Effect of NR-ANX-C (a polyherbal formulation) on haloperidol induced catalepsy in albino mice


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**Background & objectives**: Use of typical antipsychotics like haloperidol in treatment of schizophrenia is associated with a high incidence of extrapyramidal side effects. In rodents, administration of haloperidol leads to the development of a behavioural state called catalepsy, in which the animal is not able to correct an externally imposed posture. In the present study we evaluated the anticataleptic efficacy of NR-ANX-C, a polyherbal formulation containing bioactives of *Withania somnifera*, *Ocimum sanctum*, *Camellia sinensis*, triphala and shilajit in haloperidol induced catalepsy in mice.

**Methods**: Five groups (n = 6) of male albino mice were used in the study. Catalepsy was induced by ip administration of haloperidol (1mg/kg). The degree of catalepsy (cataleptic score) was measured as the time the animal maintained an imposed posture. We compared the anticataleptic efficacy of NR-ANX-C (10, 25 and 50 mg/kg) with scopolamine (1 mg/kg). The superoxide dismutase (SOD) level in brain tissue was also estimated to correlate the levels of oxidative stress and degree of catalepsy in the animal.

**Results**: Significant (P<0.01) reduction in the cataleptic scores was observed in all NR-ANX-C treated groups and maximum reduction was observed in the NR-ANX-C (25 mg/kg) treated group. Significant (P<0.05) reduction in SOD activity was observed in NR-ANX-C (25 and 50 mg/kg) treated groups and maximum reduction was observed in NR-ANX-C (25mg/kg) treated group.

**Interpretation & conclusions**: In our study, maximum reduction in cataleptic score was observed in NR-ANX-C (25 mg/kg) treated group. The maximum reduction in SOD activity was also observed in the same group. These findings suggest a possible involvement of the antioxidant potential of NR-ANX-C in alleviating haloperidol induced catalepsy.

**Key words** Antioxidants - *Camellia sinensis* - catalepsy - haloperidol - *Ocimum sanctum* - *Withania somnifera*
defined as the failure to correct an externally imposed posture. This test is widely used to evaluate the effect of drugs on extrapyramidal system\(^3\). The pathophysiological basis of catalepsy still remains obscure. Theories implicating central cholinergic dysfunction\(^2\), \(\gamma\)-amino butyric acid (GABA) deficiency\(^6\), oxidative stress\(^7\) and 5-hydroxytryptamine (5-HT)\(^8\) dysfunction have been proposed.

Haloperidol blocks dopamine D\(_2\) receptors and produces a state of catalepsy in animals by reducing dopaminergic transmission in basal ganglion. Anticholinergic drugs are most effective in counteracting the catalepsy induced by haloperidol in experimental animals\(^5\). But these anti-cholinergic drugs produce various side effects like dryness of mouth, constipation, urinary retention, etc. Hence the search for newer drugs with fewer side effects is continuing. In this context plant products which are frequently considered to be less toxic and free from side effects compared to synthetic drugs are under exploration.

The polyherbal preparation NR-ANX-C (a test drug supplied by Natural Remedies Pvt. Ltd., Bangalore) contains the extracts of Withania somnifera (Ashwagandha), Ocimum sanctum (Tulsi), Camellia sinensis (green tea), triphala and shilajit. Earlier studies have shown some of these constituents to have effects on the monoaminergic\(^9,10\), GABAergic\(^11\) and antioxidant systems in brain\(^12-15\). Since dysfunctions in these systems have been postulated to play a role in the development of catalepsy, we carried out this study to evaluate the efficacy of the polyherbal formulation NR-ANX-C in reversing haloperidol induced catalepsy in a rodent model.

**Material & Methods**

The study protocol was approved by the Institutional Animal Ethical Committee.

**Test drug:** The composition of test drug NR-ANX-C (supplied by Natural Remedies Pvt. Ltd, Bangalore), a polyherbal formulation, was as follows: *Withania somnifera* 17 per cent (water extract of root: withanolides 2.1% w/w), *Ocimum sanctum* 17 per cent (70% alcohol extract of leaves: ursolic acid 2.9% w/w), *Camellia sinensis* 33 per cent (70% alcohol extract of leaves: total polyphenols 60.1% w/w), *triphala* 25 per cent (water extract: total tannin 33.5% w/w) and *shilajit* 8 per cent (water extract: fulvic acid 52.6% w/w; humic acid 16.7% w/w).

**Animals:** Adult male Swiss albino mice (25-30 g) from our breeding stock were used for the study. They were housed in clean and transparent poly propylene cages with three animals in each cage and maintained at 27\(^\circ\)C with 12:12 h light-dark cycle for a period of 7 days prior to the study. They were fed standard rat chow and water *ad libitum*.

**Acute study:** The animals were divided into five groups (n=6). All observations were made between 10:00 h and 16:00 h. The test drug NR-ANX-C and scopolamine (as butyl bromide, German Remedies Ltd., Mumbai), were suspended in 1 per cent gum acacia solution (vehicle). All drug solutions were freshly prepared and administered orally using a feeding tube. The first group received vehicle and served as the control, the second group received 1 mg/kg body weight of scopolamine and the third, fourth and fifth groups received NR-ANX-C in doses of 10, 25 and 50 mg/kg body weight respectively. Thirty minutes after administration of vehicle/drugs, haloperidol (RPG Life Sciences Ltd., Mumbai) at the dose of 1 mg/kg body weight, in the form of an injectable solution constituted in normal saline was administered by the ip route to induce catalepsy. This dose of the neuroleptic drug was chosen from our pilot study to produce a moderate degree of catalepsy, so that either attenuation or potentiation of the phenomenon could be detected. The degree of catalepsy was measured at 30, 60, 90 and 120 min after haloperidol administration as described by Ahtee & Buncombe\(^16\). Briefly, catalepsy was measured as the time the animal maintained an imposed position with both front limbs raised and resting on a four centimeter high wooden bar. 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. If the animal maintained the imposed posture for at least twenty seconds it was said to be cataleptic and given one point. For every further twenty seconds that the animal continued to maintain the cataleptic posture one extra point was given. Based on the data obtained in our pilot study, a cut-off cataleptic score of 60 (corresponding to 20 min) was used during the recording of observations. After the recording of observations, the animals were returned to their individual cages and were maintained with a 12:12 h dark-light cycle for six more days. The same groups of animals were used for the chronic study.

**Chronic study:** NR-ANX-C (10, 25 and 50 mg/kg), scopolamine (1 mg/kg) and vehicle were administered...
orally once a day to the respective groups for six more days. Thirty minutes post oral administration, haloperidol (1 mg/kg) was administered ip to all the groups once daily for a period of six more days. Catalepsy was again measured on the seventh day at 30, 60, 90 and 120 min post haloperidol administration. Soon after the behavioural study, the animals were sacrificed by cervical dislocation, and the superoxide dismutase (SOD) activity in the whole brain was estimated by the Beauchamp and Fridovich method. The SOD activity was expressed in terms of Units/mg total protein. To get a normal range for SOD activity and to rule out the involvement of the vehicle, 6 mice were administered 10 ml/kg 1 per cent gum acacia solution for seven days. On the seventh day, they were sacrificed 150 min after vehicle administration, and SOD activity and total protein in the brain were determined.

The data were analyzed by One-way ANOVA, followed by Dunnett’s multiple comparison test. A $P < 0.05$ was considered significant.

**Results**

**Acute study** (Table I): The cataleptic score was significantly reduced after 60 min, with both, the standard drug scopolamine (1mg/kg) and the test drug NR-ANX-C at all the doses tested (10, 25 and 50 mg/kg). The reduction in cataleptic scores with NR-ANX-C 10 mg/kg and 25 mg/kg was significant throughout the period of observations, till 120 min. The reduction of cataleptic score with scopolamine and NR-ANX-C 50 mg/kg was seen only after 60 min of observation. Maximum reduction in cataleptic activity was seen in the 25 mg/kg NR-ANX-C treated group throughout the observation period.

**Chronic study** (Table II): In chronic study, NR-ANX-C in the higher doses (25 and 50 mg/kg) significantly reduced the cataleptic score right from the start of the study. NR-ANX-C 10 mg/kg and scopolamine (1.0 mg/kg) reduced the cataleptic scores significantly only after 60 min of observation. Although the reduction in catalepsy was less as compared to acute study, the reductions were significant when compared against the control (vehicle + haloperidol).

**SOD activity** (Table III): The SOD activity in the brain tissue was found to be elevated in the haloperidol treated group as compared to normal. At higher doses (25 and 50 mg/kg), NR-ANX-C significantly reduced the elevated SOD activity towards normal. Maximum reduction in SOD activity was seen in group IV (NR-ANX-C - 25 mg/kg).

**Table I.** Effect of NR-ANX-C on haloperidol induced catalepsy in albino mice - Acute study
(Data are mean ± SE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Vehicle + haloperidol</td>
<td>1mg</td>
<td>11.5 ± 1.25</td>
<td>24.5 ± 1.76</td>
<td>34 ± 3.67</td>
<td>47.5 ± 4.47</td>
</tr>
<tr>
<td>II. Scopolamine + haloperidol</td>
<td>1mg + 1mg</td>
<td>12.66 ± 0.95</td>
<td>17.00 ± 1.00**</td>
<td>21.5 ± 1.14**</td>
<td>26.83 ± 0.98**</td>
</tr>
<tr>
<td>III. NR-ANX-C + haloperidol</td>
<td>10mg + 1mg</td>
<td>5.16 ± 0.65**</td>
<td>9.66 ± 0.84**</td>
<td>13.5 ± 1.17**</td>
<td>6.65 ± 0.67**</td>
</tr>
<tr>
<td>IV. NR-ANX-C + haloperidol</td>
<td>25mg + 1mg</td>
<td>4.5 ± 0.56**</td>
<td>7.16 ± 0.60**</td>
<td>12.33 ± 0.98**</td>
<td>6.66 ± 0.66**</td>
</tr>
<tr>
<td>V. NR-ANX-C + haloperidol</td>
<td>50mg + 1mg</td>
<td>8.5 ± 0.67</td>
<td>10.5 ± 0.76**</td>
<td>13.33 ± 0.80**</td>
<td>10.13 ± 0.65**</td>
</tr>
</tbody>
</table>

Statistical analysis by One-way ANOVA followed by Dunnett’s multiple comparison test; **$P < 0.01$ compared to control

**Table II.** Effect of NR-ANX-C on haloperidol induced catalepsy in albino mice - Chronic study
(Data are mean ± SE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Vehicle + haloperidol</td>
<td>1mg</td>
<td>13.33 ± 0.80</td>
<td>18.33 ± 1.16</td>
<td>25.33 ± 0.84</td>
<td>34.16 ± 1.40</td>
</tr>
<tr>
<td>II. Scopolamine + haloperidol</td>
<td>1mg + 1mg</td>
<td>13.66 ± 0.49</td>
<td>15.33 ± 0.47*</td>
<td>18.33 ± 0.40**</td>
<td>16.66 ± 0.49**</td>
</tr>
<tr>
<td>III. NR-ANX-C + haloperidol</td>
<td>10mg + 1mg</td>
<td>11.00 ± 0.63</td>
<td>14.5 ± 0.56**</td>
<td>21 ± 0.96**</td>
<td>17.33 ± 0.55**</td>
</tr>
<tr>
<td>IV. NR-ANX-C + haloperidol</td>
<td>25mg + 1mg</td>
<td>6.33 ± 0.76**</td>
<td>9.16 ± 0.87**</td>
<td>15.5 ± 1.05**</td>
<td>13 ± 0.72**</td>
</tr>
<tr>
<td>V. NR-ANX-C + haloperidol</td>
<td>50mg + 1mg</td>
<td>9.66 ± 0.55**</td>
<td>13.33 ± 0.49**</td>
<td>19.5 ± 0.76**</td>
<td>15.13 ± 0.90**</td>
</tr>
</tbody>
</table>

Statistical analysis by one-way ANOVA followed by Dunnett’s Multiple Comparison Test; *$P < 0.05$; **$P < 0.01$ compared to control
Discussion

Neuroleptic-induced catalepsy in rodents has long been used as an animal model for screening drugs for Parkinsonism and it is a robust behavioral method for studying nigrostriatal function and its modulation by cholinergic, GABAergic, serotonergic and nitrergic systems. Evidence indicates that drugs which potentiate or attenuate neuroleptic induced catalepsy in rodents might aggravate or reduce the extrapyramidal side effects respectively. In our study, the polyherbal formulation NR-ANX-C in all the doses tested showed a significant reduction in the cataleptic scores, both on acute and chronic administration. In the higher doses tested (25 and 50 mg/kg), reduction was greater than that produced by the standard drug scopolamine. Although there was a delay in onset of action of scopolamine when compared to NR-ANX-C, the effects were evident till 120 min of observation with both the drugs. It is difficult to explain why there was delay in onset of action with scopolamine even on repeated administration. One explanation could be that in order to maintain uniformity in the dosage forms of all the drugs, a suspension of scopolamine in gum acacia was used instead of a solution. Haloperidol induced increase in SOD activity was significantly reversed by the test compound NR-ANX-C (25 and 50 mg/kg), but not by the standard drug scopolamine (1 mg/kg).

Typical neuroleptic induced catalepsy has been linked to a blockade of postsynaptic striatal dopamine D₁ and D₂ receptors. Despite this evidence, dysfunction of several other neurotransmitters such as acetylcholine, GABA, and serotonin, have also been implicated. In addition to dysfunction of various neurotransmitters in catalepsy, many clinical and preclinical studies have suggested the involvement of reactive oxygen species in haloperidol induced toxicity. In the present study also, haloperidol treated group showed an increase in oxidative stress when compared to normal vehicle treated group, suggesting the neurotoxic effect of haloperidol.

Our study showed that the decreases in haloperidol induced catalepsy by the polyherbal formulation NR-ANX-C, was comparable to the standard drug scopolamine. Moreover, the test drug had a quicker onset of action as compared to scopolamine. NR-ANX-C is a polyherbal preparation containing the extracts of Withania somnifera, Ocimum sanctum, Camellia sinensis, triphala and shilajit. The mechanism of anticataleptic activity of the test compound is not known, and at this juncture it is difficult to specify which constituents of the preparation are primarily responsible for its anticataleptic activity.

In conclusion, NR-ANX-C was found to be effective in reducing cataleptic scores in mice model of haloperidol induced catalepsy. Our study suggests that the test drug can be used as an alternative agent in preventing the haloperidol/neuroleptics induced extrapyramidal symptoms in schizophrenic patients. However, it requires further preclinical and clinical studies to prove it.

Acknowledgment

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References

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