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Effect of seabuckthorn extract on scopolamine induced cognitive impairment

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Present study involves evaluation of effects of 75% ethanolic extract of seabuckthorn [*Hippophae rhamnoides* L. (SBT)] leaves on scopolamine induced cognitive impairment in rats using three different oral doses i.e. 50, 100 and 200 mg/kg body weight through assessment of various biochemical and behavioural parameters. Scopolamine administration resulted in an increase in acetylcholinesterase (AChE) activity (approximately 9% with respect to the control group) and malonaldehyde (MDA) content. The increased AChE activity was significantly reduced in animals receiving 200 and 100 mg/kg of SBT extract. Animals treated with SBT extract showed significantly reduced MDA level in all the doses. This reduction in MDA content indicates that SBT leaf extract has potent antioxidant activities and exhibits a protective effect against oxidative damage induced by scopolamine. Behavioural studies also indicated significant improvement. The results suggest that SBT leaf extract has potential effects against scopolamine induced cognitive impairment by regulating cholinergic marker enzyme activity (AChE activity) and promoting the antioxidant system and may be explored for its use in cognitive disorders.

Keywords: AChE activity, Cognitive impairment, Scopolamine, Seabuckthorn leaf extract

Cognition is the physiological process of knowing, including awareness, perception, reasoning and judgment. Cognitive functions are mainly categorized into memory, attention, creativity and intelligence. It is subjective in nature and can be affected by a number of factors including ageing, stress, hypertension, various nervous problems such as dementia related to Parkinson's disease (PD), Alzheimer's disease (AD), schizophrenia, cancer and HIV[1,2]. On the basis of experimental as well as clinical evidences, central cholinergic system is considered as the most important neurotransmission system which is involved in regulation of cognitive functions[3,4].

A decrease in acetylcholine concentration, which is broken down by the enzyme acetylcholinesterase (AChE), results in loss of memory and other cognitive functions. Reactive oxygen species (ROS) cause neuronal damage during stress of varying types, resulting in cognitive disorders. Oxidative stress (including hypoxic stress and ischemic injury) causing free radical toxicity, radical induced mutations, autoimmunity, mineral and nutrient deficiencies etc, have all been implicated in cognitive disorders.

Usually the oxidative damage in neurons causes increased incidence of cognitive disorders. Oxidative damage occurs in the brain of subjects with mild cognitive impairment (MCI), suggesting that oxidative damage may be one of the earliest events in the onset and progression of AD[5,6]. The use of cholinergic muscarinic antagonists, such as scopolamine, in animal models to mimic the cognitive impairment is well established, and has proven to be a useful model system for understanding and developing treatment strategies for neurodegenerative diseases in humans[7].

Central cholinergic muscarinic receptor blockade produces profound cognitive impairments in human and animal subjects. Loss of cholinergic neurons, and subsequent deficits in cholinergic neurotransmission in the hippocampus and cerebral cortex, is strongly correlated with clinical signs of cognitive impairment and dementia in AD patients[8]. The effects of cholinomimetic drugs and cholinergic receptor antagonists on learning and memory tasks have been investigated[9]. The most commonly used model is based on the finding that scopolamine, a muscarinic

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receptor antagonist, induces amnesia in young healthy subjects comparable with that in old, untreated subjects. These deficits may be reversed by AChE inhibitors. Compounds that reverse these scopolamine induced deficits in experimental animals, may be considered as potential drugs to treat cognitive impairment[10].

Antioxidants may reduce or prevent the damage caused by the free radicals. Antioxidant supplements have been reported to help in stabilization and management of cognitive disorders and there appears to be a link between antioxidant intake and cognitive decline[11]. Reducing oxidative stress by antioxidants, protecting brain inflammatory lesions using anti-inflammatory drugs and facilitation of brain cholinergic neurotransmission with anticholinesterases are some positive approaches in the management of AD, which is an advanced stage of memory loss/ dementia[12].

Hippophae rhamnoides L. (Family: Elaeagnaceae), commonly known as seabuckthorn (SBT); growing in North-West Himalayas at high altitude (7000–15,000 feet), is a dwarf to tall (3–15 feet), branched, and thorny nitrogen fixing deciduous shrub, native to Europe and Asia[13]. SBT is a good source of a large number of nutrients, phytochemicals and bioactive substances[14,15]. The medicinal effects of SBT have been suggested to be due to the presence of high antioxidant contents[14,16]. SBT leaves are rich in flavonoides, tannins, and triterpenes[17]. Since SBT is an excellent source of antioxidant activity, it may help in improving the cognitive impairment by reducing oxidative stress. At present, inhibition of enzyme AChE is one of the most accepted and recognized therapeutic marker for development of cognitive enhancers[18]. As such, effect of 75% ethanolic extract of dried leaves of SBT was evaluated on scopolamine (SCOP) induced cognitive impairment in rats using various behavioural and biochemical parameters.

Materials and Methods

Plant material—SBT leaves were collected from high altitude areas of Ladakh, Western Himalayas, India, where the plant grows in the wild under natural conditions. Authentication of the material was carried out by Dr. H. B. Singh, Raw Materials Herbarium & Museum, CSIR-National Institute of Science Communication And Information Resources (NISCAIR), New Delhi.

Extract preparation—Ethanolic extract (75%) of SBT leaves was prepared by cold percolation method[13]. The powdered leaves were extracted with 75% ethanol for 24 h and filtered with 80 mesh nylon cloth. The raw material to solvent ratio used was 1:8. The extraction process was repeated 5 times. To avoid contamination, clean and sterile conditions were maintained during the extraction process. The filtrates obtained after each extraction were combined and stored at ambient temperature. The combined filtrates were again filtered with 250 mesh nylon cloth to get the liquid extract. This extract was then concentrated under reduced pressure till a solid mass was obtained.

Animals—Young male Sprague–Dawley rats (54), weighing 170 ± 20 g, were used. The animals were maintained under controlled environment at 25±1 °C. The animals were fed standard animal food pellets and water *ad libitum*. The experiments were performed after clearance from the Institutional Animal Ethical Committee. The animals (30) were divided in to 6 groups of 5 each, for behavioural and biochemical studies. Doses of SBT leaf extract (50, 100 and 200 mg/kg), selected on the basis of literature reports[19], were administered orally for 21 days. After 30 min of administration of last dose (on 21st day) scopolamine (2 mg/kg, ip)[20] was used to induce cognitive impairment in the extract treated groups. Vehicle control group was administered normal saline in place of extract. Neurotoxin group was administered scopolamine on the last day. Y-Maze Test was conducted after administration of the neurotoxin and the animals were sacrificed by cervical decapitation under light anaesthesia for biochemical assessment. Another 24 animals were used for conditioned avoidance response assessment. These animals were divided into 4 groups of 6 each for acquisition training viz. three groups treated with SBT leaf extract @ 50,100 & 200 mg/kg body weight and a control group getting normal saline. These animals were not sacrificed.

Y-Maze test—The Y-maze is a 3-arm horizontal maze (40 cm long, 3 cm wide, 12 cm high walls) in which the 3 arms are symmetrically separated at 120°. Y-Maze test was performed as per Fraser *et al*[21]. Each rat was placed at the end of one arm (arm A) facing the centre of the maze, and allowed to move freely within the maze for 6 min. The total number of arms entered and the order of arm entries were recorded. An entry was only recorded if all four paws were placed into the arm. The total number of arms

entered provides an indication of locomotor activity, and the order of arms entered provides a measure of spontaneous alternation behaviour and thus working memory. An alternation was considered as scored/positive when an animal entered three different arms consecutively. Animals performing less than five alternations in the 6-min period were excluded from the analysis of results. Since the aim of the experiment was to study the cognitive behaviour and working memory, the alternation scores were recorded using following formula:

{Positive alternations (%) = (Number of positive alternations made/ total number of arm entries – 2) × 100}.

Conditioned avoidance response (CAR)—The effect of SBT leaf extract was also evaluated by using CAR[22]. On each day, 60 min after the treatment, all the animals were subjected to a training schedule individually by placing inside the perspex chamber of the apparatus. After an acclimatization period of 5 min to the chamber, a buzzer was given, followed by a shock through the grid floor. The rat had to jump on the pole to avoid foot shock. Jumping on the pole functionally terminates the shock and this was classified as an escape while such jumping prior to the onset of the shock was considered as avoidance. The session was terminated after completion of 60 trials with an interval of 20–30 sec given for each trial. This procedure was repeated at 24 h intervals until all groups reached 95-99% avoidance. After attaining complete training of a particular group, the animals were treated with a single dose of SCOP (2 mg/kg body weight, ip), 30 min before the next day dosing. The training schedule was continued further with the daily doses of the extract and vehicle until they returned to normal level from SCOP induced amnesia.

Biochemical assessment—Following the behavioural study (Y-Maze Test), the animals were sacrificed by cervical decapitation under light anaesthesia on 21st day, 90 min after administration of the last dose of SBT leaf extract/vehicle. The whole brain was carefully removed, weighed and transferred to a glass homogenizer. Homogenization of the brain was carried out in an ice bath with 0.32 Molar sucrose buffer (10% w/v). The homogenate was centrifuged at 3000 rpm for 10 min and the resultant cloudy supernatant was used for biochemical assessment i.e. AChE activity[23] (Moles thiocholine hydrolyzed/ minute/mg protein) and MDA content[24] (nMoles/mg protein). The protein content was measured using bovine serum albumin (BSA) as standard[25].

Statistical analysis—The results are presented as means ± SE. To determine the difference in various groups/ treatments, paired *t* test was applied. Values of $P < 0.05$ were considered as significantly different. The Statistical Package SPSS 12.0 for Windows was used to analyze the data.

Results and Discussion

Y–Maze—Spontaneous alternation behaviour was used as a measure for evaluation of effect of SBT leaf extract on working memory[21]. An alternation was considered as scored/positive when an animal entered three different arms consecutively. SCOP administration reduced the positive alternations to about 70% of the control group. Administration of SBT leaf extract was found to improve cognition as evidenced by a significant ($P< 0.01$) increase in the spontaneous alternations after SCOP induced impairment in the treated groups getting SBT leaf extract @ 100 and 200 mg/kg. But a 50 mg/kg dose did not show significant improvement. This indicated that SBT leaf extract improved the working memory of the rats when administered @ 100 and 200 mg/kg orally (Fig. 1).

Conditioned avoidance response (CAR)—Acquisition (time to achieve 95% CAR) for the extract treated groups was quicker and found to be dose dependent (Fig. 2). Administration of SCOP resulted in reduction in observed CAR. However, continued treatment of SBT leaf extract produced better retention and recovery in a dose dependent manner than the vehicle treated group.

Effect of SBT leaf extract on AChE activity—For more than three decades, anti-muscarinic agents, particularly SCOP, have been used to investigate the

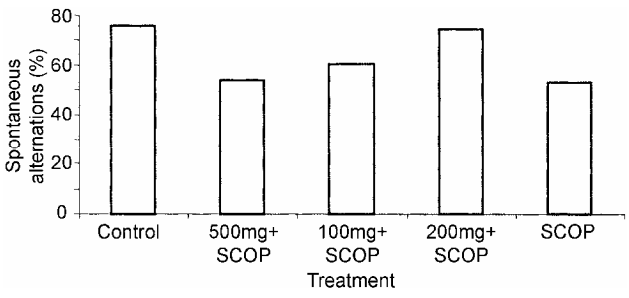

Fig. 1—Effect of SBT leaf extract on Spontaneous alternation behavior in rats on SCOP induced cognitive impairment in Y- Maze test

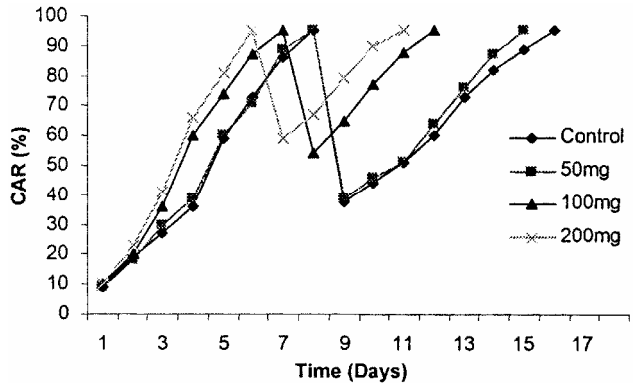

Fig. 2—Effect of SBT leaf extract on CAR before and after SCOP induced cognitive impairment

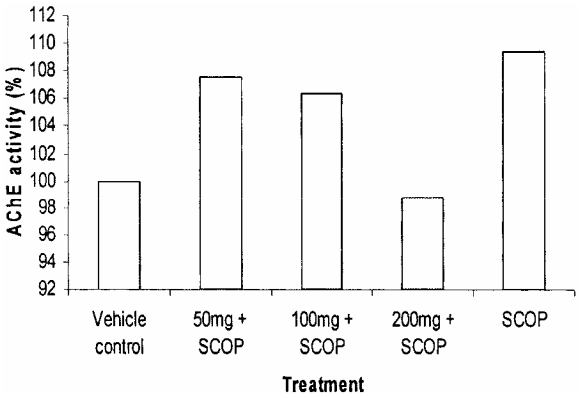

Fig. 3—Effect of SBT leaf extract on AChE activity in rats on SCOP induced cognitive impairment.

role of cholinergic system in learning and memory processes in the mammalian brain. In addition, SCOP-reversal experiments in rodents have been used extensively as an initial screening method to identify therapeutic candidates for cognitive disorders[7,8].

Acetylcholine is the most important neurotransmitter involved in the regulation of cognitive functions[26]. Cholinergic transmission is terminated mainly by acetylcholine hydrolysis through the enzyme AChE which is responsible for degradation of acetylcholine to acetate and choline in the synaptic cleft[27]. According to the cholinergic hypothesis, memory impairments in patients with senile dementia are due to a selective and irreversible deficiency in the cholinergic functions in the brain[7,11].

In the present study, SCOP administration resulted in an increase in AChE activity (approximately 9% with respect to the control group). The increased AChE activity induced by SCOP was significantly reduced in animals receiving 200 and 100 mg/kg of SBT leaf extract (approximately 10.6% and 3% respectively, when compared with SCOP treated group). However, 200 mg/kg dose ($P< 0.01$) was observed to be more effective than 100 mg/kg ($P= 0.047$) in reducing the increased AChE activity caused by SCOP administration. But, a non significant difference was observed in 50 mg/kg dose when compared with SCOP treated group. These findings indicated that SBT leaf extract possesses the AChE inhibitory activity in rats (Fig. 3) and may be considered as a natural AChE inhibitor.

Effect of SBT extract on lipid peroxidation—Lipid peroxidation is an important indicator of neurodegeneration in the brain[28]. Unlike other body membranes, neuronal membranes contain a very high percentage of long-chain polyunsaturated fatty acids because they are used to construct complex structures needed for high rates of signal transfer and data processing. ROS are generated continuously in nervous tissues during normal metabolism and neuronal activity. The brain is subjected to free radical–induced lipid peroxidation because it uses one-third of the inspired oxygen. It is rich in polyunsaturated fatty acids (targets for free radical attack), and is relatively high in redox transition metal ions but is relatively low in antioxidant capacity[29]. Antioxidant supplementation, therefore, is likely to help in reducing free radical-induced lipid peroxidation[30].

Lipid peroxidative damage occurs in the brains of subjects with amnestic MCI[31-33]. Rukhsana *et al.*[34] suggested that oxidative damage is an early event in the progression of AD and not simply a consequence of this dementing disorder. Accordingly, therapeutic strategies designed to modulate the lipid peroxidation early in the course of the disease, if not before the onset of MCI, may be promising to slow or possibly prevent AD. Memory impairment in the SCOP-induced animal models has been reported to be associated with increased oxidative stress with in the brain[35-37].

In order to evaluate the effect of SBT leaf extract on lipid peroxidation in the brain, MDA content was assessed. Brain MDA content was significantly increased by SCOP administration. Animals treated with SBT leaf extracts showed significantly reduced MDA level in all the doses. This reduction in MDA content indicates that SBT leaf extract has potent antioxidant activities and exhibits a protective effect against oxidative damage induced by SCOP (Fig. 4).

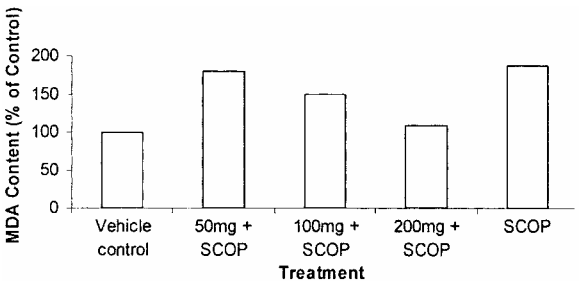

Fig. 4—Effect of SBT leaf extract on Lipid peroxidation (MDA Content) in rats on SCOP induced cognitive impairment.

Thus, it is concluded that SBT leaf extract showed potential cognitive enhancing activity by regulating cholinergic marker enzyme activity (Anti AChE activity) and promoting the antioxidant system. It can possibly be used as an effective agent to prevent the cognitive impairment, especially in the early stages of neurodegenerative disorders like MCI as in the case of amnesia caused by SCOP administration and may be explored for its use in cognitive disorders.

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