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Antidopaminergic activity of *Vitex negundo* Linn leaves

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Abstract

Objective: The objective of this study was to investigate the effect of acetone soluble fraction of methanolic extract of leaves of *Vitex negundo* on dopaminergic function. Methods: The effect of acetone soluble fraction of methanolic extract of leaves of *Vitex negundo* (50 and 100 mg/kg, i.p.) was studied on haloperidol-induced catalepsy in mice, amphetamine-induced stereotyped behavior in rats and dopamine-induced contraction of isolated vas deferens preparation of rat. Results: The acetone soluble fraction of methanolic extract of *V. negundo* significantly potentiated haloperidol-induced catalepsy, antagonized dose dependent amphetamine- induced stereotyped behavior, and also antagonized dopamine-induced contractions of rat vas deferens. Conclusion: The results suggest that the methanolic extract of *Vitex negundo* possessed antidopaminergic principles.

Key Words: Antidopaminergic, *Vitex negundo*, amphetamine, haloperidol.

1. Introduction

Vitex negundo (family: Verbenaceae), is an aromatic deciduous shrub native to China and India. [1, 2] The major constituents of leaves of *Vitex negundo* are caryophylene oxide, β-caryophylene oxide, viridifloral, globulol, sabinene, and gamma-terpinene. The leaves of the plant have aromatic, vermifuge, antiasthmatic, antiandrogenic, hepatoprotective, antiradical, antilipoperoxidase, analgesic and anti-inflammatory, cough suppressant, anti-ulcers, and antitumor activities. Leaves are used in the treatment of rheumatic disease, headache, catarrhal fever, cervical spondylitis, and convulsions [3, 4]. The *Vitex agnus* is reported to possess dopaminergic activity [5].

Therefore we investigated the effect of acetone soluble part of methanolic extract of *V. negundo* (VNE) on dopamine mediated behavior in mice and rats.

2. Materials and methods

2.1 Chemicals

Haloperidol (RPG Lifesciences, India), d-amphetamine (Sigma, USA), dopamine HCl

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(Charles Pharma Inc, Korea) and other laboratory chemicals of analytical grade were used. All drug solutions were prepared in distilled water except VNE, which was dissolved in PEG 400.

2.2 Plant material and extraction

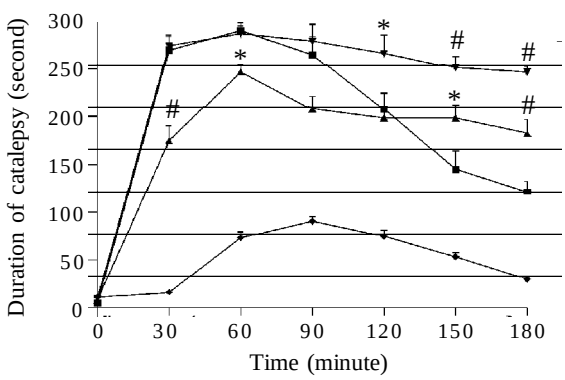
Vitex negundo leaves were collected from the local area in Nashik and were authenticated by Dr. S. C. Pal of the Pharmacognosy Dept. The leaves were sun-dried, powdered (380 g) and finally extracted with methanol by percolation. The extract was concentrated under reduced pressure and the resultant deep gummy mass (20 g) was then dissolved in acetone. The acetone insoluble part was separated by filtration. The acetone soluble part (12 g) was then concentrated by evaporation of acetone and dissolved in PEG 400 to get the solution of desired strength.

2.3 Animals

Albino Male Swiss mice (18-25 g) and Male Wistar rats (180-220 g) were housed (5 animals per cage) under the standard laboratory conditions (light period of 12 h/day, temperature 25 ± 2°C and humidity 55 ± 5%) with free access to food (standard pellets chow, Lipton, India) and water *ad libitum*. Food but not water was deprived overnight and during the experiment. The experimental procedures were carried out in strict compliance with Institutional Animal Ethics Committee regulations.

2.4 Haloperidol-induced catalepsy in mice

Haloperidol (1 mg/kg) was injected intraperitoneally (i.p.) to mice (n=5) pretreated with vehicle (PEG-4 ml/kg, i.p.) or VNE (50 and 100 mg/kg, i.p.). The vehicle or VNE was administered 30 min prior to administration of haloperidol. The duration of catalepsy was measured at 0, 30, 60, 90, 120, 150, 180 min using the Bar test [6]. Both the forepaws of mouse were placed on a horizontal bar raised 3 cm from the table and the time required to remove the forepaws from the bar was recorded as the duration of catalepsy. Between measurements, the animals were returned to their home cages.



Haloperidol (1 mg/kg, i.p)
VNE (50 mg/kg, i.p) + Haloperidol (1 mg/kg, i.p)
VNE (100 mg/kg, i.p) + Haloperidol (1 mg/kg, i.p)
VNE (100 mg/kg, i.p).

# P < 0.001, * P < 0.05, one-way ANOVA, followed by Dunnett's test.

Fig. 1.

Effect of methanolic extract of *Vitex negundo* (VNE) on haloperidol-induced catalepsy in mice n = 5.

2.5 Amphetamine-induced stereotyped behavior in rats

Male Wistar rats were divided into 4 groups each containing 5 animals. They were injected with d-amphetamine (1mg/kg, i.p.) 30 min after vehicle (PEG - 4 ml/kg, i.p.) or VNE (50 and 100 mg/kg, i.p.). The animals were observed for number of ambulation, grooming, and rearing at 0 min and 30 min after administration of amphetamine in open field model [7, 8].

2.6 Effect of VNE on dopamine-induced contraction of isolated rat vas deferens [9].

Adult male Wistar rats were sacrificed by cervical dislocation and the vas deferens was removed and kept in Krebs-Henseleit solution of the following composition (mM): NaCl, 115; KCl, 4.7; $CaCl_2$, 2; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgCl_2$, 1.2; glucose, 11.5. The effect of dopamine (10, 20, 40, and 80 mcg/ml) was observed on the vas deferens in absence and presence of VNE (0.5 ml of 25 mg/ml). The contact time between the dopamine and the tissue was maintained 60s.

2.7 Statistical analysis

Numerical results were expressed as Mean±SEM. One-way analysis of variance (ANOVA), was followed by Dunnett's test or *Student's t-test* (unpaired). $P<0.05$ being the criterion for statistical significance.

3. Results

3.1 Haloperidol-induced catalepsy in mice

In vehicle treated animals, haloperidol (1 mg/kg, i.p.) induced catalepsy and the catalepsy was maximum 60 min after haloperidol. The VNE (100 mg/kg, i.p.) *per se* also induced catalepsy. In presence of VNE (50 mg/kg, i.p.), haloperidol-induced catalepsy was slightly decreased whereas potentiation of catalepsy was observed with VNE (100 mg/kg, i.p.).

3.2 Amphetamine induced stereotyped behavior in rats

Following administration of amphetamine (1 mg/kg, i.p.), rats exhibited a stereotyped behavior. VNE (50 mg/kg and 100 mg/kg, i.p.) reduced number of ambulation, grooming, and rearing as compared to amphetamine (1 mg/kg, i.p.) treated group significantly (Table 1).

Table 1.
Effect of methanolic extract of Vitex negundo leaf on latency to amphetamine-induced stereotyped behavior in rat.

Treatment (Dose: mg/kg i.p)	**Ambulation**		**Grooming**		**Rearing With support**		**Rearing Without Support**	
	0 min	**30 min**	**0 min**	**30 min**	**0 min**	**30 min**	**0 min**	**30 min**
Vehicle + Amphetamine(1)	61.75 ± 10.25	95.5 ± 18.17	7.0 ± 0.0	8.5 ± 2.67	7.5 ± 1.67	10.4 ± 1.5	5.0 ± 1.58	13.75 ± 2.24@
VNE (50) + Amphetamine(1)	59.33 ± 1.90	59.0 ± 3.74	3.66 ± 0.72*	1.66 ± 0.72*	9.66 ± 1.76	5.66 ± 2.12*	7.0 ± 0.0	5.0 ± 1.24*
VNE (100) + Amphetamine(1)	35.66 ± 4.53*	24.66 ± 7.52*	1.66 ± 0.52*	2.0 ± 0.94*	5.66 ± 0.72	1.3 ± 0.27*@	0.0 ± 0.0	0.66 ± 0.27*@
Vehicle + VNE (100)	21.66 ± 1.51#	20.0 ± 3.88*	0.66 ± 0.17*	0.66 ± 0.24*	1.0 ± 0.4*	2.0 ± 0.64*	0.33 ± 0.17*	0.33 ± 0.17*

n = 5, The values are mean ± SEM. The dose of amphetamine was 1mg/kg i.p.
# $P < 0.001$, * $P < 0.05$, One-way ANOVA, followed by *Dunnett's test*
@ $P < 0.05$, *Student's t-test* (unpaired), compared to zero minute observation
VNE was administered 30 min before amphetamine and the effect on amphetamine-induced stereotypy was observed from 0 min after amphetamine administration.

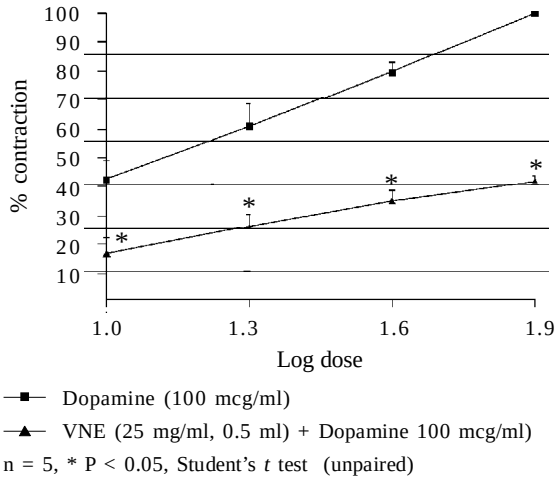


Fig. 2.

Effect of methanolic extract of *Vitex negundo* leaves (VNE) on dopamine-induced contraction on isolated rat vas deferens.

3.3 Effect on dopamine-induced contraction of rat vas deferens

Dopamine produced dose-dependent contraction of rat vas deferens. VNE reduced the amplitude of contraction produced by dopamine on rat vas deferens significantly (Fig 2).

4. Discussion

The acetone soluble fraction of methanolic extract of *Vitex negundo* (VNE) potentiated haloperidol-induced catalepsy, antagonized amphetamine-induced stereotyped behavior and inhibited contractions induced by dopamine on rat vas deferens. These results indicate that VNE possessed anti-dopaminergic activity.

The typical antipsychotic induces catalepsy in animals. This behavior has been used as a model for assessment of extrapyramidal side effects associated with antidopaminergic agents. The agents, increasing dopamine availability or transmission inhibit neuroleptic-induced catalepsy. In the present study, VNE (100 mg/kg, i.p.) per se exhibited catalepsy. The potentiation of catalepsy is indicative of the ability of drug to decrease dopamine levels in substantia nigra [7].

However, VNE (50 mg/kg, i.p.) reduced the duration of haloperidol-induced catalepsy, suggesting that VNE occupied dopamine receptors and prevented haloperidol from combining with the dopamine receptors.

The ability to block amphetamine-induced stereotyped behavior is considered to reflect the neuroleptic or antidopaminergic activity of the drug. Amphetamine is an indirect acting sympathomimetic agent which releases dopamine from its neuronal storage pools, inducing stereotyped behavior [10].

Amphe-tamine causes a dose dependent release of dopamine in both regions i.e. in nucleus accumbens and striatum. Certain components of behaviour showed a regional specific association with dopamine release in striatum [11]. In this study, VNE antagonized the action of amphetamine by reducing number of grooming, ambulation, and rearing.

Dopamine D_2 receptors are predominantly present in vas deferens [12]. Earlier studies suggest presence of dopamine receptors on rat vas deferens [13, 14]. Dopamine produced dose dependent contraction of vas deferens. Thus it is concluded that the VNE possesses antidopaminergic activity.

5. Acknowledgement

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References

1. Sivarajan VV, Balachandran Indira. (1986) *Ayurvedic Drugs and their plant sources*. 1st Edn., Oxford and IBH publishing Co. Pvt. Ltd: New Delhi; 329-331.

2. Bhattacharjee SK. (1998) *Handbook of Medicinal Plants*. 1st Edn. Pointer publication: Jaipur, India; 376-377.

3. Vidyadhar KN, Ganapaty S. (2005) *J. Nat. Rem.* 5: 75-95.

4. Tandon VR, Gupta RK. (2005) *Indian J. Physiol Pharmacol*. 49: 199-205.

5. Jarry H, Spenglu B, Wuttke W. (2000) *International Congress on Phytomedicine, Oct*. 11-13, Munich.

6. Ferre S, Guix T, Lane F. (1990) *Pharmacol. Biochem. Behaviour*. 35: 753-757.

7. Kandi CS, Metkar RB, Kasture SB. (1998) *Indian J. Pharmacol*. 51: 8-19.

8. Essman WB, Valzelli L. (1982) *Neuropharmacology Clinical Application*. 1st Edn., MTP press limited: Lancaster, England; 338-342.

9. Goyal RK. (2002-2003) *Practical in Pharmacology*. 3rd Edn. B. S. Shah Prakashan: Ahemdabad, India; 51-52.

10. Vogel HG. (2000) *Drug Discovery and Evaluation*. 2nd Edn. Springer Publication: New York; 535.

11. Sharp T, Zetterstrom T, Ljumberg T, Ungrstedt V. (1987) *Brain Res.* 401(2): 322-330.

12. Smith CF, Rowland PB. (1989) *Arch Int Pharmacodyn Ther*. 299: 144-154.

13. Relja M, Lackonic Z, Neff NH. (1982) *Life Sci*. 31(23): 2571-2575.

14. Simon M, Van Maanen EF. (1976) *Arch Int Pharmacodyn Ther*. 222(1): 4-15.