Research Article

Screening of Black spruce oil for nootropic activity in rats

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Abstract

Background: Memory failure is most common problem in dementia. The brain consists of "n" number of synapses that allow signals to travel across the brain circuits and this helps in creating cellular basis of memories. The functioning of synapses and neurons involved in transmission of acetylcholine is mainly impaired in Alzheimers Disease. **Objective:** The purpose of this study was to explore the nootropic effect of *Black spruce oil* against scopolamine induced amnesia in rats. **Materials and Methods:** Training of animals was carried out on day fourteen and retention was tested on day fifteen. The animals were treated with Mentat (100mg/kg orally) and *black spruce oil* (100 & 200mg/kg, orally) till day fourteen. Amnesia was induced using scopolamine (3mg/kg i.p.) on 14th day after acquisition trial. Retention of memory was tested on day fifteen using elevated plus maze, passive shock avoidance and morris water maze. The animals were sacrificed on 15th day for acetylcholinesterase enzyme estimation. **Results:** Significant ($P<0.001$) improvement in learning and memory was observed in animals treated with *Black spruce oil* (200mg/kg) and also amnesia induced by scopolamine (3mg/kg s.c) was found to be reversed. Levels of Acetyl cholinesterase were found to be reduced significantly ($P<0.001$) in comparison with negative control group i.e. animals exposed to scopolamine. **Conclusion:** The results indicate beneficial effect of black spruce oil on memory function and this might be due to inhibition of acteylcholinesterase enzyme activity.

Keywords: Memory Performance, Alzheimer's disease, *Black spruce oil*, Scopalamine, Acetylcholinesterase

Introduction

Dementia is often associated with Alzheimer Disease (A.D.) and leads to disturbance in learning, capacity, memory, language and judgement without altering consciousness. A.D. is neurodegenerative disorder characterized by formation of neurofibrillary tangles and senile plaques in certain areas of brain (Saini et al., 2012). People with A. D. are also often associated with symptoms such as depression, apathy and psychosis(Joshi and Parle, 2006). Neurobiological studies have revealed that cholinergic system is strongly involved in learning and memory process. Reduced cholinergic neurotransmission results in decline in cognitive function in dementia (Gindi et al., 2011). It has been found frequently that there is loss of cholinergic synapse in hippocampus and neocortex in A.D. patients. To overcome this, there is need to look for agents that regulate AChE function. AChE inhibitors such as tacrine, rivastigmine and donepezil have been approved by U.S. Food and Drug Administration for improvement of symptoms associated with A.D (Obulesu and Rao, 2011). However, due to increased incidence of side-effects associated with allopathic medicine (both nootropic & cholinesterase inhibitors) it is worthwhile to look for natural resources for management of cognitive disorders. The importance of herbal drugs containing antiradical constituents in preventing and treating oxidative stress linked disease is considerably increased (Donya and Ibrahim, 2012). *Black spruce* is a restoractive. It has strengthening and stabilizing effect on nervous system and also supports adrenal function. Therefore may have usefulness in developing stamina. Moreover *black spruce oil* finds its use in conditions such as aching joints, muscle aches & pains, strains and poor circulation. Since there are no reports available on the activity of black spruce essential oil & based on its traditional use the present study is designed to evaluate cognitive function (Damian and Damian, 1995).

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Materials and Methods

Drugs and chemicals

Mentat formulation (Himalaya, Drug Co, Bangalore, India), scopolamine (Sigma Aldrich), *black spruce oil* (New Directions Aromatics, Canada). Mentat and black spruce oil were administered orally by preparing suspension with tween 80. Scopolamine was administered subcutaneously by dissolving in distilled water.

Experimental Animals

Study was carried out using male wistar rats having body weight between 150-200 g. The animals were bought from Shri. Venkateshwara Enterprises, Bangalore and maintained under standard environmental conditions (12 hr each) light/dark cycle. The animals had free access to standard pellet and water which was given ad libitum and were allowed to get acclimatized to laboratory conditions for minimum 7 days before commencement of behavioral experiments. Study design was approved by Institutional Animal Ethics Committee (registration no. KLECOP/IAEC/Res.20-09/08/2014). Experiment on animals was carried out as per Rules and regulations mentioned under CPCSEA guidelines.

Determination of LD_{50}

The acute oral toxicity was performed by using female rats (150-200 g) as per OECD-423 guidelines. Each step was carried out using 3 animals. Dosing of animals was carried out by selecting initial dose from fixed dose levels i.e. 5, 50, 300 & 2000 mg/kg body weight p.o. Initial dose selected for dosing was 300 mg/kg body weight as per OECD recommendations (Talpate et al., 2014). *Black spruce oil* did not show any significant toxic signs of mortality at a large dose upto 2000 mg/kg, therefore 200 and 100 mg/kg p.o. were fixed as daily dose and administered by making emulsion with tween 80.

Assessment of nootropic activity

Animal protocol

Five groups of male wistar rats were made randomly. Each group comprised of six male wistar rats. Group I (Negative Control group) was given normal saline (10 ml/kg) by oral route once daily for 14 days, Group II (Positive control) scopolamine hydro bromide (3 mg/kg) by intraperitoneal route was injected on 14th day, Group III treatment was given by administering Mentat (100 mg/kg) by oral route, Group IV received *black spruce oil* (100 mg/kg) by oral route and Group V recieved *black spruce oil* (200 mg/kg) by oral route. Mentat and *black spruce oil* was administered for 14 consecutive days and scopolamine (3 mg/kg, i.p.) was administered on 14th day.

Exteroceptive behavioral models

Elevated plus maze

Elevated plus maze served as exteroceptive model for evaluating learning and memory. Learning and memory was evaluated by means of procedure described by (Joshi and Parle, 2006). The direction in which individual rat was placed was with its head facing away from central platform & transfer latency (TL) i.e. duration of time taken by the rat to enter one of the closed arms with all its four legs was noted .90 sec was considered as upper cut off time. If the rat was not able to enter one of the two covered arms within 90 sec it was gently pushed into one of the enclosed arms and transfer latency was noted as 90 sec. The rats were returned to their home cage after allowing them to explore the maze for 10sec. After 24 h of the first exposure, TL was noted on 14th day of study for determining the acquisition keeping the cut-off time of 60 sec. After acquisition trial on 14th day, scopolamine (3 mg/kg i.p.) was administered. On fifteenth day, the animals were subjected to the retention test for evaluating the transfer latency keeping the time period of 60s as cut-off criterion.

Passive avoidance paradigm

Passive avoidance task was measured using the method expressed by (Gupta et al., 1961). Animals were administered with *black spruce oil* (100 & 200 mg/kg p.o.) and mentat (100 mg/kg p.o.) for 14 days. On 14th day each animal was placed in the light compartment & the time taken by the animal to enter into the dark compartment was noted (acquition latency). Entry of animal in the dark compartment was triggred with a shock of 0.4 mA/2s onto the grid floor . The animal was then returned to its home cage and exposed to scopolamine (3 mg/kg i.p.). After a period of 24 hrs retention latency was measured. 180 sec was considered as upper cut off time of retention.

Morris water maze test

Learning and memory was assesed using morris water maze test (Gupta et al., 2012; Konathala et al., 2014; Dalla et al., 2009). During training session each animal was subjected to 4 consequtive trials each day. The gap between each trial was 5 mins. The rat was placed in water between the quadrants facing the wall of pool & was allowed to locate the hidden platform for 120 s. The drop location was changed during each trial. The rat was then allowed to stay on to the platform for 20 sec once it found the hidden platform. If hidden platform was not tracked by the animal within a time frame of 120 s it was directed towards the hidden platform and permitted to stay there for 20 s and then removed from morris water maze. The animals where

dried & than returned to their home cages. 24 hrs later (On day 15th) target quadrant was kept free of hidden platform & the mean time spent by the animal in 4th quadrant searching the hidden platform was noted as index of retrieval.

Determination of whole brain activity of biological catalyst

Determination of acetyl cholinesterase enzyme activity was carried out as per method described by Ellman et al, 1961*)*. The enzyme activity was calculated using the formula:

$R = \blacktriangle A/1.36(10^4) \times 1/(400/3120)C_0$

$= 5.74(10^{-4})\blacktriangle A/C_0$

Where, R = n moles of substrate hydrolysed at each min per gm of tissue.

$\blacktriangle A$ = absorbance change during each min.

C_0 = primary conc. of tissue (mg/ml).

Statistical Analysis

The data was subjected to one - way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison test & P< 0.05, 0.01 & 0.001 were considered significant.

Results

Acute toxicity study

Black spruce oil did not show any significant signs of mortality at large dose upto 2000 mg/kg.

Effect of *black spruce oil* on transfer latency in EPM behavioral model

Positive control group showed significant (P < 0.01; 41.33 ± 4.318) increase in TL on day 15 as compared to day 14 & and as equated with negative control group, reflecting memory damage. In the treatment group the animals exposed to scopolamine & treated with standard (Mentat), showed retrieval of memory by reducing the time taken to perform the task in EPM. Those exposed to scopolamine (3mg/kg i.p) and treated with *black spruce oil* (200 mg/kg p.o.) for 14 days protected the rats from scopolamine induced memory impairment as they showed highly significant (P < 0.001; 15.00 ± 1.713) decrease in transfer latency as equated with positive control group (Table 1).

Effect of *Black spruce oil* on Inhibitory Shock Avoidance

Positive control group i.e. animals exposed to scopolamine (3 mg/kg i.p.) produced significant (P<0.05) decrease in STL on day 15 as compared to day 14 indicating loss of memory. In the treatment group, the animals exposed to scopolamine & treated with Mentat (100 mg/kg p.o.) showed significant (P<0.001) activity by increasing the STL period. The rats exposed to scopolamine (3mg/kg i.p.) and treated with *black spruce oil*(200 mg/kg p.o) produced significant retrieval of memory as they showed significant (P<0.001) increase in STL as compared to positive control (Figure 1).

Effect of *Black spruce oil* on Escape Latency Time (ELT) using Morris Water Maze

Positive control group i.e. animals exposed to scopolamine (3 mg/kg i.p) showed highly significant (P<0.001; 20.33±0.8819) reduction in ELT on day 15 as equated to day 14 indicating significant loss of memory. In the treatment group, the animals exposed to scopolamine & treated with Mentat (100 mg/kg) showed highly significant (P<0.001; 69.83±4.578) activity by increasing escape latency. The rats exposed to scopolamine (3mg/kg i.p) & treated with *black spruce oil* (200 mg/kg) produced significant (P<0.001; 60.83±2.600) retrieval of memory as they showed significant increase in ELT as equated to positive control (Table 2).

Table 1. *Black spruce oil* outcome on Transfer Latency

Groups	Transfer Latency (in Sec) in Elevated Plus Maze Test	
	Day 14	Day 15
Normal control	28.33±0.9888	20.33±1.647
Scopolamine	19.00±0.5578	41.33±4.318##^
Mentat+SCO	30.00±0.7638	11.83±0.8724***^
B.S.O 100mg/kg+SCO	48.50±1.232	18.00±1.265**^^^
B.S.O 200mg/kg+SCO	41.33±10.16	15.00±1.713***^^^

SCO- Scopolamine; B.S.O- *Black Spruce Oil.* Characterization of values is done as Mean ± Standard Error of Mean (n=6 present in every group).

###P<0.001, ##P< 0.01, #P< 0.05- Denotes evaluation with Normal Control

***P<0.001, **P< 0.01, *P< 0.05- Denotes evaluation with Scopolamine induced

^^^P<0.001, ^^P<0.01, ^P<0.05- Denotes evaluation between day 14 & day 15.

Effect of *Black spruce oil* on Acetylcholinesterase enzyme activity

Positive control group showed significant (P<0.001) rise in Acetylcholinesterase enzyme activity as equated with negative control group. Reduction in acetylcholinesterase activity was seen in animals treated with higher dose of *black spruce oil.* Mentat being a standard drug also reversed increased AChE activity (Figure 2).

Histopathological studies

In the hippocampi of disease control group large number of pkynotic black neurons, meningeal congestion and cerebral oedema was observed and this was subsequently found to be reduced in treatment groups i.e. animals exposed to scopolamine and treated with Mentat 100 mg/kg p.o. and *black spruce oil* 200 mg/kg p.o. (Figure 3).

Discussion

A.D. is characterized by progressive pathological changes in the brain which slowly erodes memory & thinking skills, and eventually the ability to carry out simple tasks & also results in physical changes (stiffness, loss of ability to walk). The major underlying mechanism involved in A.D. is accumulation of proteins such as β amyloid & tau protein in certain areas of brain (hippocampus). Senile plaques & neurofibrillary tangles prompt the injury & death of neurons and result in memory loss & behavioral changes. Ach is the major neurotransmitter involved in regulation of learning & memory. Cholinergic neurotransmission in the basal forebrain area seems to be the most affected system in A.D. Neurochemical changes involved in A.D. is reduced brain Ach levels in hippocampus & neocortex. Estimation of acetylcholinesterase (AChE) activity is simple & valuable

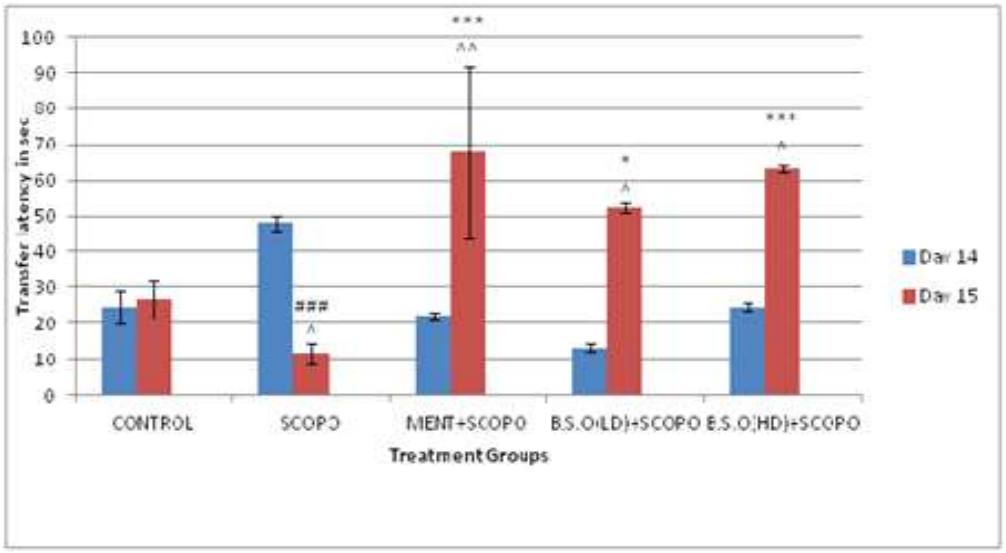

Figure 1. Effect of *Black spruce oil* on Inhibitory Shock Avoidance using Passive Avoidance Paradigm. ###P<0.001, ##P< 0.01, # P< 0.05-Denotes evaluation with Normal Control. ***P<0.001, **P< 0.01, *P< 0.05- Denotes evaluation with Scopolamine induced. ^^^P<0.001,^^P<0.01,^P<0.05- Denotes evaluation between day 14 & day 15.

Table 2. Effect of *Black spruce oil* on Escape Latency time (ELT) in Morris Water Maze test.

Groups	Escape Latency (in Sec.) in Morris Water Maze Test	
	Day 14	Day 15
Normal control	55.00±0.9309	69.17±1.682
Scopolamine	52.33±1.8017	20.33±0.8819###^^^
Mentat+SCO	41.50±1.979	69.83±4.578***^^^
B.S.O 100mg/kg+SCO	42.33±1.054	57.50±3.233***^^
B.S.O 200mg/kg+SCO	47.17±2.613	60.83±2.600***^^

SCO - Scopolamine, *B.S.O - Black Spruce Oil.* Characterization of values is done as Mean ± Standard Error of Mean (n=6 present in every group). ###P<0.001, ##P< 0.01, # P< 0.05- Denotes evaluation with Normal Control. ***P<0.001, **P< 0.01, *P< 0.05- Denotes evaluation with Scopolamine induced. ^^^P<0.001,^^P<0.01,^P<0.05- Denotes evaluation between day 14 & day 15.

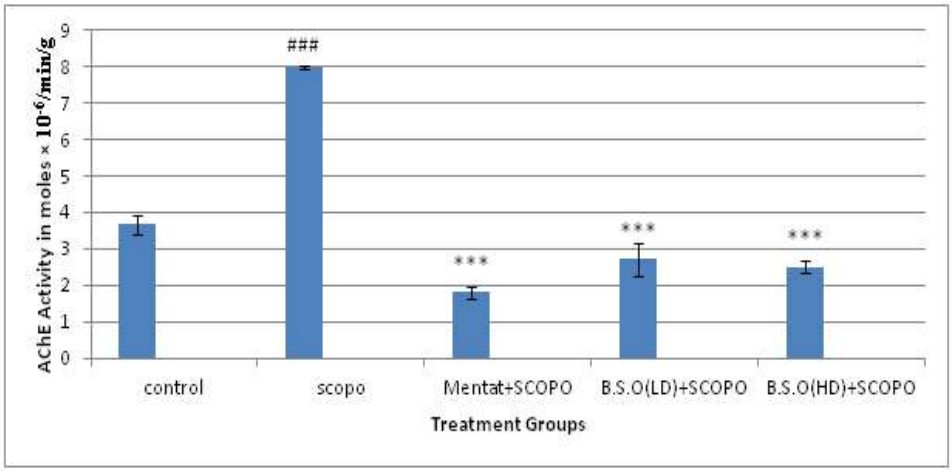

Figure 2. Effect of *Black spruce oil* on Acetylcholinesterase enzyme activity. SCO-Scopolamine, *B.S.O - Black Spruce Oil.* Characterization of values is done as Mean ± Standard Error of Mean (n=6 present in every group). ###P<0.001, ##P< 0.01,# P< 0.05- Denotes evaluation with Normal Control. ***P<0.001, **P< 0.01, *P< 0.05- Denotes evaluation with Scopolamine induced.

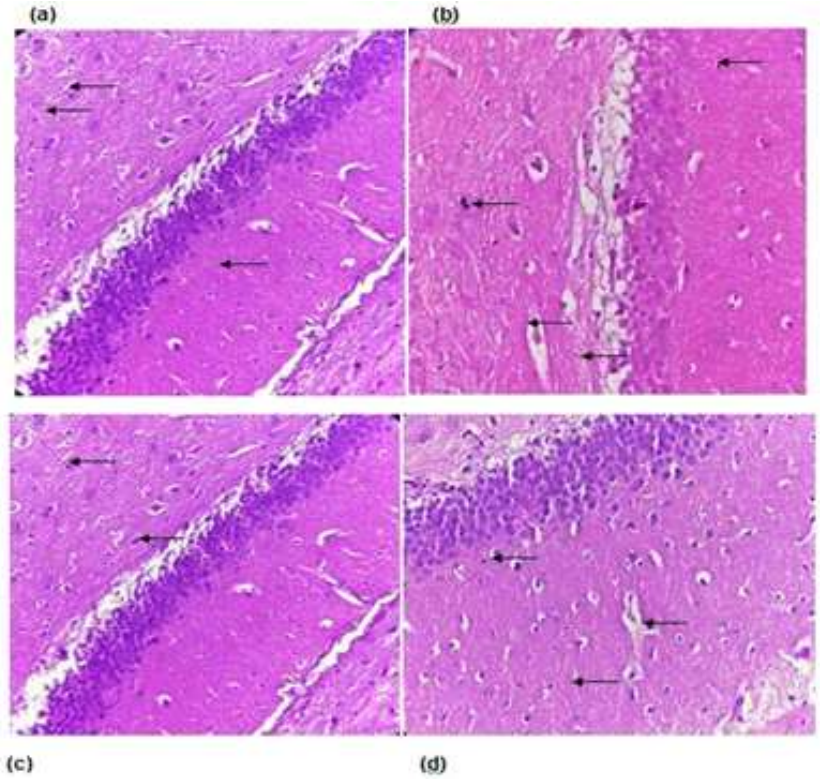

Figure 3. Histopathology of Hippocampus. (a) Negative Control, (b) Positive control, (c) Mentat + scopolamine, (d) black spruce oil 200mg/kg + scopolamine

tool for assessing cholinergic function. Moreover CNS disorders like A.D. & Parkinson's disease are mostly treated by phararmacological manipulation of cholinergic function (Naidu et al., 2013; Kalpana, 2004). Mentat also known as BR-16A contains over 20 different ingredients. Important ingredients of mentat propose to improve memory function, other ingredients of mentat are nerve tonics, putative general tonics & vitalizers(Vijay et al., 2012). In ayurveda, BR-16A is reported to improve cognition & decrease deficits involved in organic brain states (Andarde et al., 2000). In the present study scopolamine produced amnesia in experimental animals as indicated by increased TL, decreased ELT & decrease in retrieval of memory in PSA. Furthermore, treatment with black spruce oil protected the animals from learning and memory impairment produced by scopolamine (centrally acting anti-muscarinic drug) as indicated by decreased TL increased ELT & increase retrieval of memory in PSA. This finding suggest possible neuroprotective role of black spruce oil. Inhibitory shock avoidance model is used for accessing memories that are stored for lengthy period of time based on negative reinforcement. The latency to reach the

central platform of elevated plus platform is indicative of learning ability of the animals. The animal is said to have learnt if the latency to reach the central platform is reduced. MWM employed in present experiment is globally used model to test spatial learning & memory in rodents. In MWM increase in TSTQ in search of absent platform during day 15 indicates retrieval of memory (Dalla et al., 2009). Neurons involved in transmission of Ach are very crucial in memory and learning , and degeneration of cholinergic neurons is primary sign observed in A.D. Depletion of cholinergic functioning resembles cognitive deficits in patients. In the present study, black spruce oil, showed significant elevation of acetylcholine levels by significant reduction of cholinesterase activity in rats brain & ultimately improved memory.

Conclusion

Stressful lifestyle have lead to rise in incidence of A.D. in middle age group population along with elderly population. However modern system of medicine is yet to provide safer treatment for complete cure for such disease. Although many plants & their extracts are being used in animal studies & A.D. patients only few drugs are available today for management of A.D. Herbal medicine may prove to be a corner stone in treatment of such outrageous neurodegenerative disorder & since herbal medicines have wide medicinal activities, higher safety margins & lesser costs they are in great demand in developed as well as developing countries for primary healthcare. In the present study black spruce oil reverses the scopolamine induced memory deficit in E.P.M, PSA & MWM behavioral models. It elevated the acetylcholine level by significant reduction of cholinesterase activity in rat brain & hence improves memory. Hence, in the light of above it is worthwhile to utilize black spruce oil in the management of neurodegenerative disorders of a type Alzheimer's disease and further clinical evaluation is required to establish its therapeutic value.

Conflict of interest

Conflict of interest declared none.

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