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Antiepileptic activity for methanolic extract of *Vitex Negundo* leaf against different animal models

K Pavan Kumar*, G Vidyasagar[2], D Ramakrishna[1], I Madhusudhana Reddy[3] and VSSS Gupta Atyam [4]

[1]*Department of Pharmaceutical Sciences, JJT University, Jhunjhunu, Chudela, Rajasthan*
[2]*Department of Pharmaceutical Sciences, Veerayatan institute of pharmacy, Bhuj, Jakhania Kutch, Gujarat*
[3]*Department of Pharmaceutical Chemistry, Malla Reddy College of Pharmacy, Dhulapally Village, Secunderabad*
[4]*Department of Pharmacology, Joginpally B R College of Pharmacy, Yenkapally, Hyderabad, India*

ABSTRACT

The present study is to evaluate antiepileptic activity of methanolic extract of vitex negundo leaf on maximal electroshock (MES), Pentylenetetrazole, Strychnine, Picrotoxin and lithium-pilocarpine induced convulsions in mice. The extract protected animals against MES induced convulsions and showed potentiating effect against different chemical induced convulsions against standard drugs. Methanolic extract of vitex negundo may be interfering with inhibiting Na^+ Channels and interfering with gabanergic mechanism is due to the presence of flavanoids attributed to their activity in a dose dependant manner. Screening results indicate methanolic extract of vitex negundo may be showing possible efficacy potential in the treatment of epilepsy.

Key words: Vitex negundo, methanolic extract, different animal models.

INTRODUCTION

Epilepsy is a common neurological abnormality affecting about 1% of world population. A seizure means paroxysmal abnormal discharge at high frequency from aggregate of neurons in cerebral cortex. Epilepsy is a condition characterized by recurrent episodes of such seizures.

Inspite of several advancements in the field of synthetic drug chemistry plants continue to be one of the major raw materials for drugs treating various ailments of humans, plant remedies are effective and without side effects provided they are selected properly [1-2]. Herbal medicine, rather than merely curing a particular disease, aims at returning the body back to its natural state of health the phytochemical components of medicinal plants often act additively and synergistically in improvement of health [3]. Folklore systems of medicine continue to serve a large segment of population, especially those in rural and tribal areas, regardless of the advent of modern medicine, but experimental evidence to evaluate the efficacy of these phytochemical moieties as drugs should be proved by screening methods [4].

Vitex negundo is a woody aromatic shrub belonging to family verbenaceae bearing tri or penta-foliate leaves on quadrangular branches [5]. A number of pharmacological activities have been attributed to vitex negundo such as gastro protective [6], protection against human liver cells against calcium mediated toxicity [7], hepatoprotective activity [8]. Phytochemical screening results of vitex negundo leaf revealed the presence of flavanoids [9], flavones glycosides [10], diterpenes [11], triterpenes [12], and esqueterpenes [13]. Vitex negundo possess a variety of medicinal uses, on view of this the present study is to evaluate methanolic extract of vitex negundo leaf for antiepileptic activity.

EXPERIMENTAL SECTION

The leaves were collected from commercial source. The collected leaves were cleaned of extraneous matter shade dried and powdered until a constant weight was attained. The powder was macerated for 48 h in methanol; it was subjected to percolation by using methanol as a solvent. The menstrum collected and concentrated under reduced pressure. The dried extract was stored in refrigerator until ready for use.

Animals

Swiss albino mice weighing 20-25 g and male Albino Wistar rats weighing 125-150 g were selected for the present study, procured two weeks prior to the study and maintained in institutional animal house, so that animals could acclimatize to the new environment. The institutional animal ethics committee permission was obtained (1217/a/08/CPCSEA) before starting the experiments on the animals. Animals were housed in groups of 6-8 cages at a temperature of 25 ± 1^{o} C and relative humidity of 45-55%. A 12:12 (dark: light) cycle was followed during the experiments and experiments. Animals had free access to food and water however, food but not water was withdrawn 8h before and during the experiments.

Acute oral toxicity studies

Thirty Albino mice & rats of both sexes were randomly divided to four groups consisting of the control and *vitex negundo* treated groups. Mice & rats were fasted for 12 h and various doses of extract were administered orally to test groups. The mice & rats were closely observed for toxic symptoms and behavioral changes for the first 2 h after extract administration and mortality recorded within 24 h.The lethal dose that killed 50% of mice and rats was estimated after 24h

Maximum electroshock induced seizures (MES)

The maximal electroshock (MES) method was performed to induce the seizures in order to screen for antiepileptic activity [14-15]. Mice deprived of food and water ad libitium for overnight, were randomly distributed in to four groups of six animals each. Group 1 served as control(vehicle treated),Group II served as standard (received Phenytoin sodium 20 mg/kg body weight);Group III and Group IV were treated with methanolic extract of *vitex negundo* as 200 and 400 mg/kg body weight. The test extract were administered orally in 1% v/v Tween 80,1hr prior to induce the convulsion and standard drug (Phenytoin sodium 25mg/kg) was administered i.p.30 min before. Electroconvulsive shock (50 mA for 0.2 sec) was delivered through corneal electrode to induce convulsions to four groups of mice (n=6).The various phases of convulsion which were produced are Flexion, Extension, Clonus and stupor. Prior to delivery, current output was checked by multimeter. After the electric stimulation occurrence, the duration of phases were noted.

Pentylenetetrazole (PTZ) induced Seizures

Mice were divided into four groups of six animals each .Group 1 served as control (vehicle treated i.e.Tween 80, 2%), Group II served as standard received Diazepam 5mg/Kg body weight (i.p), Group III and Group IV were treated with methanolic extract as 200 & 400 mg/Kg body weight.30 min after i.p. injection of Diazepam and 60 min after oral administration of extract, 60mg/kg PTZ was injected subcutaneously. The antiepileptic activity was accessed by its ability to delay the onset of myoclonic spasms and clonic convulsions [16, 19].

Strychnine-induced Seizures

Thirty mice were divided into four groups of six mice each. Group I received 10mL normal saline per kg body weight i.p, Group II received Diazepam 5mg/Kg body weight (i.p), Group III and Group IV were treated with methanolic extract of vitex negundo at a dose of 200 & 400 mg/Kg body weight i.p. Thirty minutes later, mice in all the groups received 1 mg/kg body weight strychinine.Abolition of tonic extensor jerks of hind limbs was considered as an indicator that the testing drug could prevent strychnine induced convulsions [17].

Picrotoxin induced seizures

Mice were randomly allotted to the different control and test groups. Picrotoxin (5mg/Kg, i.p) was used to induce seizures. Procedure of strychnine induced convulsions was carried out for screening.

Lithium-pilocarpine-induced seizures

Albino rats were divided randomly into four groups each containing six animals each. Convulsions were induced by administration of pilocarpine (30 mg/kg, i.p) 24 hr after lithium sulphate (3 mEq/kg i.p). Effect of methanolic extract of vitex negundo was studied on the severity of seizures. Group I received as control, Group II received diazepam, Group III received test drug. The severity of convulsions was observed every 15 min till 90 min and thereafter 120 min, using the scoring system [18]. No response stage 0, fictive scratching stage 1, tremors stage 2, head nodding 3, forelimb clonus stage 4, rearing and falling back stage 5.

RESULTS

Antiepileptic activity of methanolic extract of *vitex negundo* leaf has been screened against different animal models. The methanolic extract does not exhibited significant decrease in different phase of epileptic seizure against MES induced seizures; at higher dose the extract was significantly effective i.e. it is showing moderate activity that was comparable to that of Phenytoin (Table-1), but in the case of PTZ induced seizures the extract has shown potentiating effect (Table-2).*Vitex negundo* extract against strychnine-induced seizure in mice has not shown prolongation effect at lower dose, but at higher dose the extract is showing prolongation of both tonic and clonic seizures and potentiating effect i.e moderate activity when compared with standard drug diazepam (Table-3),similarly against Picrotoxin-induced seizures it has shown dose dependent prolongation of both clonic and tonic seizure latencies when compared with control group(Table-4).Pretreatment of the Vitex negundo methanolic extract in doses 200 and 400 mg/kg inhibited the progression and severity of status epileptics, the extract produced a dose dependent significance when compared with control(Table-5).

Table 1: Effect of *Vitex negundo* leaf extract on MES induced convulsions in mice

Group	Drug	Flexion, sec	Extension, sec	Clonus, sec	Stupor, sec	Recovery, sec
I	Control	5.8±0.166	13.33±0.33	14.03±0.55	6.25±0.071	190.2
II	Phenytoin 20 mg/kg	3.38±0.01***	0	8.86±0.33***	1.143±0.003***	174.1
III	Vitex. negundo 200 mg/kg	2.9±0.04***	1.15±0.022***	4.88±0.06***	4.46±0.021***	119.9
IV	Vitex. negundo 400 mg/kg	2.73±0.04***	0.96±0.02***	4.46±0.02***	2.91±0.047***	111.8

Values expressed are Mean ±SEM from 6 mice; Statistical analysis was performed by using prism graph pad
****P<0.001*

Table 2: Effect of *Vitex negundo* leaf extract on PTZ induced seizures in mice

Group	Treatment	Latency(onset of colonic convulsion) sec/min	Onset of tonic convulsion sec/min	Status of animal after 30 min		Status of animal after 24 hrs	
				No of live animals	% protection	No of live animals	% protection
I	Control	48.12±0.002	785.65±0.3728	0	0	0	0
II	Diazepam 5 mg/kg	No colonus	No tonic	All	100	All	100
III	Vitex. negundo 200 mg/kg	256.06±0.042**	601.4±0.003***	5	83	5	83
IV	Vitex. negundo 400 mg/kg	222.83±2.15***	596.8±0.001***	5	83	5	83

Values expressed are Mean ±SEM from 6 rats; Statistical analysis was performed by using prism graph pad
****P<0.001 , **P<0.05 as compared to control groups*

Table 3: Effect of *Vitex negundo* leaf extract on Strychnine - induced seizures in mice

Group	Treatment and route	seizure onset	
		Clonic	Tonic
I	Control-NS (10ml/kg,i.p)	1.3±0.05	1.5±0.05
II	Diazepam (10 mg/Kg,i.p)	6.7±0.01***	7.4±0.01***
III	Vitex. negundo 200 mg/kg	5.4±0.02**	6.2±0.02**
IV	Vitex. negundo 400 mg/kg	5.7±0.003‡	6.4±0.001***

Values expressed are Mean ±SEM from 6 rats ***$P<0.001$, **$P<0.05$, ‡ $P<0.10$.

Table-4: Effect of *Vitex negundo* leaf extract on Picrotoxin - induced seizures in mice

Group	Treatment and route	seizure onset	
		Clonic	Tonic
I	Control-NS(10ml/kg,i.p)	5.2±0.05	5.4±0.02
II	Diazepam (10 mg/Kg,i.p)	17.60±0.003**	18.28 ±0.01***
III	Vitex. negundo 200 mg/kg	10.3 7±0.05**	12.2 1±0.05**
IV	Vitex. negundo 400 mg/kg	14.30±0.003**	16.89±0.004**

Values expressed are Mean ±SEM from 6 rats ***$P<0.001$, **$P<0.05$.

Table-5: Effect of *Vitex negundo* leaf extract on lithium-pilocarpine - induced seizures in rats

Group	Treatment and route	Time after pilocarpine in min							
		0	15	30	45	60	75	90	120
I	Control (10ml/kg,i.p)	0.0±0.0	1.75±0.02	.25±0.03	2.85±0.02	3.15±0.04	3.86±0.05	4.23±0.03	4.50±0.04
II	Diazepam (10 mg/Kg,i.p)	0.0±0.0	0.37±0.013*	0.57±0.007*	0.65±0.014*	0.96±0.018*	1.19±0.009*	1.4±0.049*	0.89±0.003*
III	Vitex. negundo 200 mg/kg	0.0±0.0	0.94±0.035*	1.21±0.194*	1.30±0.051*	1.91±0.271*	0.66±0.105*	0.70±0.057*	0.76±0.083**
IV	Vitex. negundo 200 mg/kg	0.0±0.0	1.20±0.063*	0.96±0.024*	0.96±0.021*	1.23±0.080*	0.73±0.114*	0.91±0.054*	0.85±0.034**

Values expressed are Mean ±SEM from 6 rats **$P<0.05$, *$P<0.10$

DISCUSSION

Antiepileptic drugs may produce their effects by the following mechanism normalization of seizure foci, prevention of the origin of seizures from the foci, prevention of post-titanic potential, elevation of excitatory synaptic threshold, potentiation of pre or post synaptic inhibition, prolongation of the refractive period. Phenytoin inhibits Na+ channels, but diazepam act through GABA (gabba amino butyric acid) producing pharmacological action. Phenytoin does not effect chemically induced seizures but prevent tonic convulsions produced by MES.Diazepam prevent chemically induced seizures and it is drug of choice of status-epilepticus, but is having sedative action and development of tolerance. *Vitex negundo*

experimental evaluation of antiepileptic activity against two different animal models has been reported; we tried an attempt to evaluate the activity against different models. The results shown are low to moderate activity we are isolating the flavanoid from vitex negundo methanolic extract and formulate, screen against different animal models in a dose dependant manner .MES screening model prevent generalized tonic clonic seizures and this model evaluates the capacity to prevent seizure spread, mainly possess an effect on voltage dependant sodium channels. The mechanism of PTZ inducing seizures is by acting as an antagonist at the GABA receptor complex, the enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance seizures respectively. Lithium does not possess general proconvulsant action in rats; it pretreatment provokes limbic seizures following administration of subconvulsant doses of pilocarpine and other cholinergic agonists.

CONCLUSION

The results from Methanolic extract of *vitex negundo* reveal it possess anticonvulsant activity either by blocking sodium channels or by enhancing GABA receptor mediated inhibitory transmission .The exact mechanism of action of Vitex negundo has to be known and biochemical evidence of neurotransmitter levels in the brain should be studied .

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