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Original Research Article

Effect of methonolic extract of *Vitex negundo* on haloperidol induced catalepsy in albino mice

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ABSTRACT

Background: Plants are being used in traditional medicine since history of mankind. The knowledge of these medicinal plants has accrued in the course of many centuries leading to medicinal systems in India such as Ayurveda, Unani and Siddha. Objective: In the present study, we evaluated the anticataleptic efficacy of *Vitex negundo*, a polyherbal formulation in haloperidol induced catalepsy in mice.
Methods: Five groups (n=6) of male albino mice were used in the study. Catalepsy was induced by i.p. administration of haloperidol (1 mg/kg). The degree of catalepsy (cataleptic score) was measured as the time the animal maintained an imposed posture. We compared the anticataleptic efficacy of *Vitex negundo* (50, 100, 200 mg/kg) with standard received Pheniramine maleate 10 mg/kg, i.p.
Results: In vehicle treated animals, haloperidol (1 mg/kg. i.p.) produced the maximum catalepsy at 180 min (46.78±3.78 min). Standard treated as Pheniramine maleate 10 mg/kg, i.p. shows maximum at 120 min. 19.24±1.32. Test herb, i.p. 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 200mg/kg, extract of *Vitex negundo* (Linn.) roots showed maximum cataleptic score 12.34±0.78, 14.43±0.43 and 15.43±0.67 min, respectively at 120 minutes in haloperidol treated animals.
Conclusions: The present study indicates that the methanolic extract of *Vitex negundo* reduces haloperidol-induced catalepsy in mice.

Keywords: Catalepsy, Haloperidol, Mice, *Vitex negundo*

INTRODUCTION

Catalepsy (tonic immobility, immobility reflex and animal hypnosis) is characterized by muscular rigidity leading to prolonged immobility and an inability to correct an externally imposed awkward posture.[1] Under physiological conditions, catalepsy can be obtained in some vertebrates as a kind of passive defensive behaviour against a predator.[2] In humans, excessive catalepsy-like dyskinesia is a pathological symptom occurring in schizophrenia, mood disorders (e.g. depression) and Parkinson's' disease. Catalepsy is a sign of extrapyramidal effect of drugs that inhibit dopaminergic transmission or increase histamine release in brain.[3]

In rodents, catalepsy can be induced by administration of the antipsychotic drug haloperidol, and such animals can be used as an appropriate model for analysis of extrapyramidal dysfunctions. Haloperidol which is used in the treatment of schizophrenia and other psychotic disorders. Antipsychotics are often associated with distressing extrapyramidal side effects.[4] Haloperidol-induced catalepsy occurs due to the blockade of dopamine (D2) receptors in the substantia nigra and

reduced dopaminergic transmission.[5] Enhanced stimulation of the intrinsic central cholinergic system has also been implicated in haloperidol-induced catalepsy as it has been reported to be intensified and antagonized by pilocarpine and atropine, respectively.[6] Evidence also suggests that the central serotonergic system modulates nigrostriatal dopaminergic transmission with 5-HT_3 antagonists reported to alleviate neuroleptic-induced catalepsy.[7] The phenomenon of cataleptic immobility induced in rodents by typical neuroleptics (e.g. haloperidol) is a robust behavioural model to study nigrostriatal function and its modulation by cholinergic, serotonergic, nitrergic and other neurotransmitter systems.[8]

*Vitex negundo (L.) (*Family: *Verbenaceae)* commonly known as monks' pepper or five leaved chest trees (Hindi- sambhalu, nirgundi). The principal constituents the leaf juice are casticin, isoorientin, chrysophenol D, luteolin, p–hydroxybenzoic acid and D-fructose and other constituents like vitamin C, carotene, β-sitosterol and C-glycoside.[9] The main constituents of the oil are sabinene, linalool, terpinen-4, β-caryophyllene, α-guaiene and globulol constituting 61.8% of the oil. The seeds contain hydrocarbons, β-sitosterol, benzoic acid and phthalic acid.[10] The *Vitex negundo* extracts have been used in Unani system of medicine as anti-inflammatory, expectorant, tranquilizer, antispasmodic, anti convalesant, rejuvenative, antiarthritic, anthelminthic, anti-fungal and antipyretic. The Ayurvedic and Unani Pharmacopoeia of India has documented the use of the leaf, seed and the root to treat excessive vaginal discharge, edema, skin diseases, pruritus, helminthiasis.[11,12]

The aim of the present study was to identify the anti-catalepsy activity of chosen herb in selected animal models. The objective of the study was to rule out the efficacy of the test compound with standard anti cataleptic drugs used Pheniramine malate i.p., available in market.

METHODS

Animals

Healthy adult mice weighing about 20-25gm were used for study. They were housed in polypropylene cages maintained under standard condition. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee and was cleared by the same before starting.

Drugs and chemicals

The following drugs and chemicals were used.

Drugs: Haloperidol and Pheniramine maleate purchased from commercial source. Chemicals: petroleum ether (60-80°C), and tween 80 AR.

Preparation of the extract

Vitex negundo (L.) plant was identified and authenticated with a botanist. Its stem bark was separated and make in course powder after shade dried, for getting thick semisolid paste by subjecting it to maceration in 80% Methanol. The obtained semisolid thick paste was stored in refrigerator.

Inclusion criteria

Preferably small, disease free animals were selected.

Exclusion criteria

The animals not responding to the study and diseased animals excluded.

Methodology

Haloperidol induced catalepsy

Haloperidol (1 mg/kg, i.p.) was injected to mice (n = 6) pre-treated 30 min before with vehicle (5 ml/kg, i.p.), and prepared extract also administered.

Experimental method

Assessment of Catalepsy was measured by the bar test. Mice were positioned so that their hindquarters were on the bench and their forelimbs rested on a 1-cm diameter horizontal bar that was 4 cm above the bench. Mice were judged to be cataleptic if they maintained this position for 30 s or more. The length of time for which the mouse maintained this position was recorded with a stopwatch with a maximum duration of 180 seconds. The durations of catalepsy were measured at 0, 30, 60, 90, 120, 150, and 180 min.[13]

Collection of plant, dose selection and acute toxicity study

Vitex negundo plant were collected from the local area in Udaipur and were authenticated by botanist. According to Borse et al, 50 mg, 100 mg and 200 mg were selected. In the acute toxicity test, extract of *Vitex negundo (Linn)* plant did not produce any detectable toxicity on oral and i.p. administration. No mortality was found, which is reflected by high LD50 of extract of *Vitex negundo (Linn)*.[14]

Experimental design

Mice were divided in 5 groups of 6 in each (n=30), Group-I (control) served as control and received Haloperidol 1 mg/kg i.p., Group-II (standard) served as standard and received Pheniramine maleate 10 mg/kg, i.p., Group-III was treated with methanol extract of *Vitex negundo* (50 mg/kg) and Haloperidol 1 mg/kg i.p., Group-IV was treated with combination of Methanol

extract of *Vitex negundo* (100 mg/kg) and haloperidol 1 mg/kg i.p. and Group-V was treatment of methanol extract of *Vitex negundo* (200 mg/kg) and haloperidol 1 mg/kg i.p.

Ethical clearance

The following experimental procedures on animals was done after getting ethical clearance from IAEC Reference No. GMCH/IAEC/2019/7677(1) of Geetanjali University, Udaipur, Rajasthan.

Statistical analysis

The results were expressed as the mean±standard error of mean (SEM) and analysed by using one-way analysis of variance (ANOVA), followed by Dunnett's Post hoc test. A $p<0.05$ was considered statistically significant.

RESULTS

In vehicle treated animals, haloperidol (1 mg/kg. i.p.) produced the maximum catalepsy at 180 min (46.78±3.78 min). Standard treated as Pheniramine maleate 10mg/kg, i.p. shows maximum at 120 min. 19.24±1.32. Test herb, i.p. 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.

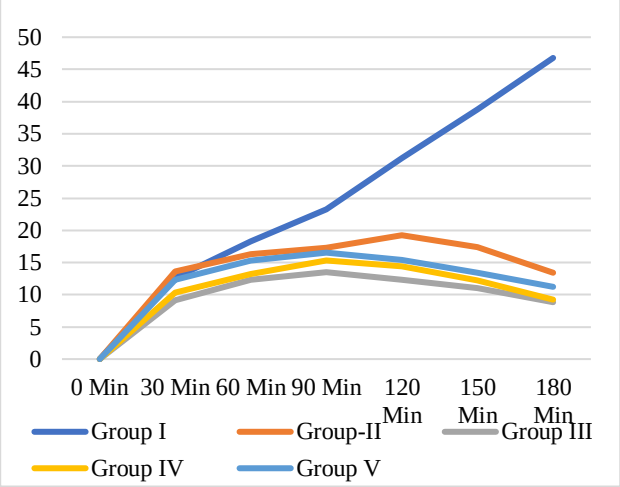

Figure 1: Effect of methanolic extract of *Vitex negundo* on haloperidol-induced catalepsy in mice (n=6).

At dose 50, 100 and 200 mg/kg, extract of *Vitex negundo (Linn)* roots showed maximum cataleptic score 12.34±0.78, 14.43±0.43 and 15.43±0.67 min, respectively at 120 minutes in haloperidol treated animals.

DISCUSSION

Neuroleptic-induced catalepsy in rodents has long been used as an animal model for screening drugs for Parkinsonism and it is a robust behavioural method for studying nigrostriatal function and its modulation by cholinergic, GABAergic, serotonergic and nitrergic systems.[15-21] Evidence indicates that drugs which potentiate or attenuate neuroleptic induced catalepsy in rodents might aggravate or reduce the extrapyramidal side effects respectively.[22]

Haloperidol-induced catalepsy: Haloperidol, typical neuroleptic produces catalepsy in rodents and extrapyramidal side effects in human.[23] Haloperidol-induced catalepsy is one of the animal models for testing the extrapyramidal side effects of antipsychotic drugs.[21] Haloperidol, (a non-selective D2 dopamine antagonist) and metoclopramide (a potent dopaminergic blocking agent) induced catalepsy is primarily due to blockade of dopamine receptors in the striatum.[24]

In vehicle treated animals, haloperidol (1 mg/kg. i.p.) produced the maximum catalepsy at 180 min (46.78±3.78 min). Standard treated as Pheniramine maleate 10 mg/kg shows maximum at 120 min. 19.24±1.32. Test herb, i.p. Methanolic extract of *Vitex negundo* (50, 100, 200mg/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 12.34±0.78, 14.43±0.43 and 15.43±0.67 min, respectively at 120 minutes in haloperidol treated 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.[25]

In present study, extract of *Vitex negundo* (Linn) roots (50, 100 and 200 mg/kg, i.p.) significantly potentiated dose dependent haloperidol-induced catalepsy. Thus, the results suggest that extract of *Vitex negundo* (Linn) roots shows antidopaminergic activity.

Present study showed that the decreases in haloperidol induced catalepsy by the polyherbal formulation *Vitex negundo*, was comparable to the standard drug scopolamine. Moreover, the test drug had a quicker onset of action as compared to scopolamine. *Vitex negundo* is a polyherbal preparation containing the extracts of *Withania somnifera*, *Ocimum sanctum*, *Camellia sinensis*. The mechanism of anticataleptic activity of the test compound shows antidopaminerigc activity are primarily responsible for its anticataleptic activity.

CONCLUSION

In conclusion, *Vitex negundo* was found to be effective in reducing cataleptic scores in mice model of haloperidol induced catalepsy. Present study suggests that the test drug can be used as an alternative agent in preventing the haloperidol/neuroleptics induced extrapyramidal symp-

toms in schizophrenic patients. However, it requires further preclinical and clinical studies to prove it.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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