EFFECT OF MEMORY PLUS, A POLYHERBAL FORMULATION, ON EXPERIMENTAL MODELS OF ALZHEIMER'S DISEASE

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Summary

The objective of the present study was to evaluate the effect of memory plus a polyherbal formulation, on experimental models of Alzheimer's disease and central cholinergic markers in rats. Cognitive impairment in rats was induced by intracerebroventricular administration of colchicine or Ibotenic acid lesion of nucleus basalis magnocellularis. Animals of the control group were subjected to the same surgical procedure but received equivalent volume of artificial cerebrospinal fluid (ACSF). The treatment was given for a period of 21 days and assessed for behavioural testing and central cholinergic markers. Colchicine and ibotenic acid induced a marked deficits of the learned active avoidance task as compared to their ACSF treated counterparts after 7, 14 and 21 days. Memory Plus (100 and 200 mg/kg p.o.) reversed the cognitive deficits after 14 days. Colchicine markedly reduced frontal cortical and hippocampal concentrations of Ach, choline acetyltransferase activity and muscarinic, cholinergic binding as compared to the ACSF administered control group. The effects were discernible by day 7 and thereafter progressively increased on days 14 and 21. Memory Plus (100 and 200 mg/kg p.o.) significantly reversed the deleterious effects of colchicine on all these biochemical parameters on days 14, 21.

Key words: Alzheimer disease, Cognitive impairment, Colchicine, ibotenic acid, cholinergic markers,

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease. It is characterized by progressive cognitive deterioration together with declining activities of daily living and behavioral changes. It is the most common type of pre-senile and senile dementia. According to the World Health Organization, 5% of men and 6% of woman of above the age of 60 years are affected with Alzheimer's type dementia worldwide (1).

Memory Plus is a polyherbal formulation containing herbs categorized as *Medharasayanas* (that which enhances the mind) in Ayurveda used to improve memory and cognitive deficits associated with chronic illness and aging (Table 1).

Table 1. Plant constituents of Memory Plus

Plant	Quantity (mg)
Brahmi (*Bacopa monniera*)	130
Brahmi (*Centella asiatica*)	70
Shankapushpi (*Evolvulus alsinoide*)	100
Turmeric (*Curcuma longa)*	100
Ashwagandha (*Withania somnifera*)	50
Tulsi (*Ocimum sanctum*)	50

Bacopa monniera (Brahmi) traditionally described with memory-enhancing, analgesic, sedative, anxiolytic and antiepileptic properties. It is reported to facilitate acquisition, consolidation and retention of newly acquired behavioural responses in animal models. The amnestic effects of scopolamine, convulsive shock and behavioural stress were also reversed with *B. monniera* extract (2).

Centella asiatica (Brahmi) is used to re-vitalize the brain and nervous system, increase attention span and concentration, and combat aging. A decoction of juice from the leaves is used as a general tonic for good health. It also has anti-oxidant properties. It is a mild anxiolytic, adaptogen, antibacterial, anti-viral, anti-inflammatory, anti-ulcerogenic, cerebral tonic, circulatory stimulant, diuretic, nervine and vulnerary. Preliminary evidence suggests that it may have nootropic effects (3).

Evolvulus alsinoide (Shankapushpi) is quoted in Charaka to be the single greatest herb for enhancing all three aspects of mind power - learning (Dhi), memory (Driti), and recall (Smriti). It helps the quality of sleep by improving mind-body coordination. Used as tranquilizer for those suffer from insomnia. Shankapushpi is also used as one of the most important ingredient in treatment of disorders/syndromes such as hypertension, hypotension, anxiety neurosis, stresses etc (4).

Curcuma longa (Turmeric) as an antioxidant, anti-inflammatory and its lipophilic action improves the cognitive functions in patients with AD. A growing body of evidence indicates that oxidative stress, free radicals, beta amyloid, cerebral deregulation caused by bio-metal toxicity and abnormal inflammatory reactions contribute to the key event in Alzheimer's disease pathology. Due to various effects of curcumin, such as decreased Beta-amyloid plaques, delayed degradation of neurons, metal-chelation, anti-inflammatory, antioxidant and decreased microglia formation, the overall memory in patients with AD has improved (5).

Withania somnifera (Ashwagandha), possesses antistress, adaptogenic immunomodulatory and GABA mimetic activity. Also its root extract reduce the enhanced brain levels of tribulin, 5HT and corticotrophin, which are linked with anxiety state (6).

Ocimum sanctum (Tulsi) possesses antistress, adaptogenic, immunostimulant and antioxidant properties. Its cortisol sparing immunomodulatory activity contributes to the behavioural disinhbitory activity (7).

Although individual components reported to possess prevention of cognitive impairment action there was no experimental and clinical evidence as formulation. The present study was undertaken to study the cognition-facilitating effect of Memory Plus in experimentally validated models of Alzheimer's disease (AD) in rats and to investigate the role of the central cholinergic system in the nootropic effect of Memory Plus.

Materials and Methods

Animals

Male, Wistar albino rats weighing 150 to 180 g (90 to 110 days old) procured from Indian Institute of Sciences, Bangalore were used for this study. They are maintained under standard conditions (temperature 22 ± 2^{o}C, relative humidity 60±5% and 12 h light/dark cycle).The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol. All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health".

Drugs

The dry powder formulation of Memory Plus provided by Srinivas Natural Remedies Mangalore, India was suspended in 0.3% carboxymethylcellulose in distilled water. The drugs administered orally in a volume of 0.5 ml/100 g body weight, once daily, in doses of 100 and 200 mg/kg, starting from day 1 just prior to surgery till day 21. Control animals received equivalent volume of vehicle through the same route, for the same time period.

Induction of cognitive deficits

Intracerebroventricular (i.c.v.) administration of colchicine (8)

The right lateral ventricle was cannulated in pentobarbitone sodium (40 mg/kg i.p.) anaesthetized rats, using the stereotaxic co-ordinates 0.6 mm posterior to bregma, 1.8 mm right lateral and 2.7 mm below cortical surface. Colchicine (15 µg/rat), dissolved in 5 µl of artificial cerebrospinal fluid (ACSF; in nM: NaCl 147, KCl 2.9, $MgCl_2$ 1.6, $CaCl_2$ 1.7 and dextrose 2.2), was slowly injected into the cannulated right lateral ventricle using a 10 µl Hamilton syringe.

Ibotenic acid lesion of nucleus basalis magnocellularis (nbm) (9)

Unilateral nbm lesion was induced by injecting ibotenic acid (10 µg/rat), dissolved in 5 µl of ACSF, in pentobarbitone sodium (40 mg/kg i.p.) anaesthetized rats, using the stereotaxic co-ordinates 1.0 mm posterior to bregma, 2.6 mm right lateral and 7.9 mm below the cortical surface.

Animals of the control group were subjected to the same surgical procedure but received equivalent volume of ACSF instead of the neurotoxins, colchicine and ibotenic acid.

Behavioural testing (10)

The apparatus consists of a shuttle box with two identical compartments, separated by a hurdle. During training, each rat was placed in one compartment and after 5 sec, a buzzer situated in the ceiling of the shuttle box was sounded (2.8 kHz, 70 dB) (conditioned stimulus, CS) for 3 sec, followed by electric shock (1.5 mA, 2 sec) (unconditioned stimulus, UCS) through the grid floor. If the rat crossed to the unelectrified safe compartment during presentation of CS, an avoidance response was recorded, otherwise UCS was applied. Each rat was given 20 trials for 5 days, with an inter trial interval of 30 sec, until it reached the criterion of 100% active avoidance response. Rats not reaching this criterion were discarded from the study. Retention of the acquired active avoidance response in the different treatment groups was assessed on days 7, 14 and 21, following lesioning with colchicine or ibotenic acid. The number of trials required to criterion of 100% active avoidance response was noted.

Biochemical studies

Rats were killed by decapitation at the predetermined time intervals and the frontal cortex and hippocampus were dissected out (11). The tissues were homogenised in 10 volumes (w/v) of ice-cold Tris-HCl buffer (pH 7.6) and divided into aliquots for estimation of acetylcholine (Ach) levels by a fluorimetric technique (12), choline acetyltransferase (ChAt) activity by a radiometric method (13) and muscarinic cholinergic binding (MCR) by the method of Ogawa *et al* (14). Protein estimation [3-quinuclidinol (QNB) binding] was done by the method of Lowry *et* a (15).

Statistical analysis

The data were initially analyzed by a one-way analysis of variance (ANOVA), which was followed by the two-tailed Student's t-test.

Results

Behavioural testing

Neurotoxins colchicine and ibotenic acid injected animals shown marked deficits of the learned active avoidance task as compared to their ACSF treated counterparts after 7, 14 and 21 days. The retention deficit was evident by day 7 and increased progressively on days 14 and 21. Memory Plus (100 and 200 mg/kg p.o.) significantly reversed colchicine and ibotenic acid induced cognitive deficits after 14, 21 day but results were non-significant on day 7 (Table 2).

Table 2: Effect of Memory Plus on the retention of an active avoidance learning

Treatments (mg/kg)	n	Number of trials required to achieve 100% avoidance response					
		Colchicine group (COL)			Ibotenic acid group (IA)		
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
ACSF	12	4.8±0.5	3.2±0.4	2.6±0.6	3.9±0.6	3.0±0.5	2.8±0.4
Memory Plus (100)	08	3.2±0.6	2.4±0.6	1.9±0.4	3.0±0.5	2.2±0.3	1.8±0.6
Memory Plus (200)	06	2.9±0.9	2.0±0.6	1.4±0.6	2.8±0.6	2.0±0.6	1.6±0.6
Lesion (COL/IA)	10	7.6±0.8^{x}	8.3±0.6^{y}	9.2±1.2^{x}	6.6±0.9^{y}	7.8±0.8^{z}	9.3±1.3^{z}
Memory Plus (100)+ Lesion	08	5.9±0.9	6.2±0.4^{a}	4.0±0.9^{c}	4.9±0.6	4.3±0.7^{c}	4.8±0.6^{b}
Memory Plus (200)+ Lesion	08	5.4±1.2	3.6±0.8^{b}	2.2±0.6c	4.4±0.9	2.1±0.4^{c}	3.6±0.8^{c}

Values are mean ± SEM. $^{x}P<0.05$, $^{y}P<0.01$, $^{z}P<0.001$, compared to ACSF treated group.
$^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, compared to COL or IA lesioned group

Biochemical parameters

Colchicine markedly reduced frontal cortical and hippocampal concentrations of Ach, ChAt activity and MCR binding as compared to the ACSF administered control group. The effects were discernible by day 7 and increased progressively thereafter on days 14 and 21. Both doses of Memory Plus reversed the deleterious effects of colchicine on all these biochemical parameters. The effect was statistically significant on days 14 and 21 but not on day 7 (Tables 3, 4 and 5).

Table 3: Effect of Memory Plus on acetylcholine concentrations of frontal cortex and hippocampus

Treatments (mg/kg)	n	Acetylcholine concentrations (nmol/g)					
		Frontal cortex			Hippocampus		
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
ACSF	08	24.6±1.2	26.2±1.6	23.2±1.9	30.5±1.4	28.4±2.2	31.4±1.3
Memory Plus (100)	06	25.2±2.9	31.6±1.8	28.4±2.6	29.3±1.7	32.6±2.9	35.9±3.1
Memory Plus (200)	06	27.4±1.8	34.6±3.2	29.9±2.9	33.8±2.1	35.2±2.8	37.6±3.3
Colchicine (COL)	10	18.2±1.6^{x}	15.3±2.9^{y}	11.6±1.2^{z}	23.9±2.1^{x}	19.6±1.9^{y}	14.2±2.9^{z}
Memory Plus (100) + COL	08	22.9±1.9	25.1±2.2^{a}	19.1±1.8^{a}	25.8±2.1	27.2±1.6^{a}	23.0±2.1^{c}
Memory Plus (200) + COL	08	23.9±1.6	30.3±1.9^{b}	24.8±2.1^{b}	28.0±2.2	33.8±2.6^{b}	31.2±1.9^{c}

Values are mean ± SEM. $^{x}P<0.05$, $^{y}P<0.01$, $^{z}P<0.001$, compared to ACSF treated group.
$^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, compared to colchicine group

Table 4: Effect of Memory Plus on choline acetyltransferase activity of frontal cortex and hippocampus

Treatments (mg/kg)	n	Choline acetyltransferase activity (nmol/mg protein/h)					
		Frontal cortex			Hippocampus		
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
ACSF	08	21.2±0.9	20.6±1.2	19.9±0.8	19.5±0.9	18.2±0.8	19.6±1.2
Memory Plus (100)	06	19.5±1.6	23.6±1.8	21.7±1.3	22.8±1.6	21.9±1.2	22.7±1.4
Memory Plus (200)	06	21.3±1.4	24.9±2.6	22.4±1.8	22.4±1.8	22.6±1.9	23.6±2.8
Colchicine (COL)	10	16.2±1.1^{x}	14.3±0.8^{z}	11.8±0.9^{z}	14.9±1.5^{x}	12.3±1.2^{y}	9.8±0.6^{z}
Memory Plus (100) + COL	08	18.4±1.6	19.2±1.4^{a}	20.6±1.9^{b}	16.8±1.2	16.2±1.1^{a}	14.3±0.9^{b}
Memory Plus (200) + COL	08	18.8±1.9	21.9±0.9^{c}	23.6±1.0^{c}	17.6±1.9	19.2±1.2b	16.9±0.8^{c}

Values are mean ± SEM. ^{x}P<0.05, ^{y}P<0.01, ^{z}P<0.001, compared to ACSF treated group. ^{a}P<0.05, ^{b}P <0.01, ^{c}P<0.001, compared to colchicine group.

Table 5: Effect of Memory Plus on muscarinic cholinergic receptors in frontal cortex and hippocampus

Treatments (mg/kg)	n	(3 H) QNB binding (pmoles/mg protein)					
		Frontal cortex			Hippocampus		
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
ACSF	08	1.52±0.06	1.69±0.09	1.58±0.08	1.38±0.07	1.43±0.08	1.41±0.08
Memory Plus (100)	06	1.46±0.09	1.56±0.09	1.79±0.08	1.46±0.04	1.54±0.06	1.69±0.07
Memory Plus (200)	06	1.58±0.08	1.68±0.09	1.96±0.12	1.59±0.07	1.67±0.06	1.84±0.11
Colchicine (COL)	10	0.82±0.06^{x}	0.64±0.04^{x}	0.49±0.05^{y}	0.71±0.07^{x}	0.52±0.06^{x}	0.41±0.40^{z}
Memory Plus (100) + COL	08	1.22±0.08	1.08±0.08^{a}	1.16±0.04^{b}	0.98±0.09	1.28±0.06^{a}	1.31±0.04^{b}
Memory Plus (200) + COL	08	1.28±0.09	1.32±0.07^{a}	1.42±0.04^{b}	1.06±0.4^{b}	1.39±0.07^{c}	1.56±0.03^{c}

Values are mean ± SEM. ^{x}P<0.05, ^{y}P<0.01, ^{z}P<0.001, compared to ACSF treated group. ^{a}P<0.05, ^{b}P <0.01, ^{c}P<0.001, compared to colchicine group

Discussion

Alzheimer's disease (AD) is characterised by degenerative changes in the brain accompanied by loss of memory, especially for recent events. It is now widely accepted, based on extensive clinical and experimental evidence that learning and memory is closely associated with the functional status of the central cholinergic system (16). The basal forebrain provides the major source of cholinergic inputs to the neocortex and hippocampus. The main cholinergic pathways in the mammalian forebrain are the projections from the medial septal nucleus and the nucleus of the vertical limb (diagonal band of Broca) to the hippocampus via the fimbria-fornix and the projection from nucleus basalis cellularis (nbm) to the neocortex. The nbm located in the ventromedial region of the globus pallidus accounts for 70-80% of the cholinergic innervation to the cortex (17). Lesions of the nbm have been proposed as an experimental model for AD, based on the observation that degenerative changes in nucleus basalis of Mynert (nbM), the human counterpart of nbm, are present in patients of AD. In addition, the nbm lesioned rat shows decrease in cholinergic markers, including release and turnover of Ach, choline uptake, ChAt, acetyl cholinesterase activity and number of muscarinic receptors in the frontal cortex similar to what reported in patients of AD (17). The nbm lesioned animals also exhibited marked cognitive deficits in a variety of experimental models (17). Similarly, i.c.v. administration of colchicine has been shown to exert a direct cholinotoxic effect, accompanied by reduction in cholinergic markers and induction of cognitive deficits in rats (18).

The polyherbal formulation Memory Plus was able to reverse cognitive deficits induced by colchicines and ibotenic acid, the effects being evident after 2 weeks of treatment. The reversal of cognitive deficits induced by colchicine was accompanied by attenuation of its cholinotoxic effects, indicating that the drug was capable of promoting cholinergic recovery. The findings support clinical and experimental observations that Memory Plus can improve memory in states of cognitive deficits (19). However, the lack of cognition facilitating effect of the drug in normal animals indicates that Memory Plus is unlikely to improve memory in normal subjects. Nootropic agents like piracetam which facilitate central cholinergic mechanisms are known to improve memory only in the presence of cognitive deficits. Nootropics restore age-related deficits of frontal cortical muscarinic cholinergic receptors (20).

The present investigations categorize Memory Plus as a nootropic agent. However, extensive and controlled clinical studies are required before the plant formulation can be considered as a valid therapy for cognitive dysfunctions, including AD and age-related memory deficits.

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Conclusion

Based on the findings detailed above, Memory Plus will lead to a promising treatment for Alzheimer's disease. However elaborative human studies are required to identify the prophylactic and therapeutic effect of Memory Plus.

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