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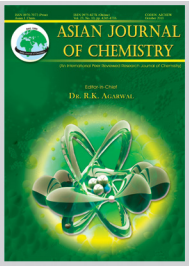

Antidopaminergic Activity of *Vitex negundo* (Linn) Roots

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The objective of the study was to investigate the effect of acetone soluble fraction of methanolic extract of roots of the vitex negundo on dopaminergic function. The effect of acetone soluble fraction of methanolic extract of roots of the *Vitex negundo* (50 and 100 mg/kg, i.p.) was studied on haloperidol-induced catalepsy in mice, amphetamine induced stereotyped behaviour in rats and dopamine-induced contraction isolated vas deferens preparation of rat. The acetone soluble fraction of methanolic extract of isolated of *V. negundo* significantly potentiated haloperidol induced catalepsy, antagonized dose dependant amphetamine-induced stereotyped behaviour and also antagonized dopamine induced contractions of rat vas deference. The result suggests that the methanolic extract of *Vitex negundo* possessed antidopaminergic principle.

Key Words: Antidopaminergic. *Vitex negundo*, Dopamine, Amphetamine, Haloperidol.

INTRODUCTION

Vitex negundo belonging to family Verbenaceae is a large aromatic shrub distributed throughout the greater part of India up to an altitude of 1500 m in the outer Himalayas[1,2]. The major constituents of roots are negundin-A, negundin-B, (+)-diasyringaresinol, (+)-lyoniresinol, vitrofolal-E and vitrofolal-F, acetyl oleanolic acid, sitosterol, 3-formyl-4.5-dimethyl-8-oxo-5*H*-6,7-dihydronaphtho(2,3-b)furan, vitexin and isovetexin. The *Vitex negundo* plant have aromatic, vermifuge, antiasthamatic, antiandrogenic, hepatoprotective, antiradical, analgesic and antinflammatory[3,4]. The leaves of *Vitex negundo* reported to possess dopaminergic activity[5]. Therefore we investigate the effect acetone soluble part of methanolic extract of *V. negundo* on dopamine mediated behaviour in mice and rats.

EXPERIMENTAL

Haloperidol, d-amphetamine, dopamine HCl and other laboratory chemicals of analytical grade were used. All drug solutions were prepared in distilled water except extract of *Vitex negundo* (Linn) roots, which was dissolved in PEG 400.

Plant material and extraction: The fresh roots of *Vitex nigundo* Linn. were collected from the medicinal garden of the college of pharmacy Shahada and were authenticated by Botanical survey of India, Koregaon road, Pune. In August and were dried in air for 15 days and finally extracted with methanol by percolation. The extract was concentrated under reduced pressure and resultant gummy mass (30 g) was then dissolved in acetone. The acetone insoluble part was separated by filtration. The acetone soluble part (15 g) was then concentrated by evaporation of acetone and dissolved in PEG 400 to get solution of desired strength.

Phytochemical screening: Phytochemical investigations of the extract were carried out using the methods described by Kokate[6], Trease and Evans[7] to check for the presence of phenolic compounds, flavonoids, tannins, triterpenes, anthocyanins, anthroquinones and sterols. The presence of alkaloids and saponins was also ascertained.

Animals: Albino male Swiss mice (18-25 g) and male Wistar rats, weighing (180-220 g). The animals were housed in colony cages and maintained under the standard environmental conditions-temperature 25 ± 2 °C, 12 h light : 12 h dark cycle and 50 ± 5 % relative humidity, with food and water *ad libitum*. The animals were deprived of food the night before the experiment and during the experiment. All experiments were carried out during the light period (08.00-16.00 h). The Institutional Animals Ethics Committee approved all the experimental protocols.

Haloperidol-induced catalepsy: Haloperidol (1 mg/kg) was injected intraperitoneally to mice (n = 5) pretreated with

vehicle (PEG-4 mL/kg, i.p.) or extract of *Vitex negundo* (Linn) roots (50, 100 and 200 mg/kg, i.p.). The vehicle or extract of *Vitex negundo* (Linn) roots was administered 30 min prior to the administration of haloperidol. The duration of catalepsy was measured at 0, 30, 60, 90, 120, 150 and 180 min, using Bar test[8]. 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. In all the experiments, the observer was blind to the treatment given to the mice. Between experiments, the animals were returned to their home cages.

Amphetamine-induced stereotyped behaviour in mice: The male Wister rats were allowed a maximum of 30 min to get acclimatized to the observation cage, prior to the experiment. Amphetamine-induced stereotypy was scored blind by an independent observer, every 5 min for 30 min[9,10]. Stereotypy scoring - 0, absence of stereotyped behaviour; 1, intermittent sniffing; 2, constant sniffing; 3, constant sniffing with intermittent licking and/or false biting; 4, constant licking or false licking; 5, constant licking; 6, constant biting and moving around; 7, constant biting and restricted to a small area in the cage; 8, rearing-was used. The animals were divided into five groups, each containing six animals. They were treated with vehicle (PEG-4 mL/kg, i.p.) or extract 50, 100 and 200 mg/kg and placed individually in the cage. Amphetamine (1 mg/kg i.p.) was given 30 min after the extract was administered. The stereotyped behaviour was recorded[11].

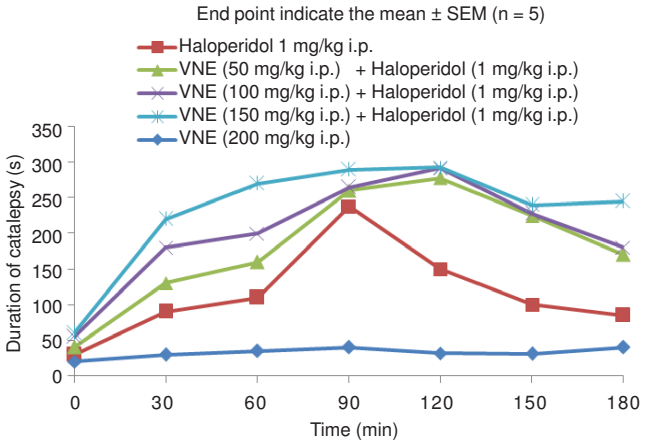
Effect of extract of *Vitex negundo* (Linn) roots on dopamine-induced contraction of isolated rat vas deferens: Adult male Wistar rats were sacrificed by cervical dislocation and the vas deferens was removed and kept in Krebs-Henseleit solution. The concentration related contraction response (CRC) to dopamine (10, 20, 40, 80 and 160 µg/mL) was recorded on Sherrington rotating drum (INCO, Ambala, India). Concentration related contraction response to dopamine was later repeated in the presence of extract of *Vitex negundo* (Linn) roots (0.5 mL of 25 mg/mL). The contact time between the dopamine and the tissue was maintained at 60 sec[11].

Statistical analysis: Results are expressed as mean ± SEM and the statistical analysis of data was done using one-way analysis of variance (ANOVA), followed by Dunnett's test. Probability level less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Haloperidol-induced catalepsy: Haloperidol, typical neuroleptic produces catalepsy in rodents and extrapyramidal side effects in human[12]. Haloperidol-induced catalepsy is one of the animal models for testing the extrapyramidal side effects of antipsychotic drugs[13]. Haloperidol, (a non-selective D_2 dopamine antagonist) and metoclopramide (a potent dopaminergic blocking agent) induced catalepsy is primarily due to blockade of dopamine receptors in the striatum.

In vehicle treated animals, haloperidol (1 mg/kg. i.p.) produced the maximum catalepsy at 90 min (238.5 ± 5.275 s). Methanolic extract of *Vitex negundo* (50, 100, 200 mg/kg, i.p.) significantly potentiated haloperidol induced catalepsy at each time interval, in a dose dependent manner. At dose 50, 100 and 200 mg/kg, extract of *Vitex negundo* (Linn) roots showed maximum cataleptic score 278.8 ± 9.998, 292.3 ± 5.852 and 293.2 ± 5.288 s, respectively at 120 min ($p < 0.01$) in haloperidol treated animals. The mice treated with extract of *Vitex negundo* (Linn) roots (200 mg/kg, i.p.) did not exhibit any catalepsy and appeared the same as the normal animals.



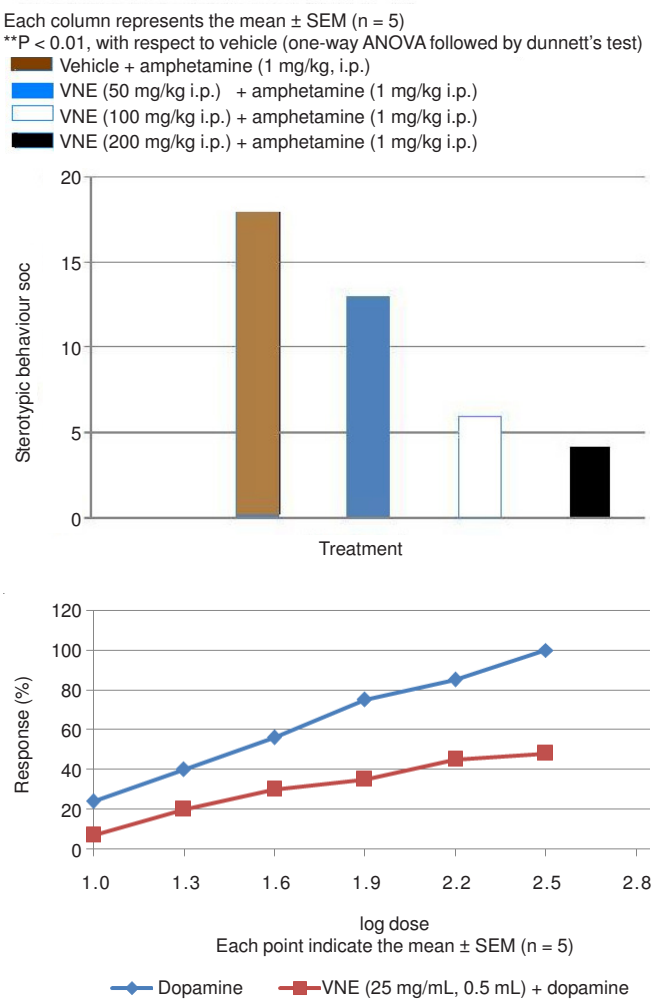
The agents increasing dopamine transmission inhibits neuroleptic-induced catalepsy. The striatum and nucleus accumbens have been implicated as the major brain structures involved in antipsychotic induced catalepsy, which appears due to the blockade of dopamine neurotransmission[14]. In present study, extract of *Vitex negundo* (Linn) roots (50, 100 and 200 mg/kg, i.p.) significantly ($p < 0.05$, $p < 0.01$) potentiated dose dependent haloperidol- and metoclopramide-induced catalepsy. Thus, the results suggest that extract of *Vitex negundo* (Linn) roots shows antidopaminergic activity.

Amphetamine-induced stereotyped behaviour in mice: Amphetamine-induced stereotyped behaviour is a well established model for schizophrenia[15]. Amphetamine is an indirectly acting sympathomimetic agent, which releases dopamine and induces characteristic stereotyped behaviour[9]. Amphetamine-induced stereotyped behaviour is a measure of dopamine D_2 receptor reactivity. It is known that amphetamine-induced stereotyped behaviour is mediated by the hyperactivity of dopaminergic mechanism in the nigrostriatal and mesolimbic pathway[11].

Amphetamine (1 mg/kg) induced a stereotyped behaviour characterized by intermittent sniffing or constant sniffing, licking, biting, moving around, restricted to a small area in the cage and rearing. Administration of extract of *Vitex negundo* (Linn) roots (50, 100 and 200 mg/kg, i.p.) significantly ($p < 0.01$) decreased amphetamine-induced stereotyped behaviour.

Methanolic extract of *Vitex negundo* (50, 100 and 200 mg/kg, i.p.) significantly ($p < 0.01$) blocked amphetamine-induced stereotyped behaviour in mice. This suggests that the extract contains antidopaminergic compound(s). A majority of antipsychotic drugs (phenothiazines, butyrophenones) used in the management of psychosis are known to have preference for D_2 receptor and abolish amphetamine-induced stereotyped behaviour. Since extract of *Vitex negundo* (Linn) roots has antidopaminergic potential, it needs further investigations in the treatment of psychosis.

Effect of extract of *Vitex negundo* (Linn) roots on dopamine-induced contraction of isolated rat vas deferens: Dopamine produced concentration related contractile response in rat vas deferens. *Vitex negundo* reduced the contraction produced by dopamine on rat vas deferens.



Earlier reports of chemical constituents and their pharmacology suggest that the plants containing flavonoids, saponins and tannins possess activity against many CNS disorders[13,16]. Phytochemical tests of extract of *Vitex negundo* (Linn) roots revealed the presence of flavonoids, saponins and tannins, which may be responsible for the antidopaminergic potential of the extract.

Dopamine D_2 receptors are predominantly present in rat vas deferens[11]. Dopamine produces dose dependent contractions of vas deferens. The result of the in vitro test indicates that extract of *Vitex negundo* (Linn) roots inhibits dopamine-induced contractions on rat vas deferens. Thus, it is concluded that extract of *Vitex negundo* (Linn) roots possesses antidopaminergic activity, mediated through dopamine D_2 receptors. In the acute toxicity test, extract of *Vitex negundo* (Linn) roots did not produce any detectable toxicity on oral and i.p. administration. No mortality was found, which is reflected by high LD_{50} of extract of *Vitex negundo* (Linn) roots.

Conclusion

The present investigation concludes that the methanolic extract of *Vitex negundo* roots contains constituents that inhibit dopaminergic neurotransmission and possibly blocks dopamine D_2 receptor. Thus, extract of *Vitex negundo* (Linn) roots possesses antidopaminergic activity. The results suggest that the leaves of *Vitex negundo* may have potential clinical application in the management of psychiatric disorders.

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