STUDY OF THE ANTICONVULSANT ACTIVITY OF ETHANOLIC EXTRACT OF SEEDS OF BENINCASA HISPIDA LINN. IN ALBINO RATS

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ABSTRACT

Objective: Seeds of Benincasahispida has been traditionally used as anticonvulsant. The aim of the study is to assess the anticonvulsant activity of Ethanolic extract of Benincasahispida (EEBH) by Maximal Electroshock seizure (MES) and Pentylenetetrazol induced seizure models on Albino (Wistar strain) rats.

Methods: Albino rats were taken and divided into five groups, each consisting of five rats both for MES and PTZ model. One group was used as control (normal saline 10 ml/kg), one as standard (phenytoin in MES model, Diazepam in PTZ model), and three groups for the test drug (EEBH in the doses of 100, 200, 400 mg/kg) treatment. In MES model Maximal electrical shock of 150 mA was passed for 0.2 seconds through ear lobe electrodes after 30 minutes of giving the drugs and normal saline. Different stages of convulsions were noted down along with time spent by the animal in each phase of convulsions. In PTZ model, Pentylenetetrazol (PTZ) was injected 30 minutes after giving the drugs and normal saline, and onset of action and severity of convulsions were noted.

Results: Data were statistically analyzed by One way ANOVA followed by multiple Dunnett’s test. EEBH dose dependently reduced the duration of tonic hind limb extension in MES model and there was increase in latency and occurrence of convulsions in PTZ model.

Conclusion: EEBH has anticonvulsant activity.

Keywords: Anticonvulsant, Maximal Electroshock, Pentylenetetrazol, Benincasahispida

INTRODUCTION

Epilepsy describes a condition in which a person has recurrent seizures due to a chronic, underlying process. This implies that a person with a single seizure, or recurrent seizures due to correctable or avoidable circumstances, does not necessarily have epilepsy. Epilepsy refers to a clinical phenomenon rather than a single disease entity, since there are many forms and causes of epilepsy. However, among the many causes of epilepsy there are various epilepsy syndromes in which the clinical and pathological characteristics are distinctive and suggest a specific underlying etiology [1].

The importance of traditional system of medicine and of certain medicinal practices has now been recognized all over the world. Today it is required to have an intelligent and pragmatic approach to evaluate selective drugs of herbal origin [2].

Benincasahispida is also known as Kushmanda in Sanskrit/ Hindi, Kumora in Assamese and Ash Gourd in English. Ash Gourd is believed to have originated in Java, Indonesia. The Chinese have been cultivating it for over 2,000 years. Its medicinal uses first appeared in 659 AD in the Materiamedica of the Tang dynasty. In Chinese medicine, the rind is used to treat urinary dysfunction and the seeds for vaginal discharge. The fruit is used to treat summer fevers. In Ayurveda, the fruit is beneficial for the management of a host of medical problems, including epilepsy, lung diseases, asthma, cough, urine retention and internal hemorrhage. It is also an excellent remedy for tapeworms.

Therapeutic constituents: Amino acids, mucins, mineral salts, starch and calcium are present in Ash Gourd. Phyto-chemical studies on the fruit indicated two triterpenes, alunsenol and mutiflorenol, which have cell stabilizing effects. The pulp of Ash Gourd is a rich source of vitamins B and C. Key therapeutic benefits are that Ash Gourd is used for diabetics and patients suffering from obesity, it combats general debility by stabilizing nerve cells. The cooling properties of its juice are helpful in treating peptic ulcers and for relieving acidity. Ash Gourd provides relief from constipation and tones the gastrointestinal tract and the seeds of Ash Gourd are anabolic and encourage tissue growth [3].

In spite of traditional use, pharmacology of its different parts has not yet been explored scientifically. As such, the present investigation was carried out to evaluate the antiepileptic activity of the ethanolic extract of seeds of Benincasahispida (EEBH) in experimental animal models [4].

MATERIALS AND METHODS

Plant: The seeds of Benincasahispida were collected and authenticated by Dr. M. Islam, Professor, Department of Life Science, Dibrugarh University. A voucher specimen (No. DU/LS/218) was deposited at Dibrugarh University.

Preparation of plant extract

The seeds were washed, air dried, powdered and then kept in percolator with 90% ethanol for 72 hours. The extract obtained from percolation was collected in a flask, and then evaporated by using controlled temperature until the solvent part was evaporated [5].

Drugs

Phenytoin obtained from Zydus Cadila Healthcare Limited, Diazepam obtained from Ranbaxy Laboratories, New Delhi and Pentylenetetrazol obtained from Sigma Aldrich India, Bangalore.

Animal
Fifty healthy Albino rats (Rattus norvegicus) weighing from 150-250 gms of either sex were taken from Central Animal House, Assam Medical College (registration no. 659/02/a/CPCSEA dated 19/05/02). The animals were housed in standard cages and maintained under normal room temperature. The rats were maintained on standard animal diet of Bengal gram, wheat, maize and carrot in sufficient quantity for the entire duration of the experiment. Water was given ad libitum during the entire period of the experiment.

Acute oral toxicity studies

Acute oral toxicity test was done according to the OECD guidelines 425 [6, 7]. Albino rats of either sex were used. A total of five animals were used. After overnight fasting they received a single oral-dose (2000 mg/kg body weight) of ethanolic extract of seeds of Benincasa hispida. Then food was withheld for further 3-4 hrs. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. At the end of the study the animals were observed for general toxic signs, morphological behaviour and mortality.

Experimental Design

MES MODEL

Twenty five numbers of Albino rats were taken and divided into four groups, 5 rats in each and treated as follows:

- **Control** - received normal saline (10 ml/kg) i.p.
- **Test 1** - received EEBH 100 mg/kg i.p.
- **Test 2** - received EEBH 200 mg/kg i.p.
- **Test 3** - received EEBH 400 mg/kg i.p.
- **Standard drug** - received Phenytoin 25 mg/kg i.p [8].

Each animal was properly held and ear lobe electrodes were placed on the ear lobes and current of 150 mA was passed for 0.2 sec. Different stages of convulsions were noted down, along with the time spent by the animal in each phase. The same procedure was repeated with other animals of control group. The current was passed 30 minutes after intraperitoneal (i.p.) injection of normal saline. For test groups, the same procedure was repeated for three different doses (100, 200, 400 mg/kg) of EEBH.

Phenytoin was also injected i.p. to all the 5 rats. After 30 minutes, the animals were subjected to electroconvulsions. The reduction in time or abolition of tonic extensor phase of MES-convulsions was recorded for all the animals [9].

PTZ MODEL

It is reported to act through benzodiazepine receptor mechanisms in the brain.

Twenty five numbers of Albino rats were taken and divided into four groups, 5 rats in each and treated as follows:

1. **Control** - received normal saline (10ml/kg) i.p.
2. **Test 1** - received EEBH 100 mg/kg i.p.
3. **Test 2** - received EEBH 200 mg/kg i.p.
4. **Test 3** - received EEBH 400 mg/kg i.p.
5. **Standard drug** - received Diazepam 4 mg/kg i.p.

Normal saline, test drugs and Diazepam were injected intraperitoneally (i.p.) to the animals and after 30 minutes Pentylenetetrazol (60mg/kg) [10] was injected subcutaneously to these animals and the onset of action (indicated by Straub’s tail, jerky movements of whole body and convulsions) and severity of convulsions due to the drug were noted.

Either delay or complete abolition of convulsions in rats treated with diazepam and EEBH were noted.

STATISTICAL ANALYSIS

The statistical significance between groups was analyzed separately using One-way analysis of variance (ANOVA), followed by Dunnett’s multiple comparison test. The significance was expressed by 'p' values, as mentioned in the tables. 'p' values of <0.05 were considered as significant.

RESULTS

Acute toxicity test

There was no mortality and no sign/symptom of toxicity reported among the animals up to 2000 mg/kg. So, the LD50 was calculated more than 2000 mg/kg body weight.

Effect on extensor phase of convulsion in induced MES model

In MES model although the test groups did not abolish the extensor phase completely like phenytoin, but there was significant (<0.01) reduction in the duration of the tonic hind limb extensor phase in a dose dependent manner. All the rats under the study recovered without any deaths.

Table 1: Effect of EEBH on extensor phase of convulsion induced by Maximal electrical shock (MES model) in albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg)</th>
<th>No. of rats</th>
<th>Hind limb extension (seconds ± SEM)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>5</td>
<td>12.04 ± 0.49</td>
<td>R</td>
</tr>
<tr>
<td>Test 1</td>
<td>EEBH 100</td>
<td>5</td>
<td>5.54 ± 0.15*</td>
<td>R</td>
</tr>
<tr>
<td>Test 2</td>
<td>EEBH 200</td>
<td>5</td>
<td>4.68 ± 0.103*</td>
<td>R</td>
</tr>
<tr>
<td>Test 3</td>
<td>EEBH 400</td>
<td>5</td>
<td>3.92 ± 0.15*</td>
<td>R</td>
</tr>
<tr>
<td>Standard</td>
<td>Phenytoin 25</td>
<td>5</td>
<td>0 ± 0*</td>
<td>R</td>
</tr>
<tr>
<td>One way</td>
<td>Diazepam</td>
<td>5</td>
<td>4.16</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ANOVA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N=5 in each group; all the values were expressed in mean ± SEM. *P<0.01 is significant when compared with control (ANOVA followed by Dunnett’s multiple comparison test)

Effect on duration of convulsion in PTZ induced model

In PTZ model there was increase in the latency to the occurrence of convulsions in the three test groups (100, 200, 400 mg/kg) of EEBH as compared to the control group, but it did not abolish the convulsions completely as was seen with the standard group of diazepam (4 mg/kg) but the increase in the latency was highly significant (<0.01) in a dose dependent manner. All the animals recovered from the convulsions after the test and there were no deaths in any of the groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg)</th>
<th>No. of Convulsions</th>
<th>Average duration of Convulsion (sec ± SEM)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>5</td>
<td>1.6 ± 0.24</td>
<td>13.20 ± 0.86</td>
</tr>
<tr>
<td>Test 1</td>
<td>EEBH 100</td>
<td>5</td>
<td>1276 ± 47.35*</td>
<td>11.46 ± 0.44</td>
</tr>
<tr>
<td>Test 2</td>
<td>EEBH 200</td>
<td>5</td>
<td>1422 ± 42.75*</td>
<td>9.28 ± 0.36</td>
</tr>
<tr>
<td>Test 3</td>
<td>EEBH 400</td>
<td>5</td>
<td>1634 ± 37.67*</td>
<td>7.80 ± 0.44</td>
</tr>
<tr>
<td>Standard</td>
<td>Diazepam</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>One way</td>
<td></td>
<td>5</td>
<td>902.4</td>
<td>167.2</td>
</tr>
</tbody>
</table>

DISCUSSION

The present study was undertaken to evaluate the anticonvulsant activity of the ethanolic extract of seeds of *Benincasahispida* (EEBH) in experimental animal models.

The following experimental designs were selected.

1. Maximal Electroshock Seizure (MES) model
2. Pentylenetetrazol (PTZ) induced seizure model

The Maximal Electroshock Seizure (MES) induced convulsions for the screening of the anticonvulsant drug is the standard experimental model for evaluating a drug in experimental animals for its anticonvulsant property, which represent grand-mal epilepsy in human beings.

The anticonvulsant effect by the MES model is determined by the effect of the drug in the tonic hind-limb extensor phase of the convulsion, by either completely abolishing it or by reducing its duration. In the present study it was found that the duration of the extensor phase in the test groups were reduced.

There was complete abolition of extensor phase in the standard group of Phenytoin. Phenytoin sodium exerts antiepileptic effect by stabilization of neuronal membrane and thus prolongation of recovery of inactivated sodium channels. In high doses, Phenytoin can also block the calcium influx during depolarization [11].

From the above findings it can be said that the EEBH reduced the extensor phase of convulsion in a dose dependent manner. The seizure produced by the Pentylenetetrazol (PTZ) induced seizure model resemble to the absence or Petit mal seizure in human beings. A drug, which causes either delay or complete abolition of convulsions in the PTZ induced seizure model, is said to have got anticonvulsant activity. It is reported to act through benzodiazepine receptor mechanisms in the brain [12].

In the present study, latency to convulsion increases in the test groups with increasing dose. In the standard group of Diazepam (4 mg/kg) there was complete absence of convulsions within the specified time of 60 minutes of observation after subcutaneous Pentylenetetrazol injection. Also the average duration of convulsions was decreased in test groups with increasing dose.

CONCLUSION

Thus, from the above two seizure models of MES and PTZ, it can be concluded that EEBH has got anticonvulsant effect in a dose dependent manner.

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REFERENCES