and menstrum is usually alcoholic, hydrochloric (in case of tincture) or may be aqueous. Drug is kept with the menstrum for a long a period. The process is carried out at ambient temperature. At the end of process the mark is either pressed or menstrum is decanted depending upon the nature of drug to be extracted in the process.

Depending upon the type of drug to be extracted by maceration two different methods are adopted. The types of drug to be extracted by maceration are either organized drugs or unorganized drugs. Organized drugs are either the parts of plant like roots, seeds, barks, etc. which have got the cellular structure. They contain the alkaloids glycosides [7].

Selection of animal and drug

Animals

A male Albino Swiss mice weighing 22-25 g weighing were obtained from Serum institute of India Pvt. Ltd., Pune. Animals were housed in groups of 6 per cage at a temperature of 25 °C ± 1 °C and relative humidity of 45-55 %. A 12:12 dark:light cycle was followed during...
the experiments and the experiments were carried out during 1200-1400h. Animals had free access to food and water however, food but not water was withdrawn 8 h before and during the experiments [8].

Drugs

Pentylenetetrazole (Sigma, USA, 80 mg/kg, i.p), diazepam (Calmpose inj. Ranbaxy, India. 4 mg/kg. i.p), were used in this study. The drugs were dissolved in water for injection and administered in a volume of 5 ml/kg to mice [9].

Acute toxicity

The acute toxicity study for ethanolic extract of Cuscuta reflexa roxb. was perform using albino swiss mice animals were fasted overnight prior to the experiment and mention under standard condition. Extract of drug administered orally in increasing dose and the animals were continuously observed for 24 hours. All animal at the end were safe without any toxic sign upto the dose of 2000 mg/kg that dose was taken as maximum tolerable dose. The 1/10th dose of maximum tolerable dose was 2000 mg/kg, on that basis 100 mg/kg, 200 mg/kg and 400 mg/kg were selected as dose level. Doses were calculated for all animals on the basis of body weight.

Assessment of anticonvulsant activity

Pentylenetetrazole induced seizure model

In each type of seizure model, the mice were divided into four groups with six animals. Group I served as solvent control, received 0.9 % w/v of saline (1 mL/kg), Group II received diazepam (4 mg/kg, i.p), treated as positive control and Group III, IV received Cuscuta reflexa Roxb. (200 and 400 mg/kg i.p.) respectively. All the drugs were administered 30 min prior to the administration of pentylenetetrazole (80 mg/kg i.p.). The animals were observed for 1 hour by placing in a separate cage. The duration of seizures (tonic-clonic convulsions) were recorded.

Maximum electroshock-induced seizure model

The mice were divided into four groups with six animals in each. Group I served as solvent control, received 0.9 % w/v of saline (1 mL/kg), group II received diazepam (4 mg/kg. i.p.), treated as positive control and Group III, IV received Cuscuta reflexa Roxb. (200 mg/kg and 400 mg/kg respectively. Both the drugs were administered orally 60 min prior to the electroshock). The electroshock induced in animal by passing a current of 45 mA for 0.2 sec duration through electroconvulsiometer (Techno India) using corneal electrodes. The incidence and duration of extensor tonus were noted. The duration of seizures (tonic-clonic convulsions) was recorded [8, 10, 11].

Discussion
The result of present study indicates that ethanol extract of *Cuscuta Reflexa* roxb, possesses anticonvulsent activity in mice. In the present study maximal electro shock produced seizures in all animals used. Antiepileptic drug that blocks MES induced tonic extension are known to blocking the seizures spread. Diazepam has anticonvulsant effect on both PTZ induced and MES induced seizures in which diazepam effects on former (100 % protection) is better than later (50 % protection).

Finally, the ethanolic extract of *Cuscuta reflexa* roxb. induced effective dose (ED$_{50}$) in mice when administered orally at doses 200 mg/kg and 400 mg/kg. However, a dose-dependent was recorded giving an LD$_{50}$ of 2000 mg/kg and 4000 mg/kg. The result content some evaluation test like ash value, extractive value, loss on drying, foreign organic matter, they may help to determine the quality and purity of crude drug. Extractive of plant may help for evaluation of the crude drug and also gives the idea about nature of chemical constituent present in the crude drug.

### Table 1. Result of Anticonvulsant activity of *Cuscuta Reflexa* Roxb. by PTZ-induced seizures in Swiss Albino mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg</th>
<th>Onset of seizures (min.)</th>
<th>Onset of death (min.)</th>
<th>% inhibition of onset of death (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Saline Water</td>
<td>3.01±0.121</td>
<td>4.23±0.324</td>
<td>0</td>
</tr>
<tr>
<td>Diazepam std.</td>
<td>2 mg/kg.</td>
<td>7.89±0.498**</td>
<td>11.45±0.909</td>
<td>89.69***</td>
</tr>
<tr>
<td>Test Comp.</td>
<td>Low Dose (200 mg/kg.)</td>
<td>5.33±0.231*</td>
<td>7.86±0.990</td>
<td>41.90*</td>
</tr>
<tr>
<td></td>
<td>High Dose. (400 mg/kg)</td>
<td>6.44±0.767**</td>
<td>9.51±0.936</td>
<td>61.25**</td>
</tr>
</tbody>
</table>

(Values are expressed as mean ± S.E.M. n=6 *** p < 0.001, ** p < 0.01, * p < 0.05 compared with vehicle control ANOVA followed by Dunnet’s t- test).
Table 2. Result of Antionvulsant activity of Cuscuta Reflexa Roxb. by MES- induced seizures in mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg</th>
<th>Duration of tonic flexion (sec.)</th>
<th>Duration of Tonic extension (sec.)</th>
<th>Latency of onset of clonus (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Saline water</td>
<td>4.30 ± 0.431</td>
<td>09.32± 0.765</td>
<td>5.12± 0.503</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Std. Drug 2 mg/kg</td>
<td>9.56 ±0.371</td>
<td>23.47±0.987</td>
<td>8.98± 0.651</td>
</tr>
<tr>
<td>Test Comp.</td>
<td>Low Dose (200 g/kg)</td>
<td>4.81 ±0.762*</td>
<td>16.66±0.878**</td>
<td>3.60± 0.912*</td>
</tr>
<tr>
<td></td>
<td>High Dose (400 mg/kg)</td>
<td>6.61 ± 0.2442**</td>
<td>21.44±0.79***</td>
<td>8.26 ± 0.7654***</td>
</tr>
</tbody>
</table>

(Values are expressed as mean ± S.E.M. n=6  *** p < 0.01, * p < 0.1, * p < 0.05 compared with vehicle control ANOVA followed by Dunnet’s t- test).

Graph 1. Calculation of Antiepileptic activity of Cuscuta Reflexa Roxb. by PTZ- induced seizures in mice.

REFERENCES