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(Research Article)

Evaluation of Anticonvulsant Activity of Ethanolic Extract of *Vitex nigundo* in Swiss Albino Rats

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ABSTRACT

Vitex-negundo Linn (Verbenaceae), a large aromatic shrub with typical five foliolate leaves pattern has been claimed to possess anticonsulvant activity apart from many medicinal properties. Maximal electroshock seizures (MES) in albino rats were used to study anticonvulsant activity of Vitex-negundo leaf extract. The ethanol leaf extract of Vitex-negundo was administered orally in graded doses (500 and 1000 mg/kg and 2000mg/kg p.o) in the experimental model and the effects were compared with Diphenylhydantoin in MES method as standard and normal saline as control. The Vitex-negundo in the doses 1000 mg/kg has significant effect and 2000mg/kg p.o showed protection against MES to a highly significant extent. Test drug in the dose (1000 mg/kg, po) showed 60% protection in clonic seizures. It also decreased number and duration of convulsions significantly.These findings suggested that Vitex-negundo possesses anticonvulsant activity against MES induced convulsions. Vitex-negundo may be useful as an adjuvant therapy along with standard anticonvulsants and can possibly lower the requirement of Diphenylhydantoin and other anti convulsant drugs, Ethanol leaf extract of vitex ningundo significantly (P<0.01) decreased the duration of tonic extensor phase in MES-induced seizures. The Vitex-negundo extract showed a maximum inhibition (80% mortality) against MES-induced seizures.Thus, it has been concluded that ethanolic extract of Vitex-negundo possesses anti-epileptic activity.

Key Words: Antiepileptic,*Vitex-negundo*, Electroconvulsiometer, Maximum Electroshock (MES), Verbenaceae

INTRODUCTION

Vitex negundo Linn. (Family : Verbenaceae) 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 thrives in humid places or along water courses in wastelands and mixed open forests and 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 15,00m in outer Western Himalayas[1]. One of the ancient use of *Vitex-negundo* documented in Ayurveda is to provide mental peace[2]. *Vitex-negundo* has been extensively studied for its anti-inflammatory[3-5] and analgesic[1,3,5,6]activities in the past but, very few studies have been done to evaluate its anticonvulsant activity and there are conflicting reports regarding anticonvulsant activity of *Vitex-negundo* and no one has previously studied anticonvulsant activity of *Vitex negundo* by oral route except one study[7].Therefore, the present study was undertaken to investigate anticonvulsant activity of ethanol leaf extract of *Vitex negundo* in comparison with standard anticonvulsant drug in sub-protective doses per orally was studied to evaluate its potential role as an adjuvant therapy.The study was carried out in the Department of Pharmacology in the month of June-July 2011.

MATERIALS AND METHODS

Plant Material

The leaves of *Vitex nigundo* were obtained from a field at Khammam district of Andhra Pradesh, India in the month of May 2011.The plants were authenticated by the Head of the Department of Botany, Government Degree College, Khammam. The voucher herbarium specimen number is 717-2011 and it has been preserved in the college museum.

Extract Preparation

The leaves of *Vitex nigudo* was dried in air, crushed to coarse powder and extracted with ethanol using Soxhlet apparatus. The extract was dried under vacuum, stored at room temperature and protected from direct sunlight in the Department of Pharmacology, Mamata Medical College, Khammam.

Drugs and Chemicals

Phenytoin (Anglo-French Drugs & Industries Ltd., Banglore) 25mg/kg and normal saline (0.9% NaCl solution) were administered in the volume of 10 ml/kg. The extracts were suspended in ethanol and subjected for anticonvulsant activity using MES models respectively. The extracts were administered orally (p. o.) in the volume of 10 ml/kg of body weight.

Experimental Animals

Swiss Albino rats of the either sex weighing 120 to150 gm were used. The animals were housed in standard cages with free access to food (standard laboratory pellet diet) and water. The animal house temperature was maintained at 23 ± 5.0^0C with a 12-h light/dark cycle. Permission from Institutional Animal Ethics Committee was taken. The guidelines for the investigation of experimental seizures in conscious animals were followed in all.

Acute Toxicity Studies

Preliminary acute toxicity study of ethanol leaf extract in albino rats by oral route carried out in a study[8] indicated it to be practically non-toxic as its LD50 dose recorded was 7.58g/kg/wt. The stomach showed no histomorphological changes in any of the dose of extract studied.Depending on this we have taken the dosages of 500mg, 1000mg and 2000mg/kg body weight and conducted the studies.

MES-Induced Seizures

Corneal electrodes were used for bilateral delivery of electrical stimulus (Maximal Electroshock Seizures, MES-50mA; 50Hz; 0.2 Sec). Convulsive shock including Hind Limb Tonic Extension (HLTE) in 99% of the animals[9] was previously determined. The time of peak effect of Phenytoin as 30 min after administration was previously established[10]. The time for the extract to reach its maximum effect was determined as 60 min after oral administration. The incidence and duration of extensor tonus was noted. A complete abolition of hind limb tonic extension (HLTE) in 99% of the animals[11] was previously determined. Intensity of stimulus was 150mA, 50Hz for 0.2 second duration was applied through corneal electrodes using electroconvulsiometer for five groups of 10 rats each, in which one control were pre-treated with normal saline (0.9% NaCl solution, 10 ml/kg p.o.), one standard with phenytoin as positive control (25 mg/kg, oral) and three groups pre-treated with 500, 1000, and 2000 mg/kg, p.o. of ethanol extract of *Vitex nigundo* leaf.Duration of various phases of epileptic attacks were recorded and compared with the control and phenytoin group. All precautions were taken to minimize animal suffering and to reduce the number of animal used.

RESULTS

Evaluation was made by electro-shock using ear electrodes after 1hr of administration of extract. Dose dependent effect of graded dose (500, 1000, 2000mg/kg, p.o.) of extract on MES-induced seizures was seen in rats. Ethnolic leaf extract of *Vitex nigundo* at 1000 mg/kg dose significantly ($P<0.01$) decreased the duration of tonic extensor phase in MES-induced seizures. Where as at 2000mg/kg dose the decrease in the duration of tonic extensor phase is highly significant ($p<0.000$) and the extract also showed a maximum inhibition (80% mortality) against MES-induced seizures. The observations are shown in Table 1.

DISCUSSION

Epilepsy is a chronic common neurological disorder that affects people of all ages. Around 50 million people worldwide have epilepsy. Nearly 90% of the people with epilepsy are found in developing regions.[12] The prevalence is 5-10/1000 persons with higher incidence in infants.[13] Epilepsy is often progressive disorder

characterized by the periodic and unpredictable occurrence of epileptic seizures which are caused by an abnormal discharge of cerebral neurons. The definition of epilepsy requires occurrence of at least one epileptic seizure.[14] Many different types of seizures can be identified on the basis of their clinical phenomena.[15] Seizures are broadly categorized into two types: partial and generalized seizures[16]. Partial (focal, local) seizures are those in which clinical or electrographic evidence exists to suggest that the attacks have a localized onset in the brain, usually in a portion of one hemisphere, while generalized seizures are those in which evidence for a localized onset is lacking. Partial seizures are further subdivided into simple partial, complex partial and partial seizures evolving to secondarily generalized seizures, while generalized seizures are categorized into absence (nonconvulsive), myoclonic, clonic, tonic, tonic-clonic and atonic seizures. In addition to classifying the seizures that occur in patients with epilepsy, patients are classified into appropriate types of epilepsy or epileptic syndromes characterized by different seizure types, etiologies, ages of onset and electroencephalographic (EEG) features.

However, despite several anti-epileptic drugs both old and novel, many patients have intractable epilepsy or epilepsy with intolerable side effects. Better understanding of processes leading to epilepsy is required to create therapies aimed at prevention of epilepsy in patients at risk. Further there is need to develop disease modifying therapies which could halt the progression of epilepsy.[17] The discovery of novel antiepileptic drugs (AEDs) relies upon the preclinical employment of animal models to establish efficacy and safety prior to the introduction of the AEDs in human volunteers. Clearly, the more predictive the animal model for any given seizure type or syndrome, the greater the likelihood that an investigational AED will demonstrate efficacy in human clinical trials.[18] Thus, many plants were known for their anticonvulsant activity. Various physiochemical and pharmacological studies have been carried out on these anticonvulsant plants[19]. Currently available anticonvulsant drugs are able to efficiently control epileptic seizures in about 50% of the patients; another 25% may show improvement where as the remainder does not benefit significantly[20]. Furthermore, undesirable side effects from the drugs used clinically often render treatment difficult,so, a demand for new types of anticonvulsants exists. One of the approaches to search for new antiepileptic drugs is the investigation of naturally occurring compound, which may belong to new classes. In the present study the antiepileptic action of *Vitex nigundo* extract was evaluated in Swiss albino rats.

Herbal medicine is the oldest form of health care known to mankind and it will not be an exaggeration to say that use of herbal drug for human health care is probably as ancient as mankind. A perfect example of medicinal plant credited with innumerable medicinal qualities validated by modern science and used since ancient times is *Vitex negundo* Linn (Family : Verbanaceae).The genus consist of 250 species of which about 14 species are found in India and some have commercial and medicinal importance. *Vitex negundo* linn, known as five leaved chaste tree or Monk's pepper (Hindi- Sambhalu Nirgundi) is used as fairly throughout greater part of India and found mostly at warmer zones and ascending to an altitude of 1500m in outer western Himalayas[1]. Plant is bitter, acrid , astringent, cephalic, stomachic, antiseptic, alterant, thermogenic, depurative, rejuvenating, ophthalmic, anti-gonorrheal, anti-inflammatory, antipyretic and useful in bronchitis, asthma and enlargement of spleen. Roots are tonic, febrifuge, anti-rheumatic, diuretic, expectorant and are useful as demulcent in dysentery, in cephalagia, otalgia, colic uropathy wound and ulcer. Bark is useful in odontalgia, verminosis and opthalmopathy. Leaves are bitter, acrid, aromatic, astringent, anodyne, anti-inflammatory, anti-pyretic, or febrifuge, tranquillizer, bronchi smooth muscle relaxant, antihelemintic, anti-arthritic and vermifuge. Flowers are cool, astringent, carminative, hepatoprotective, digestive, febrifuge, vermifuge, and are useful in hemorrhages and cardiac disorder. Fruit is nervine, cephalic, aphrodisiac, emmenagogue and vermifuge. Chemical constituents of leaves contain an alkaloid nishindine, flavanoids like flavones, luteolin-7-glucoside, casticin, iridoid glycosides, an essential oil and other constituents like vitamin C , carotene, gluco-nonital, benzoic acid, β-sitosterol and C-glycoside[21]. A studyof analgesic activity reveled that intra peritoneal (i.p.) administration of some leafs and some root extract using different solvents showed some analgesic activity[22]. The experimental studies using various animal models have demonstrated that different parts of the plants especially leaves, roots and seeds possess anti-inflammatory, anti-arthritic activity[23,24].

The plant has been studied for its anti-convulsant activity. The petroleum and butanol leaf extract have shown protection against maximum electro shock (MES) seizures. Petroleum root extract could only provide protection against leptazole induced convulsions[22]. In our study ethanol leaf extract of *Vitex ningundo* has shown protection against maximum electro shock (MES) seizures and where as some studies[6,25] suggested anticonvulsant activity of ethanolic leaf extract of this plant but also indicated that it can potentiate the effect of standard anticonvulsant, which may help to reduce dose and dose related side effects of standerd anticonvulsants.

CONCLUSION

Vitex negundo possesses numerous biological activities proved by many experimental studies. The present study revealed that ethanol leaf extract of *Vitex negundo* possess anticonvulsant effects in Swiss Albino Rats and also reduce mortality. The present work did not include the identification of the active principal and its mechanism of action. Therefore, further research should be carried out to identify the active principal and elucidate the exact mechanism of action.

Table 1: Effect of ethanol leaf extract of *Vitex negundo* on MES Induced Seizures in Rats

Treatment	Duration of tonic extension	Mortality D/N	% Protection
Group I (NS-10ml/kg)	18.8± 1.98	10/10	0%
Group II (Phenytoin-25mg/kg)	1.4±1.4 P=0.000	0/10	100%
Group III (vn-500mg/kg)	5±5 P=0.033*	6/10	40 %
Group IV (vn 1000mg/kg)	5 ± 3.52 P= 0.009*	4/10	60%
Group V (vn 2000mg/kg)	0 ± 0 P=0.000**	2/10	80 %

vn- *Vitex negundo*, NS – Normal saline, D/N – Number of death/Number of animals,
*P<0.01 –Significant , P=0.000**- Highly significant when compared to Group I.
All values are expressed as MEAN ± S.E.M.

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