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EFFECTS OF SHILAJIT ON MEMORY, ANXIETY AND BRAIN MONOAMINES IN RATS

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Summary
The effect of Shilajit was investigated for putative nootropic and anxiolytic activity, and its effect on rat brain monoamines using Charles Foster strain albino rats. Nootropic activity was assessed by passive avoidance learning and active avoidance learning acquisition and retention. Anxiolytic activity was evaluated by the elevated plus-maze technique. Rat brain monoamines and monoamine metabolites were estimated by HPLC technique. The results indicated that Shilajit had significant nootropic and anxiolytic activity. The biochemical studies indicated that acute treatment with Shilajit had insignificant effects on rat brain monoamine and monoamine metabolite levels. However, following subacute (5 days) treatment, there was decrease in 5-hydroxytryptamine and 5-hydroxyindole acetic acid concentrations and an increase in the levels of dopamine, homovanillic acid and 3,4-dihydroxyphenyl-acetic acid concentrations, with insignificant effects on noradrenaline and 3-methoxy-4-hydroxyphenylethylene glycol levels. The observed neurochemical effects induced by Shilajit, indicating a decrease in rat brain 5-hydroxytryptamine turnover, associated with an increase in dopaminergic activity, helps to explain the observed nootropic and anxiolytic effects of the drug.

Key word: Shilajit nootropic effect anxiolytic action rat brain monoamines and metabolites

Shilajit is a blackish-brown exudation, of variable consistency, obtained from steep rocks of different formations found in the Himalayas at altitudes between 1000 to 5000 metres, from Arunachal Pradesh in the East to Kashmir in the West. It is also found in Afghanistan, Nepal, Bhutan, Pakistan, China, Tibet and U.S.S.R. (Tien-Shan, Ural and Caucasus). Shilajit, apart from being used in diverse clinical conditions in Ayurveda, has been proposed to arrest aging and induce rejuvenation, and to improve memory, major attributes of Ayurvedic rasayanas and medha rasayanas\(^1\). It has been proposed that the modern equivalents of rasayanas and medha rasayanas are adaptogenic and nootropic activity, respectively\(^2\). Shilajit, long regarded as a bitumen (asphalt) or mineral resin, or as a plant fossil exposed by elevation of the Himalayas, has now been subjected to extensive chemical investigations\(^3\) and it has now been shown to contain significant quantities of organic compounds, including the bioactive oxygenated dibenzo-alpha-pyrones, tirucallane triterpenes, phenolic lipids and small tannoids. Shilajit, obtained from different sources, has now been standardized on the basis of its major organic constituents\(^3\).

While investigating the putative adaptogenic activity of Shilajit\(^4\), some behavioural characteristics emerged which suggested that the drug may have nootropic and anxiolytic activity. We, therefore, investigated these two actions of Shilajit, by using acceptable behavioural paradigms. Changes in the rat brain monoamines or their metabolite were also investigated to explain the observed pharmacological effects.

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MATERIALS AND METHODS

Standardized Shilajit was obtained from Prof. S. Ghosal, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University. Freshly prepared suspension of Shilajit in distilled water was used and the doses mentioned represent the dry weight of the drug. Shilajit was administered 60 min before behavioural testing, unless mentioned otherwise. All the experiments were done with one lot of Shilajit.

Young Charles Foster strain rats (100-150 g), of both sexes, were used for the pharmacological investigations, whereas older rats (200-220 g), of either sex, were used for the neurochemical studies. The rats were housed in colony cages at an ambient temperature of 25±2°C and 45-55% relative humidity, with a 12 hr light/12 hr dark cycle. Experiments were conducted under these ambient conditions between 0900 and 1400 hours. The rats were fed standard pellet diet (Hind Lever) and tap water was given through drinking bottles. Food, but not water, was withheld 16 hr before experimentation.

The following behavioural paradigms were used:

a. Passive avoidance learning and retention

The method used was essentially the same as described earlier5-7. Briefly, the rat was placed on the elevated platform situated in the centre of the floor of the passive avoidance test box and the latency to step down was recorded. Immediately after stepping down, the rat received electric shock (0.5 mA) of 3 sec duration through the grid floor, and was then returned to its home cage. On the following day (24 hr retention interval) the rat was once again placed on the platform and the latency to step down was recorded. Electric shock was not given on day 2. If the rat remained on the platform for the 5 min test duration, it was assigned a maximum score of 300 sec. Latency to step down was again assessed a week later on day 9 in order to evaluate the retention of passive avoidance learning. The results have been expressed as retention scores after 24 hr or one week for each rat, by calculating the ‘inflexion ratio’ by the formula:

\[
\text{Inflexion ratio} = \frac{(L_1 - L_0)}{L_0}
\]

where \( L_0 \) = initial step down latency in sec and \( L_1 \) = step down latency after 24 hr or 1 week, in sec.

b. Active avoidance learning and retention

The method used was essentially the same as described earlier, using a Sidman jumping box (Techno, Lucknow). All the rats used were trained upto 100% learning criterion of active avoidance response. During the training period, each rat was placed in one of the two chambers of the Sidman box, and after 5 sec the buzzer (conditioned stimulus, CS) was sounded for 2 sec, followed by an electric shock (unconditioned stimulus, UCS; 30v, 0.5 sec) through the grid floor. Thereafter, a rest pause of 180 sec was allowed. If the rat jumped within the CS duration to the unelectrified safe box, so as to avoid the UCS, it was allowed to rest there for 30 sec. However, if the rat did not show the avoidance response, it was removed from the shock chamber after 180 sec and was initiated for the next trial. The rats were given 10 trials daily until they reached the criterion of 100% active avoidance response. The rats were subjected to a repeat test after an interval of 15 days in order to assess the retention of the previously learned active avoidance response.

c. Elevated Plus-maze test

This test was used to evaluate anxiolytic activity.9 The plus-maze consisted of two opposite open arms, 50 X 10 cm, crossed with two closed arms of the same dimensions with walls 40 cm high. The arms were connected with a central square, 10 X
10 cm. to give the apparatus a plus-sign appearance. The maze was elevated 70 cm above the floor in a dimly lit room. Rats were individually placed in the central square facing an enclosed arm. The time spent by the rat, during the next 5 min, on the open and closed arms was recorded. An arm entry was defined when all four limbs were on the arm.

Estimation of rat brain monoamines and monoamine metabolites Whole brain concentrations of 5-hydroxytryptamine (5-HT), 5-hydroxyindole acetic acid (5-HIAA), dopamine (DA), homovanillic acid (HVA), 3,4-dihydroxyphenyl acetic acid (DOPAC), noradrenaline (NA) and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) levels were assessed by a high pressure liquid chromatographic (HPLC) technique.

RESULTS

a. Passive avoidance learning and retention

The results summarized in Table 1 indicate that Shilajit had a dose-related facilitatory effect on the retention of passive avoidance learning, though the effect was statistically significant for the higher dose (50 mg/kg, p.o.) at both retention intervals of 24 hr and 1 week.

b. Active avoidance learning and retention

The results summarized in Table 2 indicate that neither of the doses (25 and 50 mg/kg, p.o.) of Shilajit had any significant effect on the acquisition of active avoidance learning. However, Shilajit had a dose-related facilitatory effect on the retention of the previously learnt active avoidance task when tested after an interval of 15 days. The rats

Table 1. Step down latencies, represented as inflexion ratios, following pretreatment with Shilajit, in the passive avoidance test in rats (Mean ± SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Retention intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24hr</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>3.0 ± 1.7</td>
</tr>
<tr>
<td>Shilajit (25 mg/kg)</td>
<td>8</td>
<td>2.2 ± 1.4</td>
</tr>
<tr>
<td>Shilajit (50 mg/kg)</td>
<td>7</td>
<td>12.4 ± 4.1a</td>
</tr>
</tbody>
</table>

a p <0.05, b p < 0.05 as compared to control and Shilajit (25mg/kg) groups respectively (unpaired Student’s ‘t’ test).

Table 2. Effect of Shilajit on active avoidance learning and relearning in rats (Mean ± SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Active avoidance tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Learning (acquisition)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trials</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>41.2 ± 2.3</td>
</tr>
<tr>
<td>Shilajit (25 mg/kg)</td>
<td>8</td>
<td>41.2 ± 3.5</td>
</tr>
<tr>
<td>Shilajit (50 mg/kg)</td>
<td>7</td>
<td>41.4 ± 4.6</td>
</tr>
</tbody>
</table>

a p < 0.05 and b p < 0.01 as compared to control; c p < 0.05 as compared to Shilajit (25 mg/kg) group (unpaired Student’s ‘t’ test).
pretreated with *Shilajit* require significantly less trials and shocked trials to re-learn the problem, as compared to untreated controls.

c. Elevated plus-maze test

The results summarized in Table 3 indicate that *Shilajit* exhibited an anxiolytic activity as evidenced by significantly greater time spent by the rats on the open arm as compared to the closed arms. However, the effect was not dose-related, with maximal effect being induced by the lower dose (10 mg/kg, p.o.) used. The results obtained were comparable with those induced by the standard anxiolytic agent, diazepam (1 mg/kg, p.o.).

d. Rat brain monoamines and monoamine metabolites

The results, as summarized in Table 4, using two doses (25 and 50 mg/kg, p.o.) of *Shilajit* at two

| Table 3. Anti-anxiety effect of Shilajit in the elevated plus-maze test in rats (Mean ± SEM) |
|--------------------------------|----------------|----------------|
| **Groups**                  | **n** | **Time spent in enclosed arms (sec)** | **Time spent in open arms (sec)** |
| Control                     | 6    | 205.1 ± 12.6 | 94.8 ± 12.6 |
| Shilajit (10 mg/kg)          | 6    | 143.4 ± 9.2 | 156.5 ± 9.2  |
| Shilajit (25 mg/kg)          | 5    | 160.0 ±15.4 | 140.0 ±15.4 |
| Shilajit (50 mg/kg)          | 6    | 145.9 ± 20.3 | 154.1 ± 20.3 |
| Diazepam (1 mg/kg)           | 5    | 171.2 ±8.6 | 128.9 ±8.6  |

* a p < 0.05, b p < 0.01 and c p < 0.001 as compared with control (unpaired Student’s ‘t’ test)

| Table 4 Effect of Shilajit, following acute and subacute treatments, on rat brain monoamines and monoamine metabolites (ng/g, Mean ±SEM) |
|--------------------------------|----------------|----------------|
| **Monoamine Metabolites**     | **Vehicle (8)** | **Shilajit (50 mg/kg) (5)** | **Shilajit (25 mg/kg x 5 days) (6)** | **Shilajit (50 mg/kg x 5 days) (6)** |
| 5-HT                         | 186.4 ± 9.8    | 202.0 ± 8.8    | 151.0 ±6.5b | 132.1 ±5.9 |
| 5-HIAA                       | 246.2 ± 6.6    | 232.5 ±5.9     | 206.5 ±6.9  | 185.0 ±7.4 |
| Dopamine                     | 1268.2 ±32.4   | 1292.2 ±62.6   | 1433.25 ±49.8a | 1558.4 ±39.6 |
| HVA                          | 136.2 ± 8.1    | 126.6 ±5.6     | 151.3 ±6.2  | 169.9 ±7.6 |
| DOPAC                        | 172.9 ± 6.3    | 156.4 ±9.6     | 183.3 ±5.0a | 196.1 ±5.3 |
| Noradrenaline                | 606.4 ± 9.9    | 596.1 ±8.4     | 634.2 ±7.6  | 669.3 ±8.4 |
| MHPG                         | 96.3 ± 4.8     | 92.0 ±5.6      | 107.8 ±5.7  | 129.8 ±7.4 |

* a p < 0.05, b p < 0.01 and c p < 0.001 as compared with vehicle treated control. (Unpaired Students ‘t’ test) 
Figures in parenthesis indicate the number of animals.
pretreatment time intervals (60 min and 5 days), indicate that single pretreatment with the drug had insignificant effects on the rat brain concentrations of 5-HT and its metabolite 5-HIAA, DA and its metabolites HVA and DOPAC, and NA and its metabolite MHPG. However, 5-day pretreatment induced decrease in the levels of 5-HT and 5-HIAA, and increase in the concentrations of DA, HVA and DOPAC. However, the effect on NA and MHPG concentrations remained statistically insignificant.

**DISCUSSION**

Nootropics represent a new class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system, particularly on intellectual performance, learning capacity and memory. A number of drugs, including piracetam, have now been introduced in therapy to ameliorate cognitive deficits. The present studies indicate that Shilajit, an Ayurvedic medha-rasayana, can be regarded as a nootropic agent in view of its facilitatory effect on retention of acquired learning, though it had minimal effect on the acquisition of active avoidance learning. Several studies conducted in this laboratory have indicated that the proposed nootropic agents have more significant effects on learning and memory, following induction of memory deficits by undernutrition or environmental impoverishment. As such, an extended investigation on the effect of Shilajit, under induced conditions of cognitive deficits, is required to confirm its putative nootropic effect.

In the elevated plus-maze test, reduction in open arm entries, in relation to total arm entries, provides a measure of fear induced inhibition of exploratory activity which is attenuated by anxiolytic agents. Thus, the results indicate that Shilajit has significant anxiolytic activity, comparable qualitatively with that induced by diazepam, in doses lower than that required for nootropic activity.

The neurochemical basis of learning and memory remains controversial, despite extensive experimental and clinical studies. Although the role of the central cholinergic system is fairly well established, its deficiency being implicated in memory deficits, the role of the other neurotransmitter systems can not be ignored. Several studies have indicated that increase in serotonin neurotransmission can interfere with learning acquisition and memory consolidation. However, the role of 5-HT in anxiety is now well established and it has been conclusively shown that increase in central serotonergic activity invariably leads to anxiety, whereas decrease in brain 5-HT activity results in anxiolysis. Thus, the neurochemical effects induced by Shilajit can explain its nootropic and anxiolytic actions, particularly the induced decrease in 5-HT turnover, as indicated by a decrease in 5-HT and 5-HIAA levels. Conversely, the increase in DA turnover, as evidenced by the induced increase in the levels of DA and its metabolites, HVA and DOPAC, can contribute to the observed nootropic activity. Piracetam, the classical nootropic agent, has been reported to augment rat brain dopaminergic activity.

Although some newer pharmacological agents, like tianeptine, have been reported to exhibit both anxiolytic and nootropic activity, probably induced by a reduction in central serotonergic function, there is no reason to believe that improvement in memory is secondary to anxiolysis, since benzodiazepines are known to have an adverse effect on learning and memory. In the present investigations also, the doses of Shilajit exhibiting anxiolytic and nootropic actions, were at variance.

The present investigations, thus, establish the use of Shilajit in Ayurveda as rasayana and medha-
rasayana, exemplified by its anxiolytic and nootropic actions, respectively. The putative neurochemical basis for these action has also been provided. However, further investigations, using more experimental paradigms, are required before the nootropic and anxiolytic actions of Shilajit can be affirmed.

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