PHYTOCHEMICAL SCREENING AND ANTICONVULSANT ACTIVITY OF ALCOHOLIC EXTRACT OF ROOT OF VITEX NEGUNDO

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ABSTRACT

Vitex negundo (Verbenaceae), popularly known as "Nirgundi" in Hindi and "Five leaved chaste tree" in English is widely distributed throughout in India. Almost all parts of the plant are used in the Ayurvedic and Unani system of medicines. Vitex negundo is used for dispelling inflammatory swelling of joints from acute rheumatism, healing wounds, ulcers and different bacterial infections. It is also used in treatment of neuropharmacological disorders like convulsions as a traditional drug. The aim of the present study was to carry phytochemical screening for the identification of various phytoconstituents and anticonvulsant activity of alcoholic extract of root of Vitex negundo. The alcoholic extract was subjected to qualitative phytochemical screening for the identification of different phytoconstituents. The alcoholic extract showed the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, saponins and sterols. The anticonvulsant activity of alcoholic extract of root of Vitex negundo at a dose level of 250, 500 & 750 mg/kg b.w., i.p. was performed in mice by using electroshock and PTZ methods. The standard was taken as phenytoin (25mg/kg b.w., i.p.). The alcoholic extract at dose level of 750 mg/kg has shown comparable activity to that of phenytoin.

KEY WORDS : Vitex negundo, Nirgundi, Anticonvulsant activity, Maximal electroshock seizure model, PTZ, Phenytoin

INTRODUCTION

Epilepsy is a neurological disorder that affects 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, synchronous and rhythmic firing of populations of brain neurons.[1] Incidence of epilepsy in developed countries is approximately 50 per 100,000 while that of developing country is 100 per 100,000.[2] It affects approximately 50 million people Worldwide.[3] According to several publications this can amount to 70% of the people with epilepsies, with a high prevalence of about 0.8% in children below the age of seven years.[4] All the currently available antiepileptic drugs are synthetic molecules.[5] These observations have led to a shift in focus to the use of herbal remedies in the management of epileptic seizures, probably because these measures fit into the cultures of people and are not usually as expensive as the more refined orthodox drugs. Besides, these orthodox drugs possess many side effects, contraindications and possible interactions with drugs used simultaneously. The alternative drug therapy for the management of this disease can be by the use of medicinal plants and their active principles

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Vitex negundo (verbenaceae), popularly known as "Nirgundi"in Hindi and "Five leaved chaste tree" in English widely distributed throughout in India. Almost all parts of plant are used in the Ayurvedic and Unani system of medicines.[6,7] *Vitex negundo* (Verbenaceae), is used for dispelling inflammatory swelling of joints from acute rheumatism, healing wounds, ulcers and hepatic disorders. Hence it was thought worthwhile to investigate the anti epileptic activity of alcoholic extract of roots of *Vitex negundo.*

MATERIAL AND METHODS

Plant material

The roots of *Vitex negundo* were collected from Ganeshpur village, Saharanpur, India and identified by Dr. Anjula Pandey, Taxnomist, National Bureau of Plant Genetic Resources (NBPGR), Pusa campus, New Delhi. A voucher specimen (HS-19710) is

preserved in the herbarium section of taxonomic department of NBPGR, New Delhi.

Plant extract

The air dried roots (2.5 kg) were coarsely powdered and then about 2.5 kg material was defatted with petroleum ether (60-80°C) and then extracted with alcohol (95%). The extract was dried under vacuum (yield 12.6%). The alcoholic extract was screened for the anticonvulsant activity.

Preliminary Phytochemical Studies

The alcoholic extract was then subjected to qualitative phytochemical screening for the identification of different phytoconstituents. The alcoholic extract showed the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, saponins and sterols.

Preparation of suspension of extract

Dried alcoholic extract was suspended in a solution of normal saline (0.9% w/v) and tween 20 (95:5) and subjected for anticonvulsant activity.

Animals

Swiss albino mice weighing 18-25 g of either sex were used for the study. The animals were procured and housed in the animal house of Teerthanker Mahaveer College of Pharmacy, Moradabad at least 2 weeks prior to the study, so that they could adapt to the new environment. Animal house was well maintained under standard hygienic conditions, at 22 ± 2ºC, humidity (60 ± 10 %) with 12 hrs day and night cycle, with food and water *ad libitum*. Mice were housed in groups of 6 per cage. Cleaning and sanitation was done on alternate days. Paddy husk was provided as bedding material which was cleaned every day. The cages were maintained clean. The study was carried out as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) norms after obtaining approval from the Institutional Animal Ethical Committee of Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad.

ASSESSMENT OF ANTICONVULSANT ACTIVITY

Maximal electroshock seizure model

Maximal electroshock seizure model was used to evaluate the anticonvulsant activity of ethanolic extract. Seizures were induced in mice by delivering electroshock of 50 mA for 0.2 seconds by means of an electro-convulsiometer through a pair of ear clip electrodes.[8] The test animals (n=6) received 250, 500, 750 mg/kg i.p. of extract and standard group received phenytoin (25 mg/kg)[9] injected i.p. and tested 30 minutes after for MES seizure response. All the experimental groups were compared with the control treated with vehicle.

PTZ-induced seizures

PTZ at the dose of 80 mg/kg (minimal dose needed to induce convulsions) was injected i.p. to induce clonic-tonic convulsions in mice. The test animals (n=6) received 250, 500, 750 mg/kg of alcoholic extract i.p. and standard group received phenytoin (25 mg/kg)[10] injected i.p. PTZ was injected i.p. 60 min after the administration of drugs. Occurrence of HLTE and duration of seizures were noted. If no HLTE occurred during the time limit, the animals were considered protected.

RESULTS

Details of various phytochemical constituents present or absent in alcoholic extract of roots of *Vitex negundo* has been shown in Table 1, in which the extract showed the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, saponins and sterols.

Table 1: Qualitative tests of preliminary phytochemical screening of alcoholic extract of *Vitex negundo*:

S.No.	Constituents	Presence/ Absence
1	Alkaloids	+
2	Carbohydrates	+
3	Glycosides	+
4	Phenolic compounds	+
5	Proteins and Free amino acids	–
6	Saponins	+
7	Sterols	+
8	Acidic compounds	–

The anticonvulsant activity of alcoholic extract at various dose levels viz, 250, 500 and 750 mg/kg i.p. was studied by the maximum electroshock-induced and pentelenetetrazole seizure models.

The anticonvulsant activity induced by MES model of the alcoholic extract of *Vitex negundo* has

been shown in Table 2, in which the alcoholic extract at dose level of 750 mg/kg has shown comparable activity as Phenytoin (standard) whereas the alcoholic extract at 250 and 500 mg/kg has also shown activity but less significant than standard.

In PTZ induced seizures, the administration of *Vitex negundo* alcoholic extract at a dose of 750 mg/kg one hour prior to the injection of PTZ, significantly ($p<0.01$) delayed the onset of convulsions Table 3. The alcoholic extract at the dose of 250 and 500 mg/kg body weight could exert little significant protective effect on PTZ induced convulsions. Phenytoin in a dose of 25mg/ kg, totally abolished the episodes of convulsions. Alcoholic extract at the dose level of 750 mg/kg body weight showed significant antiepileptic activity.

Table 2: Effect of alcoholic extract of *Vitex negundo* on Hind limb extension in mice

S. No.	Group	Dose (mg/kg)	Hind limb extension at different dose levels (Sec)
1	Control	-	12.33 ± 1.25
2	Phenytoin	25	0.66 ± 0.49[a]
3	VNEE	250	5.83 ± 0.60[a]
4	VNEE	500	4.66 ± 0.66[a]
5	VNEE	750	2.50 ± 0.76[a]

Values are expressed as mean ± SE (n=6); [a]$p<0.001$ as compared to control

Table 3: Effect of alcoholic extract of *Vitex negundo* on PTZ induced seizures in mice.

S. No.	Group	Dose (mg/ kg)	Onset Time (Sec)	Duration of HLTE (Sec)
1	Control	-	49.40±0.24	35.68±0.28
2	Phenytoin	25	0±0[a]	0±0[a]
3	VNEE	250	53.60±0.20[a]	32.46±0.32[a]
4	VNEE	500	55.35±0.16[a]	30.80±0.53[a]
5	VNEE	750	57.31±0.20[a]	28.21±0.56[a]

Values are expressed as mean ± SE (n=6); [a]$p<0.001$ as compared to control

DISCUSSION

The observations emanated in the present study indicated that the alcoholic extract was without any lethal effect in a dose up to 750 mg/kg and possessed anticonvulsant activity against seizures induced by MES and PTZ in a dose dependent way. It was effective against MES induced seizures, since inhibition of the MES test predicts activity against generalized tonic-clonic and cortical focal seizures. Alcoholic extract of *Vitex negundo* was also active against PTZ induced seizures. PTZ induced seizure is analogous to petitmal type of seizures and human generalized seizures.[11] Drugs that are effective against petitmal seizures reduce T- type calcium currents and these types of seizures can also be prevented by drugs that enhance $GABA_A$ – BZD receptor mediated neurotransmission such as benzodiazepines and phenobarbitone.[12] Studies have shown that activation of N- methyl D- aspartate receptor (NMDA) are also involved in the initiation and generalization of PTZ induced seizures.[13,14] Anticonvulsant activity of *Vitex negundo* may be attributed to either one or more of the above mechanism.

CONCLUSION

The alcoholic extract of *Vitex negundo* exhibited anticonvulsant activity in experimental animal models. The results of this study provide support for the traditional use of *Vitex negundo* as an anticonvulsant drug. Phytochemical screening has shown the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, saponins and sterols in alcoholic extract. The potent activity may be attributed to the presence of these phytoconstituents. More detailed studies are, however, necessary to identify the active principle(s) and exact mechanism of action.

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REFERENCES

1. McNamara, J.O., 2001. Drugs effective in the therapy of the epilepsies. In: Hardman, JG, Limbird LE (eds). The pharmacological basis of therapeutics. 10th ed., NewYork, McGraw-Hill, pp. 521-539.
2. WHO, 2006. Epilepsy: Etiology, epidemiology and

prognosis.www.who.int/entire/mediacentre/factsheets/fs165/en/-25k-16jan.

3. Fisher, R., W. van Emde Boas, W. Blume, C. Elger, P. Genton, P. Lee, J. Engel, 2005. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia, 46: 470-472.
4. Ndoye N.F., 2005. Eur. J. Epilepsy, 14: 7.
5. Gauthaman, K., B. Hema, S. Bhupendra, T.S. Mohamed Saleem, 2009. Anticonvulsant Effect of *Drosera burmannii* Vahl. International Journal of Applied Research in Natural Products, 2(3): 1-4
6. Sharma P.C., M.B. Yelne, T.J. Dennis, 2005. Data base on Medicinal Plants used in Ayurveda. Published by Central Council for Research in Ayurveda Siddha. Government of India, 3: 450-453.
7. Anonymous, 2001. The Ayurvedic Pharmacopoiea of India: part-1, first edition. published by Department of Indian system of Ministry and Health and family welfare. Government of India, 3:142-144.
8. Kumar, S., Madaan R., Sharma A., 2008. Pharmacological evaluation of bioactive principle of *Turnera Aphrodisiaca*. Indian J. Pharm. Sci., 70(6): 740-744.
9. Manigauha, A., S. Patel, J. Monga, H. Ali, 2009. Evaluation of anticonvulsant activity of *Pongamia pinnata* Linn.in experimental animals. Internation J Pharma Tech. Res, 1(4): 1119-1121.
10. Thirupathi, K., D.R. Thirupathi, B. Krishna, K. Ravi, P. Tirumala Rao, G. Krishna Mohan, 2009. Anticonvulsant Activity of Pericardium Extract of *Balanites Roxburghii* Planch. in Mice. Pharmacologyonline, 1: 1150-1157.
11. Loscher, W., J.E.P. Schmid, 1988. Which animal model should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. Epilepsy Research, 2: 145-181.
12. Mcdonald, R.L., K.M. Kelly, 1995. Antiepileptic drug mechanisms of action. Epilepsia, 36: 2-12.
13. Nevis, M.E., S.M. Arnolde, 1989. A comparison of the anticonvulsant effects of competitive and non- competitive antagonists of the N-methyl D- aspartate receptor. Brain Research, 503: 1-4.
14. Velisek, L., R. Kusa, M. Kulovana, P. Mares, 1990. Excitatory amino acid antagonists and pentylene tetrazole- induced seizures during ontogenesis. I. The effects of 2- amino- 7-phosphonoheptanone. Life Science, 46: 1349-1357.