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EFFECT OF SHILAJIT AND PIRACETAM ON SCOPOLAMINE INDUCED EXPERIMENTAL AMNESIA IN MICE

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ABSTRACT

Shilajit is a herbo-mineral drug, has been referred to as 'Panacea', which means 'a cure for all diseases'. Piracetam is prescribed to treat amnesia, dementia, stroke, dyslexia, senility, and cognitive disorders. Scopolamine is known to produce short term amnesia in human and animals. The present study is designed to investigate the effect of Shilajit and piracetam on scopolamine induced experimental amnesia using elevated plus maze test in mice. Twelve groups of mice were employed and each group comprised of six mice. Scopolamine (0.4 mg kg^{-1}), shilajit (100 mg kg^{-1}), piracetam (150 mg kg^{-1}) and distilled water (as vehicle) (10 ml kg^{-1}) were injected intraperitoneally (ip) in different groups of mice 30 min before the training and immediate after the training. The each mouse was naïve to elevate 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. Scopolamine treated animals exhibit significant increase in transfer latency time. On the other hand, shilajit and piracetam treated mice shown significant decrease in transfer latency time. Simultaneously, animals treated with shilajit (100 mg kg^{-1} i.p.) and piracetam (150 mg kg^{-1} i.p.) significantly exhibit decrease in transfer latency time measured after 24 hrs in the animals previously treated with scopolamine (0.4 mg kg^{-1} i.p.). The above observations revels that scopolamine impair learning, shilajit and piracetam improve acquisition. The results also indicates that shilajit and piracetam prevent scopolamine induce learning impairment. It may be concluded that shilajit and piracetam reverse scopolamine induced amnesia by the same mechanism i.e. improvement in cholinergic or dopaminergic activity, at least in mice species.

Keywords: Shilajit, Piracetam, Scopolamine, Amnesia, Elevated plus maze, Mice.

INTRODUCTION

Charaka says, Shilajit has been referred to as 'Panacea', which means 'a cure for all diseases'. Shilajit is a herbo-mineral drug which oozes out from special types of mountains rocks in the peak summer months [1]. 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 Vindhyas it is also found in Afganisthan, Australia and Mangolia [2] (Fig.1&2). 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 secrets milky exudates which in rainy season drains with the rainy water and get adhere with the stones of mountain [3]. The plant Euphoria Royleana Boise (Euphorbiaceae) grows widely in Shilajit bearing rocks throughout the Himalayas [4]. The active constituent of Shilajit consists of dibenzo alpha pyrones and related metabolites, small peptides, some liquids, carrier molecules and several trace elements [5-9]. Shilajit has been reported to increase free radicals scavenging enzymes like superoxide dismutase, catalase and glutathion peroxide activities in rat brain striatum and

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frontal cortex [10]. The odour of shilajit (Gomutra-gandha, smell like cow's urine and kapur-gandha, smell like camphor) influence the restoration of emotional, behavioural and other biological responses which are altered by stress [11]. The literature and the folklore claim indicated that shilajit might have some action in reverting cognitive impairment. It is also reported that Shilajit enhances strength, stamina and relief stress [12]. 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 [13]. have reported that Shilajit significantly augmented learning and memory retrieval in laboratory animals. Furthermore, Rasaratna samuchchaya of Vagbhatacharya also mentions it as Medha–Rasayan (memory enhancer). It is also reported to improve cognitive function in aging rats [14]. Shilajit is used to cure various diseases in men from thousands of years including in cognitive impairment. However, the mechanisms by which Shilajit improve learning and reverse cognitive impairment is not very clear. Piracetam is frequently prescribed to treat amnesia, dementia, stroke [15], dyslexia, senility, and cognitive problems. It is a derivative of the neurotransmitter gamma-amino butyric acid and has been shown to restore cell membrane fluidity. At the neuronal level, it modulates neurotransmission and has neuroprotective and anticonvulsant property. One of its most interesting effects is the ability to promote the flow of information (via increased blood flow) between the right and left hemispheres of the brain in rats [16]. This may also account for piracetam usefulness in treating dyslexia [17]. A study suggests that piracetam may increase cholinergic receptors in the brain [18]. The jury is still out on whether piracetam is beneficial for dementia or cognitive impairment [19,20]. Therefore present study is designed to investigate the effect of Shilajit and piracetam on learning and on scopolamine induced amnesia using elevated plus maze test in mice.

MATERIALS AND METHODS

Drugs and solutions: All the drug solutions were freshly prepared prior to use. Purified shilajit (Baidhnath Ayurvedic Bhavan Ltd. Jhansi), piracetam (Brown and Burk Pharmaceuticals Ltd., 58, C/12, Singasandra Post, Hosur Road, Kudulu, Banglore-560068) and scopolamine (Merck KgaA, 64271 Darmastadt, Germany) were dissolved in distilled water.

Animals: Swiss albino mice (2-3 month of age, 25-30 gm) of either sex were used for the pharmacological investigation. The mice were housed in colony cages at an ambient temperature 25 ± 5^0C 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 clearance No. IAEC/BU/Pharm/07/58).

Apparatus: The plus maze apparatus was designed as described in hand book of experimental pharmacology by S.K. Kulkarni [21]. It consists of two open arms (16 X 5 cm) and two enclosed arms (16 X 5 X 12 cm) and an open roof with the entire maze elevated 25cm from the floor (Fig.3). The each mouse was naïve to elevated plus maze for 90 sec (Fig.4). The time taken by the animals to move from the open arms to either of two sides of enclosed arms was recorded [22].

Experimental protocol: Twelve groups of mice were employed and each group comprised of six mice. The each mice of control (Group I and II), scopolamine (Group III and IV), Shilajit (Group V and VI) and piracetam (Group VII and VIII) were treated with distilled water (10 ml kg^{-1}, i.p.), scopolamine (0.4 mg kg^{-1}, i.p.), shilajit (100 mg kg^{-1}, i.p.) and piracetam (150 mg kg^{-1}, i.p.) 30 min before the training and immediate after the training, respectively. The mice of Shilajit+Scopolamine (Group IX and X) and piracetam+scopolamine (Group XI and XII) were treated with scopolamine (0.4 mg kg^{-1}, i.p.) and after 5 min with shilajit (100 mg kg^{-1}, i.p.) and piracetam (150 mg kg^{-1} i.p.), 30 min before the training and immediately after the training, respectively.

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

RESULTS

Effect of distilled water (vehicle), scopolamine, Shilajit and piracetam on learning and memory Distilled water (10 mg 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. Scopolamine (0.4 mg 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 scopolamine impair learning. Shilajit (100 mg kg^{-1}, i.p) and piracetam (150 mg 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 piracetam facilitate cognition.

Effect of shilajit and piracetam on scopolamine induce amnesia

Shilajit (100 mg kg^{-1}, i.p.) and piracetam (150 mg kg^{-1} i.p, significantly decrease the transfer latency time measured on day 1 and after 24 hrs as compared to scopolamine (per se treated) group, in the animals previously treated with scopolamine. It indicates that shilajit and piracetam attenuated scopolamine induce learning impairment.

Table 1. Effect of distilled water, scopolamine, shilajit, piracetam, shilajit+scopolamine and piracetam+scopolamine 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	21.33±1.49	22.83±1.26
2.	Scopolamine	0.4mg	36.79±2.78*	29.37±1.19*
3.	Shilajit	100mg	18.85±3.86*	15.31±1.98*
4.	Piracetam	150mg	18.07±3.66*	14.45±1.78*
5.	Shilajit+Scopolamine	100mg+0.4mg	16.79±2.86[a]	18.70±0.89[a]
6.	Piracetam+Scopolamine	150mg+0.4mg	12.83±1.36[a]	15.70±3.48[a]

Values are expressed in mean+S.E.M. and P<0.05considered as statistically significant. *p<0.05 compared to control (vehicle treated) group and [a]p<0.05 compared to scopolamine treated group.

Fig.1. Shilajit found in Himalayan region of India

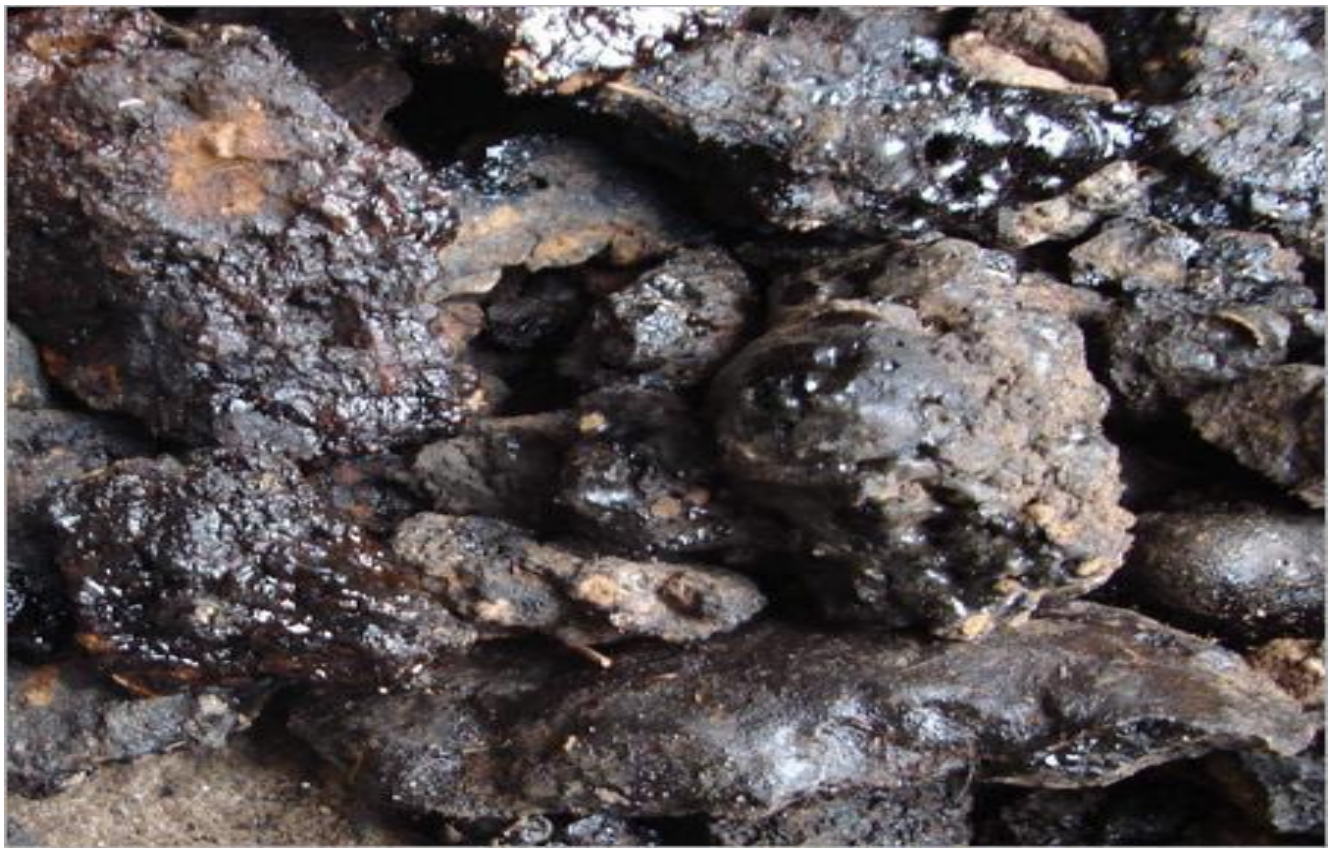

Fig.2. Purified shilajit stones

Fig.3. Elevated plus maze with mice on open arm

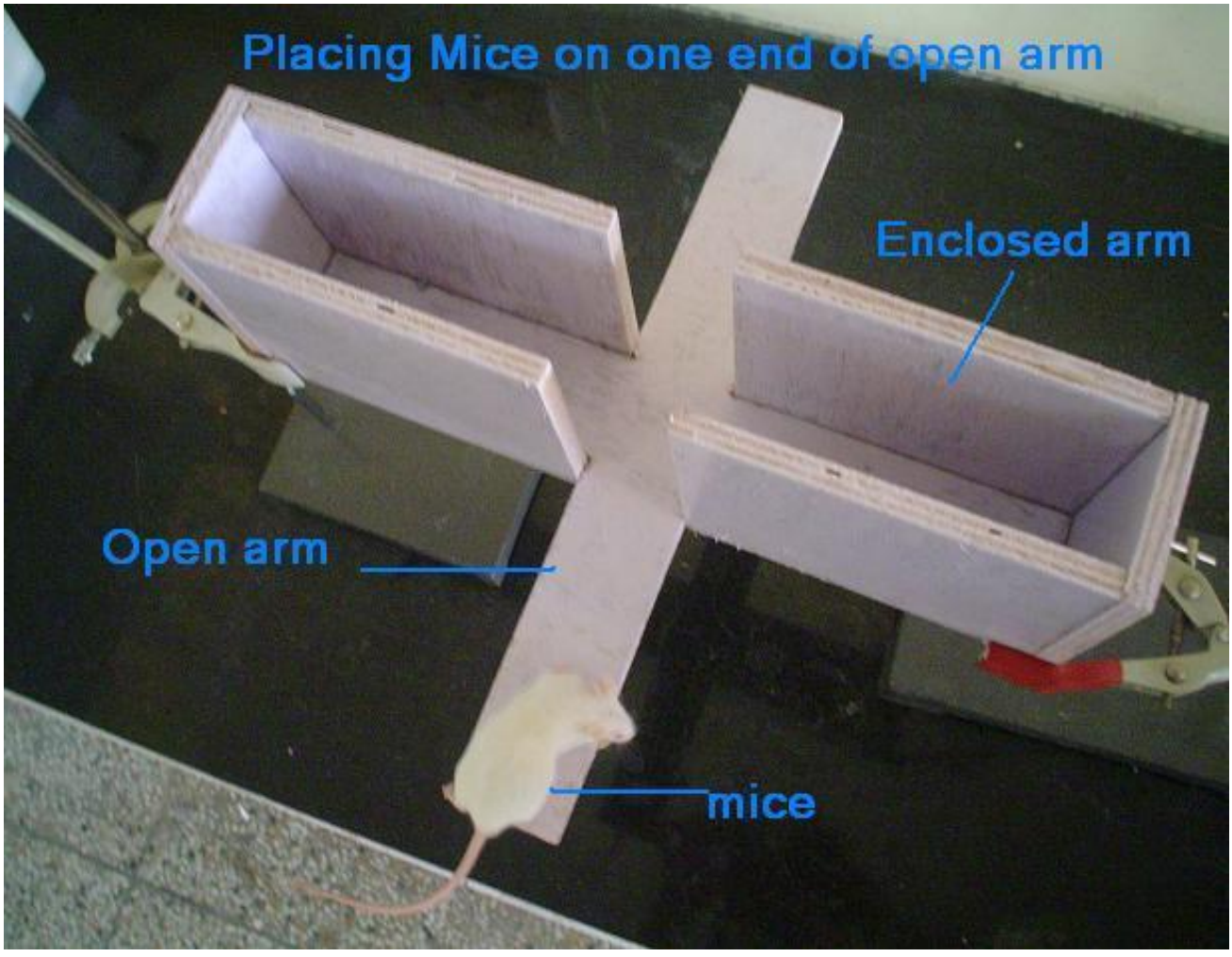

Fig.4. Elevated plus maze with mice entering in close arm.

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. Scopolamine is a well-known anticholinergic agent; impair memory by interference with cholinergic system and produce short-term amnesia in human and animals. In the present study scopolamine significantly increase the transfer latency time measured after 24 hrs. It suggests that scopolamine impair learning process. Our results are supported by the study of Dhingra et al [23]. that stated that by using plus-maze apparatus the amnesic drugs like scopolamine increase the latency of animals in reaching the enclosed arms and noortropic agents (memory enhancers) ameliorate the amnesic effect of scopolamine. Piracetam is frequently prescribed to treat amnesia, dementia, stroke [15], dyslexia, senility, and other cognitive problems. Our laboratory also observed that piracetam significantly decrease the transfer latency time measured after 24 hrs treatment. It suggests that piracetam enhance cognition. The present findings are in supports of the study of Croisile et al [24] demonstrated that piracetam slowly treats cognitive deterioration of Alzheimer's disease. In the present experimental study, Shilajit significantly decrease the transfer latency time measured after 24 hrs of treatment. It indicates that Shilajit may facilitate learning and memory. In previous studies also reported that shilajit have numerous activities including those on learning and memory [13]. Shilajit and pirecetam significantly decrease the transfer latency time measured after 24 hrs in the animals previously treated with scopolamine. It indicates both Shilajit and piracetam prevent impairment induced by scopolamine. Our observation also supports by the report which says that shilajit [14] and piracetam [20] have been recommended for the treatment of amnesia and senile dementia, to improve cognitive functions in normal individual. 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 levels. The increase in dopamine turnover as evidenced by increase in the levels of dopamine and its metabolite homovanillic acid and dihydroxylphenyl acetic acid can contribute to the observed nootropic activity. Piracetam the classical nootropic agent has been also reported to enhanced dopaminergic activity in a similar way [25].

CONCLUSION

On the basis of results it may be concluded that scopolamine induced amnesia possibly by interfering with cholinergic system. Shilajit and piracetam facilitated learning and memory and also reverse scopolamine induced

amnesia, possibly by the same mechanism i.e. elevation in cholinergic or dopaminergic activity atleast in mice species.

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