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Research Article

## Effect of Shilajit on Amnesia

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### ABSTRACT

Shilajit is a herbo-mineral drug, has been referred to as memory enhancer. donepezil is prescribed to treat mild to moderate dementia, amnesia, and cognitive disorders. Midazolam is known to produce anterograde amnesia in human and animals. The present study is framed to investigate the effect of Shilajit and Donepezil on Midazolam induced experimental amnesia using elevated plus maze test in mice. Twelve groups of mice were used and each group involved of six mice. Midazolam (2 mg kg<sup>-1</sup>), shilajit (100 mg kg<sup>-1</sup>), Donepezil (3 mg kg) and distilled water (as vehicle) (10 ml kg<sup>-1</sup>) were injected intraperitoneally (ip) in different groups of mice 30 min before the training and immediate after the training. Each mouse was naïve to elevated plus maze for 90 sec. The time taken by the animals to move from the open arms to either of two sides of enclosed arms was recorded. All the results were expressed as mean±S.E.M and P<0.05 considered as statistically significant. Midazolam treated animals exhibit significant increase in transfer latency time. On the other hand, shilajit and Donepezil treated mice shown significant decrease in transfer latency time. Simultaneously, animals treated with shilajit (100 mg kg<sup>-1</sup> i.p.) and Donepezil (3 mg kg<sup>-1</sup> i.p.) significantly exhibit decrease in transfer latency time measured after 24 hrs in the animals previously treated with Midazolam (2 mg kg<sup>-1</sup> i.p.). The above observations reveals that Midazolam impair learning, shilajit and Donepezil improve acquisition. The results also indicate that shilajit and Donepezil prevent Midazolam induce learning impairment. It may be concluded that shilajit and Donepezil reverse Midazolam induced amnesia by the same mechanism i.e. improvement in cholinergic or dopaminergic activity, at least in mice species.

**Key words:** Shilajit, Donepezil, Midazolam, Amnesia, Elevated plus maze, Mice

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### INTRODUCTION

Shilajit is a herbo-mineral drug which oozes out from special types of mountains rocks in the peak summer months<sup>1</sup>. It is pale brown to blackish-brown exudates of variable consistency from steep rocks (1000-500 M) of different formations. Apart from the Himalayas, the Aravali and the Vindhya it is also found in Afganistan, Australia and Mangolia<sup>2</sup>. The studies of C.C.R.I.H.H., research unit Ranikhet, say that Shilajit is not mountains drainage but is a vanaspati Saptdhar, which originate from the Sehund (*Euphoria royleana*) in summer season it secretes milky exudates which in rainy season drains with the rainy water and get adhere with the stones of mountain<sup>3</sup>. The plant *Euphoria royleana* Boise (Euphorbiaceae) grows widely in Shilajit bearing rocks throughout the Himalayas<sup>4</sup>. The active constituent of Shilajit consists of dibenzo alpha pyrones and related metabolites, small peptides, some liquids, carrier molecules and several trace elements<sup>5</sup>. Shilajit has been reported to increase free radicals scavenging enzymes like superoxide dismutase, catalase and glutathion peroxide activities in rat brain striatum and frontal cortex<sup>6</sup>. The active constituents such as dibenzo alpha pyrones are able to pass blood brain barrier and act as a powerful antioxidant protecting the brain and nerve tissues from free radical

damage, it also inhibits the enzyme acetylcholinesterase, which breaks down the acetylcholine. This will increase the levels of acetylcholine. The low levels of acetylcholine are associated with Alzheimer, poor memory and concentration. Ghosal *et al.*,<sup>7</sup> have reported that Shilajit significantly augmented learning and memory retrieval in laboratory animals.

The aim of the present study was to examine whether the cholinesterase inhibitors, donepezil capable of modifying short-term spatial memory and cognitive flexibility (reversal learning) impairments caused by Midazolam induced Amnesia. Past studies indicated that cholinesterase inhibitors, donepezil prevent impairment of short-term spatial memory and memory flexibility. donepezil only at higher dose prevent the cognitive flexibility impairment induced by anti-amnesic drug when given prior to the reversal learning of the task<sup>8</sup>.

### MATERIALS AND METHODS

**Drugs and solutions:** All the drug solutions were freshly prepared prior to use. Purified shilajit (Baidhnath Ayurvedic Bhavan Ltd., Jhansi), Donepezil (Cipla, India), Midazolam (Wockhardt, Aurangabad) were dissolved in distilled water.

**Animals:** Swiss albino mice (2-3 month of age, 25-30 gm) of either sex was used for the pharmacological investigation. The mice were housed in colony cages at an ambient temperature  $25\pm 5^{\circ}\text{C}$  with 12 hrs dark and 12 hrs light cycle. The animals were fed standard pellet diet (Amrut Rat and Mice feed, Pranam food industrial Area, Delhi) and the tap water was given through drinking bottle ad libitum. All the animal experiments have been carried out according to the internationally valid guidelines of "Committee for the Purpose of Control and Supervision of Experiments on animals" (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, New Delhi. (Ethical protocol No. (BU/Pharm/ IAEC/13/24).

#### Apparatus:

**Elevated Plus Maze:** Spatial memory and anti-anxiety drugs can be evaluated by Elevated plus maze apparatus. The maze was made up of wood and consisted of two open and two close arms such that two open arms were opposite to each other. The arms were connected by central platform. The principle of the test was the memory loss leads to the increase in the transfer latency of mice from open to close arm and memory enhancer drug should lead to the decrease in transfer latency. To perform the procedure each mouse was placed at the end of the open arm facing towards the environment and the transfer latency i.e. the time taken by the animal to move in one of the closed arm (all four paws inside was measured. If the mouse did not enter in close arm in 90 sec it was eliminated from the study). Mice were allowed to be in the maze for familiarization and returned to home cage. Experiment was conducted in the noon in natural light<sup>9</sup>.

**Experimental protocol:** Twelve groups of mice were employed and each group comprised of six mice. The each mice of control (Group I and II), Midazolam (Group III and IV), Shilajit (Group V and VI) and Donepezil (Group VII and VIII) were treated with distilled water (10 ml  $\text{kg}^{-1}$ , i.p.), Midazolam (2 mg  $\text{kg}^{-1}$ , i.p.), shilajit (100 mg  $\text{kg}^{-1}$ , i.p.) and Donepezil (3 mg  $\text{kg}^{-1}$ , i.p.) 30 min before the training and immediate after the training, respectively. The mice of

Shilajit+Midazolam (Group IX and X) and Donepezil +Midazolam (Group XI and XII) were treated with Midazolam (2 mg  $\text{kg}^{-1}$ , i.p.) and after 5 min with shilajit (100 mg  $\text{kg}^{-1}$ , i.p.) and Donepezil (3 mg  $\text{kg}^{-1}$  i.p.), 30 min before the training and immediately after the training, respectively.

**Statistical analysis:** All the results were expressed as mean $\pm$ S.E.M. and  $P < 0.05$  considered as statistically significant. Data was analyzed using Stat Disc Software.

## RESULTS

Effect of distilled water (vehicle), Midazolam, Shilajit and Donepezil on learning and memory Distilled water (10 mg  $\text{kg}^{-1}$ , i.p.) treatment 30 min before the training and immediate after training, did not produce any significant difference on transfer latency time. It indicates that distilled water treatment did not affect learning and memory process. Midazolam (2 mg  $\text{kg}^{-1}$ , i.p.) treatment 30 min before the training and immediate after training, significantly enhance the transfer latency time measured on day 1 and after 24 hrs as compared to control (vehicle treated) group. It suggests that Midazolam impair learning. Shilajit (100 mg  $\text{kg}^{-1}$ , i.p) and Donepezil (3 mg  $\text{kg}^{-1}$  i.p.) administered 30 min before and immediate after the training, significantly decreases the transfer latency time measured on day 1 and after 24 hrs as compared to control (vehicle treated) group. It indicates that shilajit and Donepezil facilitate cognition.

**Outcome of shilajit and Donepezil on Midazolam induce amnesia:** Shilajit (100 mg  $\text{kg}^{-1}$ , i.p.) and Donepezil (3 mg  $\text{kg}^{-1}$  i.p.) significantly decrease the transfer latency time measured on day 1 and after 24 hrs as compared to Midazolam (per se treated) group, in the animals previously treated with midazolam. It indicates that shilajit and Donepezil attenuated Midazolam induce learning impairment.

Table 1: Effect of distilled water, Midazolam, shilajit, Donepezil, shilajit + Midazolam and Donepezil + Midazolam on transfer latency time (time taken to enter in enclosed arms from the starting point of open arm) in animals treated before and immediate after training, using elevated plus maze test

S. No.	Drugs	Dose (per kg of body weight, intraperitoneally)	Transfer latency time (in sec.) measured on day 1	Transfer latency time (in sec.) measured after 24 hrs
1.	Distilled water (Vehicle)	10ml	23.33 $\pm$ 1.32	24.83 $\pm$ 1.28
2.	Midazolam	2mg	39.79 $\pm$ 2.32*	32.37 $\pm$ 1.21*
3.	Shilajit	100mg	19.21 $\pm$ 2.76*	14.23 $\pm$ 1.87*
4.	Donepezil	3mg	18.99 $\pm$ 3.26*	13.25 $\pm$ 1.66*
5.	Shilajit+ Midazolam	100mg+2 mg	17.88 $\pm$ 2.73a	20.95 $\pm$ 0.92a
6.	Donepezil + Midazolamine	3mg+2 mg	14.23 $\pm$ 1.22a	16.20 $\pm$ 3.22a

Values are expressed in mean $\pm$ S.E.M. and  $P < 0.05$  considered as statistically significant. \* $p < 0.05$  compared to control (vehicle treated) group and  $a < 0.05$  compared to Midazolam treated group.

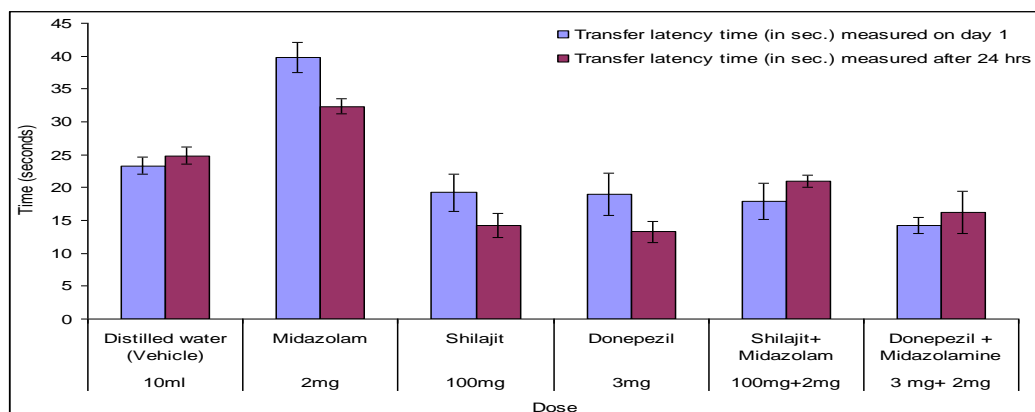


Figure 1: Effect of distilled water, Midazolam, shilajit, Donepezil, shilajit + Midazolam and Donepezil + Midazolam on transfer latency time

## DISCUSSION

Distilled water or vehicle treatment before 30 min of training and immediate after training, did not produce any significant effect on transfer latency time, that indicates the distilled water or vehicle treatment may not have any significant effect on learning and memory. (It was proved that sub hypnotic doses of Midazolam affect the acquisition mechanism of conditioned taste aversion memory in rats, resulting the suppression of CTAM. Midazolam influences active avoidance, retrieval and acquisition rate in rats) <sup>10</sup>.

In the present study, Midazolam significantly increase the transfer latency time measured after 24 hrs. It suggests that Midazolam impair learning process. These results are supported by the study of Vikram et al <sup>11</sup> that stated that by using plus-maze apparatus the amnesic drugs like Midazolam increase the latency of animals in reaching the enclosed arms and nootropic agents (memory enhancers) ameliorate the amnesic effect of Midazolam, Donepezil. Shilajit inhibits the enzyme acetyl cholinesterase, which breaks down acetylcholine. This will increase the levels of acetylcholine, low level of acetylcholine leads to poor memory and concentration, so by increasing the level of acetylcholine, shilajit treats amnesia. It is also reported that shilajit acts by decrease in 5-hydroxy tryptamine and 5-hydroxyindole acetic acid level <sup>12</sup>. It was proved that sub hypnotic doses of Midazolam affect the acquisition mechanism of conditioned taste aversion memory in rats, resulting the suppression of CTAM <sup>13</sup>.

Midazolam influences active avoidance, retrieval and acquisition rate in rats, <sup>14</sup> etc. Donepezil is known to increase extracellular ACh concentration in the hippocampus and prefrontal cortex <sup>15</sup>. In the present study, systemic (i.p.) preadministration of donepezil before Midazolam prevented Midazolam-induced spatial memory deficits in the plus maze task. These data may suggest that the cholinergic system is closely associated with the spatial memory impairment that is induced by Midazolam administration.

## CONCLUSION

On the basis of results, it may be concluded that Midazolam induced amnesia possibly by interfering with cholinergic system. Shilajit and Donepezil facilitated learning and memory and also reverse Midazolam induced amnesia, possibly by the same mechanism i.e. elevation in cholinergic or dopaminergic activity at least in mice species

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