Research Paper

# Effect of ethanolic leaf extract of *Ocimum sanctum* on haloperidol-induced catalepsy in albino mice

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**ABSTRACT**

Neuroleptic drugs used in the treatment of schizophrenia and other affective disorders are known to produce extrapyramidal side effects. Catalepsy induced by these drugs in animals has been used as a model for the extrapyramidal side effects associated with antipsychotic agents in human beings. In the present study, we have attempted to evaluate the protective effect of the ethanolic leaf extractof *Ocimum sanctum* (OS) on haloperidol (1.0 mg/kg, intraperitoneal administration)-induced catalepsy in mice by employing the standard bar test. Mice were allocated to seven groups, each group containing six animals. The effects of the test drug OS (at 1.75, 4.25 and 8.5 mg/kg doses) and the standard drugs, scopolamine (1.0 mg/kg) and ondansetron (0.5 and 1.0 mg/kg doses) were assessed after single and repeat dose administration for seven days, 30 minutes prior to the haloperidol. The results suggest that OS has a protective effect against haloperidol-induced catalepsy, which is comparable to the standard drugs used for the same purpose. Our study indicates that OS could be used to prevent drug-induced extrapyramidal side effects.

**KEY WORDS:** Catalepsy, haloperidol, *Ocimum sanctum*



Neuroleptics that are commonly used in the treatment of schizophrenia and other affective disorders[1] are often associated with distressing extrapyramidal side effects.[2,3] The phenomenon of cataleptic immobility induced in rodents by typical neuroleptics (e.g., haloperidol) is a robust behavioral model to study nigrostriatal function and its modulation by cholinergic, 5-hydroxytryptamine (5-HT, serotonergic), nitrergic and other neurotransmitter systems.[4,5] Haloperidol-induced catalepsy occurs due to the blockade of dopamine (D2) receptors and reduced dopaminergic transmission.[6] Enhanced stimulation of the intrinsic central cholinergic system has also been implicated in haloperidol-induced catalepsy as it has been reported to be enhanced and antagonized by pilocarpine and the cholinergic blocker, atropine respectively.[7] Evidence also suggests that the central serotonergic system modulates nigrostriatal dopaminergic transmission with 5-$HT_3$ antagonists reported to alleviate neuroleptic-induced catalepsy.[4] Hence, scopolamine (a known anticholinergic agent) and ondansetron (a known 5-$HT_3$ antagonist)have been used as standard drugs in this study to compare the anticataleptic effect of the test compound, OS.

*Ocimum sanctum*, popularly known as Tulsi in Hindi and 'Holy Basil' in English is one of the sacred herbs for Hindus in the Indian subcontinent. It has a versatile role in traditional medicine. The entire plant of OS has medicinal value although mostly the leaves, and sometimes the seeds, are used. Earlier studies with OS have indicated that the plant has hypoglycemic, hypolipidemic, adaptogenic, antidepressant, antiepileptic, hepatoprotective, anticancer, radioprotective, analgesic and antiinflammatory properties.[8] The ethanolic extract of OS leaves contains ≥ 2.5% ursolic acid (estimation and purity of active principle was done by the Quality Control Laboratory, M/s. Natural Remedies, Bangalore, lab reference no.0408817, dt.30-8-2004). Ursolic acid was found to have antiinflammatory, antitumor, antioxidant and antibacterial properties.[9] Preclinical psychopharmacological screening with the ethanolic leaf extract of OS has confirmed its antiepileptic and antidepressant activity.

This behavioral study has implicated the involvement of dopaminergic neurons in these activities.[10] The ethanolic leaf extract of OS has been found to increase the dopamine levels in the corpus striatum.[11] We have previously reported the anticataleptic activity of NR-ANX-C (a polyherbal product) against haloperidol-induced catalepsy in mice.[12] *Ocimum sanctum* is one of the components of NR-ANX-C. Hence, our earlier finding—evidence of involvement of the central dopaminergic system in behavioral changes as well as the known antioxidant effects of OS prompted us to investigate its anticataleptic activity.

## Materials and Methods

*Animals*

Adult male albino mice (weighing 25-30 gm), bred in the Central Animal House of Kasturba Medical College, Mangalore, were used for the study. Animals were housed under a standard 12 h:12 h light/dark cycle and were provided with food and water *ad libitum*. Animals were acclimated to laboratory conditions before testing. Each animal was used once. The experiments were performed between 10.00 and 16.00 hrs. The experimental protocol was approved by the Institutional Animal Ethics Committee and the study was conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

*Drugs and dosage*

The test drug, the ethanolic leaf extract of *Ocimum sanctum* (dry powder supplied by Natural Remedies Pvt. Ltd., Bangalore) and the standard drugs, scopolamine (German Remedies Ltd, Mumbai) and ondansetron (Cipla Ltd., Mumbai) were suspended / dissolved in 1% Gum acacia solution while haloperidol (RPG Life Sciences Ltd., Mumbai) was dissolved in distilled water. The number of animals and the treatment received by each group are shown in Tables 1 and 2. Scopolamine (1.0 mg/kg), ondansetron (0.5 and 1 mg/kg) and OS (1.75, 4.25 and 8.5 mg/kg) were given orally whereas haloperidol was given intraperitoneally.

*Experimental design*

Haloperidol-Induced Catalepsy (HIC): Catalepsy was induced with haloperidol (1.0 mg/kg i.p.) and assessed at 30 minute intervals until 120 minutes and at the end of 240 minutes by means of a standard bar test.[13] Haloperidol 1 mg/kg i.p. was chosen so that it could elicit a moderate degree of catalepsy and thus enable the detection of either attenuation or potentiation of the phenomenon.[4,5] Catalepsy was assessed in terms of the time for which the mouse maintained an imposed position with both front limbs extended and resting on a 4 cm high wooden bar (1.0 cm diameter). The end point of catalepsy was considered to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. A cut-off time of 1100 seconds was applied. Between determinations, the animals were returned to their individual home cages. All observations were made between 10.00 and 16.00 hrs in a quiet room at 23-25$^{o}$C.

Scoring method: If the animal maintained the imposed posture for at least 20 seconds, it was considered to be cataleptic and given one point. For every additional 20 seconds that the cataleptic posture was maintained, one extra point was given. The animals were tested twice at 30 minute intervals and only the greater duration of immobility was considered.[13]

In the acute study, OS, scopolamine and ondansetron were administered only once 30 min prior to the haloperidol administration. In the chronic study, these drugs were administered once daily 30 min prior to the haloperidol administration for seven days. Catalepsy was determined 30 min after haloperidol administration on the first and on the seventh day of treatment.

*Statistical analysis*

For each group, mean ± SEM was calculated and the data was analyzed by one way ANOVA followed by Dunnet Multiple Comparison test. $P < 0.05$ was considered to be statistically significant.

## Results

*Acute study [Table 1]*

In the acute study, administration of the standard drugs and all doses of the test drug gave cataleptic scores similar to that of the vehicle-treated group. However, from 60 min onwards after haloperidol administration, scopolamine (1.0 mg/kg) and ondansetron (0.5 and 1.0 mg/kg) resulted in significantly lower cataleptic scores than the vehicle-treated mice. On the other hand, the median dose and the two lower doses of OS resulted in lower cataleptic scores as compared to the vehicle group 60 and 90 min after haloperidol administration, respectively. However, from 120 min onwards until 240 min after haloperidol administration, all doses of OS significantly lowered the cataleptic scores compared to the vehicle group. In fact, 90 min post-haloperidol onwards, both 1.75 and 4.25 mg/kg doses of OS were actually more protective against haloperidol-induced catalepsy than even the standard drugs, scopolamine and ondansentron.

*Chronic study [Table 2]*

In the chronic study, administration of the standard drugs and all doses of the test drug 30 min after the last haloperidol dose on the seventh day, gave cataleptic scores similar to that of the vehicle-treated group. However, from 60 min onwards after haloperidol administration, all doses of standard and test drugs resulted in significantly lower cataleptic scores than the vehicle-treated mice. The results of the chronic study show that the highest dose of OS is as protective as the 1 ml/kg dose of scopolamine and the higher dose of ondansentron.. Unlike the acute study, the protective effect of OS against haloperidol-induced catalepsy was both dose- and time-dependent.

## Discussion

Typical neuroleptic agents such as chlorpromazine, haloperidol and reserpine induce a cataleptic state in rodents which is widely used as a model to test the extrapyramidal side effects of antipsychotic agents. Neuroleptic-induced catalepsy has been linked to a blockade of postsynaptic striatal dopamine D1 and D2 receptors.[14] Despite this evidence, several other neurotransmitters such as acetylcholine, serotonin, angiotensin, adenosine or opioids have also been implicated in the catalepsy induced by neuroleptic agents.[15] In addition to various neurotransmitters, many preclinical and clinical studies have also proposed reactive oxygen species as causes of haloperidol-induced toxicity.[16] Evidence indicates that drugs which potentiate or attenuate neuroleptic catalepsy in rodents might also aggravate or reduce the extrapyramidal signs respectively in human beings.[17]

In the present study, OS protected mice from catalepsy induced by haloperidol as effectively as the standard drugs, scopolamine and ondansetron. The protective effect of OS against HIC is consistent with our earlier report on anticataleptic effect of a polyherbal product, NR-ANX-C,[12] in which OS is one of the components. Earlier behavioral studies in rodents have suggested that OS facilitates activation of dopaminergic neurons.[10,11] Thus, the anticataleptic effect

**Table 1**

**Effects of acute administration of *Ocimum sanctum* on haloperidol-induced catalepsy**

| Time after haloperidol (min) | Control vehicle (10.0 ml/kg) | Scopolamine (1.0 mg/kg) | Ondansetron (mg/kg) | | Ocimum sanctum (mg/kg) | | | One-way ANOVA | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 0.5 | 1.0 | 1.75 | 4.25 | 8.5 | F | df |
| 30 | 11.16±1.19 | 12.80±0.9 | 10.66±1.28 | 12.66±0.68 | 14.83±1.13 | 8.82±0.44 | 13.60±1.62 | 3.42 | 6,35 |
| 60 | 24.50±1.74 | 17.00±1.00** | 15.00±1.03 | 16.16±0.70*** | 23.50±0.84 | 12.83±1.24** | 23.33±2.62 | 10.66 | 6,35 |
| 90 | 34.00±3.41 | 21.50±1.21** | 21.16±0.80** | 23.86±0.60** | 17.33±0.95** | 14.50±1.11** | 36.50±3.36 | 17.05 | 6,35 |
| 120 | 47.50±4.16 | 26.83±1.27*** | 26.83±1.27*** | 31.83±0.70*** | 12.66±1.07*** | 12.16±0.87*** | 25.66±4.12** | 24.79 | 6,35 |
| 240 | 51.33±2.43 | 17.83±1.37*** | 33.66±0.88** | 40.66±0.88** | 8.50±0.61*** | 7.50±0.56*** | 17.16±3.79** | 81.35 | 6,35 |

Values are mean ± SEM *$P$<0.05; **$P$<0.01; $P$<0.001 Vs control (Dunnett's multiple comparison)

**Table 2**

**Effects of chronic administration of *Ocimum sanctum* on haloperidol-induced catalepsy**

| Time after haloperidol (min) | Control vehicle (10.0 ml/kg) | Scopolamine (1.0 mg/kg) | Ondansetron (mg/kg) | | Ocimum sanctum (mg/kg) | | | One-way ANOVA | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 0.5 | 1.0 | 1.75 | 4.25 | 8.5 | F | df |
| 30 | 13.33±0.8 | 13.66±0.49 | 13.5±0.67 | 10.33±0.49** | 12.5±0.67 | 11.66±0.71 | 10.00±0.57 | 5.59 | 6,35 |
| 60 | 18.33±1.16 | 15.83±0.47* | 15.66±0.61** | 13.33±0.66** | 15.83±0.40* | 12.33±0.49** | 12.66±0.76** | 8.54 | 6,35 |
| 90 | 25.33±0.84 | 18.33±0.40** | 19.33±0.61** | 16.83±0.70** | 20.33±0.49** | 19.00±0.57** | 16.50±0.61** | 22.22 | 6,35 |
| 120 | 34.16±1.40 | 16.66±0.49** | 23.50±0.42** | 14.00±0.63** | 17.33±0.55** | 16.33±0.66** | 14.16±0.40** | 98.4 | 6,35 |
| 240 | 37.33±1.20 | 14.5±0.56*** | 19.16±0.30** | 11.00±0.63** | 14.66±0.42** | 13.60±0.42** | 11.33±0.49** | 207.68 | 6,35 |

Values are mean± SEM *$P$<0.05; **$P$<0.01; $P$<0.001 Vs control (Dunnett's multiple comparison)



of OS might be due to both its dopamine facilitatory and antioxidant properties. The active principle of the ethanolic leaf extract of OS contains 2.7% ursolic acid, which has antioxidant properties and gives remarkable protection against lipid peroxidation.[9] However, further studies are required to confirm the exact mechanism of action. Our results suggest that OS can be used as an alternative / adjuvant drug in preventing and treating the extrapyramidal side effects of antipsychotic agents in clinical practice.

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