INTRODUCTION

Epilepsy is a neurological disorder awaiting safer drugs with improved anticonvulsant and anti-epileptogenic effectiveness, as currently available drugs fail to provide adequate control of epileptic seizures in about one-third of patients and do not prevent progressive epileptogenic changes which are not well understood. This fact has stimulated a considerable number of researches of new antiepileptic drugs. In this regard, medicinal plants have been an important source to the development of drugs with this biological activity. The ongoing therapies and the most widely used anti-epileptic drugs are linked with several issues such as dose related chronic toxicity, side effects and teratogenic effects. It is also estimated that nearly 30% of the population suffering from epilepsy continue to have seizures with current anti-epileptic drug therapy. Occasionally it has been found that nearly 80% of the population in developing countries depends on traditional system of medicines for their primary health care. This has garnered a lot of attention being focused on traditionally used herbal drugs and formulations which are safe for prolonged usage of herbs for management of the disorder.

There are several herbs that have been proven individually for its effect on seizures.

Nardostachys jatamansi (NJ) (Valerianaceae) has been used for management of epilepsy in Ayurveda. It helps to promote physical and mental health, augment resistance of the body against disease, and has shown potent antioxidant activity. It also shows a marked tranquilizing activity, as well as, hypotensive, hypolipidemic anti-ischemic, anti-arrhythmic, hepatoprotective, anticonvulsant, and neuroprotective activities. Bacopa monnieri Linn. (BM) belonging to family Scrophulariaceae is an important constituent of several polyherbal formulations like Saraswatari, BrahmiGhrita and Mentat which are widely used for various neurocognitive. Studies have shown that it exhibits its anti convulsant properties via GABA receptors. Annona squamosa Linn. (AS) belonging to family Annonaceae, widely known as custard apple have been used by few traditional healers in case of epileptic attacks. It is reported to have varied therapeutic activities like anti diabetic, anti hyperlipidemic, expectorant, anti-cancer and insecticidal properties. Ethanolic extract of its leaves is reported for its anti convulsant activity. Thus a polyherbal formulation consisting of Jatamansi, Sitaphal leaves and Brahmi was prepared to evaluate its efficacy on seizures embarking additive effect of these drugs.
Along with this combination of extracts, a marketed formulation called Epi caps was also evaluated for its use as an anti-convulsant. It has been prescribed by local Ayurvedic and Unani practitioners for epilepsy. It has varied combination of dried extracts of Aloe vera, Basella alba, Centella asiatica, and Evolvulus alsinoides. Thus, the efficacy of this formulation was also determined for management of epilepsy and a comparison of this formulation along with the combination of extracts of AS, NJ and BM was evaluated. The combination of extracts is referred to as Test 1 (100mg/kg), Test 2 (200mg/kg).

The GABA hypothesis of epilepsy implies that a reduction of GABAnergic inhibition results in epilepsy whereas an enhancement of GABAnergic inhibition results in anti-epileptic effect. Convulsant agents that block synaptic GABA mediated inhibition amplify the dendritic spike generating mechanism that involves calcium influx. Synaptic inputs are thought to trigger and synchronize this process throughout a population of cells which then might result in epileptic fits. Thus estimation of GABA is of prime importance when evaluating anti-convulsant properties of a drug.

MATERIALS AND METHODS

Drugs and Chemicals: Pentylenetetrazole was obtained from SRL Chemicals. Bacopa monnieri extract was received as a gift sample from Sami Labs, Bangalore. Phenytoin was also received as a gift sample from Crest Healthcare, Vadodara. Epi caps was procured from a local ayurvedic store. GABA was procured from Sigma Aldrich.

Animals: Albino Swiss mice weighing 22-28g were procured from Bharat Serums Pvt Ltd., Thane and housed in polypropylene cages at 22-25°C under a 12 hour light/dark cycle. The mice were fed with standard pellets and water ad libitum. The animals were housed under these conditions and allowed for acclimatization for 7 days. The experiments were performed after obtaining approval from Institutional Animal Ethics Committee (CPCSEA/IAEC/BNCP/P-17/2014). Animals were divided into 6 groups for the study. Each group consisted of 6 animals. Group A: Control group (0.5% CMC). Group B: Phenytoin (20mg/kg), Group C Test 1 (100mg/kg), Group D Test 2 (200mg/kg), and Group E Epi caps (500mg/capsule).

Extraction procedure and preparation of drug: The leaves of AS were collected from Vasai, Palghar district, Maharashtra, India in the month of May and were shade dried. Jatamansi dried rhizomes were obtained from Pharmacognosy Laboratory of Dr. Bhanuben Nanavati College of Pharmacy. The leaves and rhizomes were identified and authenticated at Department of Botany, Mithibai College of Arts, Chauhan Institute of Science and AmrutbenJivanlal College of Commerce and Economics, Mumbai for reference Voucher MIT 009 (AS) and MIT 0069 (NJ). The dried plant materials were powdered and soxhlet extraction using 70% ethanol and 95% ethanol was performed for AS leaves and NJ roots respectively. After complete extraction, the extracts were dried using rotary vacuum evaporator. A suspension of these extracts was prepared in 0.5% CMC in the ratio of 1:1:1. The dose for administration was calculated and expressed as mg/kg of body weight.

Pentylenetetrazole (PTZ) Model to determine anticonvulsant potency: Phenytoin, Test 1 and Test 2, Epi caps were administered orally to the specific group of mice. 60 mins after oral administration of these test compounds for a period of 14 days, 60mg/kg of PTZ (in saline) was injected intraperitoneally. Each mouse was placed individually in plastic cages to record observations. The onset time and duration of each seizure was noted for all the groups. The animals were considered to be protected against seizures when no convolution or mortality occurred within 30 mins. Animals were divided into 6 groups for the study. Each group consisted of 6 animals. Group A: Control group (0.5% CMC). Group B: Phenytoin (20mg/kg), Group C Test 1 (100mg/kg), Group D Test 2 (200mg/kg), and Group E Epi caps (500mg/capsule).

Maximal Electroshock (MES) Model to determine anticonvulsant potency: MES model is preferred over other complex models as it facilitates screening of anti convulsants drugs and its development in relatively short span of time and for high number of test compounds. MES test is started after 60 mins of oral administration of Phenytoin, Test 1, Test 2 and Epi caps. Ear clip electrodes were used to deliver stimuli with the intensity of 12 mA, 50 Hz for 0.2 s to each group of mice. The duration of each of the following phase was recorded in observations: Tonic Flexion, Tonic Extension, Clonus and Stupor. The suppression of each of the phases indicates the efficacy of anti-epileptics. The control group characteristically showed these phases.

Estimation of GABA

The overnight fasted albino mice (n= 6) were euthanized, brains were dissected out immediately and washed with ice cold normal saline to remove blood/blood clots and adhering tissues, if any. Brains were dissected over an ice-cold platform and weighed. To determine total GABA content in brain,
whole brain was homogenized in methanol (for every 10 mg tissue 200 μL) using a manual tissue homogenizer. The homogenates were transferred to polypropylene tubes and centrifuged at 4500 rpm for 20 min at room temperature. The clear supernatant was then transferred into microcentrifuge tubes and filtered through 0.45-μ membrane filter (Millipore) to obtain sample solutions. This sample is stored in deep freezer (−80°C) until its estimation. Preparation of sample: The clear supernatant was allowed to evaporate to dryness. The dried material was solubilised in 0.5ml distilled water to which 2ml of Chloroform was added followed by vigorous shaking. This was centrifuged for 2 minutes at 3000g which led to separation into three layers. The topmost water layer was used for chromatography.

**Chromatography:** The qualitative separation of GABA from the mice brain supernatant was performed by 2-D paper chromatography using Whatman No 1 filter paper, whereas the quantitative determination was done using UV spectrophotometer. GABA standard solution was prepared by dissolving pure GABA in distilled water (1mg/ml). 10μl of the topmost layer obtained after centrifugation was spotted on the whatman paper using a micropipette. This was allowed to dry and placed in a TLC chamber containing the mobile phase. The mobile phase used was n-butanol: glacial acetic acid: water in the ratio of 4:1:1. After complete run of the mobile phase, the paper was dried at room temperature and then allowed to run in other direction (90° turned). This too was dried at room temperature and dipped in 0.2% Ninhydrin in acetone. The amino acids were developed on the paper by heating it at 40°C for 15 mins in the hot air oven. The colored chromatogram spots corresponding to GABA standard were cut and put separately in test tubes containing 75% ethanol with 0.05mg/ml copper sulfate. Quantitative determination: this was performed using UV spectrophotometer at 543nm.

**RESULTS**

**Statistical Analysis:** The data was analyzed using One way ANOVA followed by Tukey Post hoc test using Graph Pad In Stat 3.0 software. The time of each seizure is described in all the tables below as Mean ± SEM in seconds.

**Pentylenetetrazole model:** Pentylenetetrazole (60mg/kg) produced generalized clonic and hind limb tonic seizures in the animals. Phenytoin (20mg/kg) offered highly significant protection against seizures by causing an immense delay in the onset time as well decreasing the duration of each seizure by several manifolds when compared with control. Test 1, Test 2 and Epi caps exhibited significant protection against onset time of seizures. Seizure threshold also increased to a great extent. Thus, highly significant reduction in the mean seizure duration for Test 1, Test 2 and Epi caps in comparison to negative control was observed.

**Table 1 Effect of Phenytoin, Test 1, Test 2 and Epi caps on PTZ model**

<table>
<thead>
<tr>
<th>Group</th>
<th>Onset Time (secs)</th>
<th>Duration (secs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>301.67 ± 11.38</td>
<td>7.83 ± 1.25</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>191.83 ± 33.33***</td>
<td>24.36 **</td>
</tr>
<tr>
<td>Test 1</td>
<td>302.16 ± 19.14*</td>
<td>14.17 ± 7.83***</td>
</tr>
<tr>
<td>Test 2</td>
<td>317.5 ± 24.62**</td>
<td>129.83 ± 11.43**</td>
</tr>
<tr>
<td>Epi caps</td>
<td>337.16 ± 24.36**</td>
<td>94.83 ± 6.51***</td>
</tr>
</tbody>
</table>

In the table above, * indicates significance in comparison to control. ** P < 0.01, *** P < 0.001.

**Maximal Electroshock Model:** The combination of extracts of Test 1, Test 2 plus Epi caps showed significant reduction in tonic hindlimb flexion and extension, as well as in clonus duration. The Phenytoin group exhibited 83.33% protection against MES induced seizures. However, highly significant improvement was observed in stupor duration.

**Fig 1** Effect of Phenytoin, Test 1, Test 2 and Epi caps on mean seizure onset time PTZ model:

**Fig 2** Effect of Phenytoin, Test 1, Test 2 and Epi caps on Mean seizure duration in PTZ model:
Anticonvulsant Activity And Estimation of Gaba of Combination of Annona Squamosa, Nardostachys Jatamansi And Bacopa Monnieri Formulation And Epi Caps In Mice

The equation obtained from calibration curve of GABA standard was used to calculate the concentration of GABA in brain tissues of all the groups. The results exhibit that Phenytoin, Test 1, Test 2 and Epi cap showed highly significant changes (p<0.001) when compared to control.

### DISCUSSION

Epilepsy continues to be a major public health concern worldwide. Several times in patients suffering from refractory partial or generalized tonic clonic seizures, polypharmacy is often recommended for nearly 30% of the patients. The available anti-epileptic drugs treat symptoms rather than the disease itself. This has garnered interest in traditional approaches which includes herbal drugs and formulation. These are regarded as safe for extended usage for management of epilepsy. Similarly, the available drugs for epilepsy suggest that there is no specific satisfactory to meet the needs of the therapy. It is known in the case of epilepsy that the major cause is

### Table 2 Effect of Phenytoin, Test 1, Test 2 and Epi caps on MES model

<table>
<thead>
<tr>
<th>Group</th>
<th>Tonic Hindlimb Flexion (secs)</th>
<th>Tonic Hindlimb Extension (secs)</th>
<th>Clonus Duration (secs)</th>
<th>Stupor duration (secs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.5 ± 1.38</td>
<td>11.33 ± 1.89</td>
<td>26.16 ± 3.30</td>
<td>221.83 ± 26</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>0.33 ± 0.84 ***</td>
<td>0.67 ± 0.77</td>
<td>1.33 ± 1.33</td>
<td>17.83 ± 17.83</td>
</tr>
<tr>
<td>Test 1</td>
<td>7.5 ± 0.99 ***</td>
<td>5.5 ± 0.71</td>
<td>13.67 ± 2.23</td>
<td>104.83 ± 11.30</td>
</tr>
<tr>
<td>Test 2</td>
<td>7.16 ± 1.27 **</td>
<td>6.8 ± 0.99</td>
<td>12.17 ± 1.95</td>
<td>95.67 ± 6.75</td>
</tr>
<tr>
<td>Epi caps</td>
<td>6.67 ± 1.11 ***</td>
<td>6 ± 0.89</td>
<td>12.17 ± 1.95</td>
<td>93.67 ± 6.75</td>
</tr>
</tbody>
</table>

Table 3 Concentration of GABA in samples expressed as Mean ± SEM

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (µg/g of wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>262 ± 0.609</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>464.87 ± 11.13</td>
</tr>
<tr>
<td>Test 1</td>
<td>343.02 ± 3.71</td>
</tr>
<tr>
<td>Test 2</td>
<td>360.08 ± 8.21</td>
</tr>
<tr>
<td>Epi cap</td>
<td>354.99 ± 7.16</td>
</tr>
</tbody>
</table>

The equation obtained from calibration curve of GABA standard was used to calculate the concentration of GABA in brain tissues of all the groups. The results exhibit that Phenytoin, Test 1, Test 2 and Epi cap showed highly significant changes (p<0.001) when compared to control.

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CONCLUSION

The results evidently suggest that the combination of extracts of Anonna squamosa, Bacopa monnieri and Nardostachys jatamansi as well as Epi caps control the extent and severity of seizures in PTZ and MES models. Since the extracts and Epi caps are found to be showing protection against seizures, it can be assumed that they act on both the mechanisms, i.e. altering the functioning of voltage gated channels and glutaminergic excitability as well as acting as an agonist on the GABA- receptor. However, further studies are required to determine and validate the exact mechanism of action and the active constituents of these plants responsible for showing action.

Acknowledgement

The author is thankful to Crest Healthcare, Vadodara and Sami Labs, Bangalore for providing free gift samples. The authors are indebted to Dr. BhanubenNanavati College of Pharmacy for providing the facilities required to carry out this study.

References

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