Role of Shilajit in a murine model of haloperidol induced catalepsy


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ABSTRACT

Shilajit, an ayurvedic drug is a blackish-brown exudation obtained as a mineral resin or as a plant fossil. Experimental study has shown shilajit has nootropic and anxiolytic activities. The nootropic and anxiolytic actions have been attributed to the ability of shilajit to induce an increase in rat brain dopaminergic activity and a decrease in 5-HT turnover. Shilajit is one of the components of NR-ANX-C (a poly-herbal preparation), which has proven anticaatalpleptic activity in a previous study conducted by us. Increased dopamine turnover in the rat brain with Shilajit and the anticaatalpleptic activity of NR-ANX-C prompted us to assess the anticaatalpleptic activity of Shilajit. Inbred albino mice were divided into five groups, each containing six animals. Both, the test drug, the Shilajit and the standard drug scopolamine were dissolved in 1% gumacacca solution. Catalepsy was induced with haloperidol (1.0 mg/kg; i.p.). The first group received the vehicle (10.0 ml/kg), the second group received scopolamine (1.0 mg/kg) and the remaining three groups of animals received the test compound Shilajit (2.5, 6.25 & 12.5 mg/kg) were assessed after single and repeated dose administration for seven days, 30 minutes prior to the haloperidol. In the present study, pretreatment of aqueous extract of shilajit protected the mice from catalepsy induced by haloperidol as effectively as the standard drug scopolamine and in fact better than scopolamine on repeated administration. Our study suggests that Shilajit can be explored as an adjuvant drug in preventing and treating the extrapyramidal side effects of antipsychotic agents in clinical practice.

Keywords: Shilajit, catalepsy, haloperidol

INTRODUCTION

Shilajit, a herbomineral drug of the ancient Indian Materia Medica is extensively used by Hindu physicians even in modern times today. Early ayurvedic writings from the Charaka Samhita and Sushruta Samhita describe shilajit as a cure for all disease as well as a rasayana (rejuvenative). It has been proposed to arrest aging and induce rejuvenation. It is a blackish-brown exudation, obtained as a mineral resin or as a plant fossil composed of humus and organic plant material that has been compressed by layers of rock mixed with microbial metabolites (1,2).

Chemical analysis shows that it contains besides gums, albuminoids, traces of resin and fatty acid, a large quantity of benzoic and hippuric acids and their salts. From the medicinal point of view, the chief active substances in it are benzoic acid and benzoates (3). Ayurvedic use of shilajit as a tonic has some support from studies of the humic acids, fulvic acids, coumarins, and triterpenes that have shown anti-stress effects in animals (4). Traditional uses primarily focus on diabetes and diseases of the urinary tract, including edema, tumors, wasting, epilepsy and even insanity. Some of the most interesting research studies confirm shilajit’s uses as an analgesic & anti-inflammatory agent (5), antilcer drug (6), antidiabetic agent (7), anti-anxiety agent (8) and as a nootropic (enhancer of learning acquisition and memory retrieval) agent (9). In Swiss mice, the concomitant administration of shilajit with morphine, from day 6 to day 10, resulted in a significant inhibition of the development to morphine induced analgesia (10). Researchers evaluating the nutritive tonic effects of Shilajit suggest a better utilization of food as a cause of the weight gain seen in rats fed with Shilajit (11). Shilajit has been reported to be quite safe up to a dose of 3 g/kg in mice (24hrs mortality) (12).

Neuroleptic-induced catalepsy has long been used as an animal model for screening drugs for Parkinsonism. Catalepsy is defined as the failure to correct an externally imposed posture (13). Anti-cholinergic drugs are most effective in counteracting the catalepsy induced by haloperidol in experimental animals (14). But these anti-cholinergic drugs produce various side effects like dryness of mouth, constipation, urinary retention, etc. Hence there is an ongoing quest for new drugs with fewer side effects. 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.

Shilajit is an important constituent of polyherbal formulations like NR-ANX-C. The anticaatalpleptic effect of this herbal preparation and some of its individual constituents have been reported (15,16,17,18). Based on the above mentioned findings and contribution of Shilajit in anticaatalpleptic property of NR-ANX-C, the present study was undertaken to evaluate the efficacy of Shilajit in a murine model of haloperidol induced catalepsy.

MATERIALS AND METHODS

Animals:

Adult male albino mice (weighing 25-30gm), bred in the central animal house of Kasturba medical college, Mangalore, were used for the study. The animals were housed under standard 12h: 12h light/dark cycle and supplied with food and water ad libitum. They were allowed to acclimatize to the laboratory conditions for at least seven days prior to any experimentation. Each animal was used only once. The experiment procedures 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:

The test drug, Shilajit (Natural Remedies Pvt. Ltd, Bangalore) and the standard
Table 1: Various treatment groups and their dosages

<table>
<thead>
<tr>
<th>Groups</th>
<th>Acute study</th>
<th>Chronic study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=6)</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Control - 1% Gum acacia - 10.0ml/kg</td>
<td>Single dose Once a day for 10 days</td>
</tr>
<tr>
<td>II</td>
<td>Scopolamine 1mg/kg</td>
<td>Single dose Once a day for 10 days</td>
</tr>
<tr>
<td>III</td>
<td>Shilajit 2.5 mg/kg</td>
<td>Single dose Once a day for 10 days</td>
</tr>
<tr>
<td>IV</td>
<td>Shilajit 6.25 mg/kg</td>
<td>Single dose Once a day for 10 days</td>
</tr>
<tr>
<td>V</td>
<td>Shilajit 12.5 mg/kg</td>
<td>Single dose Once a day for 10 days</td>
</tr>
</tbody>
</table>

The drugs Scopolamine and Shilajit were administered in a single dose 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. In the chronic study, haloperidol induced catalepsy has been used as a model to test the extrapyramidal side effects of antipsychotic agents. The pathophysiological basis of catalepsy still remains obscure. Theories implicating central cholinergic dysfunction (21), gamma-aminobutyric acid (GABA) deficiency (22), oxidative stress (23), and 5-hydroxytryptamine (5-HT) dysfunction (24) have been proposed. Neuroleptic induced catalepsy has been linked to blockade of post synaptic striatal dopamine D1 and D2 receptors (25). Haloperidol is a well known neuroleptic, primarily acting as a D2 receptor antagonist in the mesolimbic-mesocortical pathway. Due to its non-selective action, it also produces blockade of post-synaptic D2 receptors in the nigrostriatal pathway leading to the development of extrapyramidal side effects in humans (24) and catalepsy in animals (26). However, from 60 minutes onwards, the standard drug significantly reduced the cataleptic score throughout the period of observations i.e. from 60 minutes onwards, the standard drug significantly reduced the cataleptic score throughout the period of observations. Though the shilajit treated groups showed significant reduction in cataleptic scores as early as 30 min after haloperidol administration (similar to the acute study), the reduction was not dose dependent. The cataleptic scores were comparably similar in all the doses used. The cataleptic scores were significantly lower than the scopolamine treated groups in all the doses of shilajit indicating a stronger action than scopolamine.

**Table 2: Acute administration of Shilajit on haloperidol induced cataleptic score**

<table>
<thead>
<tr>
<th>Treatments (ml or mg/kg)</th>
<th>30min</th>
<th>60min</th>
<th>90 min</th>
<th>120 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control - 1% Gum acacia 10.0ml/kg</td>
<td>20.6±0.8</td>
<td>29.6±2.6</td>
<td>31.4±1.4</td>
<td>31.0±3.1</td>
<td>39.1±2.9</td>
</tr>
<tr>
<td>Scopolamine 1.0 mg</td>
<td>13.1±1.1</td>
<td>17.1±0.9**</td>
<td>22.5±1.4**</td>
<td>18.1±2.4**</td>
<td>17.6±1.4**</td>
</tr>
<tr>
<td>Shilajit 2.5mg</td>
<td>12.5±2.0**</td>
<td>17.0±1.5**</td>
<td>11.5±1.0**</td>
<td>16.0±2.4**</td>
<td>14.0±2.4**</td>
</tr>
<tr>
<td>Shilajit 6.25mg</td>
<td>12.8±3.5**</td>
<td>12.5±3.0**</td>
<td>16.8±2.2**</td>
<td>16.8±3.4**</td>
<td>14.6±1.87**</td>
</tr>
<tr>
<td>Shilajit 12.5mg</td>
<td>10.5±1.9**</td>
<td>11.6±1.8**</td>
<td>13.8±0.9**</td>
<td>11.8±1.0**</td>
<td>7.8±1.07**</td>
</tr>
<tr>
<td>P-value</td>
<td>3.42</td>
<td>12.69</td>
<td>38.64</td>
<td>8.04</td>
<td>3.39</td>
</tr>
</tbody>
</table>

(Values are mean ± SEM, statistical analysis by one way ANOVA followed by Dunnet’s multiple comparison test; *P<0.05,**P<0.01 compared with control)

**Table 3: Chronic administration of Shilajit on haloperidol induced cataleptic score**

<table>
<thead>
<tr>
<th>Treatments (ml or mg/kg)</th>
<th>30min</th>
<th>60min</th>
<th>90 min</th>
<th>120 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control - 1% Gum acacia 10.0ml/kg</td>
<td>25.0±2.4</td>
<td>29.6±2.6</td>
<td>39.0±4.1</td>
<td>41.8±2.6</td>
<td>45.5±2.9</td>
</tr>
<tr>
<td>Scopolamine 1.0 mg</td>
<td>13.6±0.4**</td>
<td>15.8±0.4**</td>
<td>18.3±0.4**</td>
<td>16.6±0.4**</td>
<td>14.5±0.5**</td>
</tr>
<tr>
<td>Shilajit 2.5mg</td>
<td>6.0±0.8**</td>
<td>10.0±1.5**</td>
<td>9.0±1.6**</td>
<td>6.6±0.6**</td>
<td>8.4±0.9**</td>
</tr>
<tr>
<td>Shilajit 6.25mg</td>
<td>5.6±0.6**</td>
<td>6.3±0.7**</td>
<td>7.6±0.80**</td>
<td>9.1±1.1**</td>
<td>7.3±1.7**</td>
</tr>
<tr>
<td>Shilajit 12.5mg</td>
<td>6.5±0.7**</td>
<td>7.1±0.7**</td>
<td>10.0±0.9**</td>
<td>9.3±0.9**</td>
<td>6.3±0.7**</td>
</tr>
<tr>
<td>P-value</td>
<td>75.73</td>
<td>42.01</td>
<td>39.16</td>
<td>103.47</td>
<td>61.09</td>
</tr>
</tbody>
</table>

(Values are mean ± SEM, statistical analysis by one way ANOVA followed by Dunnet’s multiple comparison test; *P<0.05,**P<0.01 compared with control)

**DISCUSSION**

Haloperidol induced cataleptic state in rodents has been used as a model to test the extrapyramidal side effects of antipsychotic agents. The pathophysiological basis of catalepsy still remains obscure. Theories implicating central cholinergic dysfunction (21), gamma-aminobutyric acid (GABA) deficiency (22), oxidative stress (23), and 5-hydroxytryptamine (5-HT) dysfunction (24) have been proposed. Neuroleptic induced catalepsy has been linked to blockade of post synaptic striatal dopamine D1 and D2 receptors (25). Haloperidol is a well known neuroleptic, primarily acting as a D2 receptor antagonist in the mesolimbic-mesocortical pathway. Due to its non-selective action, it also produces blockade of post-synaptic D2 receptors in the nigrostriatal pathway leading to the development of extrapyramidal side effects in humans (24) and catalepsy in animals (26). Despite this evidence, several other neurotransmitters such as acetylcholine, serotonin, angiotensin, adenosine or opioids have also been implicated (26). In addition to the implications of various neurotransmitters in catalepsy reactive oxygen species have also been proposed to play a role in haloperidol induced toxicity (27). Evidence indicates that drugs which potentiate or attenuate neuroleptic induced catalepsy in rodents might aggravate or reduce extrapyramidal signs respectively in human beings (27).
In the present study, pre-treatment of aqueous extract of shilajit protected the mice from catalepsy induced by haloperidol as effectively as the standard drug scopolamine and in fact better than scopolamine on repeated administration. Moreover, the test drug also showed a quicker onset of action as compared to scopolamine in the acute study. The anticausal effect is more pronounced when shilajit was administered repeatedly than with a single dose, though no dose-dependent responses were observed. The protective effect of shilajit against HIC was consistent with our earlier reports on the anticausal activity of polyherbal product, NR-ANX-C in which shilajit is one of the components (13).

Earlier behavioral studies have suggested that shilajit increases the dopamine turnover, decreases the serotonin turnover, and exerts a significant oxidative free radical scavenging activity (8,28,29) in rodent brain. Thus the anticausal effect of shilajit might be due to both dopamine facilitatory and antioxidant activity. Research data has also demonstrated that Shilajit affects some events in cortical and basal forebrain cholinergic signal transduction cascade in rat brain (30). However, further investigation using more experimental paradigms and neurochemical analysis may provide insight into the anticausal activity of shilajit.

To conclude, the findings of the present study suggest that shilajit could be screened as a potential alternative/adjuvant drug in preventing and treating the extrapyramidal side effects of antipsychotic agents in clinical practice.

ACKNOWLEDGEMENTS

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REFERENCES