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Research Article

ASPARAGUS RACEMOSUS ROOT EXTRACT ENHANCES HIPPOCAMPAL NEUROGENESIS AGAINST SCOPOLAMINE INDUCED AMNESIA

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ABSTRACT
Asparagus racemosus commonly known as Shatawari, is classified as a Medhya Rasayana herb. It is well-known Ayurvedic medicine and key ingredient in traditional formulation. This herb exhibits a range therapeutic effects and beneficial for neurological disorders such as dementia, anxiety. Our previous studies have demonstrated the neuroprotective and antioxidant properties methanolic root extract of *Asparagus racemosus* through behavioral paradigms. The objective of current study is to examine the effects of *Asparagus racemosus* root extract on cholinergic neurotransmission and hippocampal neurogenesis in mice that have been subjected to scopolamine induced amnesic. We observed that scopolamine significantly elevated acetylcholinesterase (AChE) activity, leading to cholinergic dysfunction. The methanolic root extract of *Asparagus racemosus* was found to inhibit the hyper activation of AChE induced by scopolamine. This indicates that *Asparagus racemosus* possess anti-amnesic properties that may mitigate learning and memory impairments by modulating the cholinergic system in age related dementia. Furthermore, we noted that scopolamine suppresses neurogenesis in the dentate gyrus of the hippocampus. These finding imply that methanolic root extract *Asparagus racemosus* can effectively regulate cholinergic neurotransmission in the brain and stimulate neurogenesis in the hippocampus DG. Consequently, *Asparagus racemosus* may serve as a powerful neuro-pharmacological agent against amnesia, potentially enhancing brain plasticity.

Keywords: *Brain plasticity, BrdU, Cell Proliferation, Asparagus racemosus, dementia; Neurogenesis, Alzheimer's disease*

INTRODUCTION
Alzheimer's disease (AD) is a gradually advancing neurodegenerative condition. The estimated global number of patients reported now exceeds 57 million. (Nicols *et al.,* 2022) annual basis. It is associated with decline in memory, linguistic impairment, behavioral problems and cognitive dysfunction. (Rajaram *et al.,* 2021) The prominent hallmarks observed in AD brain are senile plaque (Aβ), neurofibrillary tangles, decreased central functioning and extensive neuronal loss (Alzheimer's Association, 2017).
The hippocampus and its functionality are notably influenced by cholinergic dysfunction (Roloff et al., 2007.) The central cholinergic system located in the basal forebrain is crucial for the regulation of learning and memory that depend on the hippocampus (Blake *et al.,* 2014).
Degeneration of cholinergic neurons results in cognitive impairment Acetylcholine (ACh) is a major excitatory neurotransmitter, released from cholinergic neurons and is crucial for both development of long-term memories and memory retention (Anand and Singh 2013, Picciotto *et al.,* 2012). Cholinergic impairment is caused by increase of Acetylcholinesterase (AChE) activity and reduction in ACh concentration in brain, which are the main cause of cognitive impairment (Choi *et al.,* 2021). Abnormalities in cholinergic neurons in the brain transmission contribute to cognitive impairments in psychiatric disorders like schizophrenia and Alzheimer's. (Muller *et al.,* 2018). Pharmacological approaches are indicated that cholinergic signaling is an important pathway controlling postnatal neurogenesis, any damage or disturbance in cholinergic system is closely associated to cell proliferation in the hippocampus (Kotani *et al.,* 2006; Yoo *et al.,* 2011). The synthesis, release, and transmission of the neurotransmitter acetylcholine

regulates adult hippocampal neurogenesis. Studies reveal direct innervation of immature neurons by ChAT neurons. (Quintanilla *et al.,* 2019). Scopolamine, which acts as muscarinic receptor antagonist, reduces the cholinergic neurotransmission and heightening oxidative stress in the brain. (Hussain *et al.,* 2022). Scopolamine also produces shrinking of neuron and initiates neurodegeneration in the hippocampus. (Safar *et al.,* 2016).

Adult hippocampal neurogenesis occurs within the mammalian brain, particularly in the dentate gyrus subregion of the hippocampus (Goncalves *et al.,* 2016). In adult, neural stem cells produce intermediate progenitor cells that subsequently differentiate into neurons, which then integrate with existing neuronal circuitry of the hippocampus, thereby enhancing cognitive functions. (Sahay *et al.,* 2011). Recent research conducted on animal models indicates that neuronal plasticity in the mice brain facilitates learning and memory, whereas the inhibition of neurogenesis in the DG region disrupts spatial pattern separation and memory retention. (Jessberger *et al.* 2009).

Asparagus racemosus is a widely recognized therapeutic medicinal plant in ancient medical systems. This plant is utilized as a rejuvenating tonic for women and offers various neuro-pharmacological advantages. The extract from its roots demonstrates health-enhancing properties, including antioxidant effects (Bhatnagar et al., 2005), immune-stimulant capabilities (Sharma et al., 2013), neuroprotective effects (Palanisamy and Manian, 2011), and anti-cancer attributes (Mitra et al., 2012). In Ayurveda, Shatavari is regarded as Medhya Rasayana, serving multiple purposes, especially in enhancing memory and intellect (Dash, 1991). The roots of *Asparagus racemosus* contain several alkaloids and steroidal saponins, identified as Shatavarin I to VI, asparoside, aspragamine, recemosal, and racemofuran, which are known to exhibit estrogenic activity (Kumeta et al., 2012; Singla and Jaitak, 2014). This research sought to investigate the neurotherapeutic impacts of the root extract from *Asparagus racemosus* on the inhibition of acetylcholinesterase (AChE) and the promotion of adult hippocampal neurogenesis within the dentate gyrus (DG) sub-region of the hippocampus.We employed a scopolamine-induced mouse model of Alzheimer's disease. BrdU immunohistochemistry was utilized to assess the formation of new neurons, conducted in thick sections through the stereological counting method (Hardman et al., 2005).

MATERIALS AND METHODS

2.1 Reagents and Antibodies

All reagents and chemicals utilized which in this research were of analytical grade. Dimethyl sulfoxide (DMSO), Acetylthiocholine iodide (ATCI), 5, 5′-di-thio-bis-nitro-benzoic acid (DTNB), Scopolamine hydrobromide and BrdU were sourced from Sigma Aldrich. Rabbit monoclonal anti-BrdU (Sigma USA) and Biotinylated goat anti Rabbit were used as primary and secondary antibody respectively. They were purchased from Vector laboratories Incorporation (USA).

2.2 Animals

Swiss albino mice (25–30g, 4 week old adult male) were utilized in this study. The mice were housed in polyutherine cages for a period of one week duration to acclimatize the laboratory conditions. Mice were provided with ad libitum access to both food and water. All the experiments procedures were conducted the hours of 0900 and 1500. Animal care and Use Committee of MLS University approved the experimental protocol, and all procedures were carried out with the guidelines set forth by the Institutional Animal Ethics Committee (IAEC Log.No.973/ac/06/CPCSEA).

2.3 Drug Preparation

Asparagus racemosus roots were procured from the local vender of herbal formulation. The coarse powder of dried roots (500gm) with 100% methanol were extracted by Soxhlet's extractor for 35h. Methanol free dry mass of filtrate was obtained by rotatory evaporator and stored in refrigerator. The dried extract was newly dissolved in distilled saline immediately prior to use.

2.4 Drug Administration

Mice were randomly classified into the four groups (Table 1): Control (Vehicle), *Asparagus racemosus* (AR- 100 mg/kg), Scopolamine (SCO- 1mg/kg scopolamine-treated), and Scopolamine with *Asparagus*

racemosus (SCO 1mg/kg + AR 100 mg/kg). AR was freshly dissolved in saline and administrated orally once daily for a duration of 14 consecutive days. Following one hour after administration of *Asparagus racemosus*, Scopolamine hydrobromide was injected intraperitoneal (i.p.) once daily for 14 days.

BrdU Treatment

To investigate neurogenesis with in the dentate gyrus (DG) of hippocampus, immunohistochemistry was performed. BrdU was dissolved in normal saline (0.9% NaCl) and sterilized before administration. Mice were given a single intraperitoneal injection (i.p.) of BrdU (150 mg/kg) on the 10^{th}, 12^{th} and 14^{th} days. All the mice were euthanized 48 hours following the final BrdU injection. After last BrdU injection. The In *vivo* experimental design is outlined in Table 1 and 2.

Table 1: Animal groups and drugs administration Table

Mice Group		Treatment
1	Control	Saline
2	AR	*Asparagus racemosus* (100mg/kg P.O.)
3	SCO	Scopolamine hydrobromide (1mg/kg I.P.)
4	SCO + AR	*Asparagus racemosus* (100mg/kg P.O.) + Scopolamine hydrobromide (1mg/kg I.P.)

Table: 2 Schematic illustration of BrdU and other drugs treatment

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
AR(P.O)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	
SCO(I.P.)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	
BrdU	-	-	-	-	-	-	-	-	-	BrdU(IP)	-	BrdU(IP)	-	BrdU(IP)	-	Mice Sacrificed

2.5 AChE Estimation

The assessment of brain AChE activities in the total homogenate was measured using Ellman's colorimetric method (Ellman *et al.* 1961).This method quantifies AChE activity by detecting the production of a yellow compound at 412 nm, resulting from the reaction between acetylthiocholine iodide and DTNB (5,5' dithio-bis-2-nitrobenzoic acid). The AChE activity was calculated using the formula: $r = 5.74 \times 10^{-4} \times \Delta A/C$, where

r represents the rate of enzymatic activity (in moles of acetyl thiocholine hydrolyzed/min/g tissue), Δ A denotes the change in absorbance/ min and C indicates the concentration of the tissue in the homogenate (mg/ml).

2.6 Immunohistochemistry

After the experiment, the mice were euthanized and their brain was removed quickly on ice. It was subsequently post-fixed in 10% neutral chilled formalin for 18 h at 4^0C before undergoing dehydration. The brain was cleared using xylene and embedded in paraffin wax. Coronal sections (10 mm) containing hippocamal sub region (bregma -1.46 mm to -2.18 mm) were prepared for Immunohistochemistry using a detailed protocol involving primary antibody (Rabbit monoclonal anti BrdU; Sigma USA) and secondary antibody (Biotinylated goat anti-Rabbit, 10 µg/ml; Vector laboratories incorporation, USA). Finally, the sections were treated with DAB to visualize BrdU-labeled cells. They were appeared brown, colour they were counted in each section in the DG region by another experimenter which were counted in the DG region by a separate experimenter.

2.7 Statistical Analysis

The results and data were analyzed with Graph Pad Prism, expressed as mean ±SEM, using a One-way ANOVA followed by Turkey's test, with statistical significance set at $P < 0.05$.

RESULTS

3.1 Effects of AR on AChE Levels in Brain

The group that was administrated solely scopolamine exhibited a significant ($p< 0.001$) increase in AChE

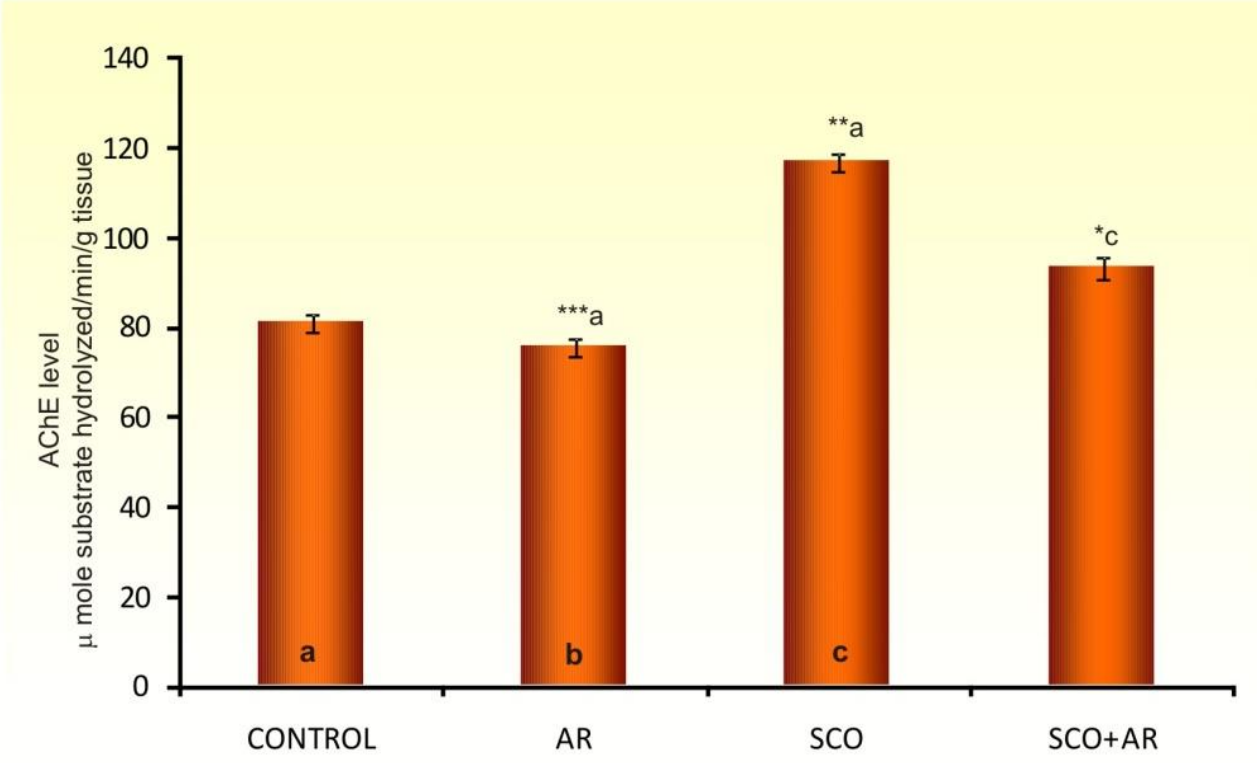

Fig. 1 Biochemical estimation of AChE activity in brain homogenate.
Value depicted as Mean ±SEM, *(P<0.05),** *(P<0.001), *(P<0.01)***

activity compared to the control group. In contrast, the SCO + AR group, which received *Asparagus racemosus* roots extract with scopolamine, exhibited a significant ($p< 0.001$) decrease in AChE activity relative to the SCO group. However, no significant ($p<0.05$) difference in activity AChE was observed between control and AR treated groups

3.2 Effects of AR on Neurogenesis and Synaptic Plasticity in the Hippocampus

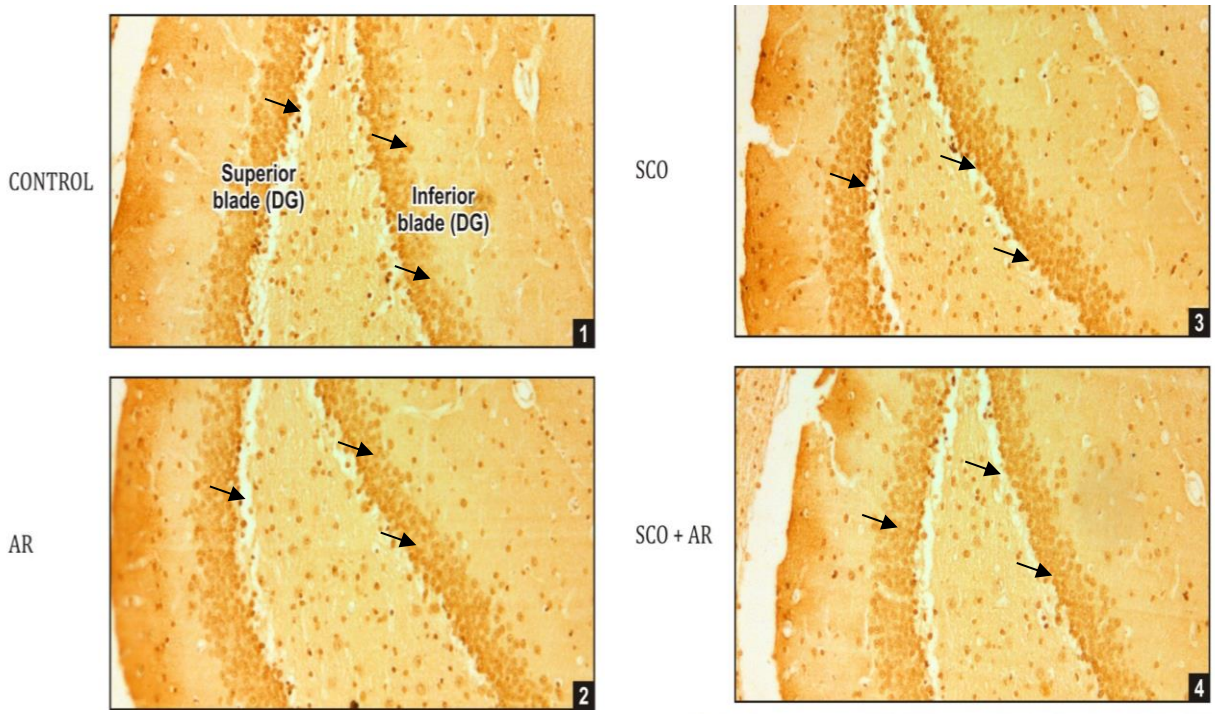

Figure 2: Representative Photomicrograph of Immunohistochemistry in the Dentate Gyrus (DG) of Hippocampus showing BrdU positive cells (Arrow 10X)

1. BrdU – positive cells in Control group 2. BrdU – positive cells in AR group

3. BrdU – positive cells in SCO group 4. BrdU – positive cells in SCO+ AR group

The functions of learning and memory are modulated by neurogenesis occurring in the hippocampus. Therefore, we investigated the impact of methonolic root extract of AR on neurogenesis**.** We assessed the effects of AR on the proliferation of newly formed cells in the DG region of the hippocampus. The BrdU positive neurons were analyzed in the DG region of the hippocampus across control, scopolamine and AR groups (**Figure 2**). Representative photomicrographs illustrating the localization of BrdU- positive cells are shown. (Fig) The administration of the muscarinic receptor antagonist scopolamine significantly suppressed neurogenesis in dentate gyrus, particularly with in the sub granular zone (SGZ), as indicated by a decrease in BrdU positive cell in this area compared to control and AR treated groups (**Figure 3**). The simultaneous treatment of AR and scopolamine in mice resulted in a notable increase in the number of BrdU positive neurons in the DG region of hippocampus when compared to those treated solely with scopolamine. However, no significant difference was noted in the number of BrdU positive neurons in the DG region of hippocampus between the control and AR treated groups.

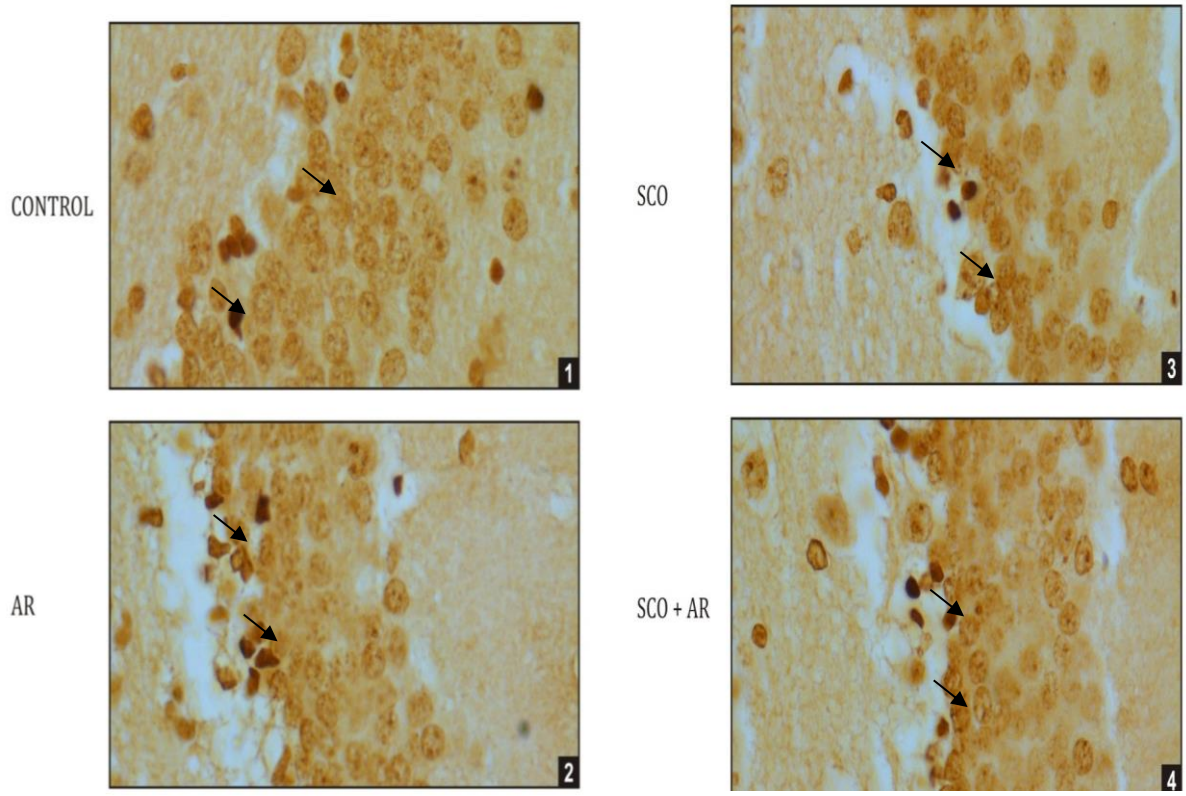

Fig. 3 Representative Photomicrograph of Immunohistochemistry in the Dentate Gyrus (DG) of Hippocampus showing BrdU positive cells (Arrow 40X)
1. BrdU – positive cells in Control group 2. BrdU – positive cells in AR group
3. BrdU – positive cells in SCO group 4. BrdU – positive cells in SCO+ AR group

DISCUSSION

Alzheimer's disease (AD) is a progressive neurogenerative disorder linked to specific brain abnormalities. (Korczyn and Grinberg, 2024.) AD produced significant neuronal loss, reduced acetylcholine level, heightened oxidative stress. (Allerton *et al.,* 2024). The cholinergic system of the brain plays a crucial role in hippocampal neurogenesis and cognitive function by modulating neurogenic processes such as BDNF and CREB. (Bruel *et al.,* 2011). Scopolamine, recognized as a non-selective muscarinic acetylcholine receptor antagonist, disrupts the cholinergic function by elevating AChE activity, and which ultimately leads to deficit in learning and memory tasks (Elvander *et al.,* 2004, Balaban *et al.*, 2017, Alvarez *et al.,* 2017). Acetylcholinesterase (AChE) serves as a key hydrolase in the cholinergic system that can cleave acetylcholine (ACh) and make it inactive, thereby maintaining the balance of Ach (Soreq and Seidman, 2001). AChE inhibitory drugs which are used in AD treatment increase the availably of acetylcholine neurotransmitter in synaptic cleft. Plant originated AChE inhibitors could be valuable alternative for AD management.

Numerous studies have demonstrated the cognitive enhancement effects of plant extract through various animal models. (Rabiei *et al.,* 2013, Rabiei *et al.,* 2014). Research indicated that AChE inhibition promotes adult hippocampal neurogenesis by activating neurogenic signaling pathway in both mice and amnesic mouse model. (Kotani *et al.,* 2006). Our research indicated that AR significantly attenuated the scopolamine induced cholinergic dysfunction which was associated with elevated AChE activity in the brain. This

indicates that AR has anti-amnesic activities that protect against learning and memory deficits by modulating the cholinergic system, thus preventing cognitive decline in patients with dementia related to aging or AD. Scopolamine treatment supress proliferation and survival of cells in the SGZ of the hippocampal DG, but AR attenuates the decline of newly generated cells. Adult hippocampal neurogenesis is closely associated to neuroplasticity (Massa *et al.,* 2011), and is crucial for thel cognitive functions of the hippocampus (Vivar, 2015). Decline in neurogenesis is also a characteristic during ageing and in neurodegenerative disease (Zhao *et al.,* 2008). Injury to the cholinergic nuclei that projecting to the hippocampus leads to a decrease in this region (Cooper- Kuhn *et al.,* 2004), that suggests cholinergic system actively involves in hippocampal proliferation (Bruel- Jungerman *et al.,* 2011).

We observed that scopolamine suppresses neurogenesis in amnesic mice while AR treatment increases the number of BrdU positive cells in the DG and facilitates neurogenesis in the scopolamine induced amnesic mice. The phytochemical and their derivatives have been discovered only in limited a number to promote neurogenesis without side effect. However, herbal medicine has been traditionally used for the treatment CNS disease since ancient times. *Asparagus racemosus*, referred to as Medhya Rasayana in ancient Ayurveda, is known to enhance brain function, memory, and intelligence. In previous research, we investigated the effects of *Asparagus racemosus* on learning and memory acquisition using behavioral paradigm. In nutshell, our study indicates that by promoting neurogenesis or inhibiting AChE activity *Asparagus racemosus* could be an effective therapeutic agent for neurodegenerative disorders such as AD.

CONCLUSION

Our research findings demonstrate that *Asparagus racemosus* (Shatavari) exhibits an anti-amnesic effect, which could be mediated by cholinergic activity and neurogenesis in the hippocampus. These results imply that the roots of *Asparagus racemosus* (Shatavari) may be beneficial for inclusion in herbal formulations for neurological disorders that involve impaired hippocampal neurogenesis and memory.

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REFERENCES

Allerton TD, Stampley JE, LiZ H, Quiariate H, Doiron JE, White G, Wigger Z, Gartia MR, Lefer DJ (2024). Nitric oxide donors rescue metabolic and mitochondrial dysfunction in obese Alzheimer's model. *Scientific Reports*, **14**(1) 26118.

Alvarez-Jimenez R, Groeneveld GJ, van Gerven JM, Goulooze SC, Baakman AC, Hay JL, Stevens J (2016). Model-based exposure-response analysis to quantify age related differences in the response to scopolamine in healthy subjects. *British journal of clinical pharmacology*, **82**(4) 1011-21.

Alzheimer's Association (2017). Alzheimer's disease facts and figures. *Alzheimer's Dement,* **13**(1) 325–373.

Anand P, Singh BA (2013). Review on cholinesterase inhibitors for Alzheimer's disease. *Archives of Pharmacal Research*, **36**(4) 375–399.

Balaban H, Nazıroglu M, Demirci K, Ovey IS (2017). The protective role of selenium on scopolamine-induced memory impairment, oxidative stress, and apoptosis in aged rats the involvement of TRPM2 and TRPV1 channels. *Molecular neurobiology*, **54**(4) 2852-68.

Bhatnagar M, Sisodia SS, Bhatnagar R (2005). Antiulcer and antioxidant activity of Asparagus racemosus Willd and Withania somnifera Dunal in rats. *Annals of the New York Academy of Science,* **1056** (1) 261-278.

Blake MG, Krawczyk MC, Baratti CM, Boccia MM (2014). Neuropharmacology of memory consolidation and reconsolidation Insights on central cholinergic mechanisms. *Journal of Physiology-Paris,* **108**(4-8) 286-291.

Bruel-Jungerman E, Lucassen PJ, Francis F (2011). Cholinergic influences on cortical development and adult neurogenesis. *Behavioral Brain Research,* **221**(2) 379–388.
Bruel-Jungerman E, Lucassen PJ, Francis F (2011). Cholinergic influences on cortical development and adult neurogenesis. *Behavioural brain research*, **221**(2) 379-88.
Choi JH, Lee EB, Jang HH, Cha YS, Park YS, Lee SH (2021). Allium hookeri extracts improve scopolamine-induced cognitive impairment via activation of the cholinergic system and anti-neuroinflammation in mice. *Nutrients* **13**(8) 2890.
Cooper-Kuhn CM, Winkler J, Kuhn HG (2004). Decreased neurogenesis after cholinergic forebrain lesion in the adult rat. *Journal of Neuroscience Research*, 77(2) 155–165.
Dash VB (1991). Materia Medica of Ayurveda. New Delhi, India B.
Doeppner TR, Dietz GP, Aanbouri M, Gerber J, Witte OW, Bahr M, Weise J(2009). TAT-Bcl-xL improves survival of neuronal precursor cells in the lesioned striatum after focal cerebral ischemia. *Neurobiology of disease*, **34**(1) 87-94.
Ellman GL, Courtney KD, Andres JV, Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology*, **7**(2) 88-95.
Elvander E, Schott PA, Sandin J, Bjelke B, Kehr J, Yoshitake T, Ogren SO (2004). Intraseptal muscarinic ligands and galanin influence on hippocampal acetylcholine and cognition. Neuroscience, **126**(3) 541-57.
Goncalves JT, Schafer ST, Gage FH (2016). Adult neurogenesis in the hippocampus from stem cells to behavior. *Cell*, **167**(4) 897-914.
Hardman MJ, Liu K, Avilion AA, Merritt A, Brennan K, Garrod DR, Byrne C (2005). Desmosomal cadherin misexpression alters β-catenin stability and epidermal differentiation. *Molecular and Cellular Biology*, **25**(3) 969–978.
Hussain H, Ahmad S, Shah SW, Ullah A, Ali N, Almehmadi M, Ahmad M, Khali AA, Jamal SB, Ahmad H, Halawi M (2022). Attenuation of scopolamine-induced amnesia via cholinergic modulation in mice by synthetic curcumin analogs. *Molecules,* 27(8) 2468.
Jessberger S, Clark RE, Broadbent NJ, Clemenson GD, Consiglio A, Lie DC, Squire LR, Gage FH (2009). Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learning & memory*, **16** (2) 147-54.
Korczyn AD, Grinberg LT (2024). Is Alzheimer disease a disease? *Nature Reviews Neurology,* **20** (4) 245-251.
Kotani S, Yamauchi T, Teramoto T, Ogura H (2006). Pharmacological evidence of cholinergic involvement in adult hippocampal neurogenesis in rats. *Neuroscience*, **142** (2) 505–514.
Kotani S, Yamauchi T, Teramoto T, Ogura H (2006). Pharmacological evidence of cholinergic involvement in adult hippocampal neurogenesis in rats. *Neuroscience*. **142**(2) 505–514.
Kumeta Y, MaruyamaT, Wakana D, Kamakura H, Goda Y (2013). Chemical analysis reveals the botanical origin of shatavari products and confirms the absence of alkaloid asparagamine A in *Asparagus racemosus*. *Journal of Natural Medicines*, **67**(1) 168-173.
Massa F, Koehl M, Wiesner T, Grosjean N, Revest JM, Piazza PV, Abrous DN, Oliet SH (2011). Conditional reduction of adult neurogenesis impairs bidirectional hippocampal synaptic plasticity. *Proceedings of the National Academy of Sciences*, **108** (16) 6644-9.
Mitra SK, Prakash NS, Sundaram R (2012). Shatavarins (containing Shatavarin IV) with anticancer activity from the roots of *Asparagus racemosus*. *Indian Journal of Pharmacology*, **44**(6) 732-736.
Muller C, Remy S (2018). Septo-hippocampal interaction. *Cell and Tissue Research*, **373** (3) 565–575.
Nichols E, Steinmetz JD, Vollset SE, Fukutaki K, Chalek J, Abd-Allah F, Abdoli A, Abualhasan A, Abu-Gharbieh E, Akram TT, Al Hamad H (2022). Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050 an analysis for the Global Burden of Disease Study 2019. *The Lancet Public Health*, **7**(2) e105-25.

Palanisamy N, Manian S (2011). Protective effects of *Asparagus racemosus o*n oxidative damage in isoniazid-induced hepatotoxic rats an in vivo study. *Toxicology and Industrial Health,* **28** (3) 238-244.
Picciotto MR, Higley MJ, Mineur YS (2012). Acetylcholine as a neuromodulator cholinergic signaling shapes nervous system function and behavior. *Neuron* **76**(1) 116–129.
Quintanilla LJ, Yeh CY, Bao H, Catavero C (2019). Assaying circuit specific regulation of adult hippocampal neural precursor cells. *Journal of visualized Experiments,* 24 (149) 10-3791.
Rabiei Z, Hojjati M, Rafieian-Kopaeia M, Alibabaei Z (2013). Effect of Cyperus rotundus tubers ethanolic extract on learning and memory in animal model of Alzheimer. *Biomedicine & Aging Pathology*, **3**(4) 185-91.
Rabiei Z, Rafieian-Kopaei M, Heidarian E, Saghaei E, Mokhtari S (2014). Effects of Zizyphus jujube extract on memory and learning impairment induced by bilateral electric lesions of the nucleus Basalis of Meynert in rat. *Neurochemical research*, **39**(2) 353-60.
Rajaram C, Kumar SN, Tabassum SS (2021). Neuroprotective Activity of the Methanolic Extract of Indigofera aspalathoides against Scopalamine induced Alzheimer's disease in Experimental Rats. *Research Journal of Pharmacy and Technology,* **14** (10) 5163–8.
Roloff EVL, Harbaran D, Micheau J, Platt B, Riedel G (2014). Dissociation of cholinergic function in spatial and procedural learning in rats. *Neuroscience,* 146(3) 875–889.
Safar MM, Arab HH, Rizk SM, El-Maraghy SA (2016). Bone marrow-derived endothelial progenitor cells protect against scopolamine-induced alzheimer-like pathological aberrations. *Molecular Neurobiology*, **53**(3) 1403-1418.
Sahay A, Scobie KN, Hill AS, O'Carroll CM, Kheirbek MA, Burghardt NS, Fenton, AA, Dranovsky A, Hen R (2011). Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. Nature, **472** (7344) 466-470.
Sharma U, Kumar N, Singh B, Munshi RK, Bhalerao S (2013). Immunomodulatory active steroidal saponins from Asparagus racemosus. *Medicinal Chemistry Research,* **22**(2) 573-579.
Singla R, Jaitak V(2014). Shatavari (asparagus racemosus wild) a review on its cultivation, morphology, phytochemistry and pharmacological importance. International Journal of Pharmacy & Life Sciences, **5**(3) 742-757.
Soreq H, Seidman S (2001). Acetylcholinesterase-new roles for an old actor. *Nature Review Neuroscence,* **2** (4), 294– 302.
Vivar C (2015). Adult hippocampal neurogenesis, aging and neurodegenerative diseases possible strategies to prevent cognitive impairment. *Current topics in medicinal chemistry*, **15** (21) 2175-92.
Yoo DY, KimW, Yoo KY, Lee CH, Choi JH, Kang IJ, Yoon YS, Kim DW, Won MH, Hwang IK (2011). Effects of Nelumbo nucifera rhizome extract on cell proliferation and neuroblast differentiation in the hippocampal dentate gyrus in a scopolamine-induced amnesia animal model. *Phytotherapy Research*, **25** (6) 809–815.
Zhao C, Deng W, Gage FH (2008). Mechanisms and functional implications of adult neurogenesis. *Cell,* **132** (4) 645-60.