Evaluation of Anticonvulsant Potential of Bryophyllum Pinnatum in Experimental Animals

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Research Article

Article Info
Recived on:24/01/2015
Accepted on: 15/02/2015
Published on: 20/02/2015

QR Code for mobile
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Open Access

ABSTRACT :

The present study was undertaken to evaluate the anticonvulsant potential of *Bryophyllum pinnatum* in mice. We studied the effects of ethanolic extract of the leaves of *Bryophyllum pinnatum* against maximal electroshock (MES) induced convulsions and Pentylenetetrazole(PTZ) seizure model in mice. Parameters observed in MES model were duration of hind limb tonic extension (HLTE), the total recovery time and percentage protection. In the PTZ model, the parameters observed were onset of clonic convulsions (latency period), duration of clonic phase, percentage reduction of clonic phase and percentage mortality. In the MES induced convulsion Phenytoin (25mg/kg) was used as standard drug and in PTZ model Diazepam (4mg/kg) was used as the standard drug. Extract was used in 200, 300 and 400 mg/kg doses. Results obtained in this study substantiate the anticonvulsant effect of ethanolic extract of *Bryophyllum pinnatum* leaves.

INTRODUCTION:

Epilepsy is a group of long-term neurological disorders characterized by epileptic seizures.[1] About 1% of people worldwide (65 million) have epilepsy, [2] and nearly 80% of cases occur in developing countries. [3] From 1964 onwards, multiple prevalence studies have been carried out in India with a range from 2.5 to 11.9 per 1000 population. There are very few incidence studies from India, and the most recent one suggests an age standardized incidence rate of 27.3/100,000 per year. [4]

As of 2014, epilepsy is defined by the International League against Epilepsy as a person who meets any of the following conditions: [5]

1. At least two unprovoked (or reflex) seizures occurring greater than 24 hours apart.
2. One unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years.
3. Diagnosis of an epilepsy syndrome.

Epileptic seizures are the result of excessive and abnormal cortical nerve cell activity in the brain. [6] Epilepsy cannot be cured, but seizures are controllable with medication in about 70% of cases.[7] Of those with generalized seizure more than 80% can be well controlled with medications while this is true in only 50% of people with focal seizures.[8] The mainstay treatment of epilepsy is anticonvulsant medications; possibly for the person's entire life.[9] The choice of anticonvulsant is based on seizure type, epilepsy syndrome, other medications used, other health problems, and the person's age and lifestyle.[10] The aim of drugs is to control and totally prevent all seizure activity at an acceptable level of side effects. With the currently available drugs, this can be achieved in about half of the patients. Another 20-30% attains partial control, while the rest remain refractory. [11]Adverse effects from medications are reported in 10 to 90% of people; depending on how and from whom the data is collected.[12]

It is therefore important to identify and evaluate available natural drugs as alternatives to current antiepileptic drugs, which are commonly associated with adverse effects. Plant extracts are some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of epilepsy.

Bryophyllum pinnatum (Crassulaceae) is a widely used medicinal plant in traditional system with a wide range of biological activities.[13] It is a cras-

doi: 10.15272/ajbps.v5i41.662

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Conflict of interest: Authors reported none

sulescent herb of about 1metre in height, with opposite, glabrous leaves (with 3–5deeply crenulated, fleshy leaflets).[14] It is a perennial herb growing widely and used in folklore medicine in tropical Africa, America, India, China, Australia and southern part of Nigeria. The plant grows all over India in hot and moist areas, especially in Bengal.[15] In common with other Crassulaceae, *Bryophyllum pinnatum* has been found to contain bufadienolide.[16] Bufadienolide compounds isolated from *Bryophyllum pinnatum* include Bryophillin A, which showed strong anti-tumor promoting activity in vitro. Bryophillin C also showed insecticidal properties.[17] The plant has considerable attention for their medicinal properties and find application in folk medicine, as well as in the contemporary medicine.[18,19] The leaves and bark are bitter tonic, astringent to bowels, analgesic, carminative, and are useful in diarrhoea and vomiting. Antimicrobial, antifungal, anti-ulcer, anti-inflammatory and analgesic activities of leaf extract were reported.[20]The juice from fresh leaves is used to treat smallpox, otitis, cough, asthma, palpitation, headache, convulsion and general debility. [21] The plant has also been employed for the treatment of edema of legs. [22] It is largely used in folk medicines for the treatment of hypertension, kidney stones,[23] pulmonary infections and rheumatoid arthritis.[24] A water extract of Bryophyllum leaves administered topically and internally has been shown to prevent and treat Leishmaniasis. [25] The plant is used traditionally for the treatment of earache, in ophthalmic preparations, sprains and in dysmenorrhoea.[26]

There is growing interest in herbal remedies because of their effectiveness, minimal side effects and relatively low cost. The results of previous studies on different properties of *Bryophyllum pinnatum* are very encouraging. As very few studies have been carried out to look for its anticonvulsant potential and its usefulness in epilepsy the present study has been undertaken.

MATERIALS AND METHODS

Experimental animals:

Swiss albino mice of either sex, weighing between 20 - 30 gm, were procured from animal house of Department of Pharmacology, Gauhati Medical College. The animals were housed at 25±2^{0}C with 12 hour light and dark cycle and allowed to feed on standard diet and water *ad libitum*. They were acclimatized to laboratory condition for 1 week before the study. The study was approved by the Institutional Animal Ethics Committee of Gauhati Medical College & Hospital. CPCSEA guidelines were adhered during the experiment.

Plant Materials:

The leaves of *Bryophyllum pinnatum* were collected from in and around Guwahati. Authentication of the plant was done in the Department of Botany, Gauhati University and a voucher specimen was preserved for further reference.

The whole plant was thoroughly washed, shade dried and then chopped to coarse powder using a mixer grinder. Powder (200 gram) was tightly packed in Soxhlet apparatus and extracted employing ethanol as solvent for 5 days at a temperature of 40-60^{0}C using a heating mantle. The extract was filtered using Whatman filter paper No.1 and the filtrate was evaporated on a water bath until it gets concentrated. The jelly like extract of the leaves was collected in a petridish. A final yield of 40.5 gm was obtained. The percentage yield of *Bryophyllum pinnatum* was 20.25% (w/w) with respect to the original dried powder. The extract was stored in a refrigerator at 4°C in labeled air-tight containers for further use.

Drugs and Chemicals:

Phenytoin , Ethanolic extract of *Bryophyllum pinnatum* (EEBP), Pentylenetetrazole(PTZ) and Diazepam.

Acute toxicity study:

Acute toxicity study was done according to OECD Guidelines. The animals were found to be alive at 2000 mg/kg per oral feeding of the ethanolic extract.

Test for anticonvulsant activity:

(1) Maximal electroshock seizure model:

A total of 30 animals were divided into 5 groups containing 6 in each. They were fasted for 24 hours before the test with free access to water. 1 hour after administration of test extracts and 30 minutes after i.p. injection of Phenytoin the animals were subjected to MES by convulsiometer with a current of 50 mA for 0.2 sec.via a pair of transauricular electrodes. Duration of hind limb tonic extension and total recovery time was noted and percentage protection was calculated for each group. Prevention or decrease in hind limb tonic extension was considered as protective action. The percentage protection was calculated as:

(Duration of HLTE in Control-Duration of HLTE in Test/Standard)/(Duration of HLTE in Control)×100

Experimental design for MES model:

GROUP I- Normal Control- Received Normal Saline at a dose of 10 ml/kg per orally +MES
GROUP II- Standard- Received Phenytoin at a dose of 25 mg/kg i.p. +MES
GROUP III- EEBP 200 mg/kg per orally +MES
GROUP IV- EEBP 300mg/kg per orally +MES
GROUP V- EEBP 400mg/kg per orally + MES

(2) Pentylenetetrazole(PTZ) seizure model:

A total of 30 animals were divided into 5 groups with 6 animals in each group. One hour after administration of the extracts and 30 min after i.p. injection of

Diazepam, the animals were given PTZ 80mg/kg i.p after dissolving in distilled water.[27] The animals were observed for a period of 1 hour.

Parameters measured were: [28]

Onset of clonic convulsions (latency period)
Duration of clonic phase
Percentage reduction of clonic phase
Percentage mortality.

The percentage reduction of clonic convulsion was calculated as:

(Duration of Clonus in Control-Duration of Clonus inTest/Standard)/(Duration of Clonus in Control)×100

Experimental design for PTZ model:

GROUP I: Normal Control- Received 10ml/kg of Normal saline per orally + PTZ
GROUP II: Standard- Received Diazepam at a dose of 4mg/kg per orally + PTZ
GROUP III: EEBP 200 mg/kg per orally + PTZ
GROUP IV: EEBP 300mg/kg per orally + PTZ
GROUP V: EEBP 400mg/kg per orally + PTZ

Statistical Analysis:

Mean ± SEM (standard error of mean) values were calculated for each group. Significant differences between the groups were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test and results were found to be significant ($p<.05$) All analysis were done using graph pad prism software version 5.01.

RESULTS AND OBSERVATION

Ethanolic extract of the leaves of *Bryophyllum pinnatum* reduced the duration of hind limb tonic extension and the duration of recovery in a dose dependent manner in maximal electroshock induced seizure model. At 200, 300 and 400 mg/kg doses of extract a significant ($p<0.01$) reduction in the duration of hind limb tonic extension was observed representing 11.33, 8.33 and 5.5 seconds respectively while the duration of recovery time also showed significant reduction at these doses representing 149.16, 109.5 and 48.5 seconds respectively.

Groups	Duration of HLTE (in sec) Mean ±SEM	Total Recovery Time (in sec) Mean ±SEM	% Protection
Group I	25.16 ± 0.83	175.83±3.00	-----
Group II	1.66±0.21#	41.66±1.17#	83.33
Group III	11.33±0.33#	149.16±0.98#	16.66
Group IV	8.33±0.40#	109.5±0.61#	33.33
Group V	5.5±0.22#	48.5±2.09#	50

Table 1: Maximal Electroshock (MES) Induced Seizures in Mice
# p < 0.05 when compared with the Control group (Group I)

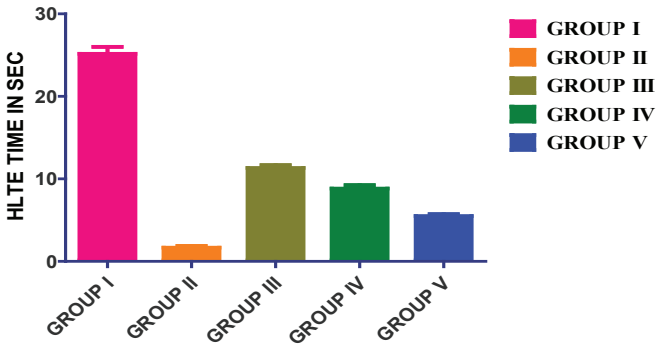

Fig 1: Duration of Hind Limb Tonic Extension

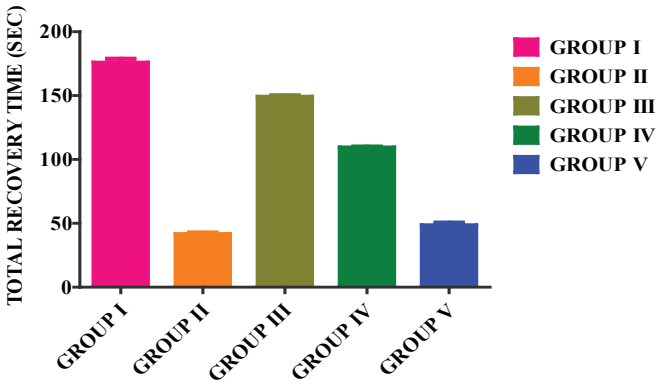

Fig 2: Total Recovery Time

Ethanolic extract of the leaves of *Bryophyllum pinnatum* increased the latency of clonic convulsion and decreased the duration of convulsion in a dose dependent manner in PTZ induced seizure model. At 200, 300 and 400 mg/kg doses of extract a significant ($p<0.01$) increase in latency of clonic convulsion was observed representing 176, 241 and 300 seconds respectively while the duration of convulsion also showed significant reduction at these doses representing 49, 29 and 19.83 seconds respectively

Groups	Onset of clonus (in sec) mean ±SEM	Duration of clonic convulsion (in sec) mean ±SEM	% Reduction of clonic convulsion	% Mortality
Group I	129.5 ± 6.82	77.16 ± 4.08	---	100
Group II	416.33 ± 8.25#	8.83 ± 0.30#	88.55	16
Group III	176 ± 5.55#	49 ± 3.04#	36.50	66.67
Group IV	241 ± 6.57#	29 ± 2.53#	64.42	50
Group V	300 ± 6.89#	19.83 ± 1.26#	74.30	33.33

Table 2: Pentylenetetrazole (PTZ) Induced Seizures in Mice
# p < 0.05 when compared with the Control group (Group I)

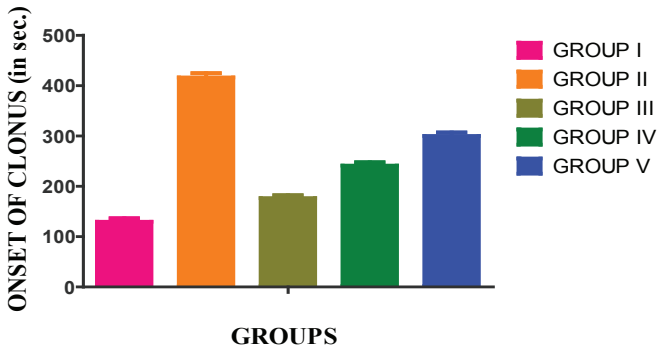

Fig 3: Onset of Clonus

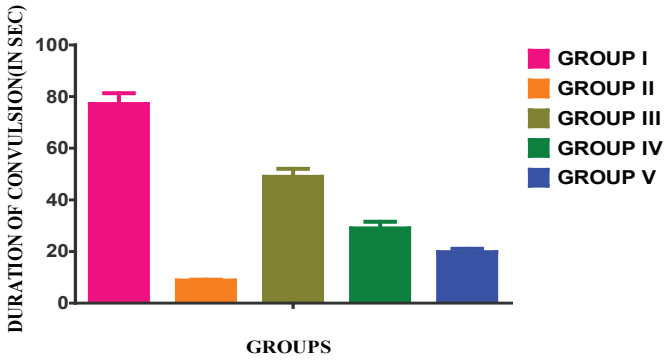

Fig 4: Duration of Convulsions (in sec)

DISCUSSION

MES test, is thought to be predictive of anticonvulsant drug effective against GTCS. The PTZ test, is thought to represent a valid model for generalized absence and/or myoclonic seizures.[29]Preliminary phytochemical investigation of different parts of plant extracts of B. pinnatum showed the presence of alkaloids, phenols, flavonoids, saponins, tannins, carotenoids, glycosides,[30,31,32] sitosterol, anthocyanins,[33] malic acid, quinines, tocopherol [34], lectins[35] coumarins[36]and bufadienolides[37,38].The above phytochemicals from other plants have been reported to have anticonvulsant property in various animal models of epilepsy like PTZ, MES, electrical kindling, etc.[39] In MES seizure EEBP at 200 mg/kg, 300mg/kg and 400 mg/kg doses showed significant decrease in HLTE and total recovery time, when compared with the control group mice. The decrease in HLTE duration and total recovery time was in a dose dependent manner of the extract. In PTZ induced convulsions EEBP at test doses increased the latency of clonic convulsion and decreased the duration of convulsion significantly when compared to the control group in a dose dependent manner. Drugs effective in PTZ induced seizure, like drugs that reduces the T-type of Ca^{++} currents or drugs that inhibit GABA mediated neurotransmission, act by elevating the seizure threshold. MES induced tonic extension seizure can be prevented either by drugs that inhibit voltage gated Na^{+} channels or by drugs that inhibit glutaminergic excitation mediated by NMDA receptors. Probable anticonvulsant mechanism may be due to potentiating of GABA-ergic inhibition or by blocking the seizure spread by inhibiting voltage gated Na^{+} channels and/or glutaminergic excitation through NMDA receptors. There is need for further investigations for identification of the active compounds and their exact molecular mechanism of action responsible for the anticonvulsant activity of *Bryophyllum pinnatum*.

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Cite this article as:

Arijit Dutta, Chimi Handique, Mangala Lahkar. Evaluation of Anticonvulsant Potential of Bryophyllum Pinnatum in Experimental Animals. Asian Journl of Biomedical and Pharmaceutical Sciences. 5(41).2015: 18-22.
