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DOSE-DEPENDENT EFFECT OF *WITHANIA SOMNIFERA* ON THE CHOLINERGIC SYSTEM IN SCOPOLAMINE-INDUCED ALZHEIMER'S DISEASE IN RATS

G. Visweswari [*1,2], Rita Christopher [1] and W. Rajendra [2]

Department of Neurochemistry [1], National Institute of Mental Health and Neuro Sciences, Bangalore - 560029, Karnataka, India.
Department of Zoology [2], Sri Venkateswara University, Tirupati - 517502, Andhra Pradesh, India.

Keywords:

Alzheimer's disease, *Withania somnifera*, Scopolamine, Donepezil, Cholinergic system, Different brain regions

Correspondence to Author:
Dr. G. Visweswari

Post-Doctoral Fellow,
Professor and Head,
Department of Neurochemistry,
National Institute of Mental Health and Neuro Sciences, Bangalore - 560029, Karnataka, India.

E-mail: visweswari.g@gmail.com

ABSTRACT: The present study was aimed to determine whether the methanol and aqueous extracts from the medicinal plant, *Withania somnifera* (root), could inhibit acetylcholinesterase (AChE) in scopolamine-induced Alzheimer's rats. The rats were randomly divided into different groups of 5 each: normal control rats treated with saline; Scopolamine (2mg/Kg b.w. i.p.) – induced Alzheimer's rats treated with saline; Scopolamine-induced Alzheimer's rats post-treated with methanol extract of three different concentrations (100, 200 and 300mg/Kg b.w. oral); Scopolamine-induced Alzheimer's rat post-treated with aqueous extract of three different concentrations (100, 200 and 300mg/Kg b.w. oral); and scopolamine-induced Alzheimer's rat post-treated with donepezil, a reference control (5mg/Kg b.w. oral) for 10 successive days. Increased AChE activity and decreased Acetylcholine content were recorded in different brain regions in scopolamine-induced Alzheimer's rats. Post-treatment with *W. somnifera* extracts caused recovery of the levels of AChE and acetylcholine (ACh) in a dose-dependent manner. These findings suggest that *W. somnifera* causes perceptible changes in the cholinergic system as one of the facets of its anti-alzheimeric activity.

INTRODUCTION: Alzheimer's disease (AD), a progressive neurodegenerative disorder with many cognitive and neuropsychiatric symptoms is biochemically characterized by a significant decrease in acetylcholine (ACh) and increase in the AChE activity, a member of the α/β hydrolase protein superfamily, hydrolyzes Acetylcholine (ACh) into acetate and choline at the central and peripheral cholinergic synapses [1].

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AChE, (EC 3.1.1.7) is found in cholinergic synapses in the brain as well as in autonomic ganglia, the neuromuscular junction, and the target tissues of the parasympathetic system [2]. This enzyme plays a major role in acetylcholine-mediated neurotransmission [3]. Loss of ACh activity in the brain correlates with the severity of AD [4].

The burden of Alzheimer's disease is continually rising globally and especially in the Asia-Pacific region. Unfortunately, the efficacy of the therapeutic strategy is still very limited. AChE is one of the most studied enzyme/protein in the field of Alzheimer's disease. Although the primary cause of AD is unclear, brain ACh deficiency, oxidative stress, and neuroinflammation may be considered the principal pathogenic factors [4, 5].

Alzheimer's disease is commonly treated by Acetylcholinesterase inhibitor (AChEI) drug therapy. Treatment of AD patients with cholinesterase inhibitors causes symptomatic benefit and seems to delay disease progression for 6-12 months [6]. Because of the varying side effects of Alzheimer's drugs, interest in the exploitation of medicinal plants as an alternative to AChEIs has greatly increased in recent years.

AChE inhibitor drugs have been designed to inhibit the breakdown of ACh in the brain and thus to increase cholinergic neurotransmitter activity in AD patients. Several types of research have been conducted to evaluate the AChE inhibitory activity of various plant species, for instance, *Tabernaemontana divaricata*, *Fumaria* sp., *Vaccinium angustifolium*, *Corydalis spp*., *Lavandula pedunculata*, *Mentha suaveolens*, and *Hypericum undulatem* [7, 8]. Nevertheless, until now no plant species has scored full marks for its potential to replace the AChEI AD drugs.

Scopolamine, a muscarinic antagonist that induces central cholinergic blockade, produces a reversible and well-described impairment in both (i) maintaining attention; and (ii) processing of information and the acquisition of new knowledge in rodents [9] and humans [10].

The cognitive deterioration observed in scopolamine-induced rats resembles the memory disturbances seen in AD. Hence, the scopolamine-induced rat has been used as an experimental model for Alzheimer's disease [11, 12].

In the present study, we determined the AChE inhibitory effect of *Withania somnifera (W. somnifera)*, a native medicinal plant which is popularly used for curing various ailments. The root extract of *W. somnifera* has been shown to have health promoting effects such as anti-stress, anti-arthritic, anti-inflammatory, analgesic, anti-pyretic, anti-oxidant, and immunomodulatory properties [13-15]. Besides these properties, it also has anti-aging, aphrodisiac, thermoregulatory, anti-peroxidative [16], anti-inflammatory [17], antitumor [18], antioxidant, hemopoietic and rejuvenating properties [19]. The root of *W. somnifera* contains several alkaloids, withanolides, and a few flavonoids and reducing sugars [20].

More than 20 active constituents have been reported to date, including withaferin A, sitoindosides VII–X, withanosides I–VII, choline, and beta-sitosterol [21]. Since, the estimation of Acetylcholinesterase activity provides valuable information on cholinergic neurotransmission, an attempt was made in the present study to investigate the neuropharmacological action and anti-alzheimeric properties of different concentrations of two different extracts of the medicinal plant *W. somnifera*, in scopolamine-induced AD concerning cholinergic neurotransmission.

In view of its broad spectrum of reported therapeutic properties, but with only limited scientific reports on the effective activity of this plant in different regions in albino rat, Cerebral cortex (CC), Cerebellum (CB), Hippocampus (HC), Hypothalamus (HT) and Pons, impairment of which leads to AD, we aimed to determine whether or not the extract from *W. somnifera* root could inhibit AChE activity in different brain regions of Scopolamine-induced AD rats. The results obtained from our study might facilitate the inhibited AChE activity in different regions of scopolamine-induced AD rat brain.

MATERIALS AND METHODS:

Collection of Plant Material: *Withania somnifera* Linn. (*W. somnifera*) (common name: Ashwagandha) is an Indian medicinal plant that grows as an evergreen shrub in dry parts of India (Ayurveda). *W. somnifera* is also one of the members of GRAS (Generally regarded as safe) category of plants that can be used for therapeutic purposes [22]. Roots of *W. somnifera* was purchased from an Ayurvedic products distributor (Indian Drugs and Stores, Bangalore) and authenticated by a botanist, Department of Botany, S.V. University, Tirupati, Andhra Pradesh. A voucher specimen was deposited in the herbarium of the Department of Botany, S.V. University, Tirupati (Voucher No. 2437).

Extract Preparation: The dried roots were ground into a coarse powder using a pulverizer. Powdered plant material was soaked in methanol for 24 hr at room temperature, and the solvent was filtered with Whatman filter paper (125 mm). This was repeated 3-4 times until the extract gave no coloration. The extract was distilled and concentrated under

reduced pressure in the Buchi Rotavapor R-114 yielding a gum-like residue. The same was repeated with distilled water. Finally, the crude extracts of methanol and water (aqueous) were collected, weighed, and stored in the refrigerator at 4 ºC for further use to treat the animals.

Experimental animals: Adult male Wistar rats, 4 months of age, with weights between 300-350g were maintained in polypropylene cages (5 in each cage at 26 ºC; 12 h light: dark cycle). The animals were fed standard pellet diet and water *ad libitum*. The animal study was performed by the guidelines provided by the Institutional Animal Ethics Committee of the National Institute of Mental Health and Neuro Sciences.

The rats were divided into the following groups and treated for 10 successive days:

Group I: Controls treated with normal saline (oral)

Group II: Scopolamine dissolved in normal saline (2mg/Kg b.w., i.p.)

Group III: Scopolamine (2mg/Kg b.w., i.p.) + methanol extract (100mg/Kg b.w., oral)

Group IV: Scopolamine (2mg/Kg b.w., i.p.) + methanol extract (200mg/Kg b.w., oral)

Group V: Scopolamine (2mg/Kg b.w., i.p.) + methanol extract (300mg/Kg b.w., oral)

Group VI: Scopolamine (2mg/Kg b.w., i.p.) + Aqueous extract (100mg/Kg b.w., oral)

Group VII: Scopolamine (2mg/Kg b.w., i.p.) + Aqueous extract (200mg/Kg b.w., oral)

Group VIII: Scopolamine (2mg/Kg b.w., i.p.) + Aqueous extract (300mg/Kg b.w., oral)

Group IX: Scopolamine (2mg/Kg b.w., i.p.) + Donepezil hydrochloride (5mg/Kg b.w., oral)

The animals from each group were decapitated after 10 days treatment, the brains quickly removed and washed in ice-cold saline. Different regions such as Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC), Hypothalamus (HT) and Pons from the brain were quickly dissected out on an ice-cold petri-dish and stored at -80 ºC till used for ACh and AChE assays.

Drugs and Chemicals: Scopolamine hydrobromide (Scopolamine), Donepezil hydrochloride (Donepezil), Acetylthiocholine iodide, 5, 5'-Dithiobis (2-Nitrobenzoic acid) (DTNB) and Hydroxylamine hydrochloride were obtained from Sigma Chemicals. All other chemicals used were analytical reagent grade.

Acetylcholinesterase (AChE) Assay: Acetylcholinesterase activity was estimated by Ellmann's method modified by Srikumar *et al*., 2004 with small modifications [23]. The tissues were weighed and homogenized in 0.1M Phosphate buffer (pH 8). The aliquot of the homogenate was added to a microwell containing 0.1M phosphate buffer and 0.01M DTNB. The contents are mixed thoroughly and measured the initial reading at 412 nm. The 0.075M substrate, *i.e.*, acetylthiocholine was added, and change in absorbance was recorded in microplate reader for a period of 10 min at intervals of 2min. Change in absorbance per minute was thus determined.

Acetylcholine (ACh) Estimation: Acetylcholine in different regions of the brain was estimated using Hydroxylamine method by Hestrin and modified by Stepankova *et al.,* [24] with small modifications. The reaction mixture was prepared and mixed with the aqueous hydroxylamine hydrochloride and 3.5M aqueous KOH (1:1 v/v). The resulting mixture was mixed 2 min. To convert ACh totally to acetohydroxamic acid. Then the pH value was changed again by adding Conc. HCl/H_20 (1:2 v/v). The reddish brown color formed after adding 0.37M ferric nitrate was read at 540nm in a microplate reader. The ACh content was calculated concerning the standard values.

Statistical Analyses: All data presented are means, standard errors and ANOVAs were calculated using SPSS statistical software.

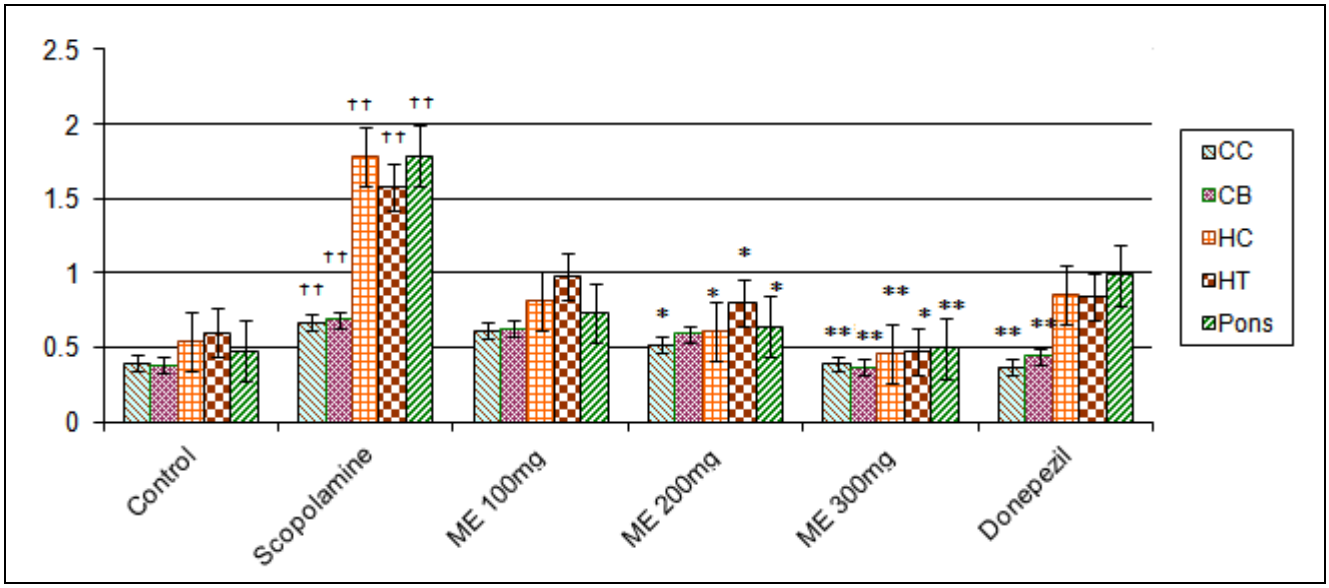
RESULTS: From the present data it was observed that scopolamine-treated rats showed significant increase in AChE activity in all the brain regions Cerebral cortex (CC), Cerebellum (CB), Hippocampus (HC), and Pons except in Hypothalamus (HT) where it showed non-significant increase in AChE activity when compared to normal saline-treated controls. In contrast to this, the rats treated with methanol

extract (ME) of *W. somnifera* 100mg, 200mg and 300mg/Kg b.w. showed decrease in AChE activity in all the brain regions in a dose-dependent manner. 200mg/Kg of ME showed significant decrease in Hippocampus (HC) whereas 300mg/Kg showed significant decrease in all the brain regions when compare to scopolamine-treated rats **Table 1**; **Fig 1**. The rats treated with an aqueous extract (AE) of *W. somnifera* also showed the decrease in AChE activity in a dose-dependent manner **Table 2**; **Fig 2**. But unlike ME, 100 and 200mg/Kg of AE showed significant anti AChE activity in CB and HC regions. Rats treated with 300mg/Kg of AE showed a highly significant decrease in AChE activity in all the brain regions except in HT, where it was insignificant. The rats treated with reference standard Donepezil Hydrochloride (DH) showed decreased AChE activity in all the regions when compare to scopolamine-treated rats but it was highly significant in CC and CB regions only.

TABLE 1: EFFECT OF DIFFERENT CONCENTRATIONS OF METHANOL EXTRACT OF *W. SOMNIFERA* ROOT POWDER ON ACHE ACTIVITY IN DIFFERENT BRAIN REGIONS OF SCOPOLAMINE-INDUCED AD RAT BRAIN (µmoles of substrate hydrolyzed/mg tissue/min)

S. no.	Name of the tissue	Normal Control	Scopolamine	Scopolamine + Methanol Extract			Scopolamine+ Donepezil
				100mg/Kg	200mg/Kg	300mg/Kg	
1	CC	0.393±0.01	0.669††±0.018	0.613± 0.02	0.518*±0.038	0.388**±0.019	0.368**± 0.005
2	CB	0.379±0.019	0.684††±0.013	0.622±0.007	0.589±0.009	0.368**±0.004	0.443**±0.033
3	HC	0.537±0.062	1.783††±0.299	0.814±0.109	0.604*±0.128	0.463**±0.052	0.854± 0.255
4	HT	0.601±0.149	1.575††±0.507	0.978±0.188	0.796*±0.246	0.472*±0.064	0.842± 0.475
5	Pons	0.475±0.048	1.786††±0.154	0.729±0.049	0.641*±0.054	0.492**±0.055	0.985± 0.088

All values are Mean ±SEM; n=5; † P≤0.05, †† P≤0.005 as compared with Control; *P ≤0.05, **P≤0.005 as compared with Scopolamine. (CC-cerebral cortex; CB-cerebellum; HC-Hippocampus; HT-Hypothalamus)

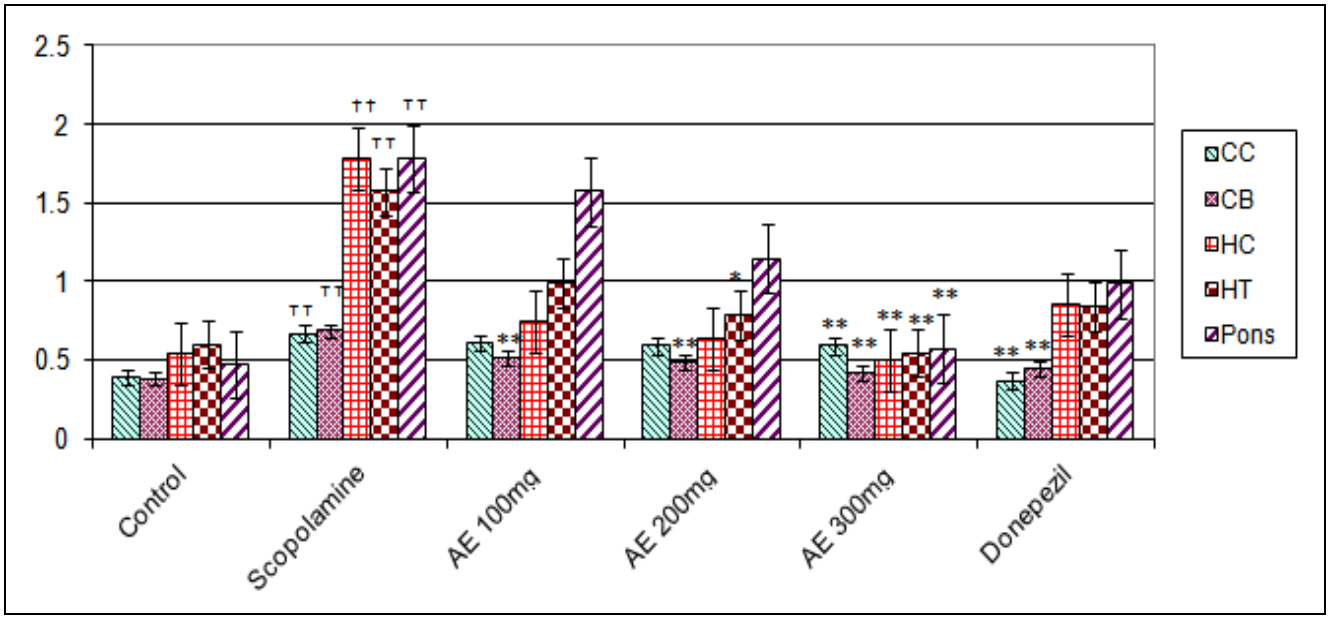


GRAPH 1: GRAPHICAL REPRESENTATION OF DIFFERENT CONCENTRATIONS OF METHANOL EXTRACT OF *W. SOMNIFERA* ROOT POWDER ON ACHE ACTIVITY IN DIFFERENT BRAIN REGIONS OF SCOPOLAMINE-INDUCED AD RAT BRAIN (µmoles of substrate hydrolyzed/mg tissue/min.)

TABLE 2: EFFECT OF DIFFERENT CONCENTRATIONS OF AQUEOUS EXTRACT OF *W. SOMNIFERA* ROOT POWDER ON ACHE ACTIVITY IN DIFFERENT BRAIN REGIONS OF SCOPOLAMINE-INDUCED AD RAT BRAIN (µmoles of substrate hydrolyzed/mg tissue/min)

S. no.	Name of the tissue	Normal Control	Scopolamine	Scopolamine + Aqueous Extract			Scopolamine+ Donepezil
				100mg/Kg	200mg/Kg	300mg/Kg	
1	CC	0.393±0.01	0.669†±0.018	0.607±0.029	0.590±0.013	0.446**±0.012	0.268**± 0.005
2	CB	0.379±0.019	0.684††±0.013	0.514**±0.031	0.485**±0.023	0.419**±0.0133	0.443**±0.033
3	HC	0.537±0.062	1.783††± 0.299	0.745±0.161	0.632±0.157	0.502**±0.048	0.854± 0.255
4	HT	0.601± 0.149	1.575††± 0.507	0.989±0.324	0.784*±0.127	0.544**±0.062	0.842±0.475
5	Pons	0.475±0.048	1.786††± 0.154	1.5730.262	1.1470.300	0.573**±0.262	0.985± 0.088

All values are Mean ±SEM; n=5; † P≤0.05, †† P≤0.005 as compared with Control; *P ≤0.05, **P≤0.005 as compared with Scopolamine. (CC-cerebral cortex; CB-cerebellum; HC-Hippocampus; HT-Hypothalamus)



GRAPH 2: GRAPHICAL REPRESENTATION OF DIFFERENT CONCENTRATIONS OF AQUEOUS EXTRACT OF *W. SOMNIFERA* ROOT POWDER ON ACHE ACTIVITY IN DIFFERENT BRAIN REGIONS OF SCOPOLAMINE-INDUCED AD RAT BRAIN (µmoles of substrate hydrolyzed/mg tissue/min.)

Acetylcholine (ACh) content in scopolamine-treated rats was decreased significantly in all the brain regions (**Table 3, 4; Fig. 3, 4**) as AChE activity showed an increase with scopolamine-treatment. In contrast to this, the rats treated with ME and AE with 100mg, 200mg, and 300mg/Kg BW showed the recovery in ACh content in all the brain regions in a dose-dependent manner.

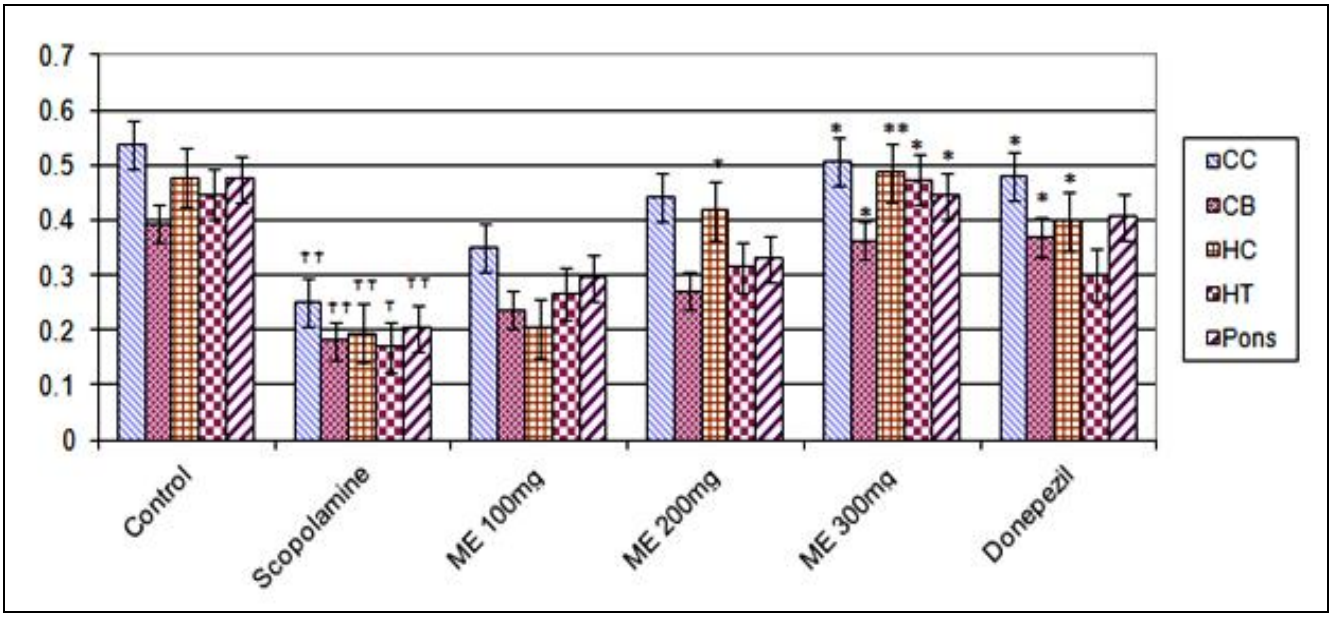
All the three doses mentioned above showed an increase in all the regions, while rats treated with 300mg/Kg b.w. ME and AE showed a significant increase in ACh content in all the regions.

At the same time, 200mg/kg BW of ME showed a significant decrease in AChE and increased in ACh in HC, and 200mg/Kg BW of AE showed a significant decrease in AChE and increase in ACh in CB and HT regions. The effect of 300mg/Kg was highly significant in all the five brain regions were 100 and 200mg/Kg showing a significant effect in limited regions. The significant response with 300mg/Kg of ME and AE in scopolamine-treated rats suggests that this dose maintains the homeostasis of the cholinergic system in all the cerebral structures discussed above.

TABLE 3: EFFECT OF DIFFERENT CONCENTRATIONS OF METHANOL EXTRACT OF *W. SOMNIFERA* ROOT ON ACH CONTENT IN DIFFERENT BRAIN REGIONS OF SCOPOLAMINE-INDUCED AD RAT BRAIN (µmoles of Acetylcholine/mg tissue)

S. no.	Name of the tissue	Normal Control	Scopolamine	Scopolamine + Methanol Extract			Scopolamine+ Donepezil
				100mg/Kg	200mg/Kg	300mg/Kg	
1	CC	0.539 ±0.044	0.252†† ±0.027	0.351 ± 0.056	0.442 ± 0.023	0.506* ± 0.048	0.480* ± 0.030
2	CB	0.393 ±0.047	0.180†† ±0.011	0.237 ± 0.028	0.271 ± 0.026	0.362* ± 0.016	0.369* ±0.033
3	HC	0.478 ± 0.014	0.194†† ± 0.021	0.204 ± 0.027	0.418* ± 0.033	0.486** ± 0.064	0.399* ± 0.017
4	HT	0.447 ± 0.051	0.169† ± 0.010	0.265 ± 0.027	0.314 ± 0.054	0.474* ± 0.060	0.300 ± 0.050
5	Pons	0.475 ± 0.019	0.203†† ± 0.044	0.295 ± 0.030	0.330 ± 0.043	0.445* ± 0.055	0.407 ± 0.025

All values are Mean ±SEM; n=5; † P≤0.05 †† P≤0.005 as compared with Control; *P ≤0.05, **P≤0.005 as compared with Scopolamine. (CC-cerebral cortex; CB-cerebellum; HC-Hippocampus; HT-Hypothalamus)

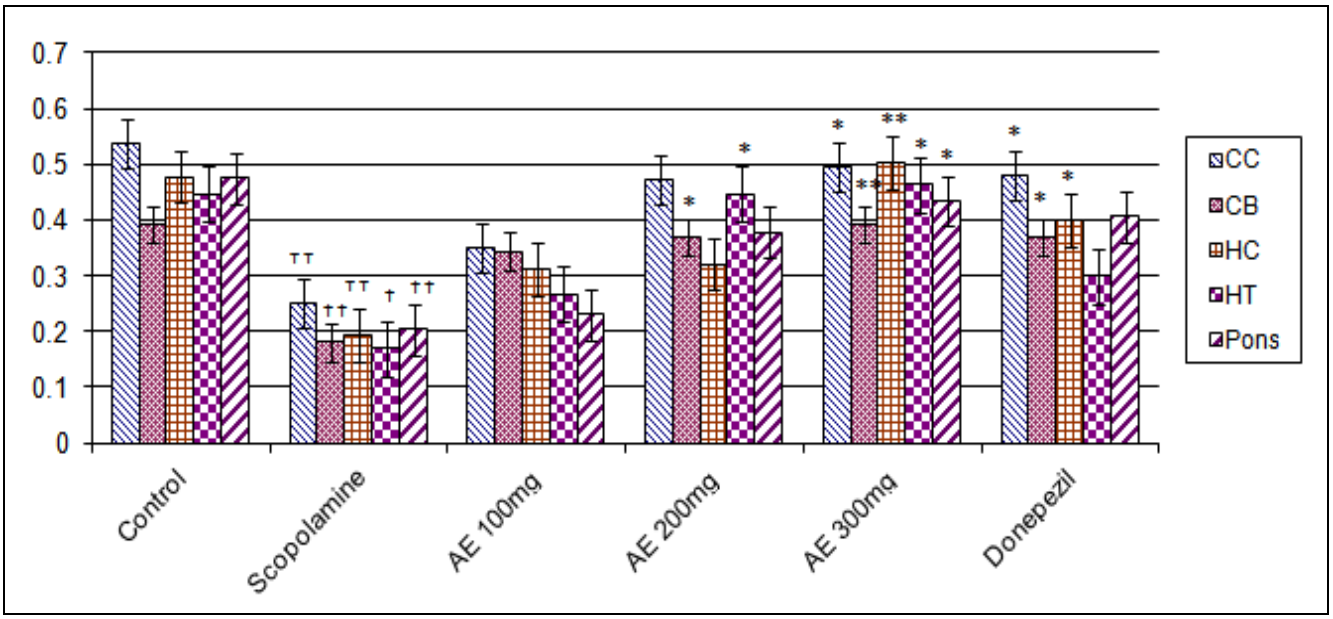


GRAPH 3: GRAPHICAL REPRESENTATION OF DIFFERENT CONCENTRATIONS OF METHANOL EXTRACT OF *W. SOMNIFERA* ROOT POWDER ON ACH CONTENT IN DIFFERENT BRAIN REGIONS OF SCOPOLAMINE-INDUCED AD RAT BRAIN (µmoles of Acetylcholine/mg tissue)

TABLE 4: EFFECT OF DIFFERENT CONCENTRATIONS OF AQUEOUS EXTRACT OF *W. SOMNIFERA* ROOT ON ACH CONTENT IN DIFFERENT BRAIN REGIONS OF SCOPOLAMINE-INDUCED AD RAT BRAIN (µmoles of acetylcholine/mg tissue)

S. no.	Name of the tissue	Normal Control	Scopolamine	Scopolamine + Aqueous Extract			Scopolamine+ Donepezil
				100mg/Kg	200mg/Kg	300mg/Kg	
1	CC	0.539±0.044	0.252††±0.027	0.351±0.048	0.473±0.016	0.495*±0.025	0.480*±0.030
2	CB	0.393±0.047	0.180††±0.011	0.344±0.044	0.369*±0.028	0.391**±0.009	0.369*±0.033
3	HC	0.478±0.014	0.194††± 0.021	0.312±0.020	0.321±0.023	0.455**±0.045	0.399*± 0.017
4	HT	0.447±0.051	0.169†± 0.010	0.267±0.324	0.446*±0.127	0.463*±0.035	0.300±0.050
5	Pons	0.475±0.019	0.203††± 0.044	0.231±0.027	0.378±0.308	0.434*±0.048	0.407±0.025

All values are Mean ±SEM; n=5; † P≤0.05 †† P≤0.005 as compared with Control; *P ≤0.05 **P≤0.005 as compared with Scopolamine. (CC-cerebral cortex; CB-cerebellum; HC-Hippocampus; HT-Hypothalamus



GRAPH 4: GRAPHICAL REPRESENTATION OF DIFFERENT CONCENTRATIONS OF AQUEOUS EXTRACT OF *W. SOMNIFERA* ROOT POWDER ON ACH CONTENT IN DIFFERENT BRAIN REGIONS OF SCOPOLAMINE-INDUCED AD RAT BRAIN (µmoles of Acetylcholine/mg tissue)

DISCUSSION: Alzheimer's disease (AD) is a disorder coupled with progressive degeneration of memory and cognitive function. Cholinesterase inhibitors are the recommended drugs for treating patients with mild to severe AD [25, 26]. The cholinergic theory assumes that memory impairment in patients with AD occurs from a shortfall of cholinergic activity in the brain. Acetylcholinesterase inhibitors can reinstate the amount of acetylcholine by inhibiting AChE. Over the years, medicinal plants have offered components with decent AChE inhibitory activity [27] and could be a potential source.

Some studies reported a very significant increase in AChE activity in scopolamine treated group [28]. The reports showed that the increase in AChE due to the scopolamine leads to the amnesia and which is reversed by *prunus amygdalus* in rats [29]. Studies on the brain from patients suffering from AD have shown reduced AChE activity in the hippocampus and cortex [30]. Biopsy studies in AD patients reported decreased AChE activity with concurrent loss of cognitive function [30]. AChE is known to have many classical functions and morphological role in a growing body. During early development, AChE expression is tightly correlated with neurite outgrowth, in addition to its role in cell survival and growth [31].

In this study, we observed the reversible action of different doses ME and AE against the scopolamine-induced hyperactivity of AChE.

Previous studies have found the anti-cholinesterase activity of methanol, and aqueous extracts from many natural fruits and vegetables in AD brain [32]. It has been reported that an aqueous extract of *W. somnifera* root inhibited AChE in a concentration-dependent manner *in-vitro* [33].

The studies on the whole brain showed that this aqueous root extract might be useful in improving memory and other cognitive functions associated with the cholinergic system [33]. Histological and biochemical studies reported that significant inhibitory effect of *W. somnifera* leaf juice on AChE in mice brain [34].

It has been already reported that *W. somnifera* leaf extract and it's component withanone shows downregulation of neuronal cell markers NF-H, MAP2, PSD-95, GAP-43 and glial cell marker, GFAP, and upregulation of DNA damage-γH2AX and oxidative stress-ROS markers in scopolamine-induced cytotoxicity in IM32 neuronal and C6 glioma cells [35]. Administration of defined extracts like sitoindosides VII-X and withaferin A i.p. for 7 days reduced the cholinergic neurons in the vertical, diagonal band whereas increased in lateral septum and globus pallidus regions in rat brain [36].

The rats administered with higher doses of aqueous and chloroform extracts along with ibotonic acid, which develops syndrome with pathological features similar to AD, significantly lowered cholinergic deterioration in rat brain [37].

Since flavonoids show AChE inhibitory activity, *W. somnifera* rich in flavonoids in its root and whole plant shows significant effects on recovery from Scopolamine - induced AD [38, 39].

From these results, it is obvious that ME and AE of *W. somnifera* extracts have conspicuous effects on recovery from Scopolamine - induced AD. The increase in AChE activity and decrease in ACh content in different regions of the brain seems to augment the anti-Alzheimeric effect of scopolamine-induced AD.

In contrast, the decreased AChE activity and increased ACh levels resulting from post-treatment with ME and AE in all regions of the brain imply their anti-Alzheimeric effect. Quite interestingly, the changes in AChE activity and ACh content occurred in all regions of the brain, whether targeted or non-targeted areas. This indicates that the anti-Alzheimeric effect of *W. somnifera* extracts proceeds through generalized changes in different brain regions in addition to their specific impact on targeted regions.

Although several synthetic cholinesterase inhibitors such as tacrine, rivastigmine, and donepezil, are being used for management of conditions such as AD, their side effects have become noticeable [40]. Some promising results have been achieved by acetylcholinesterase inhibitors, but effective therapeutic intervention in Alzheimer's disease remains an important goal.

CONCLUSION: In the present study, the changes in AChE activity and ACh content were maximal

with 300mg/Kg b.w. of ME and AE. Hence it may be concluded that these extracts with this concentration have anti-alzheimeric activity and cause perceptible changes in the cholinergic system, at least as part of their anti-alzheimeric effect.

Besides cholinergic blockade, scopolamine-induced memory loss, cognitive impairment, and oxidative stress may be associated with Alzheimer's and *W. somnifera* may serve as a potential preventive and therapeutic agent for neurodegenerative disorders. Hence further studies are warranted in this area.

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REFERENCES:

1. Block JH and Beale JM: Textbook of Organic Medicinal and Pharmaceutical Chemistry II. In: Cholinergic Drug and Related Agents. Cocolas GH and Cuter SJ, United States, Lippincott Williams & Wilkins 2004.
2. Soreq H and Seidman S: Acetylcholinesterase – new roles for an old actor. Nature Rev. Neurosci. 2001; 2: 294-02.
3. Dhingra D, Parle M and Kulkarni SK: Genetic basis of Alzheimer's disease. Indian J Pharm Sci 2005; 67: 409-13.
4. Hyde TM: Cholinergic systems and schizophrenia: primary pathology or epiphenomena? Journal of Chemical Neuroanatomy 2001; 22: 53-63.
5. Bunchaeoen W, Saenphet S and Saenphet K: Acetylcholinesterase inhibitory effect of *Pseuderanthemum palatiferum* in albino rats. Trends Research in Science and Technology 2010; 2: 13-18.
6. Aarsland D, Mosimann UP and McKeith IG: Role of cholinesterase inhibitors in Parkinson's disease and dementia with Lewy bodies. J. Geriatr. Psychiatry Neurol. 2004; 17: 164-71.
7. Papandreou, MA, Dimakopoulou A, Linardaki ZI, Cordopatis P, Klimis-Zacas, D, Margarity M and Lamari FN: Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. Behavioral Brain Research 2009; 198: 352-58.
8. Wessler I and Kirkpatrick CJ: Acetylcholinesterase beyond neurons: the non-neuronal cholinergic system in humans. British Journal of Pharmacology 2008; 154: 1558-71.
9. Zhang HT and O'Donnell JM: Effects of rolipram on scopolamine-induced impairment of working and reference memory in the radial-arm maze tests in rats. Psychopharmacology 2000; 150: 311-16.
10. Jones RW, Wesnes KA and Kirby J: Effects of NMDA modulation in scopolamine dementia. Ann NY Acad Sci 1991; 640: 241-44.
11. Jeong EJ, Lee KY, Kim SH, Sung SH and Kim YC: Cognitive enhancing and antioxidant activities of iridoid glycosides from *Scrophularia buergeriana* in scopol-amine-treated mice. Eur J Pharmacol 2008; 288: 78-84.
12. Ebert U and Kirch W: Scopolamine model of dementia: electroencephalogram findings and cognitive performance. Eur J Clin Invest 1998; 2: 944-49.
13. Gupta K, Dua A and Vohra P: *Withania somnifera* ashwagandha attenuates antioxidant defense in aged spinal cord and inhibits copper-induced lipid peroxidation and oxidative protein modifications. Drug Metabolism, Drug Interaction 2003; 19: 211-22.
14. Prakash J, Gupta K and Dinda K: *Withania somnifera* root extract prevents DMBA-induced squamous cell carcinoma of skin in Swiss albino mice. Nutrition, Cancer 2002; 42: 91-97.
15. Dhuley N: Adaptogenic and cardioprotective action of Ashwagandha in rats and frogs. Journal of Ethnopharmacology 2000; 70: 57-63.
16. Mishra C, Singh B and Dagenais S: Scientific basis for the therapeutic use of *Withania somnifera* Ashwagandha: a review. Alternative Medicine Review 2000; 5: 334-46.
17. Sudhir S, Budhiraja D, Miglani P, Arora B, Gupta C and Garg N: Pharmacological studies on leaves of *Withania somnifera.* Planta Medica 1986; 1: 61-63.
18. Sharada C, Solomon E, Devi U, Udupa N and Srinivasan K: Antitumor and radio sensitizing effects of withaferin A on mouse Ehrlich ascites carcinoma *in-vivo*. Acta Oncologica 1996; 35: 95-100.
19. Thakur S, Puri S and Husain A: Major medicinal plants of India. Central Institute of Medicinal and Aromatic Plants, Lucknow, India 1989; 50-52.
20. Visweswari G, Christopher R and Rajendra W: Phytochemical screening of active secondary metabolites present in *Withania somnifera* root: Role in traditional medicine. Int J Pharm Sci Res 2013; 4(7): 2770-76.
21. Ganzera M, Choudhary I and Khan A: Quantitative HPLC analysis of withanolides in *Withania somnifera.* Fitotherapia 2013; 7: 68-76.
22. Bolleddula J, Yanjun Z, Navindra P and Muraleedharan G: Growth inhibition of human tumor cell lines by withanolides from *Withania somnifera* leaves. Life Science 2003; 74: 125-32.
23. Srikumar BN, Ramkumar K, Raju TR and Shankaranarayana RBS: Assay of acetylcholinesterase activity in the brain. Brain and Behaviour 2004; 142-44.
24. Stepankova S, Vranova M, Zdrazilova P, Komers K, Komersova A and Cegan A: Two new methods monitoring kinetics of hydrolysis of acetylcholine and acetylthiocholine. Zeitschrift fuer Naturforschung Section C Journal of Biosciences 2005; 60: 943-46.
25. Khan H, Tariq SA, Khan MA, Inayatur R, Rukhsana, Gaffar and Saifullah: Cholinesterase and lipoxigenase inhibition of whole plant *Withanaia somnifera.* Afr J Pharm Pharmacol 2011; 5: 2272-75.
26. Sancheti S, Sancheti S and Seo SY: Evaluation of antiglycosidase and anticholinesterase activities of *Boehmeria nivea*. Pak J Pharm Sci 2010; 23: 236-40.
27. Khan M, Khan H, Khan S, Mahmood T, Khan P and Jabar A: Anti-inflammatory, analgesic and antipyretic activities of *Physalis minima* Linn. J Enz Inhib Med Chem.2009; 24: 632-37.
28. Sridharamurthy NB, Ashok B and Yogananda R: Evaluation of Antioxidant and Acetyl Cholinesterase inhibitory activity of *Peltophorum pterocarpum* in Scopolamine treated Rats. Int J Drug Dev & Res 2012; 4: 115-27.

29. Kirti SK, Kasture SB and Mengi SA: Efficacy study of *Prunus amygdalus* (almond) nuts in scopolamine-induced amnesia in rats. Indian J Pharmacol 2010; 42: 168-73.
30. Hammond P and Brimijoin S: Acetylcholinesterase in Huntington's and Alzheimer's diseases: simultaneous enzyme assay and immunoassay of multiple brain regions. J Neurochem 1988; 50: 1111–16.
31. Appleyard ME: Secreted acetylcholinesterase: non-classical aspects of a classical enzyme. Trends Neurosci 1992; 15: 485-90.
32. Szwajgier D and Borowiec K: Screening for cholinesterase inhibitors in selected fruits and vegetables. Electronic Journal of Polish Agricultural Universities 2012; 15.
33. Kumar S, Seal CJ and Okello EJ: Kinetics of Acetylcholinesterase inhibition by an aqueous extract of *Wihtania somnifera* roots. International Journal of Pharmaceutical Sciences and Research 2011; 2: 1188-92.
34. Bhatnagar M, Suhalka P, Sukhwal P, Jain A and Sharma D: Inhibition of Acetylcholinesterase and NO synthase activity in the mice Brain: Effect of a *Withania somnifera* leaf juice. Neurophysiology 2012: 44: 301-04.
35. Konar A, Shah N, Singh R, Saxena N, Kaul SC, Wadheva R and Thakur MK: Protective role of Ashwagandha leaf extract and its component withanone on scopolamine-induced changes in the brain and brain-derived cells. PLoS One 2011; 6: e27265.
36. Schliebs R, Liebman A, Bhattacharya SK, Kumar A, Ghosal S and Bigl V: Systemic administration of a defined extract from *Withania somnifera* (Indian ginseng) and shilajit differentially affects cholinergic but not glutamatergic and GABAergic markers in rat brain. Neurochemistry International 1997; 30: 181-90.
37. Ghattacharya SK, Kumar A and Ghosal S: Effects of glycowithanolides from *Withania somnifera* on an animal model of Alzheimer's disease and perturbed central cholinergic markers of cognition in rats. Phytother Res 1995; 9: 110-13.
38. Abbasi E, Nassiri-Asl M, Sheikhi M and Shafiee M: Effects of Vitexin on scopolamine-induced memory impairment in rats. Chinese J of Physiol 2013; 56: 184-89.
39. Kim JY, Lee WS, Kim YS, Curtis-Long MJ, Lee BW, Ryu YB and Park KH: Isolation of cholinesterase-inhibiting flavonoids from *Morus lhou.* J Agric Food Chem 2011; 59: 4589-96.
40. Jann MW, Shirley KL and Small GW: Clinical pharmacokinetics and pharmacodynamics of cholinesterase inhibitors. Clinical Pharmacokinetics 2002; 41: 719-39.

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