**PHARMACOLOGICAL EVALUATION OF ANTI-CONVULSANT ACTIVITY OF ETHANOLIC EXTRACT OF *VITEX-NEGUNDO LINN* IN MICE**

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**Abstract:**

The main object of our present pharmacological evaluation of anticonvulsant activity of Ethanolic leaf extract of *vitex-negundo* as well as its interaction with standard drugs in sub-protective doses orally was studied to evaluate its potential role as an adjuvant therapy. In our study we have used two methods, fresh leaves of *vitex negundo linn* was powdered and macerated in 70% v/v Ethanol as solvent, the menstruum was collected and viscous extract suspended in 1% gum acacia for the present study.

Another method is Simple Distillation method by which two liquids with different boiling points can be separated, where pure liquid collected in a round bottom flask

Twenty number of Male Albino mice weight (20-25gm) were used to study the effect of test drug on MES method, the animals were fasted overnight before the experiment.

The animals were divided into four groups with each group consisting of five animals where Group-1 treated with normal saline 10 ml/kg, Group-II treated with Diazepam 5 mg/kg,Group-III were treated with *vitex negundo*(200mg/kg), Group-IV were treated with 400mg/kg.

The animals were chosen by preliminary screening, the seizures were induced by Maximal Electro Shock(**MES**) in Albino mice with the help of Electroconvulsiometer by passing current of 45mA for 0.2 sec using ear clip electrodes, the animals were observed for the extensor phase as well as its duration and post ictal depression. The abolition of extensor (tonic phase) in drug treated group was taken as criteria for anticonvulsant activity.

The result obtained from the study suggest the Ethanolic extract of vitex negundo Linn has anti-convulsant property and verified its traditional use in Epilepsy further phytochemical studies are identify the specific active compounds in their plant responsible for anti-convulsant activity.

**Key words:**

Maximal Electro shock, Electroconvulsiometer, Simple Distillation, *Vitex negundo Linn.*

**Introduction:**

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Most of the medicinal Plants contains numerous chemical compounds for functions, including defense against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Further, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassisted by rigorous scientific research to define efficacy and safety.

Vitex-negundo Linn (Lamiaceae), a large aromatic shrub with typical five foliolate leave pattern has been claimed to possess many medicinal properties. It is found throughout the greater part of India at warmer zones and ascending to an altitude of 15,000m in outer Western Himalayas. One of the ancient use of Vitex-negundo documented in ayurveda is to provide mental peace . Vitex-negundo has been extensively studied for its anti-inflammatory and analgesic activities in the past but, very few studies have been done to evaluate its anticonvulsant activity.

Thus, there are conflicting reports regarding anticonvulsant activity of Vitex-negundo and no one has previously studied anticonvulsant activity of Vitex-negundo by oral route. Moreover, nobody has yet documented the interaction with standard anticonvulsant drugs in sub protective doses by Vitex-negundo linn for evaluating its potential role as an adjuvant therapy.

Therefore, the present study was undertaken to investigate anticonvulsant activity of ethanolic leaf extract of Vitex-negundo as well as its interaction with standard anticonvulsant drugs in sub-protective doses per orally was studied to evaluate its potential role as an adjuvant therapy.

**EPILEPSY:**

It is a neurological disorder that affected a wide range of people throughout the world. It is a Disorder of brain characterized by unpredictable and periodic occurrence of a transient alteration of behavior due to the disordered superonous and rhythamic fixing at populations at brain neurons.

**MATERIALS AND METHODS**

**Plant material :**

The plant was identified, authenticated by an expert botanist Dr.M.Syed Ali Fathima, Assistant professor &Head of the department of Botany,Sadakathullah Appa College, Tirunelveli . The whole aerial part of the plant vitex negundo were collected collected in the month of September, 2019 from local area of Kavalkinaru, Tirunelveli district , Tamilnadu

**Maceration:**

The fresh leaves of Vitex negundo were shade dried and powdered. The powder was macerated for 7 days in 70% v/v ethanol. Then it was subjected to percolation using 70% v/v ethanol as solvent. The menstrum collected was again shade dried and viscous extract suspended in 1% gum acacia for the present anticonvulsant study. Total yield of extract was 9.5%.

**SIMPLE DISTILLATION METHOD:**

Simple distillation is a procedure by which two liquids with different boiling points can be separated.

**procedure:**

• Solution mixture is heated in a flask until the mixture boils

• Pure liquid turns into a vapor and leaves the flask

• Vapors is then cooled in a condenser which changes back into liquid

• Pure liquid collected is called the distillate and is collected in a flask.

**ANIMAL STUDY:**

**Animals :**

Albino Mice(Mus musculus) of weight (20–25 g) were used to study the effect of test drug on MES method respectively. Female animals were excluded because of the fact that estrus cycle influences the electroshock threshold. They were procured from Cape Bio Lab & Research centre, Marthandam, Kanyakumari dist- 629165. The clearance for the use of 20 animals for experimental purpose was obtained from Indian Animal ethical committee constituted for the purpose at SARPC, Vadakkangulam( Tirunelveli Dist). Animals were housed in polypropylene cages (4 per cage) with dust free rice husk as a bedding material under laboratory condition with controlled environment of temperature 25° ± 2°C, humidity (60% ± 10%) and 12 h light/dark cycle as per CPCSEA guidelines. They were provided with balanced food (Lipton India Ltd. pellets) and water ad-libitum , before subjecting them to experimentation, the animals were given a week time to get acclimatized with laboratory conditions. The animals were fasted overnight before the experiment.

**Drugs :**

**Standard drug:**

Diazepam was used for the present study in volume of 10 ml/kg/wt orally.

**Control:**

Normal saline is administered orally.

**Test drug :**

Ethanolic leaf extract of Vitex-negundo was used for the present study (10ml/kg orally).

The doses are 200mg/kg and 400mg/kg.

**Experiment design:**

**Anticonvulsant activity:**

The animals were divided into four groups with each group consisting of five animals.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.No.** | **Group-I** | **Group-II** | **Group-III** | **Group-IV** |
| 1. | Normal saline  (10ml/kg) | Diazepam  (5mg/kg) | *Vitex negundo* extract  (200mg/kg) | *Vitex negundo* extract  (400mg/kg) |

**Maximal electroshock induced seizures (MES) in Albino mice:**

The animals were chosen by preliminary screening. Mice which showed extension of hind limb were included in the study. The seizures were induced by maximal electroshock in albino mice with the help of electro-convulsiometer by passing current of 45 mA for 0.2 second using ear clip electrodes. The drugs and distilled water were given one hour prior to induction of convulsions. The animals were observed for the extensor phase as well as its duration and post-ictal depression. The abolition of extensor (tonic phase) in drug treated group was taken as criteria for anticonvulsant activity.

**PRELIMINARY PHYTO-CHEMICAL STUDIES:**

The extracts were then subjected to quantitaive phytochemical screening for the identification of the phytoconstituent while petroleum ether ,benzene, chloroform does not show any appreciable tests for the presence of different phytoconstituents, ethanolic extract showed positive tests for the presence of glycosides, flavanoids and alkaloids.

**CHEMICAL CONSTITUENTS:**

**Alkaloidal :**

Qualitative analysis of alkaloids. 1ml of the extract was taken and 1ml of Mayer’s reagent was added to that in a test tube and this mixture was allowed to stand for some time to develop colour and results were recorded. Development of cream colour indicates the presence of alkaloids.

**Flavanoids:**

The stock solution (1 mL) was taken in a test tube and added few drops of dilute NaOH solution. An intense yellow colour was appeared in the test tube. It became colourless when on addition of a few drops of dilute acid that indicated the presence of flavonoids.

**Glycosides:**

Qualitative analysis of glycosides. To 1ml of the extract, 1ml of the α – napthol was added to which chloroform was added along the sides and it was looked for the development of colour and the result was recorded. Development of violet colour indicates the presence of glycosides.

**Steroids present:**

The crude plant extracts (1 mg) were taken in a test tube and dissolved with chloroform (10 mL), then added equal volume of concentrated sulphuric acid to the test tube by sides. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence.

**EVALUATION:**

Seizure reduction effect compared with standard and test:

• **Tonic flexion**

**• Tonic extension**

**• Clonic convulsion**

**• Stupor**

**• Recovery or Death**

**:**

**STATISTICAL ANALYSIS**

The data were expressed as mean ±S.E.M or percentage. One-way analysis of variance (ANOVA) followed by post hoc Dunnett’s multiple comparison test was used for data expressed in mean ± S.E.M, using sigma stat software (version 2.0, jandel scientific Inc. USA).

**RESULTS:**

As shown in the table .no.1. ethanolic extract of vitex nigundo at doses of 200 and 400 mg/kg and Diazepam 5mg/kg have shown significant reduced the onset and duration of convulsions.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **TREATMENT** | **FLEXON** | **EXTENSOR** | **CLONUS** | **STUPOR** |
| Control | 1.5± 0.8 | 1 ±0.5 | 1.5± 0.8 | 24.6 ±14.24 |
| Standard | 1.5± 0.7 | 2.1± 1.0 | 1.7 ±0.8 | 6.3± 3.1 |
| Test-1 | 0.5± 0.2 | 2.1 ±1.0 | 2.9± 1.4 | 13.1 ±6.5 |
| Test-2 | 0.5± 0.2 | 2.0 ±1.0 | 0.8± 0.4 | 8.6± 4.3 |

All values expressed as mean ± SEM; n=4, by one way ANOVA

**Table-1**

**DISCUSSION :**

It was found from the above observations that ethanolic extract of 'vitex negundo'has been shown anti-convulsant activity aganist seziures induced by MESin a dose dependent manners

It was effective aganist MES induced seziures ,since inhibition of the MES test predicts activity aganist generalised tonic flexion,tonic extension.clonic convulsion, stupor.

**CONCLUSION:**

Therefore ,the results obtained from the study ,suggest the ethanolic extract of vitex negundo linn has anti-convulsant property and the results verify its traditional use in epilepsy further phytochemical studies are in progress to isolate, characterize and identify the specific active compounds in their plant responsible for anti-convulsant activity.

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TREATMENT

FLEXON

EXTENSOR

CLONUS

STUPOR

Control 1.5± 0.8 1 ±0.5 1.5± 0.8 24.6 ±14.24

Standard 1.5± 0.7 2.1± 1.0 1.7 ±0.8 6.3± 3.1

Test – 1 0.5± 0.2 2.1 ±1.0 2.9± 1.4 13.1 ±6.5

Test - 2 0.5± 0.2 2.0 ±1.0 0.8± 0.4 8.6± 4.3

All values expressed as mean ± SEM; n=4, by one way ANOVA