Anticonvulsant activity of *Nigella sativa*, *Aegle marmelos*, and *Benincasa hispida* in Pentylenetetrazole induced seizure in Swiss albino mice

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ABSTRACT

Aims and Objectives: To test anticonvulsant properties of three medicinal plants with pentylenetetrazole induced seizure test in Swiss albino mice. Materials and Methods: Aqueous extract of *Aegle marmelos* leaves (AmAE), Ethanolic extract of *Nigella sativa* seeds (NsEE) and crude extract of *Benincasa hispida* fruit juice (BhE) were prepared. Qualitative phytochemical analysis of the test extracts were done with Preliminary chemical reaction tests, and Liquid chromatography–mass spectrometry (LC/MS). Male Swiss albino mice, 3-4 months of age, weighing 25-30g were used in the study. Extracts and vehicle were administered orally 1 hour prior to inducing convulsions with Pentylenetetrazole (PTZ) induced seizure test [60mg/kg/i.p]. Onset of first jerky movement, onset of Straub’s tail, onset of clonic convulsions, onset of tonic flexion, onset of Hind limb tonic extension (HLTE) and reduction in mortality were measured. Animals were treated with extracts at a dose of 900mg/kg orally. The results were compared with control group and test for analysis of variance and significance was done (ANOVA, Dunnett’s test). Results: Aqueous extract of *Aegle marmelos* leaves and Ethanolic extract of *Nigella sativa* seeds possess anticonvulsant property in Pentylenetetrazole (PTZ) induced seizure model, Crude extract of *Benincasa hispida* fruits did not show statistically significant anticonvulsant property. Conclusions: *Aegle marmelos* leaf and *Nigella sativa* seed possess anticonvulsant properties in pentylenetetrazole induced seizures, this is in accordance with many other published reports on the same herbs with different animal models and indicates effectiveness in human absence seizure patients. Key words: Pentylenetetrazole (PTZ) induced seizure test, Anticonvulsant, *Aegle marmelos*, *Nigella sativa*, *Benincasa hispida*, Sodium valproate, Ethosuximide, Swiss albino mice

INTRODUCTION

Indian Vedic literature, primarily Ayurvedic scripts have mentioned about many herbal preparations and are being used in the treatment of epilepsy, but most of them are not scientifically validated. Many organizations like WHO and ICMR have expressed the urgent need for validation of indigenous medicines as these could be potential alternative to fill in the treatment gap due to lack of sufficient supply and further which may help in the discovery of novel active principles and targets. In this research study we have selected three medicinal plant parts *Nigella sativa* seeds, *Aegle marmelos* leaves and *Benincasa hispida* fruits, for they are cited in ethnomedicine to possess antiepileptic activities but have no scientific validation. Hence we felt it would be worthwhile to scientifically study and validate their antiepileptic properties.

*Aegle marmelos* (Linn) correa, commonly known as bael (or bel) is a moderate-sized, slender and aromatic tree.
It is indigenous to India and is abundantly found in the Himalayan tract, Bengal, Central and South India. It is extensively planted near Hindu temples for its wood and leaves which are generally used for worship. Leaves of this plant are used along with many other plant materials for the treatment of continuous fever; convulsions; constipation and watery diarrhoea.1,2 Nomenclature:1 Botanical name: Aegle marmelos (L.) Corr., Syn.: Crataeva Marmelos Linn., Family: Rutaceae, Vernacular names: English: Wood apple, Bael tree., Sanskrit: Bilwa, Shirphal., Hindi: Shirphal, Bel. Pharmacological activities of Aegle marmelos leaves: Antifungal Activity,3-5 Anti-inflammatory activity,6 Anti-diabetic activity,7,9 Anti-diarrhoeal activity,10,11 Analgesic activity,12,14 Radioprotective effect,15 Anxiolytic and antidepressant effects.14,15 Studies on Aegle marmelos leaves aqueous extract report Pb (II) ion adsorption from aqueous solution by leaves,18 contraceptive efficacy in male albino rats,17 anti-pyretic and anti-diarrhoeal,18 Anti-Ulcer,19 Anxiolytic20 and Abortiflificent effects in albino rats.21

Benincasa hispida22-24
The Sanskrit word kusmanda literally means that, fruit, which does not contain heat at all. Benincasa hispida (Thunb.) Cogn (Cucurbitaceae) fruit is widely used as a vegetable in India and other tropical countries. The fruit B. hispida is an important ingredient of "Kushmandalehyam" (Ayurvedic medicine) which is widely used in epilepsy and other nervous disorders,25 and as anorectic.26 Some of the important isolated constituents of B. hispida were triterpenes, sterols and glycoside and volatile oils.27 Nomenclature: Benincasa hispida, (synonym – benincasa cerifera), Family - Cucurbitaceae, Genus - Benincasa. Activities reported from pharmacological studies are Gastroprotective,8,29 antinoiciceptive,29 anticomulsive.30

Nigella sativa
Nigella sativa commonly known as karayal is an annual flowering plant, native to southwest Asia. Seeds and their oil have a long history of folklore usage in various systems of medicines and are used in food as well as medicine. Leaves of Aegle marmelos Corr with the fruits of Nigella sativa and Piper nigrum could be used to treat convulsions.31,32 Nomenclature: Family: Ranunculaceae, Genus: Nigella, Species: sativa, Vernacular names: Nigella sativa commonly known as karayal, English: Small Fennel, Black Cumin Sanskrit: Kalonji, Kalajira, Kalajaji, Mugrela, Upakuncika. Pharmacological actions: Antimicrobial activity,33-35 Hepatoprotective activity,36-38 Hypolipidemic activity,37,38 Anticanceractivity,38-40 Anti-diabetic and effects in metabolic syndrome,41 Gastroprotective activity.42,43

MATERIALS AND METHODS

Ethical approval
The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC Reg.no 347/CPCSEA), Yenepoya University and care of laboratory animals were taken as per CPCSEA guidelines.

Collection of the plant materials
Green leaves of Aegle marmelos were collected locally from Someshwar, Mangalore, Karnataka, Nigella sativa seeds and Benincasa hispida fruits were procured from Central Market, Mangalore.

Authentication
The test herbs were authenticated by Taxonomist, Prof. Krishnakumar, Department of Applied Botany, Mangalore University, Karnataka, India. One voucher specimen was submitted to Department of Pharmacology, Yenepoya Medical College Mangalore, Karnataka, India.

Extraction
The collected leaves of A.marmelos were washed, air-dried, powdered and extracted with Soxhlet apparatus, in portions of 200g, with distilled water at 90°C temperature for 3 days. After exhaustive extraction, the collected aqueous extract was dried on a water bath at 60°C and kept under refrigeration. The final yield of the extraction was 12.5%.

Nigella sativa shade dried seeds were powdered and extracted with Soxhlet apparatus, in portions of 200g, with 99% ethanol at 60°C temperature for 3 days. After exhaustive extraction, the collected ethanolic extract was dried on a water bath at 50°C and kept under refrigeration. The final yield of the extraction was 25%.

Benincasa hispida fresh fruit pulp juice was filtered and dried on a water bath at 60°C.

Preparation of Test drugs and extracts: 0.2 % Dimethyl sulfoxide (DMSO) was used uniformly for all the extracts and drugs as vehicle. For control group plain 0.2% DMSO was selected.

Qualitative phytochemical analysis of the test extracts were done with Preliminary chemical reaction tests and Liquid chromatography–mass spectrometry (LC/MS).

Experimental animals
Male Swiss albino mice aged 3-4 months, weighing 25-30g were used in the study. Experimental Animals were housed (Animal house, Yenepoya University, Reg.no 347/CPCSEA) in polypropylene cages and maintained at temperature (25 ±2°C) and light (light period, 06.00–
18.00) in a controlled room with relative humidity of 50–55%. For the automated illumination control, a timer device was designed and installed in the animal house. Food and water were provided ad libitum. Experiments were carried out between 15:00 and 19:00 h.

**Grouping and dosage**

50 male Swiss albino mice were grouped into five groups, on control and three test groups (Aegle marmelos 900mg/kg, Benincasa hispida 900mg/kg, Nigella sativa 900mg/kg. Two standard groups (Sodium valproate 100mg/kg, Ethosuximide 125mg/kg). Route of administration was oral for all the treatment groups.

**Procedure**

1. Mice were brought to the laboratory and allowed them to acclimatize to the laboratory for at least 30 min before test.
2. Test extracts were diluted with 0.2 % Dimethyl sulfoxide.
3. Mice were selected, weighed, and unique identifying mark on each animal was placed. Mice were returned to their holding cages to await dosing. Care was taken not to mix mice that were housed in different home cages to avoid untoward behavioural reactions (e.g., aggression).
4. 0.5ml Vehicle (0.2 % Dimethyl sulfoxide) was administered to each mouse of the control group. Administration was staggered (1 to 3 min intervals) to maintain the same time between compound administration and testing for each animal.
5. Mice were returned to their holding cages as soon as they were treated with the test compound or vehicle.
6. After the post-treatment time (One hour) has elapsed, by grasping the mouse firmly by the nape of the neck, pentylenetetrazole at 60mg/kg/body weight intraperitoneally (i.p) was administered.
7. Following PTZ injection, mouse was immediately placed into a separate observation cage to monitor the course of the seizure.
8. The blockade of clonic convulsions or an increase in convulsion latency (of different phases) was recorded.

**Analysis of the result**

1. Latency for onset of different phases of convulsion (jerky movements, Straub’s tail, clonus, tonic flexion and tonic hind limb extension) and mortality per group were compared with control group using Dunnett’s test after ANOVA.

**RESULTS**

Preliminary phytochemical analysis indicates the presence of steroids, flavonoids, tannins, saponins, glycosides, carbohydrates, alkaloids in aqueous extract of Aegle marmelos leaves (AmAE). In ethanolic extract of Nigella sativa seeds (NsEE) glycosides, carbohydrates, alkaloids were present. In Crude extract of Benincasa hispida fruits (BhE) terpenoids, steroids, carbohydrates, alkaloids were present. (Table 1).

Liquid chromatography–mass spectrometry (LC/MS) analysis and matching of the mass charge ratio (m/z, Da) with existing databases identified the presence of Lupeollinoleate, Skimmianine, Eugenol in aqueous extract of Aegle marmelos leaves. In ethanolic extract of Nigella sativa seeds two compounds were identified, nigelidine&nigellamine-N-oxide.

Pentylenetetrazole (PTZ) induced seizure model; aqueous extract of Aegle marmelos leaves and ethanolic extract of Nigella sativa seeds possess anticonvulsant property. Crude extract of Benincasa hispida fruit did not show statistically significant anticonvulsant property. (Table 2).

As seen in the graph Ethanolic extract of Nigella sativa seeds showed effect on all the phases of Pentylenetetrazole (PTZ) induced seizure but Aqueous extract of Aegle marmelos

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**Table 1: Preliminary phytochemical analysis**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TE</th>
<th>ST</th>
<th>FL</th>
<th>TA</th>
<th>PH</th>
<th>SA</th>
<th>G</th>
<th>CA</th>
<th>AL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Am (AQ)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ns (ETH)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bh (CRUDE)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>


**Table 2: Test for anticonvulsant property in pentylenetetrazole (PTZ) induced seizure model**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>First jerk</th>
<th>Onset- straubs tail</th>
<th>Onset- clonic convulsions</th>
<th>Onset - tonic flexion</th>
<th>Onset - HLTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NsEE 900 mg/kg</td>
<td>80±6.4***</td>
<td>84±13.13***</td>
<td>156±37.14***</td>
<td>636±138.26***</td>
<td>637±138.48***</td>
</tr>
<tr>
<td>AmAE 900 mg/kg</td>
<td>56±2.24</td>
<td>54±16.88*</td>
<td>61±6.78</td>
<td>378±44.47***</td>
<td>379±43.06***</td>
</tr>
<tr>
<td>BhE 900 mg/kg</td>
<td>40±6.15</td>
<td>53±7.79</td>
<td>68±16.19</td>
<td>52±10.8</td>
<td>82±18.89</td>
</tr>
<tr>
<td>Control</td>
<td>32±1.28</td>
<td>41±5.13</td>
<td>45±4.89</td>
<td>46±12.9</td>
<td>48±13.48</td>
</tr>
<tr>
<td>Sodium valproate</td>
<td>122±12***</td>
<td>183±165***</td>
<td>1472±385***</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

NsEE: Nigella sativa Ethanolic Extract, AmAE: Aegle marmelos Aqueous Extract, BhE: Benincasa hispida crude extract. *p value<0.05, ***p value<0.001. Tests –ANOVA followed by Dunnet’s test.
leaves exhibited weak effect on delaying initial phases of Pentylenetetrazole (PTZ) induced convulsions (First jerky movement, Straub’s tail, Clonus) compared to the effect on delay in onset of tonic phase. (Figure 1).

DISCUSSION

In the year 1944 Everett and Richards demonstrated that seizures induced by pentylenetetrazole could be blocked by trimethadione and phenobarbital but not by phenytoin. A year later Lennox reported that trimethadione was effective in decreasing or preventing petimbal (i.e., absence seizures) attacks in 50 patients and that it was not effective or worsened generalised tonic clonic seizures. This established Pentylenetetrazole induced seizure test as a model for absence seizures. In a way this was reconfirmed based on the reports that drugs effective against absence seizures blocked the clonic phase of pentylenetetrazole induced seizures. In rodent models involving maximal or supramaximal convulsive stimuli, tonic extension of the forelimbs or hind limbs is the typical convulsive endpoint. During the tonic extensor phase of a seizure, the forelimbs and hind limbs are rigid and extended caudally against the ventral surface of the body, while the hind limbs are rigid and extended horizontally and away from the prone or supine body in a caudal direction. This tonic extensor phase is characteristic of seizures evoked by maximal electroshock or high doses of chemoconvulsants.10-52

Facts that would aid in logical analysis of the results from Pentylenetetrazole induced seizure test are,46,49

a) Majority of drugs effective in Pentylenetetrazole induced seizure test are found to be useful in human absence seizures, but predictive power of the model to clinical use is less compared to the Maximal Electroshock induced Seizure test, i.e. further tests specific for absence seizure before clinical trials are required.

b) Neuronal Calcium channel modulators suppress in Pentylenetetrazole induced seizures and a less potent activity by GABAergic drugs; this is similar to human absence seizures.

c) Pentylenetetrazole is reported to be GABA_A receptor antagonist which correlates with behavioural features of Pentylenetetrazole induced seizures. With increasing doses, Pentylenetetrazole is reported to induce phases of seizure in the following order, clonic convulsions, tonic convulsions, and tonic hind limb extension. The clonic phase indicates inhibitory GABAergic currents disrupted by Pentylenetetrazole and tonic phase is seen when sufficient blockade of GABAergic system is attained.

d) There are reports that activation of GABA_B receptor in the globus pallidus may gate pentylenetetrazole-induced tonic seizures. Further GABA_B receptor activation serves to inhibit globus pallidus activity, which “closes the gate,” thereby explaining the antiseizure effect on tonic seizure activity.

e) Different phases of Pentylenetetrazole (PTZ) induced seizure correlate with progressive distribution and effect of PTZ to spinal cord and different areas of brain. First jerky movements and Straub’s tail -Spinal cord below C6, Clonus – Brainstem (inferior colliculus), Tonic extensor phase – Brainstem (nucleus reticularis pontisoralis).36

Pentylenetetrazole interferes with the GABAergic inhibitory control which aids several neuronal circuits propagate action potentials leading to seizure initiation. In case of Pentylenetetrazole, the site of initiation is reported to be the Limbic system. With further blockade of more and more GABAergic inhibition by Pentylenetetrazole, seizure propagates through brain stem and probably when this reach brainstem through cerebellum the Tonic Phase characterized by spasm of muscles appears. Tonic phase appear at supra maximal doses without a clonic phase or without much demarcation between the phases in the control group where lag between clonic convulsion and tonic convulsion was only 1-4 seconds. From the time of Pentylenetetrazole administration the mean latency for clonic convulsion was 45 seconds and for tonic phase it was 46 seconds in control group. In NsEE treated group onset of clonic phase was after 2½ minutes (mean) and tonic phase was after 10½ minutes (mean). In case of AmAE clonic phase was after one minute (mean) and tonic phase was after 5 minutes (mean). This clearly shows that the extracts treated group differs in seizure behaviour from control groups.

We would propose that Aegle marmelos leaves and Nigella sativa seeds possess antiepileptic properties and could be useful in human patients. The possible mechanism of action as indicated by the seizure inhibitory pattern and theories discussed in previous paragraphs could be,
Competitive GABA_\text{A} receptor agonist with or without GABA_\text{B} receptor activation or a less probable weak calcium channel blocker activity.

The findings of the study support the traditional uses of these medicinal plants as antiepileptic medication. Ethanolic extract of *Nigella sativa* seeds possess CNS depressant effects in addition to anticonvulsant properties. Aqueous extract of *Aegle marmelos* leaves is more potent in Pentylenetetrazole induced seizure test and Ethanolic extract of *Nigella sativa* seeds in Maximal Electroshock induced Seizure (MES) test when compared to the previous MES experiments in our lab,\(^5\)\(^,\)\(^6\) this indicates the probability of Sodium/Calcium channel mediated mechanism of action for Aqueous extract of *Aegle marmelos* leaves and GABAergic Mechanism for Ethanolic extract of *Nigella sativa* seeds.

Since it is observed that *Aegle marmelos* leaves and *Nigella sativa* seeds possess anticonvulsant properties there is good scope for,

- Isolation and screening of active constituents for their anticonvulsant properties, other pharmacokinetic and pharmacodynamic properties, drug interaction profiles and toxicity profiling.
- Isobolographic analysis of drug interactions between traditional preparations or the extracts of *Aegle marmelos* and *Nigella sativa* with established drugs with detailed pharmacodynamic and pharmacokinetic parameters would be worthwhile.
- Clinical trials of traditional preparations with these plant parts or with their extracts to test the usefulness in treatment of epilepsy.
- *Benincasa hispida* fruit could be screened with 6HZ model, Kindled seizure models for antiepileptogenic effects, and with different extraction methods.

REFERENCES

22. Yoshizumi S, Murakami T, Kadoya M, Matsuda H, Yamahara J and Yoshikawa M. Medicinal foodstuffs. XI. Histamine release inhibitors from wax gourd, the fruits of *Benincasa hispida*


46. Kiwanuka A, Kitimba A and Ntwatwa M. The concept and design of the study, reviewed the literature, conducted experiment, data collection and analysis, manuscript preparation and critical revision of the manuscript.

47. Conflict of Interest: None.