INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

# A STUDY ON NEUROLOGICAL SIGNIFICANCE OF *VITEX NEGUNDO* LINN.

Sukhbir Lal Khokra [1]*, Om Prakash [1], Sandeep Jain [2]
[1]Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra-136119, Haryana, India
[2]Department of Pharmaceutical Sciences, G. J. University of Science & Technology, Hisar, Haryana, India
*Corresponding Author Email: khokrasl@gmail.com

Article Received on: 22/06/18 Approved for publication: 15/09/18

**DOI: 10.7897/2230-8407.099200**

**ABSTRACT**

The main purpose of this study was to evaluate antioxidant and anticonvulsant activity and to establish relation between two. The putative anti-convulsant activity of essential oils and ethanolic extracts of fruits, flowers, leaves and roots of *Vitex negundo* was evaluated using maximal electroshock seizures and pentylenetetrazole methods while antioxidant potential was studied using DPPH and ferric reducing assay. Ethanolic extracts of fruits, leaves, flowers and roots abolished tonic MES-induced convulsions in 40 % of test animals and reduced the mortality rate to 50 %. The leaves extract at the doses of 250 and 500 mg/kg *p.o.* produced significant decrease in duration of clonic phase and abolished the clonic phase in 60 % and 70 % of animals respectively in PTZ model. The simultaneous administration of ethanolic extract of leaves and fruit with diazepam at sub-protective doses abolished clonic phase duration significantly. Antioxidant studies showed that most of essential oils and ethanolic extracts of *V. negundo* showed significant antioxidant activity in both DPPH and ferric reducing power methods. The essential oils of leaves, flower and dry fruit showed significant antioxidant activity along with methanolic and ethanolic extract of leaves. So, it is concluded that essential oils as well as extracts especially leaves have strong antioxidant potential. These results indicate that *V. negundo* may be further explored for their use as effective anticonvulsant, which may be attributed to antioxidant properties.

**Keywords:** *Vitex negundo*, antioxidant, anticonvulsant, maximal electroshock seizures, pentylenetetrazole, phenytoin, diazepam

## INTRODUCTION

Oxidation processes are intrinsic in the energy management of all living organisms and are therefore kept under strict control by several cellular mechanisms[1]. Anti-oxidants may be defined as medical scavengers, which prevent the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, inflammation, arthritis, neurodegenration, parkinson's disease, convulsions and even dementia[2]. The high rate of oxidative metabolism, coupled with the low antioxidant defences and the richness in polyunsaturated fatty acids, makes the brain highly vulnerable to free radical damage. It highlights the importance of understanding the role of oxidative stress in pathophysiology of seizures. Widely used anti-oxidants in food products, such as butylated hydroxytoluene and butylated hydroxyanisole, phenols and amines are very effective in their use. But, their use in food products is diminishing due to their instability and suspected action as promoters of carcinogenesis[3]. These findings along with consumer interest in natural food additives have reinforced interest in natural antioxidants e.g. *Ilex paraguariensis* leaves contain many bioactive compounds, such as phenolic acids, which seems to be responsible for the antioxidant activity, both *in vivo* and *in vitro*[4]. The antioxidant activities of the essential oil from *S. oreganum* leaves and extract with essential oils from *J. Sambac* have shown *in vitro* antimicrobial and antioxidant activities, which could support the use of the plant by traditional healers to treat various infective diseases, especially neurological disorders like dementia[5-7].

Seizures are resistant to treatment with currently available anticonvulsant drugs in about one out of three patients with epilepsy. Thus there is a need for new and more effective anticonvulsant drugs for intractable epilepsy. Neuronal hyper excitability and excessive production of free radicals have been implicated in the pathogenesis of a considerable range of neurological disorders, including epilepsy. Epilepsy is disorder of CNS characterized by brief episodes (seizures) of loss or disturbance of consciousness, with or without characteristic body movements (convulsions), psychiatric phenomena[8]. Epilepsies have been majorly classified in to generalized tonic-clonic seizures and partial seizures[9-10].

In the ayurvedic system of medicine *V. negundo* is used as a drug of choice to manage pain, inflammation and other related diseases. *Vitex negundo* Linn. belongs to family Verbenaceae, known as Nirgundi in Hindi, grows gregariously in wastelands and is also planted as a hedge-plant. It is an erect, 2–5 m in height, slender tree with quadrangular branchlets distributed throughout India[11]. Although all parts *V. negundo* are used as medicine in the indigenous system of medicine, the leaves are the most potent for medicinal use. It is used for treatment of toothache, inflammation, rheumatoid arthritis, etc[12]. Among the chemical constituents, it has several flavonoids such as casticin, orientin, isoorientin, luteolin, lutecin-7-O-glucoside, corymbosin and 3-O-desmethylartemetin. Besides these many glycosidic iridoids, alkaloids, and terpenoids have also been isolated[13].

Although, many plant extracts and pure phytochemicals have been reported to have anti-inflammatory and free radical scavenging property[14-17]. However, not much work has been done to investigate the role of *Vitex negundo* in free radical management and treatment of neurological disorders[18]. ROS produced *in vivo* includes superoxide radicals ($O_2^-$) and hydrogen peroxide ($H_2O_2$) these may lead to production of the hydroxyl

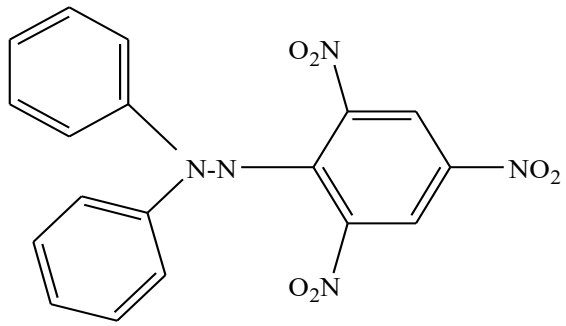
radicals ($OH^-$). These ROS can initiate or propagate many diseases, such as inflammation, cancer, liver injury, convulsions and cardiovascular diseases[19]. Although a living system possesses several natural defence mechanisms, such as enzymes and antioxidant nutrients, which arrest the chain reaction of ROS initiation. But its continuous exposure for a long time may lead to irreversible oxidative damage. Therefore, antioxidants may have a great relevance in the prevention and therapeutics of such diseases. *V. negundo* contains many polyphenolic compounds, terpenoids, glycosidic iridoids and alkaloids. Since polyphenolic compounds have high antioxidant potential, the antioxidant potency of different organic fractions of *Vitex negundo* on its free radical scavenging potential for different reactive oxygen species (ROS) was investigated. In many inflammatory and neurological disorders, there is excess activation of phagocytes and production of superoxide ($O_2^-$). Hence the agents that can scavenge these reactive species can be beneficial in the treatment of such inflammatory and neurological disorders like epilepsy. So, here we studied the antioxidant capacity of different fractions of *V. negundo* by employing various established *in vitro* methods along with anticonvulsant potency. The study was also aimed to establish a relationship between antioxidant and anticonvulsant potency of the plant.

## MATERIALS AND METHODS

### Anti-oxidant activity [20-22]

### DPPH radical scavenging activity[23]

Antioxidant activity of essential oils and along with extracts was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical and is commonly used in antioxidant assays. DPPH is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule do not dimerise, as would be the case with other free radicals. The delocalization also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution at about 517 nm.




1,1-diphenyl-2-picrylhydrazyl radical (DPPH)

***Reagents and solutions***

- DPPH solution - 100 µM of DPPH was prepared in 95% ethanol and stored in amber color bottle.
- Test samples and standard solutions of concentrations 125, 250, 500 µg/ml was prepared in ethanol

***Procedure***

After addition of equal volumes of ethanolic DPPH solution and the test solutions (essential oil/extracts)/ standard solution in amber colored test tube, mixture was kept aside for 20 minutes to allow the reaction to take place. Absorbance of test agent ($A_{TEST}$) and standard ($A_{STANDARD}$) was noted immediately after 20 minutes against 95% ethanol. Absorbance of DPPH ($A_{CONTROL}$) was measured against 95% ethanol blank.

Free radical activity was calculated on the basis of the percentage of inhibition:

$$\text{Percentage Inhibition} = \frac{A_{CONTROL}-A_{TEST}/\text{Standard X100}}{A_{CONTROL}}$$

$\mathbf{A}_{CONTROL}$ =absorbance of control sample,
$\mathbf{A}_{TEST}$ = absorbance of test sample,
$\mathbf{A}_{STANDARD}$ = absorbance of standard ascorbic acid.

Percentage inhibition of free radical generation by various concentration of the extracts and oils was compared with that of the standard ascorbic acid (ASC) & butylated hydroxy-anisole (BHA). Special care was taken to minimize loss of free radical activity of the DPPH radical stock solution. All readings were performed in duplicate. The evaluation was carried out on Systronics double beam UV/Vis spectrophotometer model 2202.

### Reducing Power Assay[24]

The determination of ferric reducing/antioxidant power is a simple direct test for measuring anti-oxidant capacity. This assay measures the change in absorbance owing to the formation of a blue colored $Fe^{2+}$ compound from a colorless oxidized $Fe^{3+}$ form by the action of electron donating anti-oxidants.

The reducing capability was measured by the transformation of $Fe^{3+}$ to $Fe^{2+}$ in the presence of different test extract at 700 nm. Increased absorbance of the reaction mixture indicates reducing power.

***Reagents and solutions***

- Potassium ferricyanide [$K_3Fe(CN)_6$] (1% ) reagent was prepared in freshly prepared Phosphate buffer (0.2 M) (pH 6.6).
- Phosphate buffer (0.2 M) (pH 6.6) was prepared by dissolving 2.38 gm of disodium hydrogen phosphate (ortho), 0.9 g of potassium dihydrogen phosphate and 8.00 gm of sodium chloride in 1000 ml distilled water.
- Trichloroacetic acid (10%) reagent was prepared in distilled water.
- Iron (III) chloride (1%) reagent was prepared in distilled water.
- Stock solutions (500 µg/ml) of test and standards was prepared in phosphate buffer (0.2 M) (pH 6.6) containing 1 % potassium ferricyanide [$K_3Fe(CN)_6$] reagent.

***Procedure***

A serial dilution of test and standards (125, 250, 500 µg/ml) was prepared from stock solutions. A mixture containing 2.5 ml of 10 % trichloroacetic acid and 5 ml of test/standard solutions was incubated at 50˚c for 20 minutes. It was then centrifuged at 3000 rpm for 10 minutes. The 2.5 ml of supernatant layer of solution thus separated was mixed with 2.5 ml of distilled water containing 0.5 ml of $FeCl_3$ (1%) solution. The absorbance of this mixture was measured at 700 nm using Systronics double beam UV/Vis spectrophotometer model 2202. Increased absorbance of the reaction mixture indicated increased reducing power.

### Anticonvulsant activity

Ethanolic extracts of leaves, flowers, fruit and root of *V. negundo* Linn. were considered for *in-vivo* anticonvulsant studies. The anticonvulsant activity was evaluated by MES and PTZ induced seizures methods[25-28]. The potentiation of anticonvulsant actions of standard drugs phenytoin and diazepam by test drugs was also

studied at sub-protective doses[25].

**Animals**

Male mice weighing 25-30 gm were used throughout this study. The approval for the use of animals for experimental purpose was obtained from Institutional Ethical Committee constituted for the purpose. Animals were housed in polypropylene cages (5 per cage) with dust free rice husk as a bedding material under laboratory condition with controlled environment of temperature 25° ± 2°C and 12 h light/dark cycle as per CPCSEA guidelines. They were fed *ad libitum* with standard laboratory rodent's chow and free access to drinking water. The animals were given a week time to get acclimatized with laboratory conditions. The animals were fasted overnight before the experiment.

**Standard and test drugs**

Pentylenetetarazole (PTZ) (85 mg/kg) was used to induce convulsions. Phenytoin sodium (25 mg/kg) and diazepam (1 mg/kg, *i.p.*) were used as standards in MES and PTZ methods respectively. All drugs were dissolved in normal saline (0.9%) and administered intraperitoneally (*i.p.*) in volumes of 0.1 ml/25 gm of body weight.

The suspensions of extracts were prepared in normal saline: tween 80 (95:5) to prepare 125, 250 and 500 mg/kg concentrations just before administration to animals. The test drugs were administered orally in the above mentioned graded doses in both the experimental models. The test drugs, normal saline: tween and standard drugs were administered in a volume not higher than 10 ml/kg of body weight for mice.

**MES method**

The Maximal electroshock seizures (MES) electrical stimulus (50mA, 50Hz, 1 sec duration) was delivered through ear-clip electrodes to induce tonic hind limb extension (THLE). The animals were chosen by preliminary screening. The mice which showed extension of hind limb were included in this study. Five groups (I-V) of six mice each, Ia - pretreated with normal saline: tween (10 ml/kg, as control), IIa - phenytoin (25 mg/kg as positive control) and IIIa,b,c,d to Va,b,c,d – (test drugs a,b,c,d at 125, 250, 500 mg/kg), received the electroconvulsive shock 30 minutes later. The time of peak effect of phenytoin was previously established. After electrical stimulation, occurrence of THLE, duration and incidence of mortality were noted. Disappearance of the hind limb extensor tonic convulsion is used as positive criterion. Percentage of inhibition of seizures relative to controls is calculated as shown in table 3.

***MES seizures interaction studies***

Four groups of animals consisting six mice in each were numbered as I, II, III, and IV. Group I received normal saline: tween 80 and served as control. Group II received sub-protective doses of phenytoin (10 mg/Kg *i.p*). Group III received sub-protective dose of test drug (100 mg/Kg, *p.o.*), which did not produce any anticonvulsant activity. Group IV was given in combination of phenytoin and extract in the above doses. The results were compared with group I.

***PTZ induced seizures method***

The minimal *i.p.* dose of PTZ at which 99% of the animals showed HLTE was pre-determined. PTZ at the dose of 85 mg/kg (minimal dose needed to induce convulsions) was injected *i.p*. to induce clonic-tonic convulsions in animals. After 30 minutes, PTZ was given to five groups (I-V) of six mice each pretreated with the test drug doses 125, 250 & 500 mg/kg per orally, diazepam (1 mg/kg *i.p*) as positive control and normal saline: tween (10 ml/kg, as control *p.o*). Mice were then observed for 30 min to detect the occurrence of general clonus, THLE and mortality.

***PTZ induced seizures interaction studies***

Four groups consisting ten mice in each were numbered as I, II, III, IV. Group I received normal saline: tween 80 and served as control. Group II received sub-protective doses of diazepam (0.5 mg/Kg *i.p*). Group III received sub-protective dose of test drug (100 mg/Kg, *p.o*), which did not produce any anticonvulsant activity. Group IV was given in combination of diazepam and extract in above doses. The results were compared with group I.

One-way ANOVA and t-test was used for data expressed in Mean±SEM and mean difference was considered significant at the 0.001 level.

**RESULTS**

The results shown in Table 1 illustrate a significant decrease in the concentration of DPPH radical due to the strong scavenging ability of extracts and essential oils. These results indicate that the test drug samples have a noticeable effect on scavenging the free radicals. The most significant inhibition was shown by methanolic extract of leaves (70%) followed by essential oil of leaves (68%) and dry fruit oil (67.6%), which were found to inhibit the free radicals most. The ethanolic root extracts inhibited free radical generation to 64 % while flower and green fruit oil only showed moderate inhibition. The standard drugs BHA and ascorbic acid (500 µg/ml) inhibited free radical generation up to 86 and 88 % respectively. Ethanolic leaves and flowers extracts were found to have moderate activity while fruit extract showed very less inhibition in free radical generation.

The reducing capacity of a drug may serve as a significant indicator of its potential antioxidant activity. The reductive capability of essential oils and extracts was compared with BHA and ascorbic acid, which were used as standards. The reducing power (absorbance) was found to increase with increase with increase in concentrations of test samples as shown in Table 2. In comparison to standards, the test drugs which showed significant and maximum increase in absorbance were dry fruit oil, flower oil, ethanolic extract of leaves and methanolic extract of leaves. Ethanolic extract of flower also showed some significant activity while others fail to show good antioxidant activity. The maximum reducing power in terms of absorbance was exhibited by dry fruit oil and methanolic extract of leaves i.e. 1.45 and 1.24 respectively in comparison to standard values 1.41 (ASC) and 1.61 (BHA).

In conclusion it was found that all oils and extracts of *V. negundo* Linn. showed either very significant or good antioxidant effects except green fruit oil and ethanolic fruit extract in both of methods.

In MES model, the standard drug phenytoin (25 mg/kg *i.p*.) showed 100 % protection in animals against MES-induced seizures. It abolished the extensor phase completely ($P<0.001$). Ethanolic fruit extract abolished tonic MES-induced convulsions in 40 % of test animals and reduced the mortality rate to 50 % at the dose of 500 mg/kg while ethanolic extract of root abolished tonic MES-induced convulsions in 30 % of test animals and reduced the mortality rate to 55 % at the dose of 500 mg/kg as shown in Table 3.

The oral administration of ethanolic extract of leaves and flowers even at the dose of 500 mg/kg showed very less protection against maximal electroshock seizures (MES) and suppressed tonic

MES-induced convulsions only in 20% to 25% of test animals, reduced the extensor phase duration to only some extent & mortality rate was 60-65%. All extracts at the dose of 125 and 250 mg/kg failed to show any protection against tonic MES-induced convulsions and test animals showed almost all phases *i.e.* extensor, clonus or stupor.

All the extracts at sub-protective doses (100 mg/kg *p.o.*), which did not exhibit any anticonvulsant activity while simultaneous administration with standard drug dose (10 mg/kg/ *i.p.*) showed abolition of tonic MES-induced convulsions in 45 to 65 % of test animals and reduced the extensor phase duration significantly (P<0.001) as presented in Table 4.

In PTZ model, the standard drug Diazepam (1 mg/kg/*i.p.*) showed abolition of clonic phase in 75% of animals and significantly decreased its duration. The ethanolic fruit extract decreased the duration of clonic phase very significantly (P<0.001) at the dose of 500 mg/kg *p.o.* and abolished the clonic phase in 75% of animals. The mortality rate was reduced to 30% equal to standard drug. The leaves extract at the doses of 250 and 500 mg/kg *p.o.* produced significant (P<0.001) decrease in duration of clonic phase and abolished the clonic phase in 60 % and 70 % of animals respectively. The mortality rate was reduced to 35-40%. The flower and root extracts failed to show any significant protection even at highest dose of 500 mg/kg *p.o.* and reduced the clonic phase to some extent only and showed minor protection against clonic convulsions. The results have been presented in Table 5.

The simultaneous administration of ethanolic extract of leaves and fruit with diazepam at sub-protective doses abolished clonic phase duration significantly. However, ethanolic extract of flower and root showed only minor protection as shown in Table 4.11.

## DISCUSSION

From these studies, it was found that most of essential oils and ethanolic extracts of *V. negundo* showed significant antioxidant activity in both DPPH and ferric reducing power methods. The essential oils of leaves, flower and dry fruit showed significant antioxidant activity along with methanolic and ethanolic extract of leaves. So, it is concluded that essential oils as well as extracts especially leaves have strong antioxidant potential. If we compare anticonvulsant activity of ethanolic extracts, extracts showed better anticonvulsant activity against clonic convulsions in PTZ method. Ethanolic extracts of leaves along with fruit showed significant protection and abolished the clonic phase duration at 250 and 500 mg/kg doses. In MES method, only ethanolic extract of fruit showed some protection against extensor phase. Sub-protective studies of anticonvulsant activity showed very significant enhancement in protection against extensor and clonic phase in both MES and PTZ methods. At sub-threshold doses all the extracts potentiated the anticonvulsant action of standard drugs in MES and ethanolic extract of leaves and fruit in PTZ. It is reported that seizures induced by PTZ, can be blocked by drugs that reduces T type $Ca^{++}$ currents, such as ethosuximide and that enhance γ-aminobutyric acid type A (GABA) receptors mediated inhibitory neurotransmission such as benzodiazepines and Phenobarbital. Hence, *V. negundo* may be useful in seizures alone or in combination with standard anticonvulsants and can also be used to lower the dose of standard anticonvulsants.

**Table 1: DPPH radical scavenging activity of essential oils & extracts of *V.negundo***

| Test Samples | % Inhibition at different concentrations | | |
|---|---|---|---|
| | 125 µg/ml | 250 µg/ml | 500 µg/ml |
| LO | 64.6 ± 1.4 | 66.0 ± 0.5*** | 68.0 ± 0.5*** |
| FLO | 44.6 ± 0.3 | 49.0 ± 0.5 | 60.6 ± 1.2 |
| GFO | 41.6 ± 0.6 | 43.0 ± 0.5 | 50.3 ± 0.3 |
| DFO | 65.3 ± 0.8** | 65.6 ± 0.8** | 67.6 ± 0.8*** |
| EEL | 48.6 ± 0.5 | 50.0 ± 0.5 | 62.6 ± 1.6 |
| EEFL | 50.4 ± 1.4 | 55.4 ± 0.8 | 61.0 ±0.7 |
| EEF | 44.0 ± 0.2 | 47.4 ± 0.8 | 50.5 ±1.2 |
| EER | 56.8 ± 0.8 | 60.0 ± 0.6 | 64.6 ± 0.4** |
| MEL | 66.6 ± 1.3*** | 68.0 ± 0.4*** | 70.0 ± 0.6*** |
| AEL | 44.6 ± 0.3 | 48.0 ± 0.4 | 60.3 ± 0.2 |
| BHA | 78.3 ± 0.8*** | 83.3 ± 0.6*** | 86.3 ± 0.7*** |
| ASC | 78.2 ± 0.5*** | 85.4 ± 0.4*** | 88.7 ± 0.8*** |

Value expressed as mean ± SEM, *P‹0.05, **P‹0.01, ***P‹0.001 compared with control (one way ANOVA followed by Dunnett's't' test)

**Table 2: Reducing power activity of essential oils & extracts of *V.negundo***

| Test Samples | Absorbance | | |
|---|---|---|---|
| | 125 µg/ml | 250 µg/ml | 500 µg/ml |
| LO | 0.556±0.01 | 0.587±0.01 | 0.660±0.02 |
| FLO | 0.525±0.04 | 0.856±0.01** | 1.110±0.01*** |
| GFO | 0.513±0.03 | 0.568±0.03 | 0.669±0.02 |
| DFO | 0.512±0.03 | 0.699±0.01 | 1.450±0.01*** |
| EEL | 0.544±0.05 | 0.896±0.03** | 1.21±0.02*** |
| EEFL | 0.585±0.08 | 0.796±0.03** | 0.895±0.01** |
| EEF | 0.544±0.05 | 0.584±0.02 | 0.652±0.01 |
| EER | 0.656±0.04 | 0.677±0.03 | 0.690±0.01 |
| MEL | 0.599±0.07 | 0.674±0.05 | 1.240±0.04*** |
| AEL | 0.541±0.05 | 0.589±0.01 | 0.689±0.03 |
| BHA | 0.849±0.05** | 1.210±0.01*** | 1.610±0.02*** |
| ASC | 0.937±0.01** | 1.070±0.03*** | 1.410±0.07*** |

Value expressed as mean ± SEM, *P‹0.05, **P‹0.01, ***P‹0.001 compared with control (one way ANOVA followed by Dunnett's't' test)

**Table 3: Anticonvulsant activity of extracts (MES) of *V.negundo***

| Group (n=6) | Treatment | Dose mg/kg (*p.o*) | MES | | |
|---|---|---|---|---|---|
| | | | % of animals showing abolition of extensor phase | Mean±SEM Duration of Extensor phase (sec.) | Mortality % |
| Ia | N.S:Tween | 10 ml/kg | 00.00 | 19.3±0.39 | 100 |
| IIa | PHT | 25 *i.p* | 100.00 | 00.0±0.00*** | 00 |
| IIIa | EEL | 125 | 00.00 | 18.8±0.28 | 100 |
| IVa | EEL | 250 | 00.00 | 17.2±0.34 | 90 |
| Va | EEL | 500 | 20.00 | 15.5±0.34 | 65 |
| IIIb | EEFL | 125 | 00.00 | 18.0±0.29 | 95 |
| IVb | EEFL | 250 | 10.00 | 17.2±0.23 | 80 |
| Vb | EEFL | 500 | 25.00 | 15.0±0.30 | 60 |
| IIIc | EEF | 125 | 00.00 | 18.5±0.34 | 100 |
| IVc | EEF | 250 | 10.00 | 16.0±0.45 | 70 |
| Vc | EEF | 500 | 40.00 | 14.4±0.44** | 50 |
| IIId | EER | 125 | 00.00 | 18.8±0.29 | 100 |
| IVd | EER | 250 | 10.00 | 16.5±0.22 | 65 |
| Vd | EER | 500 | 30.00 | 15.0±0.24 | 55 |

Animals were divided into five groups (I-V) for studying anticonvulsant effect of the essential oil, n = No. of animals, *i.p* = intraperitonial, *p.o* = per orally, N.S = Normal saline, EEL = Ethanolic extract of leaves, EEFL = Ethanolic extract of flower, EEF = Ethanolic extract of fruit, EER = Ethanolic extract of root, PHT = Phenytoin, S.E.M.= Standard Error Mean. One way ANOVA and t-test was used for data expressed in Mean±SEM and mean difference was considered significant at the 0.001 level. ***P<0.001, **P<0.01, *P<0.1

**Table 4: Subprotective activity of extracts by MES of *V.negundo***

| Group (n=6) | Treatment | Dose mg/kg (*p.o*) | MES | | |
|---|---|---|---|---|---|
| | | | % of animals showing abolition of extensor phase | Mean±SEM Duration of Extensor phase (sec.) | Mortality % |
| Ia | N.S:Tween | 10 ml/kg | 00.00 | 19.3±0.39 | 100 |
| IIa | PHT | 10 *i.p* | 10.00 | 16.8±0.32 | 50 |
| IIIa | EEL | 100 | 00.00 | 18.2±0.24 | 90 |
| Iva | PHT+EEL | 10 *i.p*+100 | 55.00 | 13.0±0.33*** | 45 |
| IIIb | EEFL | 100 | 00.00 | 18.4±0.40 | 95 |
| IVb | PHT+EEFL | 10 *i.p*+100 | 45.00 | 13.3±0.50*** | 55 |
| IIIc | EEF | 100 | 00.00 | 18.0±0.20 | 90 |
| IVc | PHT+EEF | 10 *i.p*+100 | 65.00 | 11.0±0.34*** | 35 |
| IIId | EER | 100 | 00.00 | 18.1±0.30 | 90 |
| IVd | PHT+EER | 10 *i.p*+100 | 60.00 | 12.3±0.25*** | 45 |

Animals were divided into four different groups (I-IV) for studying interaction of the essential oil of *V. negundo* with phenytoin, n = No. of animals, *i.p* = intraperitonial, *p.o* = per orally, N.S = Normal saline, EEL = Ethanolic extract of leaves, EEFL = Ethanolic extract of flower, EEF = Ethanolic extract of fruit, EER = Ethanolic extract of root, PHT = Phenytoin, S.E.M.= Standard Error Mean. One way ANOVA and t-test was used for data expressed in Mean±SEM and mean difference was considered significant at the 0.001 level. ***P<0.001, **P<0.01, *P<0.1

**Table 5: Anticonvulsant activity of extracts (PTZ) of *V.negundo***

| Group (n=6) | Treatment | Dose mg/kg (*p.o*) | PTZ induced convulsion | | |
|---|---|---|---|---|---|
| | | | % of animals showing abolition of clonic phase | Mean±SEM Duration of clonic phase (sec.) | Mortality % |
| Ia | N.S:Tween | 10 ml/kg | 00.00 | 9.0±0.39 | 100 |
| IIa | DZ | 1 *i.p* | 75.00 | 4.2±0.29*** | 30 |
| IIIa | EEL | 125 | 25.00 | 7.0±0.25 | 85 |
| IVa | EEL | 250 | 60.00 | 5.1±0.20*** | 40 |
| Va | EEL | 500 | 70.00 | 4.8±0.33*** | 35 |
| IIIb | EEFL | 125 | 00.00 | 7.8±0.34 | 100 |
| IVb | EEFL | 250 | 25.00 | 7.2±0.40 | 80 |
| Vb | EEFL | 500 | 30.00 | 6.9±0.24 | 60 |
| IIIc | EEF | 125 | 00.00 | 8.0±0.20 | 90 |
| IVc | EEF | 250 | 35.00 | 6.5±0.30 | 75 |
| Vc | EEF | 500 | 75.00 | 4.3±0.20*** | 30 |
| IIId | EER | 125 | 00.00 | 8.8±0.30 | 90 |
| IVd | EER | 250 | 20.00 | 7.7±0.20 | 85 |
| Vd | EER | 500 | 30.00 | 6.8±0.38 | 60 |

Animals were divided into five groups (Ia-Va) for studying anticonvulsant effect of the essential oil, n = No. of animals, *i.p* = intraperitonial, *p.o* = per orally, N.S = Normal saline, EEL = Ethanolic extract of leaves, EEFL = Ethanolic extract of flower, EEF = Ethanolic extract of fruit, EER = Ethanolic extract of root, DZ = Diazepam, S.E.M.= Standard Error Mean. One way ANOVA and t-test was used for data expressed in Mean±SEM and mean difference was considered significant at the 0.001 level. ***P<0.001, **P<0.01, *P<0.1

**Table 6: Subprotective activity of extracts (PTZ) of *V.negundo***

| Group (n=6) | Treatment | Dose mg/kg (*p.o*) | PTZ induced convulsion | | |
|---|---|---|---|---|---|
| | | | % of animals showing abolition of clonic phase | Mean±SEM Duration of clonic phase (sec.) | Mortality % |
| Ia | N.S:Tween | 10 ml/kg | 00.00 | 9.0±0.39 | 100 |
| IIa | DZ | 0.5 *i.p* | 00.00 | 8.8±0.29 | 100 |
| IIIa | EEL | 100 | 00.00 | 7.9±0.29 | 95 |
| Iva | DZ+EEL | 0.5 *i.p*+100 | 60.00 | 4.8±0.25*** | 30 |
| IIIb | EEFL | 100 | 00.00 | 8.8±0.20 | 100 |
| IVb | DZ+EEFL | 0.5 *i.p*+100 | 40.00 | 6.0±0.25 | 65 |
| IIIc | EEF | 100 | 00.00 | 7.9±0.20 | 90 |
| IVc | DZ+EEF | 0.5 *i.p*+100 | 65.00 | 4.5±0.43*** | 35 |
| IIId | EER | 100 | 00.00 | 7.9±0.29 | 100 |
| IVd | DZ+EER | 0.5 *i.p*+100 | 40.00 | 6.0±0.45 | 65 |

Animals were divided into four different groups (Ib-IVb) for studying interaction of the essential oil of *V. negundo* with diazepam, n = No. of animals, *i.p* = intraperitonial, *p.o* = per orally, N.S = Normal saline, EEL = Ethanolic extract of leaves, EEFL = Ethanolic extract of flower, EEF = Ethanolic extract of fruit, EER = Ethanolic extract of root, DZ = Diazepam, S.E.M.= Standard Error Mean. One way ANOVA and t-test was used for data expressed in Mean±SEM and mean difference was considered significant at the 0.001 level. ***P<0.001, **P<0.01, *P<0.1

**ACKNOWLEDGMENT**

Authors are thankful to Forest Research Institute, Dehradun for identification and authentication of plant and its different parts as voucher specimen of the sample (Sr. No. 160/Flora of Haryana) deposited in the NWFP Herbarium collection at Forest Research Institute and College, Dehradun, India. Authors are also thankful to IAEC, GJUST, Hisar and IAEC, Kurukshetra University, Kurukshetra for giving approval to carry out animal experimentation.

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**Cite this article as:**

Sukhbir Lal Khokra *et al*. A study on neurological significance of *Vitex negundo* Linn. Int. Res. J. Pharm. 2018;9(9):130-136 http://dx.doi.org/10.7897/2230-8407.099200

Source of support: Nil, Conflict of interest: None Declared