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Evaluation Of Nootropic Activity Of Polyherbal Formulation Against Scopolamine-Induced Amnesia In Rats

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Abstract:
The present study was designed to assess the nootropic activity of Polyherbal formulation (PHF) using Radial arm maze on scopalamine induced amnesia in rats. Ayurvedic Polyherbal formulation (PHF) consists of plant ingredients of Brahmi (Bacopa monniera), Shatavari (Asparagus racemosus), Ashwagandha (Withania somnifera) and Vacha (Acorus calamus). This Ayurvedic polyherbal preparation can be extensively used in the treatment of loss of memory and memory enhancement both in children and adults. In the current investigation, the nootropic and anticholinesterase action of plant extract of PHF at doses (200 and 400 mg/kg, p.o.) was assessed using animal models. The PHF treated group showed a dosage dependent substantial decline in the radial arm maze when compared to the corresponding control group. For memory intoxication, sodium nitrite and scopolamine were utilised. Anxiolytic activity in the treatment group was shown by the considerable (p0.001) increase in social engagement time. Additionally, as compared to the control group, the treated group demonstrated a substantial (p0.001) decline in anticholinesterase activity. Therefore, an improvement in spatial reference and spatial working memory corresponds to a drop in radial arm performance and a rise in the proportion of memory retention of PHF against scopolamine-induced dementia. As a result, polyherbal preparation demonstrated to be a viable treatment for cognition disorders.

Keywords: Radial arm maze, Anticholinesterase, Polyherbal formulation, Nootropic.

Introduction

The World Health Organization estimates that 450 million people worldwide experience a mental or behavioural problem. Memory is a complex brain function that uses several neural connections to register sensory stimuli, events, and knowledge and then keep it for short or extended periods of time. Cognitive illnesses like schizophrenia, Alzheimer's disease, forgetfulness, and depression may accompany learning and memory impairments. These illnesses place a heavy weight on the delayed recall, reduced retention, and poor memory that characterize today's stressed and competitive lifestyle.

A small number of nootropic drugs, which fall within the category of psychotropic

pharmaceuticals, are used to treat cognition issues. Giurgea first used the term "nootropic" in 1972, combining the Greek words "noon" (thinking) and "tropos" (turn). Nootropics are also known as "smart medications" since they enhance cognitive abilities including memory as well as the flow of blood and oxygen to the brain. While Donepezil®, Rivastigmine, and Galantamine, three regularly prescribed AChE inhibitors, were widely given to treat cognition deficit, clinical evaluation of these medications has demonstrated the incidence of relapses, side effects, and drug interactions. This has served as the justification for the development of novel nootropics, including herbal medications. Herbal preparations that can counteract the negative effects of synthetic medications are being sought after by researchers. Numerous plants have been thoroughly investigated and are said to have memory-boosting qualities.

Due to its focus on health promotion and disease prevention, Ayurveda, an ancient system of medicine from India, is becoming more and more popular throughout the world. Four traditional herbs, including Bacopa monnieri Linn, Withanea somniferra Linn, Asparagus racemosus Linn, and Acorus calamus Linn, were combined to create an Ayurvedic Polyherbal formulation for the current study. The aforementioned herbs were chosen because of their traditional medical usage for improving cognition and memory. Brahmi, or Bacopa monnieri Linn, is a member of the Plantaginaceae family. It is a significant medicinal plant from the Scrophulariaceae family that is used in conventional medicine to treat a variety of nerve illnesses and to enhance cognition and memory. One of the herbs that is frequently utilised in the Indian traditional medical system is ashwagandha (Withania somnifera: Solanaceae), more popularly known as Indian ginseng. Ashwagandha is also used as a general tonic and as a "adaptogen" to assist the body handle daily stress. It is used as an anthelmintic, diuretic, sedative, hypnotic, stress reliever, and immunomodulator. Asparagus Racemosus is a member of the Asparagaceae family and is utilised for its neuroprotective, adaptogenic, and anti-epileptic effects. Acorus calamus is used as an antioxidant, antidepressant, neuroprotective, anti-obesity, etc.

Because memory deficit disorders like schizophrenia, dementia, Alzheimer's, and autism have neurodevelopmental roots, the memory-improving effectiveness of Polyherbal Formulation (PHF) was assessed in this study using a variety of behavioural models. Additionally, to estimate the concentration of the metabolic enzyme acetylcholinesterase in the brain in order to assess the biochemical level of the polyherbal formulation (AChE).

Drugs/Chemicals

Piracetam (Dr. Reddy's Labs, India), Scopalamine butyl bromide (German remedies, Mumbai), Dithiobis nitrobenzoic acid (DTNB), Metrifonate (Sigma-Aldrich, USA), Acetylthiocholine iodide (ATCI). All other reagents and solvents used in the experiment were of standard analytical grade.

Animal Care and Selection

Male Wistar rats typically weighed 150–200 g. All the experimental animals were maintained under standard husbandry conditions. The temperature in the room housing

the experimental animals was maintained at 22°C (±3°C). Under laboratory conditions with a 12-hour-long cycle of alternate light and dark, the rats were divided into experimental and control groups. Animals were housed in cages made of sterile polypropylene. Standard pellets were utilised as a base diet for feeding, and water was supplied. Three groups—the control, the standard, and the treatment—were made up of healthy rats. The protocol was accepted by the Institutional Animal Ethics panel (IAEC), approval no. CPCSEA/IAEC/ PT-01/01-2K22

Experimental Design and Drug Administrations

The effect of Bacopa monnieri Linn, Withanea Somniferra, Asparagus Racemosus and Acorus calamus extracts and their formulation was evaluated in experimental models of memory impairment due to Scopalamine administration. The test drug (PHF) along with standard and vehicle were giving for 14 days. Intraperitoneal (IP) Scopalamine was given prior to test drug administration.

Acute Oral Toxicity

Healthy adult wistar rats (150- 200g) were subjected to acute toxicity studies as per guidelines (AOT 423) suggested by the OECD-2000 (OECD Guideline). The rats were monitored continuously immediately after dosing up to first 4 h for behavioral, neurological and autonomic profiles. The animals were under supervision after 24 hours and 14 days for any sign of toxicity or mortality. Thus, effective oral dose 200 and 400 mg/kg of polyherbal formulation (PHF) was exposed to animals for pharmacological activity.

Radial Arm Maze Model

Olton and Samuelson created the radial arm maze in 1976 to test rats' capacity for spatial learning and memory. It has an exteroceptive behavioural model and an octagonal central hub with eight radial arms that measures 36 cm in diameter. Small plastic food containers were installed on each arm's (45 x 15 cm and 12 cm sides) sides, 30 cm from the hub's centre, and 50 cm above the floor. Working memory and reference memory are two memory types that are evaluated while rats complete this activity. When the rats only visit the arms of the labyrinth that hold the reward, reference memory is evaluated. Reference memory errors will occur if this is not done. Re-entry into the arms would result in working memory error.

Experimental Screening Procedure

The rats were trained to choose any arm they wanted to receive food from, with a 300 second time limit, prior to medication administration. In order to produce food-motivated performance during the experiment, 85% of the average daily food intake for the animals was provided. When the animal had visited each of the eight arms, the training session was said to be over. Performance on the radial maze test was evaluated based on the amount of time it took each rat to discover the food and the number of times it tried again. The

animals were split into five groups of six animals each when the training was successfully completed.

Table 1. Experimental table: five groups containing 6 animals each

Group-I	Control received normal saline	Saline (10 ml/kg)
Group-II	Normal saline against Scopolamine	Scopolamine (1 mg/kg, i.p.)
Group-III	Standard drug Piracetam (400 mg kg, p.o) against scopolamine (1 mg/kg, i.p.)	Piracetam (400 mg kg, p.o) Scopolamine (1 mg/kg, i.p.)
Group-IV	Polyherbal formulation (PHF) (200 mg/kg, p.o) against scopolamine (1 mg/kg, i.p.)	PHF (200 mg/kg, p.o) Scopolamine (1 mg/kg, i.p.)
Group-V	Polyherbal formulation (PHF) (400 mg kg, p.o) against scopolamine (1 mg/kg, i.p.)	PHF (400 mg/kg, p.o) Scopolamine (1 mg/kg, i.p.)

Animals were given the usual medications Piracetam and Polyherbal Formulation (PHF) (200 and 400 mg/kg) over the course of ten consecutive days. Scopolamine (1 mg/kp, i.p.) was then administered to produce amnesia 24 hours following the last dose on day 11 of treatment. Following the administration of scopolamine for 60 minutes, the individual animals in the group were exposed to radial arm. As a measure of the drug's impact on the learning and memory process, the number of days needed to train the rats, their latency to find food, and how many of their initial right entries were noted.

Estimation of Brain Acetyl Cholinesterase (AChE) Level

For seven days straight, the various animal groups received treatments of vehicle (normal saline), the reference medicine Metrifonate (50 mg/kg, p.o.), and the test drug Polyherbal Formulation (PHF), 200 and 400 mg/kg. After 60 minutes of the last dosage of therapy, scopolamine (1.4 mg/kg, i.p.) was administered. On the eighth day, the animals were killed by cervical decapitation while being given only a mild anaesthetic; the complete brain was then removed, weighed, and homogenised by adding 10 litres of regular saline. In order to estimate the amount of cholinesterase, the homogenate was centrifuged at 3000 rpm for 10 minutes.

The method of Ellman et al. (1961) was used to quantify the brain cholinesterase activity with a few minor modifications. In a nutshell, 0.5 ml of the brain homogenate's supernatant was pipette out into a 25 ml volumetric flask, and DTNB (10 mg in 100 ml of Sorenson phosphate buffer, pH 8.0) solution was used to make a dilution. 4 ml of the solution were transferred to two test tubes from the volumetric flask. Two drops of serine solution were introduced to one of the test tubes in order to alter the zero. Both test tubes received 1 ml of the substrate solution (75 mg of acetyl choline iodide in 50 ml of distilled water) and

were incubated for 10 minutes at 30 degrees Celsius. The resulting yellow color was measured by using spectrophotometer at 420 nm.

Effect on Radial Arm Maze Performance

This model is frequently used to examine spatial working memory and spatial reference in animals. Scopolamine (1 mg/kg i.p.) - induced amnesia mice performed the radial arm maze in 194.12 seconds longer than the vehicle control group (137.85 seconds), demonstrating impaired cognition. Scopolamine's effects were dramatically reversed in a dose-dependent manner in the Polyherbal Formulation (PHF) 200 and 400 mg/kg treated group. When compared to the scopolamine-treated group, the performance of the radial arm maze task was significantly (p 0.001) worse in the piracetam (400 mg/kg) group. When compared to the normal vehicle group (94.95%), the percentage of memory retention in the scopolamine-treated group was significantly lower (36.32%).

Standard medication Piracetam, Polyherbal Formulation (PHF) 200, and 400 mg/kg treated group showed 84.75, 61.96, and 72.22% of memory retention percentages, respectively. Therefore, improvement in spatial reference and spatial working memory corresponds to a drop in radial arm performance and an increase in the percentage memory retention of Polyherbal Formulation (PHF) against scopolamine-induced dementia.

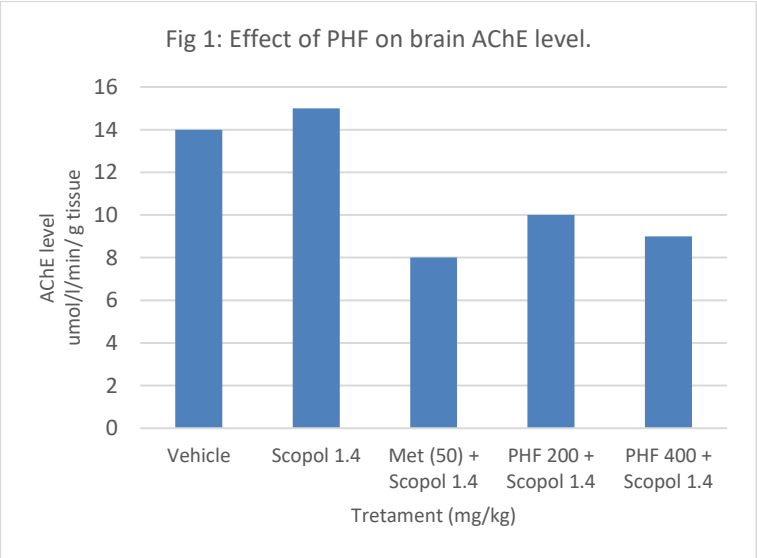
Table 2: Effect of Polyherbal Formulation (PHF) on radial arm maze performance

Treatment (mg/kg)	**Before scopolamine**		**After scopolamine**	**Percentage memory retention**
	Average time taken (sec)	Average days taken to learn	Average time taken (sec) (11 day)	
Vehicle control (normal saline)	131.21 ± 1.24	6.9	137.85 ± 1.14	94.95 %
Scopolamine (1)	118.45 ± 1.17	7.6	194.12 ± 1.35	36.32 %
Piracetam (400) + Scopolamine (1)	37.81 ± 1.08	4.4	43.67 ± 2.01***	84.75 %
PHF (200) + Scopolamine (1)	93.65 ± 1.21	6.3	129.28 ± 1.99**	61.96 %

PHF (400) + Scopolamine (1)	87.53 ± 1.32	6.0	111.85 ± 1.41***	72.22 %
Values represent Mean ± SEM (n=6), one way ANOVA followed by Dunnet's t-test. ###p<0.001 as compared to vehicle control, **p<0.01 and ***p<0.001 as compared to scopolamine group.				

Effect on Brain Acetyl Cholinesterase (AChE) Level

Acetylcholine is crucial for memory processes in both rats and humans. Additionally, scopolamine, a muscarinic antagonist, interferes with muscarinic action to produce memory impairment and excessive phosphorylation of tau proteins, which results in the production of -amyloid. Memory is improved by cholinergic or muscarinic agonists that are known to stop the production of beta-amyloids by acting through the GSK-3 enzyme pathway. AChE inhibitors were therefore developed as a new type of medication in the early stages of AD and serve as the basis for its symptomatic treatment. The results of the current study demonstrated that the AChE levels in the scopolamine-treated group were considerably (p 0.01) higher than those in the vehicle control group. The impact of scopolamine was successfully countered by metrifonate (50 mg/kg). PHF 200 and 400 mg/kg demonstrated dose-dependent suppression of AChE level compared to group treated with scopolamine. PHF's anticholinesterase activity may therefore help with its nootropic potential (Fig 1).



Discussion

Amnesia is frequently brought on by a distressing emotional event which can be treated by nootropics. Nootropics are medications that are also referred to as smart drugs, memory boosters, neuroenhancers, cognitive boosters, and IQ boosters. According to epidemiological research on the Indian population, dementia is mostly a problem that is not widely known. The majority of smart medications have a specific site of action on brain

and memory receptors and are connected to psychotic drugs. Scopolamine caused a memory loss, which was treated with the usual medication piracetam. Piracetam is a medication that is frequently prescribed to cure amnesia and is also used to treat Alzheimer's disease.

Due to widespread use of specific folk remedies and phytotherapy, the Ayurvedic Polyherbal Formulation (PHF) was used for memory enhancement in experimental behavioural animal models. The purpose of the study's protocol was to determine if these herbs—Bacopa monnieri L., Withania somnifera L., Asparagus racemosus L., and Acorus calamus L.—in the form of PHF were effective brain tonics against mental diseases and neurological problems. The use of herbs has presented numerous potential to develop novel medications for memory impairments.

In the present study, we observed that, Polyherbal Formulation (PHF) significantly improved learning and memory. It also exhibited potential anxiolytic action and significantly reduced the level of AChE. Thus, Polyherbal Formulation (PHF) was found to be effective in the treatment of cognition deficient and psychobehavioural disorders.

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