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# 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 anticataleptic activity in a previous study conducted by us. Increased dopamine turnover in the rat brain with Shilajit and the anticataleptic activity of NR-ANX-C prompted us to assess the anticataleptic 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% gumacacia 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], antiulcer 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 of tolerance 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].

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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 anticataleptic 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 anticataleptic 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**

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

drug Scopolamine (German Remedies Ltd., Mumbai) were suspended/ dissolved in 1% gum acacia solution and administered orally. Haloperidol (RPG Life Sciences Ltd., Mumbai) was dissolved in distilled water and was given by the intraperitoneal route. Groups of animals and the doses of drug received by each group are shown in table 1.

**Experimental design**:

**Haloperidol induced Catalepsy (HIC):** Thirty minutes after administration of vehicle/drugs, haloperidol (1mg/kg body weight) was administered by the intraperitoneal route to induce catalepsy. This dose of haloperidol was chosen to produce a moderate degree of catalepsy so that attenuation or potentiation of the phenomenon could be detected [19]. The degree of catalepsy was measured at 30, 60, 90, 120 and 240 min after haloperidol administration by using a method similar to the standard bar test[20].

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 4cm high wooden bar (1.0cm 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 during the recording of observations[17]. The animals were returned to their individual home cages in between determinations. All observations were made between 10.00 and 16.00 hrs in a quiet room at 23-25º 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. One extra point was given for every additional period of 20 seconds that the cataleptic posture was maintained. The animals were tested twice at 30 minute time intervals and only the greater duration of immobility was considered[19].

In the acute study, Shilajit and scopolamine 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 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's multiple comparison test. P<0.05 was considered to be statistically significant. The statistical package used for the analysis was SPSS version 11.0.

**RESULTS**

**Acute study:**

In the acute phase of the study (Table 2), prior administration of scopolamine (1mg/kg) did not alter the cataleptic score when observed at the end of 30 minutes. However, from 60 minutes onwards, the standard drug significantly reduced the cataleptic score throughout the period of observations i.e. till 240 minutes. On the other hand, Shilajit at all the doses tested (0.8mg/kg, 2mg/kg and 4mg/kg) significantly reduced the cataleptic score throughout the period of observations i.e. from 30th minute to 240th minutes.

**Chronic Study:**

In the chronic study (Table 3), administration of scopolamine (1mg/kg) and shilajit at all the doses (0.8mg/kg, 2mg/kg and 4mg/kg) significantly reduced the cataleptic score when compare to vehicle treated group 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**

| Treatments (ml or mg/kg | Cataleptic score at the end of (minutes) 30min | 60min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|
| Control 1% Gum acacia 10.0ml/kg | 20.6±0.8 | 29.6±2.6 | 31.4±1.4 | 31.0±3.1 | 39.1±2.9 |
| Scopolamine 1.0 mg | 13.1±1.1 | 17.1± 0.9** | 22.5±1.4** | 18.1±2.4** | 17.6±1.4** |
| Shilajit 2.5mg | 12.5±2.0* | 10.0±2.0** | 11.5±1.0** | 14.0±2.4** | 14.0±2.4** |
| Shilajit 6.25mg | 12.8±3.53* | 12.5±3.0** | 16.8±2.2** | 16.8±3.4** | 14.6±1.87** |
| Shilajit 12.5mg | 10.5±1.9** | 11.6±1.8** | 13.8±0.9** | 11.8±1.0** | 7.8±1.07** |
| F value | 3.42 | 12.69 | 38.64 | 8.04 | 33.96 |

*(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**

| Treatments (ml or mg/kg) | Cataleptic score at the end of (minutes) 30min | 60min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|
| Control 1% Gum acacia 10.0ml/kg | 25.0±2.4 | 29.6± 2.6 | 39.0±4.1 | 41.8 ± 2.6 | 45.5± 2.9 |
| Scopolamine 1.0 mg | 13.6±0.4** | 15.8±0.4** | 18.3±0.4** | 16.6±0.4** | 14.5±0.5** |
| Shilajit 2.5mg | 6.0±0.8** | 10.0±1.5** | 9.0±1.6** | 6.6±0.6** | 8.5±0.9** |
| Shilajit 6.25mg | 5.6±0.6** | 6.3±0.7** | 7.6±0.80** | 9.1±1.1** | 7.3±1.7** |
| Shilajit 12,5mg | 6.5±0.3** | 7.1±0.7** | 10.0±0.9** | 9.3± 0.9** | 6.3±0.7** |
| F value | 75.73 | 42.01 | 39.16 | 103.47 | 61.09 |

*(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 [13]. The pathophysiological basis of catalepsy still remains obscure. Theories implicating central cholinergic dysfunction [14], gamma-amino butyric acid (GABA) deficiency[21], oxidative stress [22], and 5 - hydroxy tryptamine (5-HT) dysfunction [23] have been proposed. Neuroleptic induced catalepsy has been linked to blockade of post synaptic striatal dopamine D1 and D2 receptors [13]. 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 [13]. Despite this evidence, several other neurotransmitters such as acetylcholine, serotonin, angiotensin, adenosine or opioids have also been implicated [25]. 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[26]. 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 anticataleptic 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 anticataleptic activity of polyherbal product, NR-ANX-C in which shilajit is one of the components [15].

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 anticataleptic 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 anticataleptic 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.

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**REFERENCES**

1. Ghosal S, Lal J, Singh SK, Goel RK, Jaiswal AK Bhattacharya SK. The need for formulation of Shilajit by its isolated active constituents. Phytotherapy Res 1991, 5, 211-216.
2. Nadkarni, KM. Indian Materia Medica, 3rd edn. Vol 2, Popular Prakashan Private Ltd. Bombay, India, 1954, pp. 23.
3. Chopra RN, Chopra IC, Handa KL, Kapur LD. Chopra's Indigenous Drugs of India. 2nd Edn. Academic Publishers, Calcutta India, 1958.
4. Ghosal S, Singh SK, Kumar Y, Srivatsava R. Antiulcerogenic activity of fulvic acids and 4-metoxy-6-carbomethyl biphenyl isolated from shilajit. Phytother Res 1988, 2,187-191.
5. Acharya SB, Frotan MH, Goel RK, Tripathi SK, Das P.K. Pharmacological actions of Shilajit. Indian J Exp Biol 1988, 26(10), 775-757.
6. Goel RK, Banerjee RS, Acharya SB. Antiulcerogenic and antiinflammatory studies with shilajit. J. Ethnopharmacol 1990, 29(1), 95-103.
7. Trivedi NA, Mazumdar B, Bhatt JD, Hemavathi KG. Effect of shilajit on blood glucose and lipid profile in alloxan induced diabetic rats. Indian J Pharmacol 2004, 36 (6), 373-376.
8. Jaiswal AK, Bhattacharya SK. Effects of Shilajit on memory, anxiety and brain monoamines in rats. Indian J Pharmacol 1992, 24,12 - 17.
9. Mukherjee, Biswapati. Traditional Medicine, Proceedings of an International Seminar. Nov. 7-9 1992, Hotel Taj Bengal, Calcutta India. Oxford & IBH Publishing, New Delhi, 1992, pp 308-319.
10. Tiwari P, Ramarao P, Ghosal S. Effects of Shilajit on the development of tolerance to morphine in mice. Phytother Res 2001, 15(2), 177-9.
11. Gupta SS, Seth CB, Mathur VS. Effect of Gurmar and shilajit on body weight of young rats. Indian J Physiol Pharmacol 1966, 9(2), 87-92.
12. Frotan, MH, Acharya, SB. Pharmacological studies of shilajit. Indian J Pharmacol 1984, 16, 45.
13. Sanberg PR. Haloperidol induced catalepsy is mediated by postsynaptic dopamine receptors. Nature 1980, 284, 472-473.
14. Klemm WR. Evidence for a cholinergic role in haloperidol induced catalepsy. Psychopharmacology (Berl) 1985, 85,139-142.
15. Arjuman A, Nair V, Gopalakrishna HN, Nandini M. Evaluation of the antioxidant potential of NR-ANX-C (a polyherbal formulation) and its individual constituents in reversing haloperidol induced catalepsy in mice. Indian J Pharmacol 2007, 39(3), 151-154.
16. Pemminati S, Nair V, Dorababu P, Gopalakrishna HN. Effect of ethanolic extract of leaves of *Ocimum sanctum* on haloperidol-induced catalepsy in albino mice. Indian J Pharmacol 2007, 39(2), 87-89.
17. Pemminati S, Nair V, Dorababu P, Gopalakrishna HN, Pai MRSM. Effect of aqueous fruit extract of *Emblica officinalis* on haloperidol induced catalepsy in albino mice. Journal of Clinical and Diagnostic Research 2009, 3(4),1657-1662.
18. Gopala Krishna HN, Pemminati S, Dorababu P, Pai MRSM, Colaco N, Vineetha V. The effect of acute and chronic administration of the aqueous extract of Triphala on haloperidol induced catalepsy in mice. Journal of Clinical and Diagnostic Research 2010, 3, 2134-2138.
19. Ferre S, Guix T, Prat G, Jane F, Casas M. Is experimental catalepsy properly measured? Pharmac Biochem Behav 1990, 35, 753-777.
20. Ahtee L, Buncombe G. Metoclopramide induces catalepsy and increases striatal homovanillic acid content in mice. Acta pharmacol et toxicol 1974, 35,429-432.
21. Somani RS, Kasture VS, Kasture SB. Haloperidol inhibits (-) bicuculline-induced seizures and bicuculline potentiates haloperidol-induced catalepsy in mice. Indian J Pharmacol 1999, 31, 434-436.
22. Kedar NP. Can we prevent Parkinson's and Alzheimer's disease? J Postgrad Med 2003, 49, 236-245.
23. Silva SR, Futuro NHA, Pires JGP. Effect of 5-HT3 receptor antagonists on neuroleptic-induced catalepsy in mice. Europharmacology 1995, 34, 97-99.
24. Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C, Sedvall G. Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine: Relation to extrapyramidal side effects. Arch Gen Psychiatry 1992, 49, 538-544.
25. Osswska K. Neuronal basis of neuroleptic-induced extrapyramidal side effects. Polish J Pharmacol 2002, 54, 299-312.
26. Polydoro M, Schroder N, Lima MN, Caldana F, Laranja DC, Bromberg E., *et al*. Haloperidol and clozapine induced oxidative stress in the rat brain. Pharmacol Biochem Behav 2004, 78,751-766.
27. Marti-Masso JF, Poza JJ, Lopez de Munain A. Drugs inducing or aggravating Parkinsonism: A Review. Therapie 1996, 51, 568-577.
28. Ghosal S, Bhattacharya SK. Antioxidant defense by native and processed Shilajit: a comparative study. Indian J Chem 1996, 35B, 127-132.
29. Bhattacharya SK, Sen AP, Ghosal S. Effects of Shilajit on biogenic free radicals. Phytother Res 1995, 9, 56-59.
30. Schliebs R, Liebmann A, Bhattacharya SK, Kumar A, Ghosal S, Bigl V. Systemic administration of defined extracts from *Withania somnifera* (Indian Ginseng) and Shilajit differentially affects cholinergic but not glutamatergic and GABAergic markers in rat brain. Neurochem Int 1997, 30(2),181-190.

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