An Experimental Evaluation of Anticonvulsant Activity of Ethanolic Extract of *Vitex negundo* Linn. on Validated Animal Model

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ABSTRACT

*Vitex negundo* Linn, belonging to the verbenaceae family and commonly known as 'Nirgundi', is a significant medicinal plant. This woody, aromatic shrub can grow into a small tree and typically features 3 or 5 leaves on quadrangular branches. It produces bluish-purple flowers arranged in branched tomentose cymes. In addition to its various medicinal properties, it is reputed to have anticonvulsant effects. The objective of this study is to analyze the anti-convulsant effects of an ethanolic extract of *Vitex negundo* (EEVN) with model of Maximum Electroshock-induced Seizure (MES) induction. 30 albino rats, each weighing between 120-150 grams, were divided into five groups of 6 animals each. Group I is administered normal saline (0.5 ml p.o), Group II received Phenobarbitone (10 mg/kg body weight, i.p), and Groups III, IV, and V were given different dosages of EEVN (50, 100, and 200 mg/kg body weight, p.o respectively). Convulsions were induced in all groups by applying a maximal electric shock of 150 mA for 0.2 seconds using an electro-convulsiometer, 1 hour after administering the control, standard, and test drugs. The onset and duration of tonic hind limb extension (THLE) and the percentage of protection were recorded. In the MES model, EEVNF at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg significantly (p<0.001) reduces the onset and reduced the duration of THLE compared to the standard drug. The *Vitex negundo* demonstrated anticonvulsant properties in the maximal electric shock-induced convulsions in experimental animals.

Keywords: *Vitex negundo* Linn, THLE, Neuroimaging studies, Phenobarbitone, Neuronal Hyperexcitability, Seizure.

INTRODUCTION

Epilepsy, a complex neurological disorder characterized by recurrent seizures, represents a significant global health burden affecting millions of individuals across all age groups.[1] This introduction provides an overview of epilepsy, encompassing its epidemiology, etiology, clinical manifestations, impact on patients' lives, current treatment modalities, and ongoing research efforts. Epilepsy is one of the most common neurological conditions worldwide, with an estimated prevalence of approximately 70 million individuals globally.[2] It transcends geographical, cultural, and socioeconomic boundaries, affecting people of all ages, races, and backgrounds. Despite its widespread prevalence, epilepsy remains poorly understood and often stigmatized, leading to misconceptions and discrimination against affected individuals. In conclusion, epilepsy encompasses a diverse array of disorders characterized by recurrent seizures, with focal and generalized epilepsy representing major categories based on seizure type and electroclinical features.[3] The classification, diagnosis, and management of epilepsy require a multidisciplinary approach, integrating clinical expertise, neuroimaging studies, EEG findings, and patient preferences. By understanding the various types of epilepsy and their underlying etiology, healthcare providers can tailor treatment strategies to individual patients, optimizing seizure control and improving quality of life for those living with epilepsy.[4]
Epilepsy is a complex neurological disorder characterized by recurrent, unprovoked seizures resulting from abnormal electrical activity in the brain. The etiology of epilepsy is multifactorial, meaning it arises from a combination of genetic, structural, metabolic, immune, and environmental factors. These diverse underlying mechanisms can vary widely among individuals, making epilepsy a highly heterogeneous condition. Despite this variability, several common themes in the pathophysiology of epilepsy have been identified, which help to explain the recurrent nature of seizures and provide targets for therapeutic intervention.

Neuronal hyperexcitability is a hallmark of epilepsy, where neurons become excessively responsive to stimuli, leading to spontaneous and synchronized firing that can trigger seizures. This hyperexcitability can result from genetic mutations, acquired brain injuries, or alterations in the regulation of neuronal activity. Ion channel dysfunction is closely related to neuronal hyperexcitability. Ion channels, which control the flow of ions across the neuronal membrane, play a critical role in maintaining the electrical stability of neurons. Mutations in ion channels, such as sodium, potassium, and calcium channels, can disrupt this balance, leading to increased neuronal excitability and seizures.

Another crucial factor in the pathophysiology of epilepsy is the imbalance between excitatory and inhibitory neurotransmission. The brain relies on a precise balance between excitatory neurotransmitters like glutamate and inhibitory neurotransmitters like gamma-aminobutyric acid (GABA) to regulate neuronal activity. In epilepsy, this balance is often disrupted, with either an excess of excitatory activity or a deficiency in inhibitory control, which can precipitate seizure activity. Additionally, abnormalities in synaptic plasticity—the ability of synapses to strengthen or weaken over time in response to activity—can contribute to the development and persistence of epilepsy. Changes in long-term potentiation (LTP) and long-term depression (LTD) can enhance network excitability and promote the recurrence of seizures.

Neuroinflammation is increasingly recognized as a significant contributor to epilepsy. Inflammatory processes within the brain can be triggered by infections, autoimmune responses, or brain injuries, leading to changes in neuronal excitability and network function. Pro-inflammatory cytokines, microglial activation, and other immune responses can exacerbate seizure activity and contribute to the chronicity of the disorder. Genetic predisposition also plays a critical role in epilepsy. Certain genetic mutations can affect the function of ion channels, neurotransmitter receptors, and other proteins essential for neuronal function. These genetic factors can increase an individual’s susceptibility to seizures, either through direct inheritance or complex interactions with environmental factors.

Structural abnormalities in the brain, such as cortical dysplasia, tumors, or scarring from previous injuries, can serve as focal points for seizure activity. These structural changes disrupt normal neuronal networks and create localized areas of hyperexcitability that can initiate and propagate seizures. Understanding these diverse mechanisms is crucial for developing effective treatments for epilepsy. Current therapeutic strategies include antiepileptic drugs (AEDs) that aim to reduce neuronal hyperexcitability, surgical interventions to remove epileptic foci, dietary therapies like the ketogenic diet, and emerging therapies such as gene therapy and targeted molecular interventions.

The complexity of epilepsy arises from the interplay of various genetic, molecular, and environmental factors that contribute to abnormal neuronal activity and seizure generation. By understanding the
specific mechanisms underlying different types of epilepsy, researchers and clinicians can develop more targeted and effective therapeutic approaches, ultimately improving seizure control and the quality of life for individuals living with epilepsy.

**Mechanisms of Epilepsy**[7]

I. Neuronal Hyperexcitability: Epilepsy is characterized by abnormal and excessive neuronal activity in the brain, leading to seizures. This hyperexcitability can arise from various factors, including alterations in ion channel function, neurotransmitter imbalance, and synaptic dysfunction.

II. Ion Channel Dysfunction: Dysfunction of ion channels, particularly voltage-gated sodium, potassium, and calcium channels, can disrupt normal neuronal excitability and contribute to the generation and propagation of epileptic discharges. Mutations in ion channel genes have been implicated in certain forms of genetic epilepsy syndromes.

III. Excitatory/Inhibitory Imbalance: The balance between excitatory (e.g., glutamate) and inhibitory (e.g., gamma-aminobutyric acid, GABA) neurotransmission plays a crucial role in regulating neuronal activity. Imbalances favoring excessive excitation or reduced inhibition can lead to hyperexcitability and seizures.

IV. Synaptic Plasticity: Abnormalities in synaptic plasticity, including long-term potentiation (LTP) and long-term depression (LTD), may contribute to the development and maintenance of epilepsy. Dysregulation of synaptic strength and connectivity within neuronal networks can promote seizure generation and epileptogenesis.

V. Neuro-inflammation and Glial Activation: Inflammatory processes in the brain, involving activated microglia and astrocytes, have been implicated in epilepsy pathogenesis. Neuroinflammation can exacerbate neuronal hyperexcitability, promote synaptic dysfunction, and contribute to epileptogenesis.

VI. Neurovascular Coupling: Dysfunction in neurovascular coupling, the tight coupling between neuronal activity and cerebral blood flow, may play a role in epilepsy. Altered blood flow regulation and impaired oxygenation of brain tissue can contribute to seizure generation and propagation.

VII. Genetic Factors: Genetic mutations and alterations in gene expression have been implicated in various forms of epilepsy, particularly in idiopathic and genetic generalized epilepsy syndromes. These genetic factors may affect ion channel function, neurotransmitter release, synaptic plasticity, and other neuronal processes.

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**Fig. 2: Abnormal and excessive brain neuronal activity is the hallmark of epilepsy**

VIII. Structural Abnormalities: Structural abnormalities in the brain, such as cortical dysplasia, hippocampal sclerosis, tumors, and vascular malformations, can predispose individuals to epilepsy by disrupting normal neuronal circuits and promoting hyper excitability.

**Description of Plant Vitex Negundo**[8]

*Vitex negundo*, commonly known as the five-leaved chaste tree, is a large aromatic shrub or small tree native to the Indian subcontinent and Southeast Asia. Here are some key points about it:

**Description:** It typically grows up to 10 meters in height and has compound leaves with five lance-shaped leaflets. The flowers are small and bluish-purple in color, arranged in spikes.

**Traditional Uses:** In traditional medicine systems like Ayurveda, different parts of *Vitex negundo* are used for various medicinal purposes. Its leaves, seeds, and roots are believed to have therapeutic properties and are used to treat a wide range of
ailments such as fever, inflammation, respiratory disorders, skin diseases, and menstrual disorders.

**Phytochemicals:** The plant contains various phytochemicals such as flavonoids, alkaloids, terpenoids, and essential oils, which contribute to its medicinal properties.

**Pharmacological Activities:** Scientific studies have shown that extracts from *Vitex negundo* possess various pharmacological activities including anti-inflammatory, analgesic, antimicrobial, antioxidant, antidiabetic, and immunomodulatory effects.

**Cultural Significance:** In addition to its medicinal uses, *Vitex negundo* holds cultural significance in many regions where it grows. It is sometimes planted near temples and is considered sacred in some traditions.

**Horticultural Use:** Apart from its medicinal and cultural significance, *Vitex negundo* is also cultivated ornamentally for its attractive foliage and flowers.

**MATERIALS AND METHODS**

**Plant Material**

Fresh powder of *Vitex negundo* Linn was procured from Yucca Chemicals Mumbai showing in Figure 3. The authenticity of the plant powder material was confirmed by Agricultural Department of Manglaytan University, which is located in Aligarh. The specimen, with the voucher number Manglaytan/Pharma 2701, is preserved for reference in the herbarium of the Department of Pharmacy at Manglaytan University Aligarh.

![Fig. 3: Fresh powder of plant Vitex negundo Linn.](image)

**Drugs and Chemicals**

Inducing agent is Phenobarbitone and normal control group have normal saline 0.9% v/v.

**Instruments**

The instruments used in this study included an electroconvulsio meter, digital weighing balance, stopwatch, ear electrodes, feeding tube, insulin syringe, mouth gags, tuberculin syringe, Ryle's tube, beaker, glass jar, and glass rod.

**Preparation of Plant Extract of Vitex negundo**

The flower powder of *Vitex negundo* was extracted with 70% ethanol using a Soxhlet extraction apparatus and evaporated to dryness at 50°C. From 10 g of flower powder, 2 g of crude extract was obtained. The solid residues were stored in an airtight container and preserved in a refrigerator at −10°C. Fresh preparations were made from this stock as needed.

**Experimental Animals**

The animals used in this study were procured from a registered vendor of CCSEA. Albino Wistar rats of either gender, weighing between 120-150 g, were selected for the experiment. Prior to and during the study, the animals were maintained under standard animal house conditions, including a 12:12 hour dark-light cycle, a temperature of 24±2°C, humidity ranging from 32-55%, and other environmental conditions as recommended by the Committee for the Control and Supervision of Experiments on Animals (CCSEA). They were housed in polypropylene cages covered with stainless steel wire mesh and a paddy husk bed, with sufficient provision for feed and water. The study commenced after obtaining approval from the Institutional Animal Ethics Committee (IAEC/NIRTH/Reg No.1671/GO/RBi/S/12/CPCSEA).

**Phytochemical Screening of Vitex negundo Linn.**

The freshly prepared extract of *Vitex negundo* flowers was subjected to phytochemical screening tests to detect various constituents.

**Acute Toxicity Study**

The animals were treated with increasing doses of EEVNF. Toxicity studies were conducted in
accordance with the Organization for Economic Co-operation and Development (OECD) 423 guidelines. All treated animals were observed for any abnormal or toxic manifestations and mortality.

**Evaluation of Anticonvulsant Activity**

*Maximal Electroshock-Induced Seizures (MES)*

The model developed by Merritt and Putnam in 1938 is a well-established method for screening drugs effective against primary and secondary generalized tonic-clonic seizures. In this experimental setup, animals were divided into five groups, each consisting of six subjects. Group I, serving as the control, received normal saline (0.5 ml/kg). Group II, designated as the standard group, was administered Phenobarbitone (10 mg/kg, i.p.). Groups III, IV, and V were treated with three graded doses of the test drug (EEVNF) at 50, 100, and 200 mg/kg orally, respectively, in the maximal electroshock (MES) experimental models.

Convulsions were induced in all groups by applying a maximal electric shock of 150 mA for 0.2 seconds using an electroconvulsiometer, one hour after the administration of the control, standard, and test drugs. The onset and duration of hind limb extension were meticulously recorded. The complete abolition of hind limb tonic extension was considered as 100% seizure protection, serving as the primary efficacy endpoint for the test drug in comparison to the control and standard treatments. This model provides a robust framework for evaluating the anticonvulsant potential of new pharmacological agents.

The percentage protection was calculated using the following formula:

\[
\text{Percentage protection} = \left( \frac{\text{Number of animals protected}}{\text{Total number of animals}} \right) \times 100
\]

**Statistical Analysis**

The data were expressed as Mean ± SEM (Standard Error of the Mean) and statistically analyzed using One-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison tests. A 'p' value of less than 0.05 was considered statistically significant for all tests.

**RESULTS AND DISCUSSION**

Epilepsy affects approximately 60 million people worldwide and is characterized by recurrent seizures, necessitating effective treatment options. While synthetic anticonvulsant drugs are available, they often come with accessibility issues, high costs, and significant adverse effects. As a result, there is growing interest in exploring natural alternatives, such as herbal products, which may offer safer and more affordable treatment options for epilepsy.

**Phytochemical Screening of powder of *Vitex negundo***

Phytochemical screening of EEVN revealed the presence of tannins, alkaloids, terpenoids, flavonoids, sterols, phenolic compounds, and proteins. These compounds have been previously associated with various pharmacological activities, including anticonvulsant effects.

**Acute Oral Toxicity Study**

No adverse effects or mortality were observed in albino Wistar rats administered up to 1 g/kg of EEVN. Throughout the 14-day observational period, all animals remained alive, healthy, and active. Therefore, the LD50 was considered greater than 1000 mg/kg.

**Anticonvulsant Activity using MES-Induced Seizure Model**

The anticonvulsant activity of EEVN in albino Wistar rats was investigated using the MES-induced seizure model. EEVN administered at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg significantly delayed the onset of tonic hind limb extension (THLE) with means of 3.32 ± 0.14, 5.15 ± 0.13, and 5.97 ± 0.27, respectively (p<0.001). It also shortened the duration of THLE with means of 20.38 ± 0.32, 16.07 ± 0.24, and 13.17 ± 0.22, respectively (p<0.001), compared to the standard drug (10.67 ± 0.21 and 6.81 ± 0.39, respectively). The percentage protection of EEVN at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg was 65.5%, 85.5%, and 80.5%, respectively, while the standard drug showed 100% protection. These findings indicate that EEVN exhibits protection against maximal electroshock-induced seizures.
## Table 1: Anticonvulsant data of EEVN in albino Wistar rats using the MES-induced seizure model

<table>
<thead>
<tr>
<th>Groups</th>
<th>Onset of THLE (M ± SEM)</th>
<th>Duration of THLE (M ± SEM)</th>
<th>Percentage protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>2.54 ± 0.21</td>
<td>19.42 ± 0.70</td>
<td></td>
</tr>
<tr>
<td>Group II (Phenobarbitone 10mg/kg)</td>
<td>10.67 ± 0.21</td>
<td>6.81 ± 0.39</td>
<td>100%</td>
</tr>
<tr>
<td>Group III (EEVN 50mg/kg)</td>
<td>3.32 ± 0.14***</td>
<td>20.38 ± 0.32***</td>
<td>65.5%</td>
</tr>
<tr>
<td>Group IV (EEVN 100mg/kg)</td>
<td>5.15 ± 0.13**</td>
<td>16.07 ± 0.24***</td>
<td>85.5%</td>
</tr>
<tr>
<td>Group V (EEVN 200mg/kg)</td>
<td>5.97 ± 0.27***</td>
<td>13.17 ± 0.22***</td>
<td>80.5%</td>
</tr>
</tbody>
</table>

All values are presented as Mean ± SEM (Standard Error of the Mean) and compared with the standard, with n = 6 animals per group. Statistical significance is denoted as *p < 0.05, **p < 0.01, ***p < 0.001.

### Implications and Future Directions

The findings underscore the potential of *Vitex negundo* Linn as a source of natural anticonvulsant agents. Further research is warranted to elucidate the specific mechanisms underlying its anticonvulsant properties and to explore its efficacy in other models of epilepsy. Moreover, clinical trials are necessary to validate its efficacy and safety in human subjects, paving the way for its potential use as a complementary or alternative therapy for epilepsy management.

### CONCLUSION

In conclusion, *Vitex negundo* Linn extract demonstrated promising anticonvulsant activity in the MES model, highlighting its potential as a natural alternative to synthetic anticonvulsant drugs. Given its phytochemical composition, safety profile, and efficacy observed in this study, EEVN warrants further investigation as a potential therapeutic option for epilepsy. This research contributes to the ongoing quest for affordable, effective, and safe treatments for epilepsy, addressing the current limitations associated with synthetic drugs.

Based on the results of the present study, it is concluded that EEVNF exhibits protective effects against maximal electroshock-induced seizures, indicating a potential interaction with the GABAergic system. Given the efficacy demonstrated in the MES model, EEVNF warrants further preclinical evaluation to validate its utility in generalized tonic-clonic seizures (GTCS).

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This study received no funding from any sources.

### Conflict of Interest

The authors declare no conflicts of interest.

### Ethical Approval

The study was approved by the Institutional Animal Ethics Committee.

### Acknowledgement

NA

### REFERENCES


