Anticonvulsant effect of the ethanol extract of Caesalpinia pulcherrima (L.) Sw., Fabaceae, leaves

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ABSTRACT: In this study, ethanol extract of Caesalpinia pulcherrima (L.) Sw., Fabaceae, leaves (CPEE) was investigated for anticonvulsant effect against maximal electroshock (MES) and pentylenetetrazole (PTZ) induced seizures in rats and mice at dose levels 200 and 400 mg/kg, i.p. respectively. Diazepam (3 mg/kg, i.p.) was used as a standard anticonvulsant drug for comparison. CPEE was found to be safe up to the dose of 4000 mg/kg in mice, when administered intraperitoneally. The extract at 400 mg/kg dose produced significant (p<0.01) anticonvulsant effect w.r.t. control against PTZ-induced clonic seizures. In MES-induced seizure model, there were no significant alterations in the onset as well as duration of hind limb extension seizures as compared to control at a dose of 200 mg/kg when administered intraperitoneally. However, the extract (CPEE, 400 mg/kg i.p.) significantly (p<0.01) delayed the onset as well as decreased the duration of hind limb extension seizures (HLES) as compared to control. However, the extract, CPEE, percentage protection of the animals was increased at higher dose (200 mg/kg) in both the models. The results of the study suggest that ethanol extract of Caesalpinia pulcherrima (L.) Sw. leaves possess anticonvulsant effect.

Keywords: Caesalpinia pulcherrima, ethanol extract, anticonvulsant effect, maximal electroshock, pentylenetetrazole.

INTRODUCTION

Epilepsy is a common neurological disorder characterized by recurrent unprovoked seizures (Guidelines, 1993; Blume et al., 2001; James, 2001). Approximately 5% of the world population develops epilepsy in their lifetime whereas overall prevalence rate of epilepsy in India is 5.59 per 1000 population (Sander & Shorvon, 1996; Bharucha, 2003). Currently, many synthetic drugs like carbamazepine, ethosuximide, gabapentin, oxcarbazepine, phenobarbital, phenytoin, valproic acid, felbamate etc. are used as potent antiepileptic agents, but they are not free from marked side effects. Approximately 30% of the patients continue to have seizures with current antiepileptic drug therapy (Smith & Bleck, 1991; Samren et al., 1997; Poole et al., 2000). Hence, there is a need to address a potent alternative...
as antiepileptic agent with minimal side effects. Several plants have been traditionally used in the treatment of epilepsy. A number of studies have been carried out on medicinal plants or plants based products and revealed good results when screened for anticonvulsant activity and many such plants are yet to be scientifically explored (Hosseinizadeh & Parvardeh, 2004; Quintans-Júnior et al., 2008a, b).

**Caesalpinia pulcherrima** (L.) Sw., Fabaceae, is a leguminous, perennial shrub or small tree and native of South America. In India, it is cultivated as ornamental plant. Commonly, it is known as Gulutura, Gulutura (Hindi), Peacock flower, Barbados pride (English), Ratnagandhi (Sanskrit). It is a small tree, 3.7-4.3 m in height. Prickles are sparse on the branches, bark is grey in color. Leaves are abruptly bipinnate, leaflets in 13-20 pairs, 1.3-1.9 cm long. Flowers are red or yellow, fragrant. Flowering season of this plant starts from September to November and fruits from March to April.

Traditionally, the flowers, leaves, barks and roots of *C. pulcherrima* have been in clinical use in India since ancient times. Leaves are traditionally used as purgative, tonic, antipyretic and emmenagogue whereas roots have folkloric use in convulsions, intermittent fevers, lungs and skin diseases (Chatterjee & Prakashi, 2006; Pullaiah, 2006). Moreover, the plants of genus *Caesalpinia* are reported to have anticonvulsant activity (Adesina, 1982). Therefore, based upon the reported uses of plants of genus *Caesalpinia* and traditional use of roots of *C. pulcherrima* in convulsions, *C. pulcherrima* leaves were selected for the investigation of anticonvulsant activity.

**MATERIAL AND METHODS**

Experimental protocols and procedures used in this study were approved by the Animal Ethics Committee of Kurukshetra University, Kurukshetra, India and conform to the guidelines of ‘Committee for the Purpose of Control and Supervision on Experiments on Animals’ [Reg. No. 562/02/a/CPCSEA].

**Procurement and identification of plant materials**

Fresh leaves of the plant were collected from University College, Kurukshetra University, Kurukshetra during the month of October 2008 and authenticated by Dr. B. D. Vashistha, reader in Botany department of this university, as *Caesalpinia pulcherrima* (L.) Sw., Fabaceae. Voucher specimen (No. IPS/KUK/CP-1/2008) of the plant has been preserved in the herbarium of the Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra.

**Preparation of extract**

Leaves of *Caesalpinia pulcherrima* were dried in shade, powdered and stored in an air tight container at room temperature. Dried leaf powder (1690 g) was extracted with ethanol (95%) using soxhlation method. The extract was concentrated to dryness using Rotary evaporator, giving yield as 14.12% w/v and preserved in a refrigerator. Aliquot portions of the ethanolic extract of *Caesalpinia pulcherrima* leaves (CPEE) were weighed and suspended in an appropriate volume of Tween 80 (2% v/v) for use on each day of our experiments. Doses of the extract were prepared according to body weight of the animals.

**Phytochemical screening**

Various phytochemical tests (Khandelwal, 2007; Kokate, 2005) were used to screen the *Caesalpinia pulcherrima* ethanol leaf extract.

**Animals**

Albino rats (125-140 g) and mice (25-32 g) of either sex were selected for experimental study. They were obtained from Haryana Agriculture University, Hisar, Haryana, India. The animals were kept and maintained under laboratory conditions of temperature (21.5±2 °C), humidity (60±1%) and 12 h light/dark cycle; and were allowed free access to food (standard pellet diet) and water *ad libitum*.

**Acute toxicity study of the extract**

Adult albino mice (25-30 g) were divided into five groups each containing ten mice. The mice were fasted for 6 h with only access to water *ad libitum* before experimental study. Group I, II, III and IV animals were administered with various dose of CPEE i.e. 500, 1000, 2000, 3000 and 4000 mg/kg. Group V received only vehicle (TWEEN 80, 2% v/v in saline). All the doses and vehicle were administered intraperitoneally. The animals were observed for 72 h for mortality (Ravichandran et al., 2007; Sonavane et al., 2002).

**Evaluation of anticonvulsant activity**

**Pentylenetetrazole (PTZ)-induced seizure**

Albino mice of either sex (20-25 g) were selected for the study and divided into four groups of six animals each. Standard convulsant agent, pentylenetetrazole (PTZ, 80 mg/kg i.p.) was used to induce convulsions in the mice whereas Diazepam (DZP, 3.0 mg/kg i.p.) was used as reference anticonvulsant drug for comparison. Group I (control) received only vehicle. Groups II and III received CPEE at doses of 200 and 400 mg/kg, respectively whereas Group IV was treated with DZP. All the treatments were done intraperitoneally (i.p.), 30 min before administration of PTZ. The animals were individually placed in plastic
cases and observed immediately after PTZ injection for 30 min. The onset and duration of myoclonic jerks/convulsions as well as the percentage of protection against mortality were recorded (Hosseinzadeh & Khosravan, 2002; Sayyah et al., 2002; Sonavane et al., 2002).

**MES-induced seizures**

Albino rats of either sex weighing between 100-150 mg/kg were used in this experiment. The electrical stimulus (50 mA, 50 Hz, 1 s duration) was applied through ear-clip electrodes using electroconvulsiometer. Group I (control) animals received only vehicle (Tween 80) whereas Group II and III received CPEE at the doses of 200 and 400 mg/kg, respectively. Group IV received reference anticonvulsant drug, DZP 3.0 mg/kg. All the treatments were given intraperitoneally 30 min before electrical stimulus applied. Following induction of convulsions in the ‘test’ rats (due to electrical stimulus), the animals were observed for 30 min for signs of neurological deficits, especially hind limb tonic-clonic seizures or convulsions. Hind-limb tonic extensions of the rats were regarded as manifestations of seizures. The onset and duration of hind limb tonic-clonic seizures or convulsions as well as the percentage of protection against mortality were recorded (Hosseinzadeh & Khosravan, 2002; Sayyah et al., 2002; Sonavane et al., 2002).

**Statistical analysis**

The Dunnett’s test was employed for statistical comparison. In all the cases, value of \( p < 0.05 \) was considered significant. All values have been presented as mean±S.E.

**RESULTS**

**Phytochemical screening**

Phytochemical screening of *Caesalpinia pulcherrima* (L.) Sw., Fabaceae, leaves ethanolic extract showed the presence of flavonoids, glycosides and tannins as shown in Table 1.

**Table 1. Phytochemical screening of *C. pulcherrima* leaves ethanolic extract.**

<table>
<thead>
<tr>
<th>Tests/Reagents</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorff’s/Mayer’s reagent</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Lead acetate/Sodium hydroxide</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
</tr>
<tr>
<td>Steroids</td>
<td>Salkowski/Liebermann-Burchard reaction</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride/Bromine solution</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>Keller-Kilani/Legal test</td>
</tr>
</tbody>
</table>

**Table 2. Effect of CPEE on PTZ induced seizures in mice.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (dose)</th>
<th>Animals used</th>
<th>Onset of myoclonic jerks (sec.) Mean±SEM</th>
<th>Duration of myoclonic jerks (sec.) Mean±SEM</th>
<th>animals convulsed</th>
<th>deaths</th>
<th>% Protection against mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>6</td>
<td>69.25±3.411</td>
<td>15.62±1.13</td>
<td>6/6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>CPEE (200 mg/kg)</td>
<td>6</td>
<td>92.28±2.826</td>
<td>10.28±1.16</td>
<td>6/6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>CPEE (400 mg/kg)</td>
<td>6</td>
<td>135.66±3.383*</td>
<td>4±0.23*</td>
<td>6/6</td>
<td>4</td>
<td>33.3</td>
</tr>
<tr>
<td>IV</td>
<td>Diazepam (3 mg/kg)</td>
<td>6</td>
<td>Absent</td>
<td>Absent</td>
<td>3*/6</td>
<td>1</td>
<td>83.3</td>
</tr>
</tbody>
</table>

*Significance in relation to control: \( p < 0.01 \). Six mice were used in each group and diazepam was taken as standard.

**Table 3. Effect of CPEE on maximal electroshock (MES) induced seizures in rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (dose)</th>
<th>Onset of HLES (sec.) Mean±SEM</th>
<th>Duration of HLES (sec.) Mean±SEM</th>
<th>Number of rats convulsed/ No. used</th>
<th>Number of deaths</th>
<th>% Protection against mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>12±0.91</td>
<td>25.12±1.85</td>
<td>6/6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>CPAE (200 mg/kg)</td>
<td>13±1.15</td>
<td>17.25±1.56</td>
<td>6/6</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>III</td>
<td>CPAE (400 mg/kg)</td>
<td>23.33±2.4 *</td>
<td>15.8±1.83*</td>
<td>5/6</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>IV</td>
<td>Diazepam (3 mg/kg)</td>
<td>Absent</td>
<td>Absent</td>
<td>2*/6</td>
<td>2</td>
<td>75</td>
</tr>
</tbody>
</table>

*Significance in relation to control: \( p < 0.01 \). Diazepam was taken as control.
Acute toxicity study of the extract

The CPEE was found to be safe at the escalated doses used and there was no mortality up to the dose of 4000 mg/kg of extract when administered intraperitoneally.

Pentylenetetrazol-induced seizures

Single dose of Pentylenetetrazole (PTZ, 80 mg/kg i.p.) produced hind-limb tonic seizures in all the six mice used. The plant extract (CPEE, 200 mg/kg i.p.), did not significantly delay the onset and decreased the duration of seizures. The plant extract (CPEE, 400 mg/kg i.p.), however, significantly (p<0.01) delayed the onset of, and antagonized, PTZ-induced clonic seizures as well as decreased the duration of clonic convulsions. The CPEE (400 mg/kg, i.p.) exhibited 33.3% of protection against mortality whereas it was 83.3% in Diazepam treated group. The effect of CPEE on PTZ induced seizures in mice is shown in Table 2.

Maximal electroshock (MES) induced seizures in rats

The effects of CPEE on MES induced seizures in rats are summarized in Table 3. There were no significant alterations in the latency of convulsions as compared to control at a dose of 200 mg/kg of the extract. However, the extract (CPEE, 400 mg/kg i.p.) significantly (p<0.01) delayed the onset as well as decreased the duration of hind limb extension seizures (HLES) as compared to control. The extract also exhibited 25 and 50% protection against mortality at doses of 200 and 400 mg/kg respectively whereas it was 75% in diazepam treated rats.

DISCUSSION

Some plants of genus Caesalpinia are reported to have anticonvulsant activity (Adesina, 1982). The present study was carried out based on the ethnomedicinal use of the Caesalpinia pulcherrima (L.) Sw., Fabaceae, in convulsions (Chatterjee & Prakashi, 2006; Pullaiah, 2006).

The most popular and widely used animal seizure models are the traditional PTZ and MES tests. Prevention of seizures induced by PTZ in laboratory animals is the most commonly used preliminary screening test for characterizing potential anticonvulsant drugs. The MES test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures. Generally, compounds with anticonvulsant activity in petit mal epilepsy are effective in PTZ induced seizure model (Löschler et al., 1991).

The extract (CPEE, 200 mg/kg i.p.) slightly delayed the onset, decreased the duration of myoclonic seizures induced by PTZ, but mortality at this dose level was 100%. The extract, however, significantly delayed the onset of as well as decreased the duration of myoclonic seizures induced by PTZ. The protection percentage of animals was also increased to 33% at a dose of 400 mg/kg. Therefore, the extract at 400 mg/kg dose produced significant (p<0.01) anticonvulsant effect w.r.t. control against PTZ-induced clonic seizures.

In MES-induced seizure model, there were no significant alterations in the onset as well as duration of HLES as compared to control, suggesting that Caesalpinia pulcherrima extract does not have anticonvulsant effect at a dose of 200 mg/kg when administered intraperitoneally. However, the extract (CPEE, 400 mg/kg i.p.) significantly (p<0.01) delayed the onset as well as decreased the duration of hind limb extension seizures (HLES) as compared to control. Protection percentage of animals was also increased at higher dose level i.e. 400 mg/kg.

Phytochemical screening of plant leaves ethanolic extract showed the presence of saponins, flavonoids and tannins. Based on this knowledge of the extract, it is not possible to attribute with certainty its anticonvulsant effect to one or several active principles among those detected in the screening. However, triterpenoidal saponins are reported to possess anticonvulsant activity in some experimental seizure models such as MES and PTZ (Chauhan et al., 1988; Kasture et al., 2002). Some flavonoids are also reported to have protective effects against PTZ, picrotoxin and NMDLA-induced convulsions (Johnston et al., 2004).

In conclusion, the results of the present study demonstrate, for the first time, that the ethanolic extract obtained from C. pulcherrima leaves possess anticonvulsant properties in PTZ and MES treated animals. Such pharmacological effects confirm and justify, at least in part, the popular traditional use of this plant to treat convulsions. However, the mechanism by which the plant extract exerts its anticonvulsant effect is still unclear. Therefore, this plant part must be further investigated to isolate the phytoconstituent responsible for anticonvulsant effect as well as its mechanism.

REFERENCES


Chatterjee A, Prakashi SC 2006. The treatise on Indian medicinal plants. New Delhi: NISCAIR.


