Experimental Study of Anticonvulsive Effects of Euphorbia Pulcherrima in Mice

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Abstract

Background: Euphorbia pulcherrima belongs to the family: Euphorbiaceae and Genus: Euphorbia. Many species of Euphorbia have been reported as having beneficial properties like anticonvulsive effect. However, little study has been done and published on Euphorbia pulcherrima (Lalupate). In the present study we evaluated the anticonvulsant properties of Euphorbia pulcherrima by using various convulsive experimental models.

Objective: To observe and evaluate anticonvulsive effects of Euphorbia pulcherrima in mice.

Methods: Experiments were performed on adult albino mice (n = 100) weighing 20-30g. Maximal Electroshock Seizure test model and Pentylenetetrazole induced seizure model were used to assess the anticonvulsant effect of Euphorbia pulcherrima in mice and rats after oral administration of Euphorbia pulcherrima crude dried extracts in three different doses (250, 500 and 1000 mg/kg).

Results: EP crude extract reduce the duration of tonic hind limb extension in Maximal Electroshock Seizure test model in mice treated with EP (250, 500 and 1000 mg/kg). There was also an increase in the latency to convulsions with the use of EP in three different doses (250, 500 and 1000 mg/kg) in Pentylenetetrazole induced seizure model. EP in all three doses (250, 500 and 1000 mg/kg) significantly increased the latency and decrease the incidence of convulsions induced by Pentylenetetrazole induced seizure model.

Conclusion: This study showed EP crude dried extracts to possess anticonvulsant properties in Maximal Electroshock Seizure test model and Pentylenetetrazole induced seizure model. In the present study provides evidence supporting anticonvulsive properties of other related Euphorbia species.

Introduction

Euphorbia pulcherrima belongs to the family: Euphorbiaceae and Genus: Euphorbia. (English name: Poinsettias [Christmas star], Nepali name: Lalupate.[1,2] It is also easily found all over Nepal especially in the Hilly region. Poinsettias are shrubs to small trees, typically reaching a height of 0.6 to 4 m (2 to 16 ft). The plant bears dark green dentate leaves that measure 7 to 16 cm (3 to 6 inches) in length. The top leaves, known as bracts, are flaming red, pink, or white and are often mistaken as flowers. The actual flowers are grouped within the small yellow structures found in the center of each leaf bunch, which are called cyathia.[3] In a study involving evaluation of psychopharmacological profile of hydro-alcoholic extract of Euphorbia neriifolia provided protection against maximal electro-shock-induced convulsion. [4] Interestingly one placebo controlled single blind study in 72 epileptic patients of whom 22 had intractable epilepsy, alkaline extract of Euphorbia fisheriana produced significant antiepileptic effect compared with placebo.[5] Toxicity profile of Euphorbia pulcherrima: People have misconception about Euphorbia pulcherrima. Public and some health professionals thought that it is a much-maligned plant as well as it is extremely toxic to human. It was also recognized as a poisonous plant.[6] Studies have accounted for 22,793 cases of Poinsettia exposures to the American Association of Poison Control Centers but surprisingly there were no fatalities among all Poinsettia exposures and 98.9% were accidental in nature, with 93.3% exposures involving children. The majority of exposed patients (96.1%) did not require treatment in a health care facility and 92.4% did not develop any toxicity related to their exposure to the Poinsettia. Most patients do not require any type of therapy and can be treated without referral to a health care facility. [6] Few studies have reported the allergic potential of Euphorbia Pulcherrima like rhinitis and asthma induced by Euphorbia Pulcherrima but patients were able to tolerate it.[7] Also there were few reported cases of contact dermatitis due to latex of Euphorbia pulcherrima.[8,9] Since many of the Euphorbiaceae family action was related anticonvulsive action, objective of present study was to investigate the anticonvulsant activity of Euphorbia pulcherrima against the seizure induced by Maximal Electroshock Seizure and Pentylenetetrazole in the mice.
Methods

Study type and study design: Quantitative experimental study in the mice.
Place and duration of study: Laboratory of Department of Clinical Pharmacology and Therapeutics, BPKIHS, Dharan. Duration of study was one year.
Drugs and chemicals:
Phenytoin (M-Toin, Medopharm, India); Sodium valproate (Encorate, Sun Pharmaceuticals, India); Pentylenetetrazole (Sigma Chemicals, USA); Pentobarbital (LobaChemie, India).

Animals:
Experiments were performed on adult albino mice (n = 100) weighing 20-30g. Animals were produced in the laboratory breeding house of the Department of Clinical Pharmacology and Therapeutics. The animals were maintained under controlled room temperature (25 ± 2°C) and light and dark (12:12 hr) conditions and were given food pellets and water ad libitum. Animals were fasted overnight before the experiment. Before conducting the experiment, ethical clearance was obtained from the local Ethical committee on Animal Research.

Each experiment consisted of five groups of animals of ten in each group. Group I (vehicle control, vehicle treated); Group II (standard control), Group III (test drug, Euphorbia pulcherrima[EP] 250mg/kg), Group IV (test drug, EP 500mg/kg) and Group V (test drug, EP 1000mg/kg).
Drugs preparation:
Euphorbia pulcherrima plant was identified with the help of available literature and resources. Milky latex of plant was collected from leaf and stem in Petri dish which was allowed to dry in room temperature for 15 days. After 15 days, it was recovered from Petri dish with the help of new scalpel blade and finally grinded to fine powder with mortar and pestle. Vehicle for the study was distilled water. EPcrude dried latex suspension was made by mixing with distilled water. Pentylenetetrazole, Phenytoin and Valproic acid were dissolved in distilled water.

Test drug, standard control drugs and vehicle was given through oral route with the help of orogastric tube. Distilled water (10ml/kg) was vehicle control in the study. The Standard controls for anticonvulsant effect were Phenytoin 10mg/kg (maximal electroshock seizure) and Sodium valproate 300mg/kg (Pentylenetetrazole induced seizure).
Acute toxicity:
The crude extract of EP was administered in doses of 1000 and 1500 mg/kg orally to groups of mice, each containing 10 animals and mortality was observed after 24 hrs.

Experimental models:
Maximal Electroshock Seizure (MES) test:
The maximal electroshock seizure pattern was induced in animals by using a convulsiometer (Techno, India) to give an alternating current of 150 mA for 0.2 sec. After 45 minutes of post dosing, mice was subjected to MES of 150 mA of alternating current from a convulsiometerfor 0.2 sec through a pair of electrodes attached to each ear.[10] The duration of the tonic hind limb extensor phase, clonic phase and the number of animals protected from convulsions was noted. Phenytoin in doses of 10 mg/kg PO was be used as standard control.
Pentylenetetrazole (PTZ) induced seizures:
This assay was used to evaluate antiepileptic drugs. Pentylenetetrazole was used in a dose of 40 mg/kg, intraperitoneally. This is the dose that produces clonic seizures in all the animals without mortality. The test drug was administered 45 minutes prior to pentylenetetrazole administration. The latency to first convulsion and the no. of mice which exhibited seizure was observed immediately after the test drug injection for a period of 30 minutes.[11]

Statistical analysis:
Values reported are means±SEM (number of animals). The significance of difference with respect to controls was evaluated using the Mann–Whitney U test. A probability (p-value) level less than 0.05 were considered as significant.[12]All analyses was conducted using SPSS 17.00 for Windows.

Results

Acute toxicity: The EP was found to be safe in the doses used and there was no mortality in a dose of 1500/kg orally.
Anticonvulsant effect of dried latex of the aerial parts of Euphorbia pulcherrima (250mg/kg, 500mg/kg and 1000mg/kg) were evaluated in this study and the effects were compared with vehicle control and standard control.

In Maximal Electroshock Seizure Test, treatment with EP in three different doses (250, 500 and 1000 mg/kg) reduced the number of convulsing animals, suggestive of protective effect in mice against the maximal electroshock induced convulsion. The duration of tonic hind limb extension in mice treated with EP (250, 500 and 1000 mg/kg) was significantly (p0.05) as compared to Phenytoin at 10mg/kg (Table 1).
Likewise, there was also an increase in the latency to
convulsions with the use of EP in three different doses (250, 500 and 1000 mg/kg) in Pentylenetetrazole induced seizure. EP in all three doses (250, 500 and 1000 mg/kg) significantly (p<0.05) as compared to Valproic acid 300 mg/kg (Table 1).

Discussion

The present study demonstrates the anticonvulsive effect of crude dried latex of Euphorbia pulcherrima (EP) in mice by using convulsive experimental models. The outcome of the present study demonstrates that EP produced anticonvulsive effect in convulsive experimental models.

In preliminary experiments, the toxicity of crude extract of EP was tested, and judging from high doses (1500mg/kg) that were tolerated without significant overt mortality or signs of toxicity. It was estimated that the doses used in the experiment may reflect the low concentration of the active compound present in the crude extract.

This is probably the first report on this aspect on EP. However, there are previous reports on the CNS depressing activity of other related Euphorbia species. For example, Euphorbia neriifolia leaf extract has anti-convulsant action. Euphorbiafisheriana reportedly has produced significant antiepileptic effect in patients having intractable epilepsy.

In the present study, EP was studied for its anticonvulsive activity in experimental models. To evaluate anticonvulsive properties of EP, PTZ induced seizure and MES model were used. PTZ is the most frequently used substance, as well as an acute experimental model, in a preliminary screening to test potential anticonvulsant drugs. The mechanism by which PTZ is believed to exert its action is by acting as an antagonist at the GABAA receptor complex. Drugs protecting against tonic–clonic seizures induced by PTZ are considered to be useful to control myoclonic and absence seizures in humans.

Ethosuximide, a blocker of T-type Ca2+ currents, can prevent PTZ-induced seizures and is effective in controlling absence epilepsy, most commonly in childhood. The drugs which inhibit the MES induced seizures mostly inhibit the sodium current and block the repetitive firing of neurons. It has been often stated that seizures induced by PTZ, can be blocked by drugs that enhance γ-aminobutyric acid type A (GABAA) receptor-mediated inhibitory neurotransmission, such as benzodiazepines and phenobarbital.

In the present study, EP significantly inhibited MES induced seizures and PTZ induced seizures in mice. This may suggest that the anticonvulsant action of EP is mediated by the channel of GABAA/benzodiazepine receptor complex. The formulation may act by increasing GABA concentration in brain because PTZ is a known GABAA receptor antagonist. The benzodiazepine site in the GABAA receptor and even T type Ca2+ currents could be targets for future studies to know mechanisms of action of the EP extract and/or its components. In our experiment, the extract of EP replicated the effect of this antiepileptic drug delaying the presence of seizures and reducing tonic convulsions and mortality.

The prolongation of the onset time of PTZ convulsions by EP extract indicates anti-convulsant effect of the plant, which again involves the inhibition of excitatory mechanisms in the CNS, although the parameter, i.e. onset time of convulsions, decreased duration of tonic clonic convulsion used for evaluation of anti-convulsant activity in the present study is not conclusive. However, it gives a preliminary indication as to the anti-convulsant effect of EP extract. Further studies are needed to answer the questions like the differential effects on tonic and clonic convulsions; effect on mortality following the PTZ induced convulsions, preventive effects of the plant on convulsions on chronic administration.

Conclusion

Thus, in conclusion, this study showed EP crude dried extracts to possess anticonvulsant properties. This study also provides evidence supporting anticonvulsive properties of other related Euphorbia species. This is probably the first experimental study for EP to characterize its anticonvulsive profile. Despite showing good pharmacological effects, there is still a need for more precise studies to determine and separate the active compounds and elucidate its mechanisms of action where possible. So, further studies are needed to determine the exact mechanism(s) of action for anticonvulsant action of the various compounds in the crude extract of EP.

Limitation

This is experimental study based in crude dried latex of Euphorbia pulcherrima (EP) in mice. There is needed to extract the pure compound responsible for this activity.

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Reference(s)

Illustrations

Illustration 1

Table 1: Effect of Euphorbia pulcherrima (EP) on convulsion.

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Duration of tonic hind limb extension in MES (sec)</th>
<th>Latency to convulsion in PTZ (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>13.33±0.33</td>
<td>2.92±0.21</td>
</tr>
<tr>
<td>II</td>
<td>0.50±0.50</td>
<td>23.50±4.27</td>
</tr>
<tr>
<td>III</td>
<td>3.17±2.00*</td>
<td>23.00±4.45*</td>
</tr>
<tr>
<td>IV</td>
<td>1.17±1.16*</td>
<td>23.83±3.95*</td>
</tr>
<tr>
<td>V</td>
<td>1.00±1.00*</td>
<td>25.67±4.33*</td>
</tr>
</tbody>
</table>

Values are expressed as (mean±SEM) from 10 animals in each group. Group I: Vehicle Control (Distilled water 10mg/kg), Group II: Standard control, Group III: EP (250mg/kg), Group IV: EP (500mg/kg) and Group V: EP (1000mg/kg).

*p value <0.05 when compared to vehicle control.
Illustration 2

Electroconvulsiometer for MES test

Illustration 3

Tonic hind limb extension in MES test
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