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ORIGINAL ARTICLE

Neuroprotective Potential of a Polyherbal Formulation in a Scopolamine-Induced Rodent Model of Alzheimer's Disease

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Abstract

Introduction: Alzheimer's disease (AD) is a Commence neurodegenerative disorder typified by progressive deterioration of cognitive function, memory dysfunction, and behavioral irregularities. Contemporary pharmacological options such as cholinesterase inhibitors provide only transient symptomatic reprieve and are frequently accompanied by undesirable consequences. **Objective:** This investigation assessed the potential neuroprotective properties of a polyherbal composition of plant *Withania somnifera*, *Azadirachta indica*, and *Hibiscus rosa-sinensis* in a scopolamine-induced rodent. **Methodology:** Plant specimens underwent hydroalcoholic extraction through cold maceration and were subjected to phytochemical screening, confirming the presence of alkaloids, flavonoids, and steroids. Adult male Wistar rats were stratified into five cohorts: Normal control, disease control (scopolamine 10 mg/kg intraperitoneal), polyherbal mixture (PHM) I (150 mg/kg), PHM II (300 mg/kg), and a standard group receiving donepezil (10 mg/kg). Cognitive profiling was conducted using Morris Water Maze and Y-Maze paradigms over a 14-day duration. **Results:** Data were analyzed using one-way analysis of variance, followed by Tukey's *post hoc* test for multiple comparisons. Exposure to scopolamine produced notable impairments in spatial memory, as reflected by increased alternation percentages and prolonged escape latency. Polyherbal interventions, particularly PHM II demonstrated appreciable cognitive restitution in a dose-responsive fashion. Donepezil exhibited the most pronounced therapeutic effect. The significant values are $***P < 0.001$, $**P < 0.01$, $*P < 0.05$ for Donepezil (standard), PHM II, and PHM I, respectively. **Conclusion:** The polyherbal formulation imparted significant anti-amnesic benefits, likely mediated through its antioxidative and cholinergic-enhancing phytoconstituents. These findings underscore its promise as an adjunctive or alternative modality for AD management, meriting additional molecular and translational studies.

Key words: Alzheimer's disease, *Azadirachta indica*, *Hibiscus rosa-sinensis*, polyherbal formulation, scopolamine, *Withania somnifera*

INTRODUCTION

Alzheimer's disease (AD) is an advancing neurological disorder that results in recall deficits, cognitive impairment, and psychological alterations.[1] It primarily affects areas of the brain responsible for retention, communication, and logic functions.[2] The emergence of AD can be categorized into early-onset and late-onset variants. Early-onset AD often impacts individuals younger than 65 and is linked with hereditary mutations and pronounced neurodegeneration.[3] In contrast, late-onset AD, the more prevalent form, typically occurs in the elderly and stems from an unidentified cause.[4] Clinical manifestations commence with mild remembrance lapses and progressively weaken core cognitive abilities. As the condition worsens, patients may exhibit diminished judgment, impaired choice-making, and difficulty managing everyday routine activities. In its most

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advanced stages, individuals often become fully reliant on caregivers and forfeit their interactive capabilities.[5] At the molecular level, AD is marked by the build-up of amyloid-β plaques and tau-protein tangles, resulting in neuronal dysfunction, neuroinflammation, and cerebral shrinkage. These pathological changes arise from a complex synergism of oxidative stress, metal ion dyshomeostasis, mitochondrial disruption, and cholinergic deficiency. Oxidative stress is exacerbated by the brain's intense oxygen utilization, which renders it especially vulnerable to lipid degradation and DNA injury.[6] According to the cholinergic hypothesis, a reduction in acetylcholine contributes significantly to mental deterioration and is the primary target of current therapies.[7] Although Food and Drug Administration (FDA)-approved medications such as donepezil, rivastigmine, and galantamine offer transient relief from symptoms, they do not halt the disease course. Additionally, these treatments are associated with various unwanted side effects, reinforcing the necessity of more effective, integrated approaches.[8] In this context, herbal medicine has garnered attention for its polyvalent capabilities. Numerous botanicals, including *Withania somnifera* (Ashwagandha),[9] *Azadirachta indica* (Neem),[10] and *Hibiscus rosa-sinensis*,[11] possess brain-supporting and antioxidant activities. This investigation evaluates the anti-Alzheimer's effectiveness of a polyherbal formulation using computational, laboratory, and animal studies. Through this comprehensive strategy, the study aims to uncover the curative efficacy of natural therapeutic interventions in the treatment of Alzheimer's.

MATERIALS AND METHODS

Plant collection and authentication

Fresh leaves of *W. somnifera* (Ashwagandha),[9] *A. indica* (Neem),[10] and *H. rosa-sinensis* (Hibiscus)[11] were collected from the institutional botanical garden of Vignan Institute of Pharmaceutical Technology, Visakhapatnam. Each specimen was taxonomically verified by Dr. S. B. Padal, a Plant taxonomist, AU, Visakhapatnam, and voucher samples were archived for future reference. The compounds of the polyherbal mixture (PHM) are given in Table 1.

Preparation of polyherbal extract

The harvested materials were thoroughly cleansed, air-dried under ambient conditions, and finely pulverized using a mechanical grinder. Equal portions of each dried sample were subjected to cold maceration in a hydroalcoholic solvent mixture (Methanol: Water, 70:30 v/v) for a duration of 7–10 days. The resulting macerates were filtered through Whatman No.4 membranes and concentrated using a rotary evaporator. Final residues were dried, placed in desiccators, and appropriately labeled for further analysis. The composition of PHMs was given in Table 2.[12]

Phytochemical screening

Preliminary qualitative investigations were performed to determine the presence of major phytochemical classes using standard chemical protocols. The polyherbal extract's initial phytochemical screening is given in Table 3.[13]

Animals and ethical statement

Adult male albino Wistar rats (150–250 g) were procured from a certified supplier (Albino Research Centre, Hyderabad). The subjects were housed under standardized experimental conditions (temperature 26 ± 2°C, relative moisture 45–55%, 12-h light/dark cycle) with unrestricted access to nutritional resources and water. All procedures were evaluated and formally sanctioned by the Institutional Animal Ethics Committee, Registration No: 2003/PO/Re/S/18/CPCSEA.

Experimental design

The animals were divided into five experimental groups. Group I served as the normal control (NC) and received saline orally. Group II served as the disease control (DC) and was administered scopolamine at a dose of 10 mg/kg by the intraperitoneal route to induce memory impairment. Groups III and IV received scopolamine along with PHM I at 150 mg/kg and PHM II at 300 mg/kg, respectively, by

Table 1: Poly herbal mixtures

Plant name	Scientific name	Part used	Major phytochemicals	Medicinal uses (Pharmacological activities)
Hibiscus[11]	*Hibiscus rosa-sinensis*	Leaves/ Flowers	Flavonoids, anthocyanins, alkaloids, quercetin- 3,5-diglucoside, cyanidin derivatives	Neuroprotective (via antioxidant and anti-inflammatory pathways), wound healing, antibacterial, antipyretic
Neem[10]	*Azadirachta indica*	Leaves	Limonoids, flavonoids, nimbins, polyphenols, gallic acid, catechins, phytosterols	Antioxidant, anti-inflammatory, antibacterial, antiviral, neuroprotective, anti-apoptotic, inhibition of lipid peroxidation
Ashwagandha[9]	*Withania somnifera*	Roots	Withanolides (A–Y), withaferin A, alkaloids, beta-sitosterol, sitoindosides	Memory enhancement, antioxidant, anti-inflammatory, anti-tumor, anti-stress (adaptogenic), neuroprotective

oral administration. Group V served as the standard treatment group and received scopolamine along with donepezil at a dose of 10 mg/kg orally. The treatments were administered according to the experimental protocol to evaluate the potential effects of PHM I and PHM II on scopolamine-induced memory impairment. The normal control and disease control groups were used for comparison with the treatment groups given in Table 4.

Behavioral models

Y-Maze test

The Y-maze apparatus is utilized to assess mnemonic performance and exploratory tendencies in rodents, based on their inherent inclination to investigate novel environs.

Procedure

Each subject was situated at the extremity of arm B and permitted to traverse all three arms for a duration of 5 min. An alternation was classified as consecutive ingressions into three distinct arms (ABC, CAB, etc.) given in Figure 2.

Calculation

Percentage alternation = (Number of alternations/Total arm entries - 2) × 100

Table 2: Composition of PHM s

PHM	Hibiscus (*Hibiscus rosa sinensis*)	Neem (*Azadirachta indica*)	Ashwagandha (*Withania somnifera*)
PHM1	1	1	1
PHM2	2	1	1
PHM3	1	2	1

PHM: Polyherbal mixture

Interpretation

Elevated alternation percentages indicate superior spatial mnemonic functioning. Scopolamine induces deficits, whereas reversal through test extract administration denotes cognitive recuperation.[14]

Morris water maze test

The Morris water maze serves as a quintessential paradigm for evaluating spatial acquisition and retention in rodent analogues of Alzheimer's pathology.

Apparatus

A cylindrical pool (height: 60 cm, diameter: 45 cm) filled with aqueous medium (~28°C) was employed. A submerged platform was concealed beneath the surface within a designated quadrant.

Procedure

Rats underwent acclimatization and sequential trials to identify the concealed platform using extrinsic cues affixed around the periphery. The escape latency time (ELT), signifying the duration to locate the platform, was systematically logged, given in Figure 1.

Retention test

On the final assessment day, the platform was extracted, and the subject reinstated into the pool to evaluate mnemonic retention, quantified by time spent in the prior platform quadrant.

Interpretation

Diminished ELT and augmented time within the targeted region suggest enhanced cognitive performance. Conversely, scopolamine-induced amnesia prolongs latency, mitigated significantly by the polyherbal regimen.[15]

Table 3: Initial phytochemical analysis of polyherbal extract

Phytochemical class	Test performed	Procedure summary	Result	Indication/Interpretation
Alkaloids	Mayer's test	5 mg extract+1% HCl, gently heated→red color formation	Positive	Red color indicates the presence of alkaloids (nitrogen-containing bases)
	Wagner's test	5 mg extract+0.5 mL Wagner's reagent→reddish-brown coloration	Positive	Reddish-brown color confirms alkaloids
	Dragendorff's test	5 mg extract+Dragendorff's reagent→orange-red precipitate	Positive	Orange-red precipitate confirms alkaloid content
	Hager's test	5 mg extract+saturated picric acid→yellow precipitate	Positive	Yellow precipitate indicates alkaloids are present
Flavonoids	Alkaline reagent test	5 mg extract+2 mL 2% NaOH→yellow color that turns colorless with dilute acetic acid	Positive	Yellow color change confirms presence of flavonoids (polyphenolic compounds)
Steroids	Liebermann-Burchard test	Cholesterol+acetic anhydride+conc. H_2SO_4 in chloroform→green/blue ring formation	Positive	Green/blue ring indicates presence of steroids or triterpenoids

Statistical analysis

All experimental data were expressed as mean ± standard error of the mean and subjected to rigorous inferential evaluation using parametric analytical approaches. The intergroup comparisons were conducted employing one-way analysis of variance, followed by *post hoc* Tukey's multiple comparison test to elucidate significant differences among treated and control cohorts.

A confidence threshold of $P < 0.05$ was considered statistically significant. Graphical and numerical representations were derived utilizing GraphPad Prism software, ensuring precise visualization and accurate inferential reporting. Data were assessed for normality and homogeneity prior to statistical interrogation to validate the appropriateness of parametric methods.[16]

The significant results were obtained (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). In the order Standard > PHMII > PHMI.

RESULTS

Y Maze

The Y-Maze test evaluates spatial memory and exploratory behavior in rats by measuring the percentage of alternations between three arms of the maze. A higher percentage indicates better cognitive function. The effect of PHM on the Y-Maze behavioral test in scopolamine-induced rats is given in Table 5.

Disease control (DC) increased % alternation, indicating memory impairment. PHM I and PHM II reduced % alternation, showing cognitive improvement, with PHM II (300 mg/kg) being more effective than PHM I (150 mg/kg), suggesting a dose-dependent effect. Donepezil, the standard drug, showed the best results, nearly restoring memory to control levels.

Table 4: Grouping of animal and treatment

Group	Treatment	Dose	Route of administration
Group I (NC)	Saline	—	Oral
Group II (DC)	Scopolamine	10 mg/kg	Intraperitoneal
Group III (PHM I)	Scopolamine+ PHM I	150 mg/kg	Oral
Group IV (PHM II)	Scopolamine+ PHM II	300 mg/kg	Oral
Group V (Standard)	Scopolamine+ Donepezil	10 mg/kg	Oral

NC: Normal control, DC: Disease control, PHM: Polyherbal mixture

Water Maze

The Morris Water Maze assesses spatial learning and memory by measuring the time it takes for a rat to locate a hidden platform (ELT). Lower escape latency indicates better memory and learning ability. The effect of polyherbal extract on the Water Maze behavioral test in rats given scopolamine is given in Table 6.

DC showed increased escape latency, confirming impaired spatial memory. Both PHM I and PHM II improved memory,

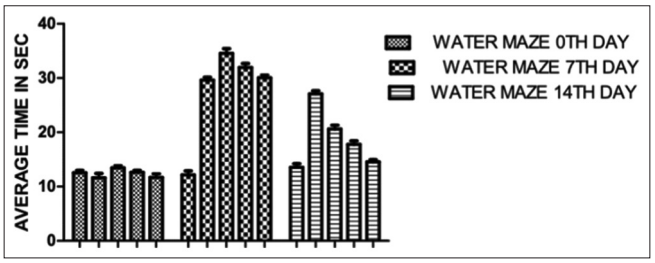


Figure 1: Water Maze test

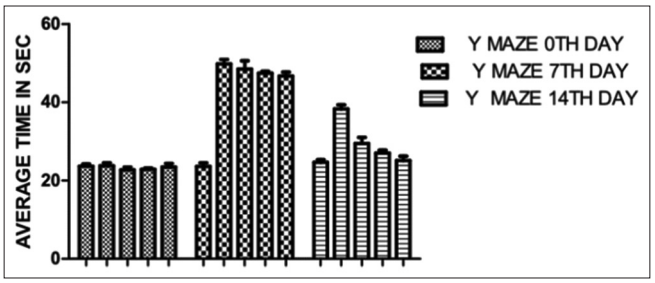


Figure 2: YMAZE test

Table 5: Effect of PHM on Y-Maze behavioral test in scopolamine-induced rats.

Groups	Treatment	Basal values of Y Maze		
		0th Day	7th Day	14th Day
Group I (NC)	Saline	23.66±0.55	23.60±0.92	24.66±0.81
Group II (DC)	Scopolamine (10 mg/kg) I.p.	23.77±0.74	49.83±1.14	38.32±1.04
Group III (PHM I)	Scopolamine+PHM I (150 mg/kg) oral	22.71±0.71	48.55±2.08	29.49±1.54
Group IV (PHM II)	Scopolamine+PHM II (300 mg/kg) oral	22.88±0.36	47.49±0.41	27.05±0.70
Group V (Standard)	Donepezil (10 mg/kg) oral	23.49±0.91	46.71±1.03	25.10±1.12

NC: Normal control, DC: Disease control, PHM: Polyherbal mixture, I.p.: Intraperitoneal

Table 6: Effect of polyherbal extract on Water Maze behavioral test in scopolamine-induced rats

Groups	Treatment	Basal values of Water Maze		
		0th Day	7th Day	14th Day
Group I (NC)	Saline	12.55±0.44	12.16±0.73	13.55±0.65
Group II (DC)	Scopolamine (10mg/kg) I.p.	11.60±0.79	29.60±0.55#	27.09±0.57#
Group III (PHM I)	Scopolamine+PHM I (150mg/kg) oral	13.45±0.40	34.56±0.85*	20.63±0.68*
Group IV (PHM II)	Scopolamine+PHM II (300mg/kg) oral	12.63±0.35	31.96±0.70**	17.78±0.64**
Group V (Standard)	Donepezil (10mg/kg) oral	11.68±0.65	30.06±0.48***	14.45±0.39***

Data was analysed by two way ANOVA,followed by Bonferroni post hoc test.When disease control was comapared with standard, PHMII, PHM I ,The significant results were obtained (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$) respectively. NC: Normal control, DC: Disease control, PHM: Polyherbal mixture, I.p.: Intraperitoneal

with PHM II showing greater effectiveness, indicating a dose-dependent benefit. Donepezil was the most effective in reversing amnesia, as expected for a standard Alzheimer's treatment.

Histograms

The histogram depicts mean escape latency across treatment days, with scopolamine groups exhibiting heightened latency indicative of cognitive decline. Polyherbal and standard treatments attenuated this effect by Day 14, suggesting potential neuroprotective efficacy.

The histogram portrays mean temporal allocation within the novel arm, where scopolamine-exposed cohorts demonstrated exacerbated durations, while polyherbal and standard therapeutics facilitated cognitive restitution by instigating a decrement toward baseline exploratory intervals.

DISCUSSION

The investigation scrutinizes the anti-Alzheimer potential of a polyherbal composition comprising *W. somnifera* (Ashwagandha), *A. indica* (Neem), and *H. rosa-sinensis*. Preliminary phytochemical appraisal corroborated the existence of alkaloids, flavonoids, and steroids compounds renowned for their antioxidative, anti-inflammatory, and neuroprotective attributes. The extract underwent evaluation in scopolamine-induced amnesic rodent models employing behavioral paradigms such as the Y-Maze and Morris Water Maze to gauge cognitive performance. In the Y-Maze paradigm, scopolamine markedly elevated the percentage alternation index, signifying compromised spatial cognition. Administration of the polyherbal concoction, particularly at the elevated dosage (PHM II, 300 mg/kg), attenuated the alternation percentage, implying enhancement of cognitive faculties. This observation underscores a dose-responsive phenomenon, wherein Donepezil – the reference standard – manifested optimal efficacy by nearly reinstating mnemonic integrity to baseline levels. Analogously, within the Morris Water Maze construct, scopolamine prolonged escape latency duration, emblematic of spatial memory deterioration. Both dosages of the botanical formulation augmented mnemonic retention, with PHM II exhibiting superior efficacy relative to PHM the significant results were obtained (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$).

Donepezil once again exhibited preeminent mitigation of cognitive deficits. Collectively, these outcomes imply that the polyherbal preparation demonstrates considerable anti-amnesic activity, plausibly attributable to its bioactive components that potentiate cholinergic transmission, alleviate oxidative burden, and safeguard neuronal architecture. Although marginally less efficacious than Donepezil, the herbal amalgam exhibits promise as an adjunctive or alternative therapeutic modality in the treatment of Alzheimer's. Further inquiry is warranted to elucidate its chronic safety profile, molecular mechanisms, and translational viability in human clinical settings. This research substantiates the application of multi-herbal strategies in mitigating cognitive deterioration and accentuates the necessity of harmonizing traditional ethnobotanical knowledge with contemporary pharmacotherapeutic methodologies in the domain of neurodegenerative pathologies.

CONCLUSION

The extant inquiry meticulously scrutinizes the anti-Alzheimeric capacity of a polyherbal composition incorporating *W. somnifera* (Ashwagandha), *A. indica* (Neem), and *H. rosa-sinensis*, utilizing both phytochemical and behavioral methodologies within scopolamine-induced amnesic rodent models. Qualitative phytochemical profiling substantiated the existence of alkaloids, flavonoids, and steroids bioactive entities possessing well-documented antioxidant, anti-inflammatory, and neuroprotective attributes. Behavioral evaluation through the Y-Maze and Morris Water Maze assays disclosed that scopolamine administration profoundly compromised spatial cognition and intellectual performance in experimental subjects. Nevertheless, intervention with the polyherbal preparation, particularly at the elevated dosage (PHM II, 300 mg/kg), appreciably mitigated these impairments, illustrating a dose-dependent neuropsychological augmentation. The reference pharmaceutical donepezil manifested the most

salient restoration of mnemonic dysfunction, consistent with expectations for an FDA-sanctioned acetylcholinesterase inhibitor. These observations imply that the polyherbal concoction elicits substantial anti-amnesic efficacy, plausibly facilitated through the synergistic interplay of its phytocomponents in regulating oxidative distress, potentiating cholinergic signaling, and safeguarding neuronal viability. Although marginally less potent than donepezil, the botanical amalgam manifests potential as an adjunctive or alternative therapeutic modality in the amelioration of Alzheimer's pathology. The significant results were obtained (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). This investigation accentuates the remedial significance of harmonizing traditional ethnopharmacological wisdom with contemporary pharmacotherapeutic frameworks to engineer multi-mechanistic, naturally derived therapeutics for neurodegenerative afflictions. Further exploration is indispensable to elucidate the exact molecular underpinnings, isolate bioactive constituents, and appraise chronic safety profiles alongside clinical translatability within human populations.

The polyherbal formulation exhibits remarkable nootropic potential, reinforcing its prospective utility in adjuvant Alzheimer's treatment and underscoring the relevance of botanical agents in confronting multifaceted neurodegenerative disorders.

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