Indian J Physiol Pharmacol 2005; 49 (2) : 199–205

AN EXPERIMENTAL EVALUATION OF ANTICONVULSANT ACTIVITY OF VITEX-NEGUNDO

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(Received on November 15, 2004)

Abstract : Maximal electroshock seizures (MES) in albino rats and pentylenetetarazole (PTZ) induced seizures in albino mice were used to study anticonvulsant activity of *Vitex-negundo* leaf extract. The ethanolic leaf extract of *Vitex-negundo* was administered orally in graded doses (250, 500 and 1000 mg/kg p.o) in both the experimental models and the effects were compared with diphenylhydantoin in MES method and valporic acid in PTZ induced seizures method as standard control respectively. The *Vitex-negundo* in the doses (250, 500 and 1000 mg/kg, p.o) did not show protection against MES to any significant extent but significant post-ictal depression was observed in the dose of 1000 mg/kg body weight in comparison to control. However, sub-protective dose of test drug (100 mg/kg, p.o) potentiated the anticonvulsant action of diphenylhydantoin. The test drug in the dose (1000 mg/kg, po) showed 50% protection in clonic seizures and 24-hour mortality against PTZ induced seizures. It also decreased number and duration of convulsions significantly. *Vitex-negundo* potentiated anticonvulsant activity of valporic acid. The anticonvulsant activity of *Vitex-negundo* has not been found equi-effective with standard drugs. These findings suggest that *Vitex-negundo* possesses anticonvulsant activity particularly against PTZ induced convulsions. Moreover, the potentiation of diphenylhydantoin and valporic acid by *Vitex-negundo* indicates that it may be useful as an adjuvant therapy along with standard anticonvulsants and can possibly lower the requirement of diphenylhydantoin and valporic acid.

Key words : vitex-negundo maximal electroshock seizure (MES) pentylenetetarazole (PTZ) anticonvulsant sub-protective potentiation

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INTRODUCTION

Vitex-negundo Linn (Verbenaceae), 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 15,00m 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 (3, 5, 6) activities in the past but, very few studies have been done to evaluate its anticonvulsant activity. In these studies, Ravishankar et al (5) did not notice any protection against MES and PTZ induced seizures by an ethanolic extract of *Vitex-negundo*. On the contrary Gupta et al (6) recorded protection against PTZ induced seizures. 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* 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.

METHODS

The plant material and preparation of extract: The plant was identified, authenticated by an expert (botanist) and collected in the month of September, 2001 from local area of Sevagram, district Wardha, Maharashtra. The fresh leaves of *Vitex negundo* were shade dried and powdered. The powder was macerated for 24 hrs 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%.

Animals:

Male albino rats (Wistar strain) and swiss mice of, weight (125–160 g) and (25–35 g) were used to study the effect of test drug on MES and PTZ induced seizures respectively. Female animals were excluded because of the fact that estrus cycle influences the seizure threshold. They were procured from National Institution of Nutrition, Hyderabad. The clearance for the use of 120 animals for experimental purpose was obtained from institutional ethical committee constituted for the purpose at MGIMS, Sevagram, (Wardha). 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 weeks time to get acclimatized with laboratory conditions. The animals were fasted overnight before the experiment.

Drugs :

Test drug : ethanolic leaf extract of *Vitex-negundo* was used for the present study in volume of 10 ml/kg wt/orally. The doses were selected by preliminary trial.

Diphenylhydantoin : suspension (Parke Davis India) was given p.o in a volume of 10 ml/kg body weight in dose of 50 mg/kg as a standard drug for comparison and 20 mg/kg to see the potentiation effect of test drug in MES method.

Valporic acid was dissolved in sterile saline and administered intraperitoneally in the dose of 300 mg/kg in volume of 10 ml/kg of body weight as a standard drug for comparison and 50 mg/kg/i.p dose in the same volume for potentiation study in PTZ induced seizures method.

Pentylenetetrazole (Bochemzer-knoll, Ltd.) was prepared in distilled water just before use.

Experiment design and drug treatment :

Anticonvulsant activity : The animals were divided into five groups with each group consisting of six animals. Group-I_a received distilled water and served as control. Group-II_a, III_a and IV_a were administered three graded doses of test drug i.e 250, 500 and 1000 mg/kg, orally in both the experimental models. Group-V_a received diphenylhydantoin (50 mg/kg, orally) and valporic acid (300 mg/kg, i.p) as standard controls in MES method and PTZ induced seizures method respectively. The observations were compared with control (Group-I_a).

Interaction of Vitex-negundo extract with diphenyhydantoin and valporic acid : The animals were divided into four groups with each group consisting of six animals. Group-I_b received distilled water and served as control. Group-II_b received sub-protective doses of diphenlyhydantoin (20 mg/kg, orally) in MES induced seizures method and valporic acid (50 mg/kg, i.p) in PTZ induced seizures method. Group-III_b received 100 mg/kg, orally, the dose of test drug which did not produce any anticonvulsant activity in both the experimental models. Whereas, group-IV_b was given combination of diphenylhydantoin/valporic acid and *Vitex-negundo* in above doses in respective experimental models. The results were compared with group-I_b.

Two methods were employed to evaluate anticonvulsant activity as well as potentiation effect of diphenylhydantoin and valporic acid by Vitex-negundo extract.

1. Maximal electroshock induced seizures (MES) in rats : The animals (x = y) were chosen by preliminary screening. Rats which showed extension of hind limb were included in the study. The seizures were induced by maximal electroshock in albino rats with the help of electroconvulsiometer by passing current of 45 mA for 0.2 second using ear clip electrodes. The drugs and distilled water were given one our 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.

2. Pentylenetetrazole (PTZ) induced seizures in mice : The albino mice were selected two weeks prior to conducting the experiment by injecting the pentylenetetrazole in a dose of 30 mg/kg subcutaneously in the scruff of neck. Only those mice which showed clonic convulsions within 30 minutes during preliminary examination were chosen for the present study. After one hour drug treatment, PTZ (80 mg/kg subcutaneously) was given in the scruff of neck. Animals were observed for clonic convulsions, number of convulsions in 30 minutes, duration of convulsions and 24 hour mortality. Absence of clonic convulsions in drug treated groups was taken as criteria for anticonvulsant activity.

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). Differences between means were considered to be significant at $P<0.01$. Whereas, z-test was applied for the data expressed in percentage and in this case the 'P' value less than 0.05 was considered significant when compared to control.

RESULTS

Anticonvulsant effect of Vitex-negundo on maximal electroshock induced seizures in albino rats : Oral administration of *Vitex-negundo* extract in 250, 500 and 1000 mg/kg doses did not show anticonvulsant activity to any significant extent against maximal electroshock seizures (MES), however post-ictal depression was increased by *Vitex-negundo* extract significantly ($P<0.01$) in a dose of 1000 mg/kg in comparison to control. The standard drug diphenylhydantoin showed 100% protection in animals against MES. It abolished the extensor phase completely ($P<0.001$), as well as prolonged post-ictal depression which however was not-significant in comparison to control (Group-I_a) as shown in Table–I.

Interaction of Vitex-negundo extract with diphenyhydantoin (by MES method) : Oral administration of sub-protective doses of diphenylhydantoin (20 mg/kg) and Vitex-negundo extract (100 mg/kg) did not exhibit any anti-convulsant action. The simultaneous administration of both, significantly ($P<0.01$) showed anti-convulsant activity in 66.66% of animal and reduced extensor phase duration ($P<0.001$) ,indicating potentiation of anticonvulsant action of diphenylhydantoin. Though,it prolonged post-ictal depression duration but it was statistically non-significant in comparison to control (Group–I_b).The results are shown in Table–I.

Anticonvulsant action of Vitex-negundo extract on pentylenetetrazole induced convulsions in mice : The *Vitex-negundo* extract only in the dose of 1000 mg/kg showed 50% protection against clonic convulsions and 24 hour mortality which was statistically significant ($P<0.05$). It also decreased number of clonic convulsions and duration of clonic phase which was statistically ($P<0.01$) significant in the above mentioned dose in comparison to control. Whereas, in the doses of 250 and 500 mg/kg *Vitex-negundo* failed to show any anticonvulsant activity. However, the standard drug valporic acid (300 mg/kg/i.p) provided 83.33% protection against

TABLE I: Anticonvulsant effect of *Vitex-negundo* extract and its interaction with diphenylhydantoin (by MES method in albino rats).

Group (n=6)	*Drug*	*Dose (mg, ml•/kg) (p.o)*	*% of animals showing abolition of extensor phase*	*Duration of (Mean±S.E.M)*	
				Extensor Phase (sec.)	*P.I.D (min.)*
I_a	D.W	10•	0.00	11.33±0.71	1.91±0.08
II_a	VNE	250	0.00	11.66±0.42	2.00±0.22
III_a	VNE	500	0.00	11.00±1.03	3.41±0.59
IV_a	VNE	1000	16.66	10.33±2.08	3.58±0.50*
V_a	D.P.H	50	100.00†††	0.00±0.00**	3.16±0.10
I_b	D.W	10•	0.00	11.33±0.71	1.91±0.08
II_b	D.P.H	20	0.00	10.33±0.66	2.16±0.44
III_b	VNE	100	0.00	10.33±0.55	2.00±0.28
IV_b	D.P.H+VNE	20+100	66.66††	2.66±1.69**	3.83±0.74

Animals were divided into five groups(I_a–V_a) for studying anticonvulsant effect of Vitex-negundo and into four different groups (I_b–IV_b) for studying interaction of *Vitex-negundo* extract with diphenylhydantoin, n = No. of animals; P.O = Per orally; D.W = Distilled water; PID = Post-ictal depression; VNE = Vitex-negundo; D.P.H = Diphenylhydantoin S.E.M = Standard error mean, †P<0.05; ††P<0.01; †††P<0.001 (test of significance between two proportions by z-test) in comparison to control. One-way ANOVA followed by Dunnett's test was used for data expressed in mean±S.E.M and mean difference was considered significant at the 0.01 level. *P<0.01, **P<0.001 compared to control.

TABLE II: Anti-convulsant action of Vitex-negundo extract and its interaction with valporic acid (by PTZ induced convulsions in mice).

Group (n=6)	*Drug*	*Dose (mg, ml•/kg) (p.o)*	*% of animals showing abolition of clonic phase*	*% of protection in 24 hrs mortality of animals*	*Clonic convulsions (Mean±S.E.M)*	
					No. of clonic convulsions (in 30 min)	*Duration of clonic phase (sec)*
I_a	D.W	10•	0.00	0.00	7.00±0.36	16.16±1.38
II_a	VNE	250	0.00	16.67	6.83±0.30	13.50±2.13
III_a	VNE	500	0.00	33.33	5.83±0.47	11.00±1.63
IV_a	VNE	1000	50.00†	50.00†	3.33±1.52*	6.83±3.11*
V_a	VA	300 (I.P)	83.33†††	66.66††	0.50±0.50**	1.67±1.67**
I_b	D.W	10•	0.00	0.00	7.00±0.36	16.16±1.38
II_b	VA	50 (I.P)	0.00	0.00	6.83±0.40	16.50±1.41
III_b	VNE	100	0.00	16.67	7.33±0.42	17.16±1.52
IV_b	VA+VNE	50(I.P)+100	33.33	66.66††	2.83±0.98**	6.50±2.25**

Animals were divided into five groups(I_a–V_a) for studying anticonvulsant effect of Vitex-negundo and into four different groups (I_b–IV_b) for studying interaction of *Vitex-negundo* extract with diphenylhydantoin, n = No. of animals; P.O = Per orally; I.P. = Intraperitoneally; D.W = Distilled water; VNE = Vitex-negundo; V.A. = Valporic acid; S.E.M = Standard error mean, †P<0.05; ††P<0.01; †††P<0.001 (test of significance between two proportions by z-test) in comparison to control. One-way ANOVA followed by Dunnett's test was used for data expressed in mean±S.E.M and mean difference was considered significant at the 0.01 level. *P<0.01, **P<0.001 compared to control.

pentylenetetrazole induced convulsions which was statistically significant (P<0.001). It also provided statistically significant (P<0.01) protection against 24-hrs mortality in 66.66% animals as well as decreased number and duration of convulsions in comparison to control(Group-I_a)significantly (P<0.001) as shown in Table–II.

Interaction of *Vitex-negundo* extract with valporic acid (by pentylenetetrazole induced convulsions in mice)

The results are shown in Table–II. The sub-protective doses of valporic acid (50 mg/kg/i.p) and *Vitex-negundo* extract (100 mg/kg/p.o) did not show anticonvulsant activity. Whereas simultaneous administration of both drugs, abolished clonic phase in 50% of the animals which was statistically significant ($P<0.05$).It showed significant ($P<0.01$) protection against 24 hour mortality in 66.66% animals. It also significantly ($P<0.001$) decreased the number of convulsions and duration of clonic phase in comparison to control (Group–I_b). Hence indicating potentiation of anticonvulsant action of valporic acid.

DISCUSSION

The present study indicated that, *Vitex-negundo* extract though did not show any significant protection against maximal electroshock induced seizures but it significantly increased post-ictal depression, thereby indicating its central nervous system depressant action. However, *Vitex-negundo* in sub-threshold dose potentiated the anticonvulsant action of sub-threshold dose of diphenylhydantoin. Ravishankar et al (5) used various extracts of *Vitex-negundo* to study the effect against maximal electroshock seizures but none of the extracts used by them modified seizures pattern except petroleum ether and butanol extract. Ethanol extract also failed to modify seizures pattern. Their study is not in agreement with our study. This, discrepancy might be due to different experimental set up as they studied effect of *Vitex-negundo* by intraperitoneal route, used 30 mA for 0.2 sec to study MES, used mice and extraction of Vitex-negundo was carried out with 90% ethanol. However, the potentiation of anticonvulsant action of diphenylhydantoin by *Vitex-negundo* in the present study, suggests that it may be useful as an adjuvant therapy in the treatment of generalised tonic clonic seizures, as it is well known fact that drugs which provide protection against seizures induced by maximal electroshock method are generally effective against generalised tonic clonic seizures (8–10).

Vitex-negundo showed significant protection against pentylenetetrazole induced convulsions as well as 24-hr mortality in mice in higher dose. It potentiated the anticonvulsant action of valporic acid in our study. The present findings are in agreement with the findings of Gupta et al (6) who showed protection by increasing survival time against pentylenetetrazole induced convulsions on intraperitoneal administration of *Vitex-negundo*. On the contrary, Ravishankar et al (5) did not notice any protection against pentylenetetrazole induced seizures. This might be due to different experimental setup as they used a higher dose of PTZ (100 mg/kg) to evaluate PTZ induced seizures and studied the effect of *Vitex-negundo* extract by intraperitoneal route. The seizures induced by chemoconvulsant (pentylenetetrazole), is useful in identifying drugs that are effective against absence seizures (8). Hence, *Vitex-negundo* may be useful in absence seizures in higher dose as well as it can decrease the dose of valporic acid when used in combination.

It is well documented that

pentylenetetrazole induced convulsions (11, 12) are produced due to diminution of brain GABA (Gamma Aminobutyric Acid) level. Moreover, *Vitex-negundo* showed significant protection, particularly against PTZ induced convulsions in present study. Therefore, it is likely that *Vitex-negundo* might possibly be producing anticonvulsant action by increasing level of (GABA), an inhibitory neurotransmitter in the central nervous system. However, several mechanisms are involved in anticonvulsant activity, it is very premature at this stage of the study to say that anticonvulsant action appears to be due to increased level of GABA. Thus this aspect/hypothesis requires further investigation in future.

In conclusion, these findings suggest that *Vitex-negundo* possesses anticonvulsant activity particularly against PTZ induced convulsion. Moreover, the potentiation of diphenylhydantoin and valporic acid by *Vitex-negundo* indicates that it may be useful as an adjuvant therapy along with standard anticonvulsant and can lower the requirement of diphenylhydantoin and valporic acid.

ACKNOWLEDGEMENTS

Authors are extremely thankful to the Department of Pharmacology, M.G.I.M.S, Sevagram, Wardha, Maharashtra, India for sponsoring this research project vide grant number 248/10092001. Authors also extend sincere thanks to Dr. Parmil Kumar, M.Sc, Phd (Lecturer, Statistics), Department of Statistics, University of Jammu for his help in statistical work.

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