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ANTICONVULSANT ACTIVITY OF ETHANOLIC EXTRACT OF VITEX NEGUNDO FLOWERS IN EXPERIMENTAL ANIMALS

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ABSTRACT

Vitex negundo Linn (Family: Verbenaceae), locally known as 'Nirgundi' an important medicinal plant is a woody, aromatic shrub growing to a small tree. It commonly bears tri- or penta-foliate leaves on quadrangular branches, which give rise to bluish-purple coloured flowers in branched tomentose cymes. It has been claimed to possess anticonvulsant activity apart from many medicinal properties. **Objectives:** The aim of the present study was to evaluate the anticonvulsant activity of ethanolic extract of Vitex negundo flowers (EEVNF) in Maximum Electroshock induced Seizure (MES) model in experimental animals. **Methods:** 30 albino wistar rats of either sex weighing 150-200 gms were randomly divided into 5 groups of 6 animals each. Group I received Normal saline (0.5 ml p.o), Group II received Phenobarbitone (10mg/kg body weight) i.p, Group III,IV,V received different dosages of EEVNF(200,400,800/mg/kg body weight p.o respectively). Convulsions were produced in all groups by giving maximal electric shock of 150 mA for 0.2sec using electroconvulsiometer after 1 hour of giving control, standard and test drugs. Onset and duration of tonic hind limb extension (THLE) and percentage protection were noted. **Results:** In Maximal electric shock induced seizure (MES) Model, EEVNF in doses of 200mg/kg, 400mg/kg and 800mg/kg significantly ($p<0.001$) delayed the onset of THLE and shortened the duration of THLE when compared to the standard drug. **Conclusion:** Vitex negundo flowers showed anticonvulsant property in Maximal electric shock induced convulsions in experimental animals.

KEYWORDS: Convulsions, Vitex negundo, Phenobarbitone, Maximal electricshock, Anticonvulsant.

INTRODUCTION

Epilepsy is the third most common neurologic disorder after dementia and stroke. Approximately 1% of world's population has epilepsy. Epilepsy is a heterogenous symptom complex-a chronic disorder characterized by recurrent seizures. Seizures are finite episodes of brain dysfunction resulting from abnormal discharge of cerebral neurons.[1] The underlying abnormality of epilepsy is poorly understood, but it may be associated with an imbalance between excitatory and inhibitory neurotransmitters in the brain.[2] In the management of epilepsy the introduction of anticonvulsant therapy has contributed significantly. With the use of conventional anti-epileptic drugs (AED) 60-70% of patients with epilepsy achieve control of their seizures. However seizure control is not achieved in nearly one-third of the epileptic patients, even with the continued use of AED.[3] Moreover the use of AEDs are associated with a vast array of side effects, dose related and chronic toxicity as well as teratogenic effects.[4] As such, there is an increased need to discover drugs which are effective in refractory epilepsy and have lesser adverse effects. The use of medicinal plants offer important sources of new chemical substances with potential therapeutic benefits. So many herbal products have been tried in the treatment of epileptic models to prove their action against epilepsy. One among them is *Vitex negundo Linn* belongs to the family Verbenaceae.

Vitex negundo Linn (Family: Verbenaceae) is a woody, aromatic shrub growing to a small tree. Some common names are in Hindi nirgundi and in Sanskrit as sindhuvara.(Figure 1). It commonly bears tri- or penta-foliate leaves on quadrangular branches, which give rise to bluish-purple coloured flowers in branched tomentose cymes. It thrives in humid places or along water courses in wastelands and mixed open forests.It is found throughout the greater part of India at warmer zones and ascending to an altitude of 15,00m in outer Western Himalayas.[5] All parts of the plant from root to fruit possess a multitude of phytochemical secondary metabolites which impart an unprecedented variety of medicinal uses to the plant.[6,7] *Vitex negundo* has been investigated for antipyretic,[8] antiinflammatory,[9-11] anti-convulsant,[9,12] hepatoprotective[13] and bronchial

relaxant.[14] Very few studies have been done to evaluate its anticonvulsant activity and no study was done on anticonvulsant activity of flowers of *Vitex negundo* .Therefore, the present study was undertaken to investigate anticonvulsant activity of ethanolic extract of flowers of *Vitex negundo.*

Figure 1: Vitex negundo Linn.

MATERIALS AND METHODS

Plant material

Fresh flower powder of *Vitex negundo Linn* was brought from AGHP Enterprises, Chennai in the month of June 2016 which was auntheticated by Dr.D.Aravind, Assistant Professor, Botany, National Institute Of Siddha,Chennai. The specimen (Voucher number: SNMC/Pharma 009), is preserved for reference in the herbarium of department of Pharmacology, S.Nijalingappa Medical College, Bagalkot.

Drugs and chemicals: Phenobarbitone, normal saline were used in this study.

Instruments: Electroconvulsiometer(Figure 2),Digital weighing balance, Stopwatch, ear electrodes, Feeding tube, Insulin syringe, Mouth gags, Tuberculin syringe, Ryle's tube, beaker, glass jar, glass rod.

Figure 2: Electroconvulsiometer.

Preparation of Plant extract

The material was extracted with 80% ethanol using soxhlet extraction apparatus (Figure 3) and it was evaporated to dry at 60°C. Flower powder (20 g) of *Vitex negundo* yeilded 4 g of crude extract. The solid residues were stored in airtight container and preserved in the refrigerator at −20°C.[15] From this stock, fresh preparations were obtained whenever required.

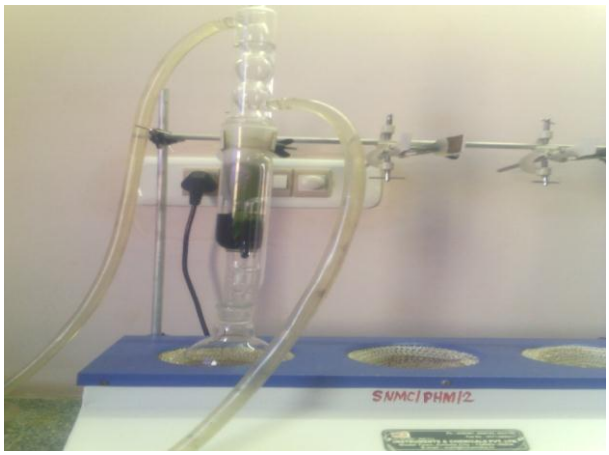

Figure 3: Soxhlet Appartus.

Experimental animals

All the animals were procured from the Central Animal house, S. Nijalingappa Medical College, Bagalkot. *Albino wistar rats* of either gender weighing 150-250 g were selected for the experiment. Pregnant rats, animals with an infection, animals with injuries, deformities were excluded from the study. Prior to and during study, all the animals were maintained under standard animal house conditions at 12:12 hrs dark: light cycle, at temp 25±2°C, humidity 35-60% and other micro and macro environment conditions as suggested by Committee for the Purpose of Control and Supervision of Experiments on Animals(CPCSEA). All animals were housed in a polypropylene cage covered with a stainless steel wire mesh and a paddy husk bed, with adequate provision for feed and water. All the animals were maintained on standard laboratory diet (VRK Nutritionals, Pune) and water was provided *ad libitum*. The study was started after getting the Institutional Animal Ethics Committee approval (IAEC/ S.Nijalingappa Medical College, Bagalkot Reg No.829/AC/04/CPCSEA).

Phytochemical screening

The freshly prepared extract of the flowers of *Vitex negundo* was subjected to phytochemical screening tests for the detection of various constituents.[16]

Acute toxicity study

The animals were treated with increasing doses of EEVNF. The toxicity studies were conducted according to the Organization for Economic co-operation and development(OECD) 423 guidelines.[17] All the treated animals were observed for any abnormal or toxic manifestations and mortality.

Evaluation of Anticonvulsant activity
Maximal Electro Shock Induced Seizures (MES)
This model was developed by Merritt and Putnam in 1938.[18] This model is helpful in the screening of drugs effective against primary and secondary generalized tonic-clonic seizure.[18-19]

The animals were divided into five groups with each group consisting of six animals. Group-I received normal saline 0.5ml/kg served as control, Group-II received Phenobarbitone (10 mg/kg, ip) as standard, Group-III, IV,V were administered three graded doses of test drug (EEVNF) i.e.200, 400, 800mg/kg, orally in MES experimental models. Convulsions were produced in all groups by giving maximal electric shock of 150 mA for 0.2sec using electroconvulsiometer after 1 hour of giving control,standard and test drugs (Figure 4). The onset and duration of hind limb extension was noted. A complete abolition of hind limb tonic extension was considered as 100% seizure protection. Percentage protection was calculated by the formula

%Protection=100-[(Number of animal showed seizures /Total number of animals used)×100]

Figure 4: Rat Showing Convulsive activity by MES method using ear electrode.

Statistical Analysis
The data was expressed as Mean±SEM and statistically analyzed using One way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. For all the tests a 'p' value of less than 0.05 was considered as statistical significance.

RESULTS

Phytochemical screening
Phytochemical screening of EEVNF showed that the crude extract contained tannins, alkaloids, terpenoids, flavonoids, sterols, phenolic compounds and proteins.

Acute oral toxicity study
No adverse effect or mortality was detected in albino wistar rats at 2 g/kg of EEVNF. All the animals were alive, healthy and active during the observational period of 14 days. So the LD 50 was considered as >2000 mg/kg.

MES-induced seizure model
The results of the anticonvulsant activity of EEVNF on *albino wistar rats* were depicted in Table-1 and Figure - 5. Present study has shown EEVNF when given in doses of 200 mg/kg,400mg/kg and 800mg/kg significantly delayed the onset of THLE with mean of (3.42 ± 0.15, 5.05 ± 0.11,and 6.97 ± 0.27 ; p<0.001) and shortened the duration of THLE with mean of(19.39 ± 0.30, 15.47 ± 0.25, 12.57 ± 0.32; p<0.001)respectively in MES induced seizure model (Table 1 & Figure 5) when compared to standard (10.67 ± 0.21; 6.81 ± 0.39). The percentage protection of EEVNF in doses of 200 mg/kg, 400mg/kg and 800mg/kg was 66.5%83.5%, 83.5% and that of standard drug was 100%.Thus EEVNF has protection against Maximal electro shock induced seizures.

Table 1: Time duration of phases of MES – induced convulsion.

Groups	Onset of THLE (M ± SEM)	Duration of THLE (M ± SEM)	Percentage protection (%)
Group I(Control)	2.54 ± 0.21	19.42 ± 0.70	-
Group II (Phenobarbitone 10mg/kg)	10.67 ± 0.21	6.81 ± 0.39	100%
GroupIII (EEVNF 200mg/kg)	3.42 ± 0.15***	19.39 ± 0.30***	66.5%
Group IV (EEVNF 400mg/kg)	5.05 ± 0.11***	15.47 ± 0.25***	83.5%
Group V (EEVNF 800mg/kg)	6.97 ± 0.27***	12.57 ± 0.32***	83.5%

All the values are expressed as Mean ± SEM compared with standard, (n= 06), SEM: Standard Error of Mean *p<0.05, **p<0.01, ***p<0.001.

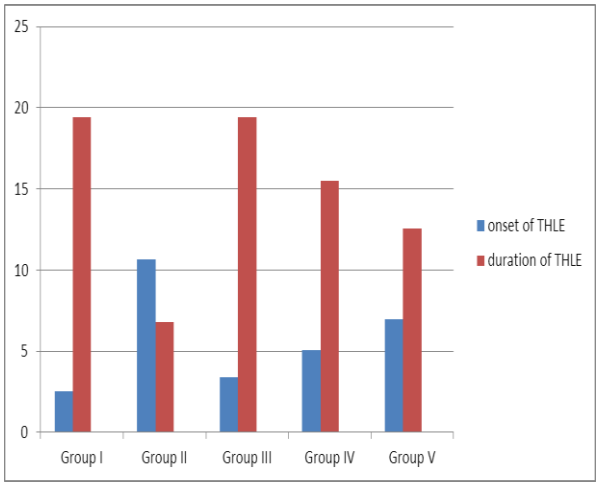

Figure 5: Graph representing anticonvulsant activity of ethanolic extract of Vitex Negundo flowers in maximal electric shock induced seizure induced convulsions in Wistar albino rats.

DISCUSSION

Approximately 50 million people worldwide have Epilepsy making it one of the most common neurological disease globally. There are a number of synthetic anticonvulsant drugs currently available for use in the management, control and treatment of individuals with epilepsy. However, most of the synthetic drugs are not only inaccessible and unaffordable, but also possess many toxic adverse effects. Therefore, there is a great need for the development of cheap, effective and safe anticonvulsant agents from plants and other sources. So many herbal products have been tried in the treatment of epileptic models to prove their action against epilepsy. One among them is *Vitex negundo Linn* belongs to the family Verbenaceae.

Previous studies have shown significant anticonvulsant activity of EEVN leaves.[20] This study is the first report regarding anticonvulsant activity of EEVN flowers.

In the present study, the anticonvulsant activity of EEVNF was evaluated by MES induced seizure test in albino wistar rats. The MES is probably the best-validated method for assessment of anti-epileptic drugs in generalized tonic- clonic seizures(GTCS).[21] MES causes several changes at the cellular level, which can disrupt the signal transduction in the neurons. One of the most important mechanism by which it causes cellular damage is the facilitation of Ca2+ entry into the cell in a large amount and thus, prolonging the duration of convulsion.[22] Apart from Ca2+ ions, MES also facilitates the entry of other positive ions such as Na+, blockade of which can prevent the MES-induced tonic extension.[23] Currently available anticonvulsant drugs such as valproate and phenytoin act by modulation of these ions channels.[24] On the other hand, potentiation of gamma-aminobutyric acid (GABA) receptors is also reported to protect against MES-induced seizures.

Present study has shown EEVNF when given in doses of 200 mg/kg, 400mg/kg and 800mg/kg significantly delayed the onset of THLE and shortened the duration of THLE respectively in MES induced seizure model when compared to standard.

Preliminary phytochemical analysis of EEVNF showed that the crude extract contained tannins, alkaloids, terpenoids, flavonoids, sterols, phenolic compounds and proteins.

Several studies have indicated that plants containing flavonoids and saponins have significant anticonvulsant activity. Many flavonoids and phytosteroids have been found to be ligands for the GABA-A receptors and hence can act like benzodiazepine like molecules.[25,26] Therefore, these phytoconstituents may be responsible for the anticonvulsant activity of EEVNF. However, further research work is needed to establish the active constituent(s) of the extract and the exact mechanism of action.

CONCLUSION

Based on present study results, we conclude that the EEVNF has shown protection against maximum electroshock seizures induced convulsions and also suggests the possibility of a GABA- ergic interaction to be responsible for the observed effect. As MES model is useful for screening drugs effective against GTCS, the EEVNF can be considered for further preclinical evaluation to substantiate its use in GTCS.

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2. **Conflict of interest:** None declared

3. **Ethical approval:** The study was approved by the Institutional Animal Ethics Committee.

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