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EFFECT OF HIPPOPHAE RHAMNOIDES ON COGNITIVE ENHANCEMENT *VIA* NEUROCHEMICAL MODULATION IN SCOPOLAMINE INDUCED SPRAGUE DAWLEY RATS

Shivapriya Shivakumar [1], K. Ilango [1], Aruna Agrawal [2] and G. P. Dubey [*2]

Interdisciplinary School of Indian System of Medicine [1], SRM University, Kattankulathur - 603203, Tamil Nadu, India

Faculty of Ayurveda [2], Institute of Medical Science, Banaras Hindu University, Varanasi - 221005, Uttar Pradesh, India.

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Correspondence to Author:
Dr. G. P. Dubey

Faculty of Ayurveda, Siddhant Dharsan, Institute of Medical Science, Banaras Hindu University, Varanasi - 221005, Uttar Pradesh, India.

E-mail: spsk99@gmail.com

ABSTRACT: *Hippophae rhamnoides*, is known well for its cardioprotective, immunomodulatory, and antioxidant property; hence an effort was taken to evaluate its neuroprotective effect on scopolamine-induced neurochemical impairment and cognitive dysfunction. Male Sprague Dawley rats were induced with 2 mg/kg body weight of scopolamine as a single dose for 14 days, along with test formulation for four weeks and its efficacy was assessed on the cholinergic and other neurotransmitter systems according to standard laboratory method**.** It was found that the neurotoxin scopolamine elicited marked decrease in the level of neurotransmitters and cognitive function. A significant increase was found in the level of neurotransmitters, ChAT enzyme activity, and active avoidance learning score after the treatment of with test formulation showing values of $P<0.05$ & $P<0.001$. The study thus provides evidence for the potential effect of *H. rhamnoides* in improving the cognitive functions by stimulating the cholinergic system. Hence, it may be useful in the future as an alternative medicine for neurodegenerative disorders especially those of Dementia and Alzheimer's after further confirmations and clinical trial.

INTRODUCTION: Conventional drugs cause multiple side effects on long-term use; hence, there is an urgent need for alternate sources. Recently, scientists have been interested in using plants and their bioactive compounds as an alternate therapy for many diseases.

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Studies have been carried out on plants and their parts, which are highly beneficial for health; there is evidence, that edible plants can reduce the incidence of non-communicable diseases such as cardiovascular diseases, diabetes, cancer, and stroke. *Hippophae rhamnoides* L. (Elaeagnaceae), known as sea buckthorn, is edible and has its origin from Europe and Asia, they are nitrogen-fixing actinomycetes, producing yellow-orange berries at the end of summer [1]. It has been used as medicine in Russia, China, and Tibet since ancient times. The word *Hippophae* is derived from the Latin word 'Hippo' meaning 'horse' and phaos – shine.

Pharmacological uses of the plant have been recorded in books such as Sibu Yidian from the Tang Dynasty and Jing Zhu Ben Cao from the Qing Dynasty. The use of this plant by Tibetans was as early as 900 A.D, which are documented in ancient texts, including "the RGyud Bzi" (The Four Books of Pharmacopoeia) dated to the times of Tang Dynasty (618–907 AD) [1, 2].

Scopolamine, a neurotoxin is a tropane alkaloid. The anticholinergic hallucinogen which blocks the muscarinic acetylcholine (ACh) receptors, inducing dysregulation of cholinergic activity and cognitive impairment, which results in deficits in the learning, acquisition, and short term retention of spatial memory tasks [3]. Scopolamine research has played an active role to understand and alleviate the symptoms of Alzheimer's, and it is seen to be the best model for Alzheimer's [4].

Neurodegenerative disease affects millions of individuals worldwide, particularly Alzheimer's disease (AD) and Parkinson's disease (PD). Progressive decline in cognitive function, due to the degeneration of the cholinergic nervous system is the characteristic feature of Alzheimer's, which is one of the most common forms of dementia.[5] Alzheimer's has also been correlated with the loss of cholinergic neurons and a decrease in the levels of acetylcholine (ACh) and choline acetyl-transferase (ChAT).

Cholinergic deficits are a major neuropathological feature associated with memory loss and have been closely correlated with the severity of cognitive dysfunction. In aging and neurodegenerative disorder along with acetylcholine and many other neurotransmitters also show decreases, including serotonin, norepinephrine, dopamine, cortico-trophin releasing factor and in some studies also reported gamma-aminobutyric acid [6]. As *H. rhamnoides* is well known for its various other therapeutic properties, we in the present study opted to determine the neuroprotective effect of *H. rhamnoides.* With the known fact that there is a decline in the neurotransmitter system, especially cholinergic in cognitive dysfunction diseases like Alzheimer's and dementia we have thus focused on using scopolamine-induced model and find the efficacy of the plant to maintain the neurochemical balance and improve the cognitive function.

MATERIALS AND METHOD:

Chemicals: Scopolamine hydrobromide was purchased from (Sigma-Aldrich chemical Co. India). The chemicals, reagents, and solvents, required were procured from Biotech India private Ltd., and were of analytical grade.

Plant material: Plant materials of *H. rhamnoides* (Leaves and fruits) were collected from the forest of north India, Himachal Pradesh in September 2006. It was authenticated by Professor N. K. Dubey. Professor of Plant Taxonomy, Dept of Botany, Banaras Hindu University. Voucher number HR: 0706, SH 18, the specimen copy has been deposited in the department.

Preparation of Hydroalcoholic Plant Extracts: Dried leaves and fruits of *H. rhamnoides* were powdered, and ten grams of each plant material was cold macerated with hydro-alcohol ethanol: water in 60:40 proportions, for 48 h. The extract was filtered through Whatman no. 1 filter paper. The extracts were then dried using rotor evaporator and stored in the freezer until use.

Test Drug Formulation: The hydro-alcoholic extract of *H. rhamnoides* fruit's 125mg/kg and leaves 125mg/kg were mixed. Then the test formulation was suspended in distilled water and administered orally in three different dose range of 100, 200 and 300 mg/kg body weight, per oral (P.O) and given twice a day for four weeks.

Animals and Care: Adult male Sprague Dawley rats, weighing 240-260 gm have been procured from the central animal house of Banaras Hindu University. The animals were housed in stainless cages with four rats kept per cage in the limited-access facility. The animal house was maintained at 23ºC ± 2ºC with relative humidity at 50 ± 10%. Artificial light in the cage was used for 12 hours per day. Sterilized drinking water and standard chow diet (ad libitum) were given to animals during the whole period of the experiment. The guide for the care and use of laboratory animals was adopted from the NIH guidelines to carry out the present animal study.

Institutional animal ethical committee (IAEC) was taken. The animals were acclimatized in laboratory condition before the experiment.

Experimental Design: The rats were randomly divided into five following groups; eight rats were kept in each group.

Group I: Normal control group – received vehicle (saline water only).

Group II: Animals induced with Scopolamine, this group served as a disease control group.

Group III: Scopolamine induced rats treated with 100mg/kg/day test formulation for four weeks.

Group IV: Scopolamine induced rats treated with 200mg/kg/day test formulation for four weeks.

Group V: Scopolamine induced rats treated with 300mg/kg/day test formulation for four weeks.

The animal has injected 2 mg/kg body wt. of scopolamine for 14 days as a single dose per day (through intra-peritoneal root) and scopolamine were dissolved in physiological saline solution before administration.

Detection of Neurotransmitters and RAAL: After the treatment with test formulation, the following tests were conducted at the given interval period (14th day and 28th day) following standard protocol. Acetylcholine content was detected following method [7], which is based upon the formation of the acetylcholine-iodine complex followed by its absorption on cationic exchange column and separation as a fluorescent material, acetylhydrazylsalicylhydrazone, with salicyl-aldehyde. The choline acetyltransferase (ChAT) activity was measured by radiometric method [8].

The contents of 5-HT, nor-adrenaline, and dopamine were measured as described previously [9], using high-performance liquid chromatography (HPLC) with fluorescence detection with minor modifications. Briefly, brain cortex was homogenized in 0.1 M perchloric acid, and the homogenate was centrifuged at 14,000 g (4ºC) for 20 min.

The supernatant was filtered through a 0.45 µm filter membrane and injected into HPLC pump. The mobile phase consists of sodium acetate (0.02 M), methanol (16%), heptanes sulphonic acid (0.1375%), EDTA (0.2 mM) and dibutylamine (0.01%). The flow rate was set to 1 ml/min. After separation, the catecholamines were detected at the excitation wavelength of 280 nm and an emission wavelength of 315 nm and expressed as ng/gm wet tissue.

After one hour recipients of the test drug, the rats were tested for Retention of Active Avoidance Learning (RAAL) by following method [10].

RESULTS: Statistical tools were used to compare the results of the experimental groups. The variability of the result is expressed as the mean ± SD. Statistical analysis was done using GraphPad Prism software version 6.0. The mean difference between the experimental groups is done with by using ANOVA followed by post-Dunnett's test. A p value of less than 0.05 was considered to be significant.

Effect of Scopolamine the Level of Neurotransmitters and the Efficacy of *H. rhamnoides*: The reduction in the level of the neurotransmitters acetylcholine, noradrenaline, serotonin in both frontal cortex and hippocampus of the rat's brain on induction with scopolamine, along with test formulation treatment was measured on the 14th and 28th day of the experiment.

Acetylcholine is one of the prime neurotransmitters required for normal cognitive functioning which is found to be decreased at the time of aging and dementia; the test formulation is efficient to increase and maintain the level of acetylcholine in scopolamine-induced rats as seen in **Fig. 1**.

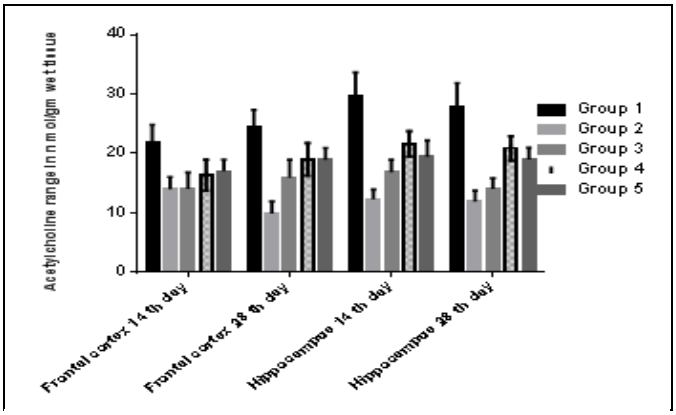

FIG. 1: LEVEL OF ACETYLCHOLINE IN FRONTAL CORTEX AND HIPPOCAMPUS ON TREATMENT WITH *H. RHAMNOIDES*. Acetylcholine level (n mol/gm) in the frontal cortex and hippocampus regions on 14th and 28th day. Values are given in mean ±SD (n=8). P values <0.05 and above were considered significant.

The plant is also capable of enhancing the level of noradrenaline and serotonin in scopolamine-induced rats, as seen in **Fig. 2, 3**. When comparing the three treated groups with Group II, we can see that the test formulation administration has maintained the level of neurotransmitters (acetylcholine, noradrenaline, and serotonin) and stopped its further decline. The P values were also significant in all treated groups compared to Group II.

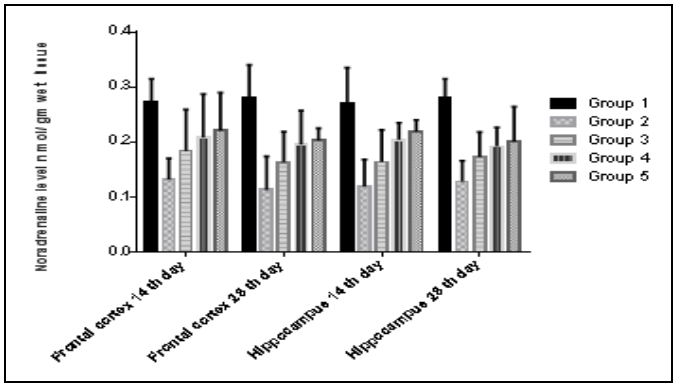

FIG. 2: LEVEL OF NOR-ADRENALINE IN FRONTAL CORTEX AND HIPPOCAMPUS ON TREATMENT WITH *H. RHAMNOIDES*. Noradrenaline level (n mol/gm) in the frontal cortex and hippocampus regions on 14th and 28th day. Values are given in mean ±SD (n=8). P values <0.05 and above were considered significant.

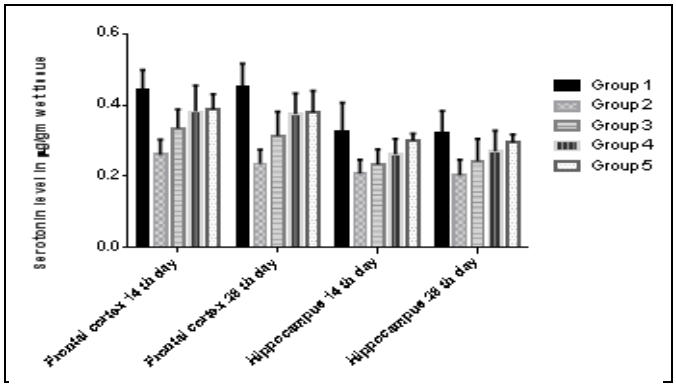

FIG. 3: SEROTONIN LEVEL IN FRONTAL CORTEX AND HIPPOCAMPUS ON TREATMENT WITH *H. RHAMNOIDES*. Serotonin level (n mol/gm) in the frontal cortex and hippocampus regions on 14th and 28th day. Values are given in mean ± SD (n=8). P values <0.05 and above were considered significant.

Effect of Scopolamine and Test Formulation on the Level of ChAT Enzyme: Fig. 4 shows that the level ChAT enzyme activity in the frontal cortex and Hippocampus region, on the 14th and 28th day. ChAT enzyme is required for the proper regulation of neurotransmitter acetylcholine, which plays a major role in cognitive dysfunction.

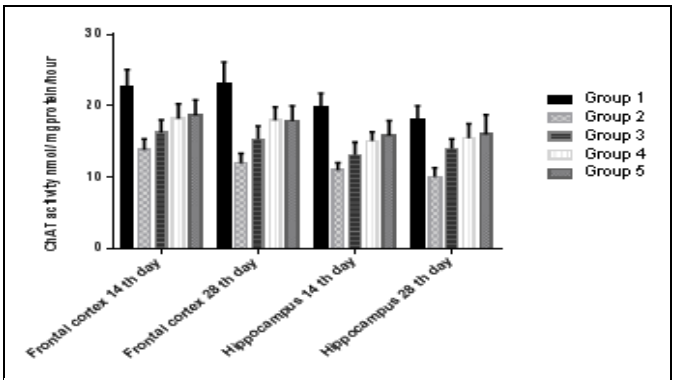

FIG. 4: CHAT ENZYME ACTIVITY IN FRONTAL CORTEX AND HIPPOCAMPUS ON TREATMENT WITH *H. RHAMNOIDES*. ChAT enzyme level (n mol/gm) in the frontal cortex and hippocampus regions on 14th and 28th day. Values are given in mean ±SD (n=8). P values <0.05 and above were considered significant.

Administration of the test formulation has improved the regulation of enzyme activity in treated groups compared to group II, also the level of significance was P<0.001 in treated groups III, IV, and V, which is highly significant while compared to group II having P>0.05.

Effect of Scopolamine and Test Formulation on the Learning Ability of Rats: Retention active avoidance learning test (RAAL) was performed to test the cognitive function of the rats.

Fig. 5 show that scopolamine reduces the retention level in rats by blocking the muscarinic receptors, hence altering the acetylcholine level, which leads to cognitive dysfunction in scopolamine-induced rats. When comparing the normal and treated groups, the RAAL score was better in treated groups III, IV, and V with P value <0.001 significantly higher when compared to group II having P value >0.05.

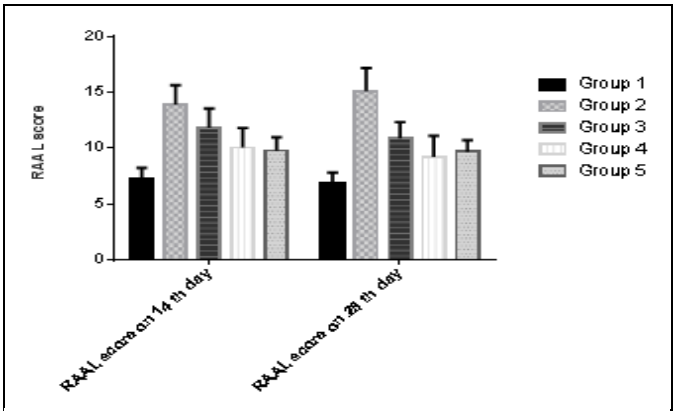

FIG. 5: RAAL SCORE ON TREATMENT WITH *H. RHAMNOIDES*. RAAL score observed on 14th and 28th day. Values are given in mean ±SD (n=8). P values <0.05 and above were considered significant.

DISCUSSION: Acetylcholine plays an important role in learning, memory, and attention processes [11, 12,] and that, anticholinergic drugs impair learning and memory in long term use [13]. Studies have shown that the biochemical and behavioral changes on scopolamine administration lowers the level of central cholinergic transmission [14], and leads to dementia associated with AD. Scopolamine, when administered in animals transiently produced deficits in learning and short-term memory, which are considered to be characteristic features of cholinergic deficits in (AD) or senile CNS dysfunction [15]. In the present study, it is revealed that chronic administration of scopolamine (intraperitoneal), affected the cholinergic system which reduced the level of acetylcholine in the hippocampal region leading to severe deficits in cognitive function implying neurodegeneration in the brain. This was clearly understood with the reduced learning capacity.

Apart from cholinergic disruption, several pieces of evidence revealed significant monoaminergic deficits and reduction in the level of other brain neurotransmitters with progressive aging [16] and Alzheimer's disease. In the present study also scopolamine induction leads to neurotransmitter modulation, which was noted by a reduced level of Norepinephrine and serotonin in the cortex and hippocampus region of the rat's brain. There was also a decrease or dysregulation in the level of ChAT enzyme activity.

In the present study, the effect of scopolamine was reversed with the treatment of different doses of *H. rhamnoides* formulation. It was able to stop the further decline of neurotransmitters and also enhanced to a certain level in comparison to the non-treated group. Recent literature also reveals that *H. rhamnoides* is rich source of antioxidants, like phenols, flavonoids, phytosteorls, tocopherols, vitamins, organic acids, Coumarins, Carotenoids, triterpenes, minerals and essential oil which show a wide spectrum of pharmacological effects of *H. rhamnoides* like antioxidant, immunomodulatory, anti-atherogenic, anti-stress, hepatoprotective, radioprotective and tissue repair [17-27].

The overall results obtained in the current study shows that neurotoxin scopolamine has produced severe neuro-chemical impairment in association with cognitive decline in experimental animals, and the treatment with the test formulation containing the leaf and fruit extracts of *H. rhamnoides* has enabled to enhance the level of the neurotransmitters and improve the memory or learning capability. Thus, the result implies that the extract may significantly alleviate age-related neurodegenerative complications by modulating the neurotransmitter system.

CONCLUSION: We conclude from the present study that with the plant *H. rhamnoides* being edible with its proved pharmacological activities like hypertensive, cardio-vascular, *etc*., may be used in the treatment of cognitive decline.

The plant may be used as an alternative therapy or combined therapy for dementia and AD, to improve memory and to balance the level of neuro-transmitters.

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CONFLICT OF INTEREST: The authors declare that we have no conflict of interest.

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