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EVALUATION OF MEMORY ENHANCING ACTIVITY OF SR-105 IN EXPERIMENTAL ANIMALS

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ABSTRACT

The learning and memory is closely associated with the functional status of the central cholinergic system and others monoamines. Based on literature in ayurveda, SHRUSHTI a Herbal Pharma Industry of Bangalore has come out with the Polyherbal formulation SR-105 for Memory enhancing activity; consisting of plant ingredients like *Convolvulus miorophyllus, Celastrus paniculata, Acorus calamus* and *Bacopa monniera*. Hence in the present work an effort has been made to identify the Memory enhancing activity of SR-105 in experimental animals studies i.e., scopolamine-induced amnesia on active avoidance paradigm and inhibition of cholinesterase activity in rats brain. The LD_{50} studies of SR-105 were conducted according to OECD guidelines No.425; up to 2000 mg/kg the formulation had not produced any mortality. Piracetam and the different doses of polyherbal formulation SR-105 treated groups had shown decreased the time spent in shock zone and number of errors on active avoidance paradigm and also shows dose dependent inhibition of cholinesterase enzyme activity. In the light of above, it may be worthwhile to explore the potential of this SR-105 polyherbal formulation in the management of Alzheimer's disease.

KEYWORDS: Polyherbal formulation, Scopolamine, nootropic activity, anticholinesterase activity.

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INTRODUCTION

Neuropathologic changes in Alzheimer's disease (AD) include cerebral atrophy, neurotic plaques, and neurofibrillary tangles. Neurons that use acetylcholine are critical to memory and learning and it is primarily cholinergic neurons that show changes and degeneration in Alzheimer's disease. The decrease in cholinergic function correlates closely with cognitive deficits in patients.

The major neurotransmitter change in the brains of patients with Alzheimer's disease is a 30% to 90% decrease of the biosynthetic enzyme choline acetyltransferase in the cerebral cortex and hippocampus. Biopsy analyses have suggested that this cholinergic marker is reduced even in the first years of symptoms.

The basal forebrain, the major source of cholinergic innervation to the neocortex and hippocampus, shows progressive neuronal loss in Alzheimer's disease. Relative preservation of postsynaptic muscuranic receptors suggests that cholinergic stimulation may be effective in restoring function. Increased levels of acetylcholine with the use of acetylcholinesterase inhibitors produce a modest improvement in cognitive function for some patients. Piracetam is one of the widely used Nootropic agents, but the resulting chemophobia associated with it and other similar agents has made their use limited. So it is worthwhile to explore medicines from the traditional system in the treatment of these cognitive disorders.

The Indian system of medicine is replete with medicinal plants claimed to promote learning, memory and intelligence[1]. Plants like *Bacopa monniera*[2], *Azadirachta indica*[3], *Withania somnifera*[4], *Hypericum perforatum*[5], *Albizzia lebbeck*[6], *Vitis vimifera*[7], *Ginseng*[8], *Desmodium gangesicum*[9] as well as *Ocimum sanctum*[10] have been investigated for their effect on cognitive functions of the brain. Considering the available literature in ayurveda, we and **SHRUSHTI** a Herbal Pharma Industry of Bangalore had planned to study the Memory enhancing activity of SR-105, an Indian ayurvedic poly-herbal formulation. The reversal effect of SR-105 against memory deficits induced by scopolamine was evaluated on active avoidance paradigm, as well as estimation of cholinesterase activity in same rat's brain.

MATERIALS AND METHOD

Drug and Chemicals

Piracetam(200mg/kg) ('Neurocetam syrup', Brown & Burk.India) used as standard drug, Scopolamine ('Hyoscine' German Remedies, India), as inducing agent, 5,5-dithio bis (2-nitrobenzoic acid) (Shah Scientific, India), S-acetylthiocholine Iodide (NR Chem, India) and SR-105 (SHRUSHTI a Herbal Pharma Industry of Bangalore) in the form of tablets. SR-105 is a polyherbal formulation contains *Convolvulus microphyllus, Cellastrus paniculta, Acrorus calamus* and *Bacopa monniera.* All drugs were dissolved in distilled water and administered orally.

Animals

Group of adult Swiss albino rats 180-220gm and albino mice 20-30 g are used. Animal studies were performed as per rules and regulations in accordance to guideline of CPCSEA with registration number 557/02/c/CPCSEA,18.2.2002.The SR-105 with different doses was administered for 14 days to experimental animal for evaluation of memory enhancing activity.

Determination of Acute Toxicity (LD_{50})

The LD_{50} studies of SR-105 were calculated according to OECD guidelines No.425 by using albino mice of either sex (20-30 g) and there is no mortality during 48 h study period. LD_{50} of polyherbal formulation SR-105 is more than of 2000 mg/kg. So $1/20^{th}$, $1/10^{th}$ and $1/5^{th}$ doses of 2000mg/kg were selected as low (100 mg/kg), medium (200 mg/kg) and high doses (400 mg/kg) and were tested in the present study to explore memory enhancing activity.

Treatment Schedule

The memory- impairing dose of Scopolamine (1.0 mg/kg p.o.) daily for 14 days to induce impairment of memory through muscuranic system and the selected dose of polyherbal formulation SR-105 and Piracetam for 07 days i.e. on 8^{th} to 14^{th} day and the parameters like number of shock and time spent in shock zone was noted. Group I with Scopolamine alone (1.0 mg/kg p.o.) daily once for 14 days. Group II with Piracetam (200 mg/kg, p.o.) which served as standard, Groups III, IV, V were treated with different doses of SR-105 (100,200 and 400 mg/kg p.o.) a polyherbal formulation respectively daily once for 7 days as mentioned above.

Active Avoidance Paradigm (Shuttle Box)

Group of adult male albino rats 100-150g each consisting of 6 animals was divided in to Six groups and animals are fasted overnight prior to the test but water was supplied ad libitum. All groups of rats were trained upto 100% learning criterion of active avoidance response. During the training period, each rat was placed in one of the two chambers of the Sidman box, and after 5 sec the buzzer (conditioned stimulus, CS) was sounded for 2 sec, followed by an electric shock (unconditioned stimulus, UCS; 30v, 0.5 sec) through the grid floor. Thereafter, a rest pause of 180 sec was allowed. If the rat jumped within the CS duration to the unelectrified safe box, so as to avoid the USC, it was allowed to rest there for next 30 sec. However, if the rat did not show the avoidance response removed from the shock chamber after 180 sec and was initiated for the next trial. The rat was given 10 trials daily until they reached the 100% criterion of active avoidance response[5,11-15]. After an interval of 15 and 16 days the rats was subjected to a repeat test with treatment of different dose of the polyherbal formulation SR-105 in order to assess the relearning and retention of the previously learned active avoidance response. Similarly Nootropic activity of standard drug was evaluated.

Estimation of Acetylcholinesterase Activity in Rat's Brain

1. Dissection: Adult Male Wistar rats (250-300g body weight) are used in above experiment. The rats are decapitated after 60 min of treatment with vehicle, piracetam (200 mg/kg) and SR-105 (100, 200, 400 mg/kg); brains are removed quickly and placed in ice-cold saline. Frontal cortex, hippocampus and septum (and any other regions of interest) are quickly dissected out on a petri dish chilled on crushed ice.
2. The tissues are weighed and homogenized in 0.1M Phosphate buffer (pH 8).
3. 0.4ml aliquot of the homogenate is added to a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8) and 100µl of DTNB.
4. The contents of the cuvette are mixed thoroughly by bubbling air and absorbance is measured at 412 nm in a photoelectric colorimeter (H2 grade). When absorbance reaches a stable value, it is recorded as the basal reading.
5. 20 ml of substrate i.e., acetylthiocholine is added and change in absorbance is recorded for a period of 10 mins at intervals of 2 mins. Change in the absorbance per minute is thus determined[15,16].

Calculations

The enzyme activity is calculated using the following formula;

$$R = 5.74x\ 10\text{-}4\ x\ A/CO$$

Where,

R = Rate in moles of substrate hydrolyzed / minute / gm tissue

A = Change in absorbance / min

CO = Original concentration of the tissue (mg / ml).

Statistical Analysis

Values are expressed as mean ± SEM. Statistical differences between means were determined by performing one-way ANOVA followed by Dunnet's't' test. P <0.05 were considered as significant. All the statistical analysis was performed using demo version of Instat® software (Graph pad Inc., Santabarbara, CA)

RESULTS

Effect of SR-105 on Active Avoidance Learning and Retention in Rats

In active avoidance paradigm apparatus piracetam and different doses of SR-105 treated groups had shows significant reduction in time spent in shock zone and number of errors. **(Table 1)**

Anti-acetylcholinesterase Activity in Rat's brain

Scopolamine treated group had shown $9.635x10^{-7}$ µmol/min/g tissue of acetyl Cholinesterase activity in rat's brain. Prior treatment with piracetam and different doses of SR-105 100, 200, 400 mg/kg had showed decreased the acetyl Cholinesterase activity $5.453x10^{-7}$, $6.844x10^{-7}$, $6.095x10^{-7}$, $5.4545x10^{-7}$ respectively. However, a significant effect was observed with Piracetam and all doses of SR-105 as compared control group. **(Table 2)**

DISCUSSION

Dementia is generally defined as the "loss of intellectual abilities, in dementia, memory capacity to solve problems of day-to-day living, performance of learned motor, social skills and control of emotions are primarily affected.

Alzheimer's disease (AD) is characterized by degenerative changes in the brain accompanied by loss of memory, expressly for recent events. The learning and memory is closely associated with the functional status of the central cholinergic system. 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 projection 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 to the neocortex[17]. Despite the severity and high prevalence of this disease, Allopathic system of medicine is yet to provide a satisfactory remedy. Therefore, we were motivated to explore the Indian traditional system to come up with a promising solution to manage this deadly disease (AD).

The active avoidance paradigm used in this was based on Pavlovian fear conditioning. A large body of evidence suggests that the amygdale is a likely site of the plasticity underling memory storage of conditioned fear as well as an unconditioned one. The impairment of emotional event memory in Alzheimer's disease is related to intensity of amygdala damage[18]. In this model, the intensity of amygdala is impaired through foot electric shock by using shuttle box model causes impairment of memory.

Active avoidance learning is a fundamental behaviour phenomenon. As in other instrumental conditioning paradigms, the animal learns to control the administration of the unconditioned stimulus (UCS) by appropriate reaction to the conditioned stimulus (CS) preceding the noxious stimulus. The first stage of avoidance learning is usually escape, whereby a reaction terminates the UCS[5]. The active avoidance is induced by a sequence of conditioned and unconditional stimuli to the animal. In response, the animal must relocate to the adjoining compartment within a preset time in order to avoid the mild electric shock. The latency from stimuli onset to escape of subject after the pretraining is related to the retention of memory task[19].

The impairment of learning and memory induced by scopolamine (1.0mg/kg) an anticholinergic agent, was reflected by increased no of shocks and time spent in shock zone. The polyherbal formulation SR-105 (100mg, 200mg and 400 mg/kg) and piracetam (200mg/kg) have reversed the amnesia induced by scopolamine, i.e. decreased no of shocks and time spent in shock zone indicates that they are acting on Ach receptors because they had shown nootropic activity in presence of scopolamine which is a muscuranic receptor antagonist[20]. In the present study Polyherbal formulation SR-105, showed elevation of acetylcholine level by significant reduction of cholinesterase activity in rat's brain and ultimately improved memory.

CONCLUSION

In the light of above, it may be worthwhile to explore the potential of this polyherbal formulation SR-105 exhibited nootropic actitivity and useful in management of Alzheimer's disease.

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Table:-1 Effect of SR-105 on Active Avoidance Learning and Retention in Rat (Mean ± SEM)

TREATMENT	NUMBER OF SHOCK			TIME SPENT IN SHOCK ZONE (in secs)		
	Learning (acquisition)	Relearning	Retaining	Learning (acquisition)	Relearning	Retaining
	1st day	15th day	16th day	1st day	15th day	16th day
Scopolamine 1.0 mg/kg	7.333 ± 1.740	8.667 ±1.406	7.333 ± 1.022	15.283 ±3.534	13.775 ±2.089	10.153 ±1.628
Piracetam 200 mg/kg	7.000 ±1.565	1.833** ±0.6009	0.3333** ±0.2108	12.917 ± 3.048	4.553** ±1.066	1.993** ±0.5148
SR-105 100 mg/kg	1.165 ±0.2395	2.500** ±0.8466	1.833** ± 0.4773	4.107 ±0.5999	5.275** ±1.197	3.505** ±0.9537
SR-105 200 mg/kg	1.055 ±0.05500	2.167** ±0.6540	1.167** ±0.4014	4.718 ±0.5803	3.628** ±0.8171	3.135** ± 0.2451
SR-105 400 mg/kg	5.583 ±1.535	1.000** ±0.4472	0.3333** ±0.3333	11.667 ±2.848	5.052** ±1.277	1.700** ±0.5260

n=6 in each group. Data is expressed as mean ±SEM. Statistical analysis by one-way ANOVA followed by Dunnett's 't' test Significance at P<0.05*, P <0.01** and ns-not significant vs control group.

Table:-02. Effect of SR-105 on Acetyl Cholinesterase (AchE) Activity in Rat's Brain

Group No	Treatment	Dose mg/kg	AchE Activity µmol/min/g tissue(Mean±SEM)
I	Normal control (Scopolamine)	1.0 mg/kg	$9.635x10^{-7}$±$0.2452x10^{-7}$
II	Piracetam	200mg/kg	$5.453x10^{-7**}$ ±$0.2131x10^{-7}$
III	SR-105	100 g/kg	$6.844x10^{-7**}$ ±$0.1455x10^{-7}$
III	SR-105	200mg/kg	$6.095x10^{-7**}$±$0.0514x10^{-7}$
IV	SR-105	400mg/kg	$5.4545x10^{-7**}$±$0.1151x10^{-7}$

n=6 in each group. Data is expressed as mean ±SEM. Statistical analysis by one-way ANOVA followed by Dunnett's 't' test. Significance at P<0.05*, P <0.01** and ns-not significant vs control group

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