ANTICONVULSANT ACTIVITY OF ROOTS AND RHIZOMES OF *GLYCYRRHIZA GLABRA*

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**ABSTRACT**

**Objective:** To study the anticonvulsant activity of ethanolic extract of *Glycyrrhiza glabra* in albino rats and mice.

**Methods:** The anticonvulsant activity of ethanolic extract of roots and rhizomes of *Glycyrrhiza glabra* (10, 30, 100 and 500 mg/kg, i.p.) in mice was assessed using maximum electroshock seizure (MES) test and pentylenetetrazol (PTZ) using albino mice. The lithium-pilocarpine model of status epilepticus was also used to assess the anticonvulsant activity in rats.

**Results:** The ethanolic extract of *G. glabra* did not reduce the duration of tonic hindleg extension in the MES test even in the dose of 500 mg/kg. However, the extract significantly and dose-dependently delayed the onset of clonic convulsions induced by pentylenetetrazol. The dose of 100 mg/kg afforded protection to all animals. The extract also protected rats against seizures induced by lithium-pilocarpine.

**Conclusion:** The ethanolic extract of *G. glabra* inhibits PTZ and lithium-pilocarpine-induced convulsions but not MES-induced convulsions.

**KEYWORDS** Anticonvulsant, *glycyrrhiza glabra*, lithium-pilocarpine, MES, PTZ

**INTRODUCTION**

In the traditional system of medicine, the roots and rhizomes of *Glycyrrhiza glabra* (family: Leguminosae) have been in clinical use for centuries. Roots have demulcent, antacid, anti-ulcer, anti-inflammatory, expectorant, tonic, diuretic, laxative, and sedative properties. They also possess antipyretic, antimicrobial, antiherpes, and anxiolytic activities. Glycyrrhizin, a triterpene saponin, possesses antiviral activity. In the traditional system of medicine *G. glabra* also known as liquorice is recommended for the treatment of epilepsy.

Attempts to find out a common neurochemical basis for human or experimental epilepsy have been disappointing. An imbalance between the excitatory and inhibitory neurotransmitters is responsible for seizures. At neuronal level, seizure activity often occurs when glutamatergic excitatory neurotransmitters overrides gamma-aminobutyric acid (GABA) mediated inhibition. Several animal models of convulsions have been developed to evaluate anti-seizure activity. Many drugs that increase the brain contents of GABA have exhibited anticonvulsant activity against seizures induced by maximum electroshock (MES), pentylenetetrazol (PTZ) and lithium-pilocarpine (Li-Pilo). The MES is probably the best-validated method for assessment of anti-epileptic drugs in generalized tonic-clonic seizures. The PTZ-induced seizures are similar to the symptoms observed in the absence seizures and drugs useful in treatment of absence seizures suppress PTZ induced seizures. The status epilepticus induced by lithium-pilocarpine increases brain contents of acetylcholine. The reproducibility of the responses to this treatment makes this a very useful model to investigate various facets of seizures.
The objective of the present study was to investigate anticonvulsant activity of ethanolic extract of *G. glabra* (EE) against the seizures induced by MES, PTZ and combination of lithium sulphate and pilocarpine nitrate.

**MATERIALS AND METHODS**

**Preparation of extract:** The roots and rhizomes of *Glycyrrhiza glabra* were purchased from commercial source. The roots and rhizomes (1.0 kg) were crushed to a coarse powder and extracted with ethanol (70% v/v) using Soxhlet’s extractor for 24 h. The extract was concentrated under reduced pressure and then dried in air (yield -170 g). The extract was stored in a refrigerator and reconstituted in water for injection just before use.

**Animals:** Male Albino Swiss mice weighing 22-25 g and male Albino Wistar rats weighing 125-150 g were obtained from National Institute of Toxicology, Pune. Animals were housed in groups of 6-8 per cage at a temperature of 25±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 1200-1400 h. Animals had free access to food and water however, food but not water was withdrawn 8 h before and during the experiments. The Institutional Animal Ethics Committee approved the protocol of the study.

**Drugs:** Pentylenetetrazol (Sigma, USA), lithium sulphate (Glenmark Pharmaceuticals, India), Pilocarpine nitrate (FDC Limited, India), diazepam (Calmpose inj. Ranbaxy, India), were used in this study. The drugs were dissolved in water for injection and administered in a volume of 5 ml/kg to both rats and mice.

**Acute toxicity:** The ethanolic extract was administered in doses of 600 and 1000 mg/kg, *i.p.*, to groups of mice, each containing ten animals and mortality was observed after 24 h.

**Assessment of anticonvulsant activity**

**Maximum electroshock-induced seizures:** The EE was administered to groups of mice (n = 6) in doses ranging from 10-500 mg/kg, *i.p.*, 30 min before application of electric shock (42 mA, 0.2 sec) using corneal electrodes*. The duration of tonic hindleg extension was noted.

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Onset of clonic convulsions (mean±SEM)</th>
<th>Incidence of convulsions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>140.3 ± 13.8</td>
<td>100</td>
</tr>
<tr>
<td>EE 10</td>
<td>664.5 ± 93.0*</td>
<td>66.6</td>
</tr>
<tr>
<td>30</td>
<td>780.0</td>
<td>16.6</td>
</tr>
<tr>
<td>100</td>
<td>A</td>
<td>0</td>
</tr>
<tr>
<td>Diazepam 2</td>
<td>3.9</td>
<td>0</td>
</tr>
<tr>
<td>d.f.</td>
<td>2.15</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

n = 6 in each group. *P<0.001 when compared to the vehicle treated group (ANOVA followed by Dunnett's test).

**Pentylenetetrazol-induced seizures:** The EE was administered *i.p.* in varying doses (10-100 mg/kg) 30 min before the subcutaneous injection of PTZ (80 mg/kg) and mice were observed for onset of myoclonic spasm and clonic convulsions*. One group received vehicle while other group received diazepam (2.0 mg/kg, *i.p.*) as a reference standard. The animals were observed for onset of convulsion upto 30 min after PTZ. Each group contained six animals.

**Lithium-pilocarpine-induced status epilepticus:** Albino rats were divided randomly into five groups each containing six animals. Status epilepticus was induced by administration of pilocarpine (30 mg/kg, *i.p.*) 24 hr after lithium sulphate (3 mEq/kg, *i.p.*). The effect of EE (10, 30 and 100 mg/kg, 30 min before injection of pilocarpine nitrate) was studied on the severity of status epilepticus. One group received only vehicle while the other group received diazepam (1.0 mg/kg). The severity of status epilepticus was observed every 15 min till 90 min and thereafter every 30 min till 180 min, using the scoring system described earlier*: No response-stage 0, fictive scratching-stage 1, tremors-stage 2, head nodding-stage 3, forelimb clonus-stage 4, rearing and falling back-stage 5.
ANTICONVULSANT ACTIVITY OF G. GLABRA

Table 2: Effect of ethanolic extract of *Glycyrrhiza glabra* (EE) on lithium-pilocarpine-induced status epilepticus in rats.

<table>
<thead>
<tr>
<th>Time after Pilocarpine in min.</th>
<th>Treatment EE in mg/kg, i.p.</th>
<th>Diazepam (1 mg/kg)</th>
<th>H</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.3 ± 0.2*</td>
<td>0.8 ± 0.4</td>
<td>0.5 ± 0.2*</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.0 ± 0.5</td>
<td>1.5 ± 0.4*</td>
<td>0.7 ± 0.2*</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>2.3 ± 0.4</td>
<td>2.1 ± 0.5</td>
<td>1.5 ± 0.4*</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>3.0 ± 0.0</td>
<td>2.1 ± 0.5</td>
<td>1.5 ± 0.4*</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>4.0 ± 0.0</td>
<td>0.6 ± 0.2*</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>4.3 ± 0.2</td>
<td>0.8 ± 0.3*</td>
<td>1.3 ± 0.2</td>
</tr>
</tbody>
</table>

n = 6 in each group. H and P are values of Kruskal Wallis ANOVA and Probability respectively (df = 4). The values are mean ± SEM of the scores indicating the severity of seizures.

*P<0.05 as compared to vehicle treated group (Dunn's test).

Statistical analysis: The data are presented as mean±SEM. The data of PTZ test were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test and the data of lithium-pilocarpine-induced seizures were analyzed using Kruskal- Wallis ANOVA followed by Dunn's test. The differences were considered significant at 5% level.

RESULTS

Acute toxicity: The EE was found to be safe in the doses used and there was no mortality in a dose of 1 g/kg, i.p.

Assessment of anticonvulsant activity

Maximum electroshock test: The duration of tonic hindleg extension in mice treated with vehicle was 15.66±1.33 sec. The EE in doses of 10, 30, 100 and 500 mg/kg did not protect animals from seizures and duration of hindleg extension was not reduced. The vehicle treated mice showed tonic hindleg extension for 15.66±1.33 sec whereas mice treated with the EE (10, 30, 100, and 500 mg/kg) exhibited hindleg extension for 14.33±1.14, 18.33±1.89, 16.33±1.43, and 18.8±2.45 sec respectively.

Pentylenetetrazol-induced seizures: In animals treated with vehicle, clonic convulsions appeared 140.3±13.8 sec after PTZ and all animals died after seizures. The EE significantly and dose dependently inhibited the onset and incidence of convulsions. The convulsions were completely abolished by the dose of 100 mg/kg. The EE in a dose of 10 mg/kg exhibited seizures in 66.6% of mice and all animals exhibiting seizures died within 30 min. No mortality was observed in the groups treated with EE 30 and 100 mg/kg even after 24 h. Diazepam (2 mg/kg) inhibited seizures completely (Table 1).

Lithium-pilocarpine-induced status epilepticus: Rats treated with lithium and pilocarpine showed stage 4 seizures in all animals 75 min after pilocarpine. The EE diminished the intensity of seizures and none of the animals exhibited stage 4 seizures
and the animals were normal in behavior after 180 min. The EE in a dose of 100 mg/kg was more effective than other two doses in inhibiting the seizures till 45 min. Thereafter the effects of the three doses were almost similar. The observations are given in Table 2.

**DISCUSSION**

The observations emanated in the present study indicated that the EE was without any lethal effect in a dose upto 1 g/kg and possessed anticonvulsant activity against seizures induced by PTZ and lithium-pilocarpine in a dose dependent way. However it was not effective against MES induced seizures. Since inhibition of the MES test predicts activity against generalized tonic-clonic and cortical focal seizures, lack of activity against MES induced seizures suggests that the EE is not useful in suppressing generalized tonic-clonic seizures. Several drugs are thought to inhibit seizures by regulating GABA-mediated synaptic inhibition through an action at distinct sites of the synapse.

Although lithium does not possess general proconvulsant action in rats, its pretreatment provokes limbic seizures following administration of sub-convulsant doses of pilocarpine and other cholinergic agonist. The combined treatment with lithium and pilocarpine results in accumulation of acetylcholine and inositol monophosphate and reduction in cortical inositol that are about ten times greater than the effects obtained with either drugs alone. Phenobarbitone, sodium valproate, diazepam and trimethadione prevent the limbic seizures induced in rats by pilocarpine, however, phentoyin and carbamazepine are ineffective. Studies from our laboratory have shown that blockade of post-synaptic 5-HT receptors and inhibition of serotonergic transmission inhibited lithium-pilocarpine-induced seizures and also suppressed PTZ-induced seizures (unpublished data).

Thus, in conclusion, *G. glabra* possesses anticonvulsant property against the PTZ and lithium and pilocarpine induced seizures. Further research is in progress to isolate the compound responsible for the activity.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


**COL. R.N. CHOPRA**

(17.8.1882 - 13.6.1973)

Fifty paise stamp issued on his 101st birth anniversary

Dr. R.N. Chopra, hailing from Jammu & Kashmir had his early education at Lahore. He took Natural Sciences Tripos from Cambridge University. After completing his medical studies at St. Bartholomew's Hospital, London he returned to Cambridge to work with Prof. W.E. Dixon on his thesis on “ciliary movements” and in 1908 he was awarded an M.D. degree. In 1909, he joined the Indian Medical Service with the 19th Punjab Regiment in North West Frontier Province and served in East Africa in World War I. He was awarded ScD (Cantab) in 1927. After several years in general medical duties he joined the Calcutta School of Tropical Medicine (STM) and simultaneously was Professor of Pharmacology at the Calcutta Medical College. He initiated several programs on clinical and experimental research including that on Indian indigenous drugs. His pioneering publications include Indigenous Drugs of India, Glossary of Indian Medicinal Plants, Poisonous Plants of India and Drug Addiction in India. He introduced research on *Rauwolfia Serpentina*. He was a Director of STM and Drug Research Institute, Srinagar. From 1958 onwards he held the post of Scientific Advisor, RRL, Jammu. He was made Brevet Colonel in 1935 and knighted by the Queen in 1941.

The birth of the Indian Pharmacological Society in 1969 received his blessings. He was the first president of IPS. Dr. R.N. Chopra oration which was started as a memorial tribute by IPS following his demise, is an integral part of the Annual Conference of IPS each year.

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