EVALUATION OF ANTICONVULSANT ACTIVITY ON LEAVES OF ALCOHOLIC EXTRACT OF BACOPA MONNIERI LINN.

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ABSTRACT
Phyto medicines are used by the traditional healers, in the management, control and/or treatment of convulsions. Traditionally, the Bacopa monnieri leaves extract is widely used in India for the treatment of epilepsy. The present report is an investigation of anti-seizure activity of Bacopa monnieri (L), belonging to the Scrophulariaceae (family). The alcoholic extract of Bacopa monnieri was tested for anticonvulsant activity in albino rats, and Swiss mice using different convulsive models. The Pentylenetetrazole (PTZ) and the maximal electroshock seizure (MES) models were used for assessing the anticonvulsant effects of the leaves of alcoholic extract in mice and rats. The alcoholic extracts of Bacopa monnieri (orally) was administered in mice and rats at graded doses of 40 mg/kg and 80mg/kg p.o. The extracts suppress the duration of hind limb extension induced by MES in mice and also exhibited protector effect in PTZ–induced seizures in rats at the doses of 40 mg/kg and 80mg/kg p.o. Since the alcoholic extract if Bacopa monnieri (L) Delayed the occurrence of MES convulsions and PTZ induced Convulsions. The study in Pentylenetetrazole induced seizure test, onset of myoclonic spasm and clonic convulsions were delayed in test groups. It is concluded that, it interferes with gabaer mechanisms to exert their anticonvulsant effects. The alcoholic extract of leaves produced significant anticonvulsant activity for all the different models studied.

Keywords: Bacopa monnieri, anticonvulsant, antiseizure activity.

INTRODUCTION
A number of studies suggest that oxidative stress plays an important role in the etiology of epilepsy. Epilepsy is among the most prevalent of the serious neurological disorders, affecting from 0.5 to 1.0% of the world’s population¹. Seizures are the most common neurological disorder worldwide. The etiology of seizures comprises various pathophysiological conditions. Change of gamma aminobutyric acid (GABA) levels in the brain is highly correlated with seizure development in humans³. Epilepsy is mental neurological and behavioural disorders are common to all countries and cause immense suffering. People with these disorders are often subjected to social isolation of poor quality of life and increased mortality. Seizures are considered to be the result of an unbalance between excitatory and inhibitory systems, and it is believed that anticonvulsants exert their actions through enhancement of inhibitory mediated transmission, the reduction of excitatory mediated transmission or a combination of both⁴. Antiepileptic drugs exert their action by different mechanisms. They include an enhancement of the GABAₐ neurotransmission, effects on neuronal voltage-gated sodium and/or calcium channels⁵. The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose related and chronic toxicity, teratogenic effects and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy traditional systems of medicine are popular in developing countries and up to 80% of the population relies on traditional medicines or folk remedies for their primary health care need. The use of herbal medications by physicians in Europe and Asia is becoming more common and researchers are exploring the traditional remedies to find a suitable cure for this mind affecting diseases⁶. Bacopa monnieri (L.) family (Scrophulariaceae) is a small, creeping perennial herb, common in marshy places throughout in India. The drug is present throughout the entire dried plant, but principally concentrated in leaves and stems. It has a wide range of CNS activity, but is most popularly considered a memory enhancer⁷. Other pharmacological properties of the extracts include sedation, cardiotonic, vasoconstriction, anti-inflammatory activity, and astringency⁸. The entire plant is used in indigenous medicine as a nervous tonic. It has been referred in Ayurveda since centuries as a "Madhya Rasayan". Bacopa’s main chemical constituents include alkaloids, brahmine, herpestine and nicotine, saponin, monierin, hersaponin, bacoside A₁, A₂, A₃ and B and four saponin bacoegenin A₁ to A₄. It is known to be safe and well tolerated in humans⁹. The present study was undertaken to investigate the anticonvulsant activity of an alcoholic extract of dried leaves of Bacopa monnieri (BM), in different models of convulsions. Several animal models of convulsions have been developed to evaluate anti-seizure activity. The MES model is probably the best validated method for assessment of anti-epileptic drugs in generalised tonic-clonic and cortical seizures⁹. The PTZ induced seizures are similar to the symptoms observed in the absence seizures¹⁰. The objective of the present study was to investigate anticonvulsant activity of alcoholic extract of B.monnieri against the seizures induced by MES and PTZ.
MATERIALS AND METHODS

Collection and authentication of plant material

The leaves of *Bacopa monnieri* (L.) were procured from local market of Mumbai and it was authenticated at Nicholus Piramal Pvt Ltd. India. The leaves were shade dried and powdered and stored in air tight container till the use.

Preparation of extracts

Air-dried leaves of *Bacopa monnieri* (L.) were defatted with Pet. ether (60-80°C) by using Soxhlet extraction apparatus for 48 hours. All the solvents were removed from the Pet. ether (60-80°C) extracts under reduced pressure. All the defatted leaves were air dried and then used individually for alcoholic extraction. Individual alcoholic extraction was carried out by using Soxhlet extraction apparatus for 48 hours. All the extracts were removed by using reduced pressure and a crude solid mass was dried and stored in desiccators for further studies. The preliminary phytochemical analysis was undertaken to detect the presence of major phytoconstituents in the extract. All the extracts were freeze dried and stored in air tight container.

Preliminary phytochemical screening

The preliminary phytochemical investigation was carried out with alcoholic extract of *Bacopa monnieri* for qualitative identification of phytochemical constituents. Phytochemical tests were carried out by standard methods13. Freshly prepared alcoholic extract of the leaves of *Bacopa monnieri* was subjected to phytochemical screening tests for the detection of various constituents. All the chemicals and reagents were used of analytical grade.

Experimental animals

Healthy Swiss mice of either sex, weighing about 25-30 g and Wistar rats of either sex, weighing about 150-200 g were used in experiments. They were housed in polypropylene cages and maintained under standard laboratory conditions with a (12 hours light / dark cycle; 25°C) and had free access to standard rat feed and water ad libitum. They were acclimatized to laboratory conditions for 10 days before starting the experiment14. The experimental protocol was approved by the institutional animal ethical committee (ref. no:837/CPCSEA 29th Dec.2009).

Drugs and Chemicals

Pentylentetrazole (PTZ; Sigma Chemical Co. USA), and 5, 5-diphenylhydantoin sodium salt (Phenytoin, PHNY; Sigma Chemical Co. USA) were Purchased.

Experimental Design

Study Design and drug treatment

The animals were randomly allocated into four groups of six each. Rats and mice were used for the PTZ and MES experimental epileptic models respectively. Group I received 10ml/kg of 1% NaCMC p.o. and served as control. Group-II received phenytoin sodium (25 mg/kg, orally) as standard controls in MES method and PTZ induced seizures method respectively. Group- III and IV were administered two graded doses of test drug i.e. 40mg/kg and 80 mg/kg, respectively, p.o. in both the experimental models. Percentage protection of animals was recorded as percentage reduction in duration of seizures by considering control group as 100 %. The observations were compared with control (Group-I).

Experimental Epileptic Models

Two methods were employed to evaluate anticonvulsant activity in Wistar albino rats and Swiss albino mice.

a) Electrically- induced Seizures in albino mice

b) Pentylentetrazole (PTZ) induced seizures in rats

**Electrically- induced Seizures in albino mice**

Maximal electroshock induced seizures (MES) in mice was used to evaluate the anticonvulsant activity of alcoholic extract of *Bacopa monnieri*. Twenty-four male mice were allotted into four groups of six animals each and were treated as follows.

Group I (control): 1 % Na CMC (10 ml/kg p.o.).

Group II: Phenytoin sodium (25 mg/kg i.p.).

Groups III: alcohol extract (40 mg/kg p.o.)

Group IV: alcoholic extract (80 mg/kg p.o.) were administered. After a pre-treatment time of 30 minutes, the seizures were induced by maximal electroshock in albino mice with the help of electroconvulsio meter by passing current of 50 mA for 0.2 second using corneal electrodes. A drop of electrolyte solution 0.9% sodium chloride with lignocaine was applied to the corneal electrodes, which ensures better contact and the mortality rate to zero. This current intensity elicited complete tonic extension of the hind limbs in control mice. For recording various parameters, mice were placed in a clear rectangular polypropylene cage with an open top, permitting full view of the animal motor responses to seizure the pilot study of various phases of convulsions, like tonic flexion, extension, stupor and mortality due to convulsions were observed. The animals were observed for 2 minutes. The onset of tonic hind limb extension and number of animals protected was recorded11.

**Pentylentetrazole (PTZ) induced seizures in rats**

Four groups, each containing six rats were used to test for the effect of alcoholic extract of *Bacopa monnieri* on PTZ-induced seizures. They were treated as follows;

Group I (control): 1% NaCMC (10ml/kg p.o.).

Group II: Phenytoin sodium (25 mg/kg i.p.).

Groups III: alcohol extract (40 mg/kg p.o.)

Group IV: alcoholic extract (80 mg/kg p.o.) were administered orally. PTZ at the dose of 90mg/kg (minimal
dose is needed to induced convulsions) was injected i.p. to induced tonic-clonic convulsions in rats. The test animals group III and group IV (n=6) received 40mg/kg and 80mg/ kg of alcohol extract orally as a suspension prepared in 1% sodium CMC solution and standard group received phentoyin sodium (25mg/kg) orally in the form of suspension. After a pre-treatment time of 60 minutes, PTZ (90 mg/kg i.p.) was administered to the four groups of animals. The onset of convulsions, number of animals that convulsed and number of animals that were protected from clonic convulsions were recorded.

Statistical analysis

The data are expressed as mean ± S.E.M. The data were statistically analysed using One-Way Analysis of Variance (ANOVA), followed by Dennett’s Multiple Comparison Test. Values of p < 0.05 were considered significant.

RESULTS

Preliminary phytochemical studies

The phytochemical screening of the alcoholic extract of *Bacopa monnieri* revealed the presence of alkaloids, glycocides, carbohydrates, tannins, phenolic compounds, proteins, amino acids, saponins, flavonoids and bacosides. The alcoholic extract of leaves of *Bacopa monnieri* were administered orally to different groups of rats and mice with two different dose levels (40 mg/kg and 80 mg/kg).

Effect on MES induced convulsions on mice

In MES model, different doses of alcoholic extract of *Bacopa monnieri* i.e.40mg/kg and 80 mg/kg) were administered daily once for 7days. It was found that lower and higher dose had produced a significant anti-convulsant effect in above parameters. The alcoholic extract of *Bacopa monnieri* exhibited a dose dependent reduction in various phases of epileptic seizure on comparison with the control group. There was also a significant reduction in the time required for the righting reflex (recovery) in the extract treated groups compared with control group (Table 1). Phentoyin and different doses of *Bacopa monnieri* decreased Hind Limb Tonic Extension as compare to control group. The standard drug Phentoyin showed 100% protection in animals against MES. It abolished the extensor phase almost completely.

PTZ induced convulsions

40 mg/kg and 80mg/kg of alcoholic extract of *Bacopa monnieri* exhibited a significant anticonvulsant effect by increasing latency, onset of clonic convulsions and decreases onset of tonic seizures. After 30 min of interval, 67 % and 84 % of animals survived at a dose of 40 mg/kg and 80mg/kg respectively. Intraperitoneal administration of PTZ induced tonic-clonic convulsions with 100% mortality in the control group. It was found that lower and higher dose of *Bacopa monnieri* had produced a significant delayed the onset of clonus as compared to control group. Standard drug Phentoyin sodium had exhibited significantly anticonvulsant activity and gave 100% protection. PTZ induced tonic-clonic convulsions with100% mortality in control group. The alcoholic extract of *Bacopa monnieri* (40 mg/kg p.o.) and (80 mg/kg p.o.) (Significantly (p < 0.05) increased the onset of clonic convulsion in rats and decreases onset of tonic convulsions dose dependently in rats showed dose dependant activity. All the results are incorporated in table 2.

**Table 1: Effect of alcoholic extract of Bacopa monnieri on MES induced convulsions model**

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Bacopa monnieri results of MES Induced seizure (sec /min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flexion</td>
</tr>
<tr>
<td>Control</td>
<td>10.29</td>
</tr>
<tr>
<td>Standard (Phenytoin)</td>
<td>3.28</td>
</tr>
<tr>
<td>(40 mg/kg)+PTZ</td>
<td>8.19</td>
</tr>
<tr>
<td>(80 mg/kg)+PTZ</td>
<td>5.28</td>
</tr>
</tbody>
</table>

**Figure 1:** Graph of Tonic extension phase of MES Induced Convulsion
In the present study, Pentylenetetrazole (PTZ) and MES were shown to induce seizures in all the rats and mice. Pentylenetetrazole is an antagonist of Gama Amino Butyric Acid (GABA) at GABAA receptors which has been widely implicated in epilepsy. Furthermore, drugs which protect animals against the generalized clonic seizures induced by PTZ are effective in protection and management of petitmial epilepsy. MES induced seizure can be prevented either by drugs that inhibit voltage gated sodium channel such as phenytoin or by drugs that inhibit glutaminergic excitation mediated by NMDA receptors. In addition, drugs that are effective in protecting animals against the tonic clonic extensor spasm induced by MES are effective in the management of and/or protecting against grandmal epilepsy. Phytocchemical screening of the plant showed that the plant contains alkaloids, flavonoids, sterols, glycosides and saponins, bacosoides, to which the anticonvulsant activity of the plant extracts may be attributed. There are a number of synthetic anticonvulsant drugs available in the market for use in the management, control and/or treatment of individuals with epilepsy. However, most of these synthetic drugs are not only inaccessible and unaffordable, but they possess many toxic effects. It is therefore necessary to look inward for the development of cheap, effective and safe anticonvulsant agents from plants and other natural resources. This implies that Bacopa monnieri may be effective as an anticonvulsant medicinal plant and its anticonvulsant effect may involve gabergic inhibitory and glutaminergic excitatory mechanisms or inhibition of the voltage gated sodium channel. In Pentylenetetrazole induced seizure, test parameters like latency, onset of tonic convulsions, clonic convulsions and % protection were observed in the test groups showed strong antiepileptic effect. The death rate was 100% in Group I. Group II(25 mg/kg) of phenytoin, prevents tonic-clonic convulsions and offered 100% protection. 40 mg/kg and 80mg/kg of alcoholic extract of Bacopa monnieri exhibited a significant anticonvulsant effect by increasing onset of clonic convulsion and by decreasing onset of tonic seizures. The present study reveals that the anticonvulsant effect of alcoholic extract of Bacopa monnieri using MES and PTZ induced convulsions in mice and rats models and it seems that this effect increased dose dependently. Seizures and mortality protection almost increased dose dependently. However, the potentiation of anticonvulsant action of Phenyltoin in the present study, suggests that it is useful in the treatment of generalised tonic clonic seizures. It is well known fact that drugs which provide protection against seizures induced by maximal electroshock method are generally effective against generalised tonic clonic seizures. Bacopa monnieri showed significant protection against pentylenetetrazole induced convulsions. The seizures induced by chemoconvulsant (pentylenetetrazole), is useful in identifying drugs that are effective against absence seizures. Therefore, it is likely that Bacopa monnieri might possible by producing anticonvulsant action by increasing level of (GABA), an inhibitory neurotransmitter in the central nervous system. Phenyltoin at 25mg/kg body weight is protected the animal from PTZ induced convulsion with very less clonic convulsions occurring within the period of observation. Bacopa monnieri at 40 mg and 80 mg/Kg body weight gave 67 and 84% protection for PTZ induced convulsions. The probable protection induced models is mediated either by first one is the separation of Ca2+ conductance across low threshold T-type channels or second enhancement of GABAa receptor mediated Cl- conductance. B. monnieri at 40 mg/kg, 80 mg/kg body weight produce significant effect in the phases of extension, minute and recovery compare to the control.
The dose of *B. monnieri* did not stop hind limb extension but decreased it by nearly half the extension time in control. This indicates its ability to prevent the spread of seizure in the CNS. The MES model has served to identify antiepileptic drugs that are functionally similar to phenytoin and most of these compounds display the same ability to inactivate voltage dependent Na+ channels in a used dependent fashion. Such compounds have been shown to suppress sustained repetitive firing in cultured neurons. Thus *B. monnieri* is expected to have a similar type of mechanism, and may be effective against partial and secondary generalized seizures.

### CONCLUSION

In conclusion, *Bacopa monnieri* extracts may have potential anticonvulsant activity which may be due to the presence of certain active phytoconstituents. The anticonvulsant activity of *Bacopa monnieri* may involve gabergic transmission and glutaminergic transmission or sodium channel blockage. Further studies are however needed to isolate the active principle(s) of the plant and to enlighten the mechanism underlying its anticonvulsant effect.

### REFERENCES


