

Memory Enhancing Efficacy of an Ayurvedic Polyherbal Formulation on Scopolamine-Induced Memory Deficit Experimental Models

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ABSTRACT

The present study was performed to determine the efficacy of an ayurvedic polyherbal formulation (PHF) in the learning and memory process of animal models. The prepared PHF was a suspension of three plant extracts i.e. *Alpinia galanga* (AG), *Glycyrrhiza glabra* (GG) and *Convolvulus pluricaulis* (CP) along with other excipients. Learning and memory assessment of PHF was performed using different behavioral models in scopolamine-induced memory impairment animals. Biochemical estimation was performed to determine the enzymatic level of MDA, GSH and AChE level in rats. Results indicated that the time of social investigation trial 2 (SIT2) was reduced significantly compared to SIT1 due to the treatment of PHF. In the water maze test, plant extracts significantly decreased the latency time in the 4th and 5th sessions comparing to session 1. Administration of AG, CP, GG, and PHF caused amelioration of scopolamine-induced memory impairment in the MWM test. The extracts and PHF were also effective in reducing latency time in the pole climbing test. Biochemical parameters exhibited a reduction in MDA and AChE levels with an increase in GSH level after treatment with extracts and PHF. It can be concluded from the study that PHF is an effective formulation for learning and memory processes against scopolamine-induced memory impairment. Further, the isolation of active constituents is desired from different plant extracts.

Key words: *Alpinia galanga*, *Glycyrrhiza glabra*, *Convolvulus pluricaulis*, Pole climbing test, Social recognition test, Latency time.

INTRODUCTION

Memory and learning are crucial mental processes occurring in the cerebrum of the brain. Memory is an important factor for all types of learning due to gathering and retrieving information after the learning process.¹ Learning and memory processes based on electrochemical signaling within the neuronal networks in the brain.² Synaptic plasticity is a connection at the synapse of neurons that hypothetically to be modified during learning and memory disturbance.³ It is expected that modification of synapse function may lead to remodeling of neurons signaling as cognitive behavioral therapy.⁴ Cognitive behavioral therapy (CBT) helps in improving mental health by providing cognitive information and causing training-induced neuroplasticity.⁵

Medicinal Plants have been used since ancient times in the treatment of several disorders.⁶ Natural medicines are now attracting researchers in the development of several formulations due to low side effects and higher efficacy.⁷ The traditional medicines are used worldwide in the form of crude herbal extracts or herbal formulation in alleviating and curing nervous disorders.⁸ *Alpinia galanga* (L.) wild belongs to family Zingiberaceae, it is a well-known medicinal plant containing mild spicy fragrance, has been used in the Asian countries for treating various diseases and disorders such as diabetes mellitus, inflammation, oxidative stress,

diarrhea, ulcer, stomach ache, spasm, and microbial and insecticidal infection.⁹⁻¹³ It is used as a nervine tonic in India. The plant of *A. galanga* is a class of the edible group and its rhizome possesses several active constituents in the form of essential oils such as p-hydroxycinnamaldehyde, galangin, galanganol B, methyl cinnamate, trans-p-coumaric acid, alpinin, kampheride, and cincole.¹⁴

Glycyrrhiza glabra L. (liquorice) belongs to family Leguminosae.^{15,16} It is an ayurvedic herb traditionally used in the treatment of insomnia and anxiety.¹⁷ It possesses several biological activities including neuroprotective, sedative-hypnotic, anticancer, immunomodulatory and hepatoprotective activities.¹⁸⁻²⁰ This plant possess bioactive compounds such as glycyrrhizin, glycyrrhetic acid, octadecane, octanoic acid and paeonol, benzaldehyde and 4-terpineol.^{21,22} It is a widely used food industry due to its sweet taste.

Convolvulus pluricaulis Linn. is a native plant of India belongs to family Convolvulaceae, it has been used traditionally as a memory enhancer and neuroprotective herb^{23,24} and in the treatment of several disorders such as liver diseases, diabetes, ulcer, anxiety, and oxidative stress.²⁵⁻²⁸ It is used as a tonic of the brain, reduces age-related degradation of neurons and also performs the anti-stress activity.²⁹ The plant possess numerous active constituents including shankhpushpine, scopoletin, and 29-oxodotriacontanol.³⁰

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Thus, due to wide utilization of selected folk medicines and phytotherapy as well, the protocol of the study was designed to evaluate the efficacy of these herbs in the form of polyherbal formulation (PHF) as brain tonic against Alzheimer's disease, mental problems and other brain disorders including Schizophrenia, and Autism. Biochemical analysis was performed to determine the level of acetylcholinesterase due to treatment with PHF.

METHODOLOGY

Drugs and reagents

Donepezil, Scopolamine, Propylparaben, Methylparaben, Tween 80 and Sodium carboxymethyl cellulose were procured from Sigma Aldrich, USA. All other solvents were of analytical grade and distilled water was used throughout the study.

Preparation of polyherbal formulation (PHF)

The prepared PHF containing 0.5014 g, 0.3343 g and 0.3343 g of *Alpinia galanga* (AG), *Glycyrrhiza glabra* (GG) and *Convolvulus pleuricaulis* (CP) extracts, respectively; further, the addition of parabens, tween 80 and a little amount of sodium carboxymethyl cellulose then uniformly triturated to form a smooth paste. The paste was rinsed with distilled water (100 ml) and vortexed using a mechanical stirrer (500 rpm) to get a suspension.

Experimental design and drug administrations

The effect of *Alpinia galanga* (AG), *Glycyrrhiza glabra* (GG) and *Convolvulus pleuricaulis* (CP) extracts and their PHF was evaluated in-memory impairment models induced by scopolamine.

Scopolamine induced memory impairment

Scopolamine was given on 5th day to induce social recognition impairment. The memory function test was determined by using different experimental models such as Elevated plus maze, Morris water maze, Pole climbing test, and Social recognition test. Animals were divided into eleven different groups i.e. Group – 1 Control, Vehicle treated, orally (p.o); Group – 2 Toxic group, Scopolamine (i/p); Group – 3 Piracetam (200 mg/kg, i/p), Group – 4 AG1 extract (100 mg/kg, p.o), Group – 5 AG2 extract (200 mg/kg, p.o), Group – 6 GG1 extract (100 mg/kg, p.o), Group – 7 GG2 extract (200 mg/kg, p.o), Group – 8 CP1 extract (100 mg/kg, p.o), Group – 9 CP2 extract (200 mg/kg, p.o), Group – 10 PHF1 extract (100 mg/kg, p.o), Group – 11 PHF2 extract (200 mg/kg, p.o). Animals were administered for consecutive five days and memory function was evaluated from 5th day onwards.

Assessment of learning and memory

Social recognition test (SRT)

Male adult rats weighing 225-250 g were used to evaluate the efficacy of PHF Scopolamine induced social recognition memory impairment. Scopolamine testing was performed in the cages on the 5th day of drug administration in scopolamine (1.25 mg/kg)³¹ models. The social stimuli were generated using small rats weighing 50-60 g. The time interval of 5 min was recorded between adult rats and juvenile rats as social interaction between them (T1). The juvenile rats were removed from cages and re-introduced after 2 h to again record the social interaction time (T2).

Morris water maze (MWM) test

In this and afterward animal models, the highest dose of individual plant extracts and PHF i.e. 200 mg/kg was selected based on its efficacy in SRT. Male adult rats (225-250 g) were subjected to the Morris water maze (MWM) test from the 5th day in the scopolamine model (1 mg/kg).³² The water pool of 45 cm diameter and 26 cm height with four

different starting points- N-E-SE-NW was used in this model. Animals were placed from anyone starting point of pool and any animal failed to escape from the pool within 120 s were placed at the side and allowed to stay for 30 s. The index of learning was determined by escape latency time in the water maze.

Pole climbing test (PCT)

Pole climbing test was performed to evaluate the efficacy of PHF, it is an apparatus enclosed with a chamber (25 cm × 25 cm × 40 cm) with light and sound arrangement. This chamber containing a grid floor to provide electricity. The rat jump to the pole to avoid foot shock while delivering an electrical stimulus, it was recorded as escape and electricity were terminated, however, another response 'avoidance' was noted when jumping occurs due to the sound of the buzzer. The experiment was terminated after 10 trials with intervals of 30 s. The animals were given the respective treatment and again subjected to the test procedure for 5 consecutive days after the completion of training. Reduction in escape latency time was considered as successful retention of avoidance memory.³³

Elevated plus maze (EPM) test

Efficacy of PHF was studied on scopolamine-induced memory impairment using the EPM test. The apparatus containing open and closed arms in all four directions with a central platform. The height of the maze is approximately 25 cm from the floor. Animals were placed on the open arm and latency time was noted when the animal moves to a closed arm. latency time was assigned 90 s if one of the two arms was pushed gently. Examination of memory retention was done after a 24 h post-first-day trial.³⁴

Biochemical analysis

Evaluation of MDA level in scopolamine-induced amnesic rat brain

Malondialdehyde (MDA) level was measured using a thiobarbituric acid reaction (TBAR) colorimetric assay. Briefly, TBA reagent was mixed with brain homogenate and were mixed in a test tube followed by incubation at 90°C for 60 min. The test tube was then placed on ice cubes for cooling, it was centrifuged at 1000 RPM for 10 min. The supernatant was measured at 534 nm in a microplate reader.³⁵

Evaluation of GSH level in scopolamine-induced amnesic rat brain

In this method, GSH was measured by preparing brain homogenate, it was precipitated in trichloroacetic acid in the concentration of 10% followed by centrifugation at 12,000 rpm for 5 min. The supernatant was incubated and absorbance was measured at 425 nm.³⁶

Evaluation of AChE level in scopolamine-induced amnesic rat brain

The acetylcholinesterase (AChE) level was measured as Ellman reagent (100 µL) was filled in an individual well of 96 well plates. Brain homogenate (10 µL) was added in each well containing Ellman reagent and the absorbance was measured at 456 nm in microplate reader followed by determination of protein content in brain homogenate.³⁵

RESULTS

Effect of PHF in SRT behavioral model

Results exhibited that SIT2 was significantly decreased compared to SIT1. It indicated successful learning due to the administration of PHF. However, the scopolamine-induced group without treatment with test drug showed no change in the time during trial 2 comparing to trial 1. A significant reduction in SIT2 was noted in the piracetam (200 mg/

kg) administered group. Treatment with AG, CP and GG extracts at 200 mg/kg each have shown a significant reduction in SIT2 comparing to SIT1. It indicated the prevention of Scopolamine induced memory impairment (Figure 1). PHF was more effective than individual plant extracts in preventing scopolamine-induced memory impairment. Further, there was a significant difference in the recognition index of control, vehicle and Scopolamine group, confirming impairment of memory (Figure 2). The recognition index was significantly lower in piracetam, individual herbal extracts (200 mg/kg) and PHF (200 mg/kg) treated groups.

Effect of PHF in MWM behavioral model

A significant decrease in latency time during the 4th and 5th sessions was observed in control [F (4, 20) = 7.0] and vehicle [F (4, 20) = 9.1] groups comparing to session 1 (Figure 3). The administration of scopolamine caused memory impairment throughout all water maze sessions. Treatment with piracetam caused a significant decrease [F (4, 20) = 6.5] in latency time during the 4th and 5th sessions in comparison to the first session. Plant extracts (200 mg/kg) caused amelioration of memory impairment in animals. Administration of AG [F (4, 20) = 9.5], CP [F (4, 20) = 8.2] and GG [F (4, 20) = 7.1] extracts caused amelioration of scopolamine-induced memory impairment. No, any change was noted between the latency times of session 1 and session 2 of all groups (Figure 3). The PHF also significantly reduced [F (4, 20) = 6.9] latency from session 3 onwards in Scopolamine-induced memory deficit rats indicating successful learning of the MWM task.

Effect of PHF in pole climbing behavioral model

Control [F (4, 20) = 15.7] and vehicle [F (4, 20) = 9.4] groups exhibited significant reduction in the latency time on days 4th and 5th, whereas, in the scopolamine group, no significant [F (4, 20) = 1.3] reduction was seen throughout all days. Administration of piracetam 200 mg/kg caused significant [F (4, 20) = 8.5] reduction in the latency time from day 3 onwards indicating the prevention of scopolamine-induced memory impairment. Treatment with AG [F (4, 20) = 3.8], CP [F (4, 20) = 5.2] and GG [F (4, 20) = 4.9] extracts caused reduction of latency time on day 4 and 5. No, any change was noted between the latency times of day 1 of all groups (Figure 4). The PHF also significantly reduced [F (4,

20) = 10.5] latency time from day 3 onwards in scopolamine-induced rats.

Effect of PHF in EPM behavioral model

The effects of plant extracts and PHF in the EPM test were evaluated on days 5th and 6th. As shown in Figure 5, the transfer latency time in the retention trial was significantly lower than the acquisition trial in control and vehicle groups. However, the scopolamine group showed no significant change in retention latencies in comparison to the acquisition trial. Piracetam at 200 mg/kg significantly reduced latency time during retention trial in comparison to acquisition trial indicating the prevention of memory impairment. Treatment with AG, CP, and GG extracts caused amelioration of scopolamine-induced memory impairment as indicated by significantly lower latency time in retention trial (Figure 5). The PHF also significantly reduced latency time during the retention test in scopolamine-induced rats.

Biochemical estimations

Effect of PHF on MDA level in scopolamine-induced amnesic rat brain

The MDA level was raised significantly in the cortex and hippocampus of scopolamine-treated rats in comparison to the control and vehicle groups. Preventive treatment with piracetam significantly reduced MDA levels in both brain regions. Administration of 200 mg/kg of plant extracts significantly decreased MDA levels in the cortex and hippocampus of scopolamine-treated rats. PHF treatment also caused a significant decrease in MDA level in brain regions (Figure 6).

Effect of PHF on GSH level in scopolamine-induced amnesic rat brain

GSH level was observed using the calibration curve obtained with different concentrations of glutathione. Scopolamine caused a significant reduction in GSH levels in brain regions in comparison to control and vehicle groups. As shown in Figure 7, piracetam significantly prevented the scopolamine-induced reduction in GSH levels in the cortex and hippocampus. The administration of plant extract prevented the scopolamine-induced reduction in GSH levels. AG, CC, and GG

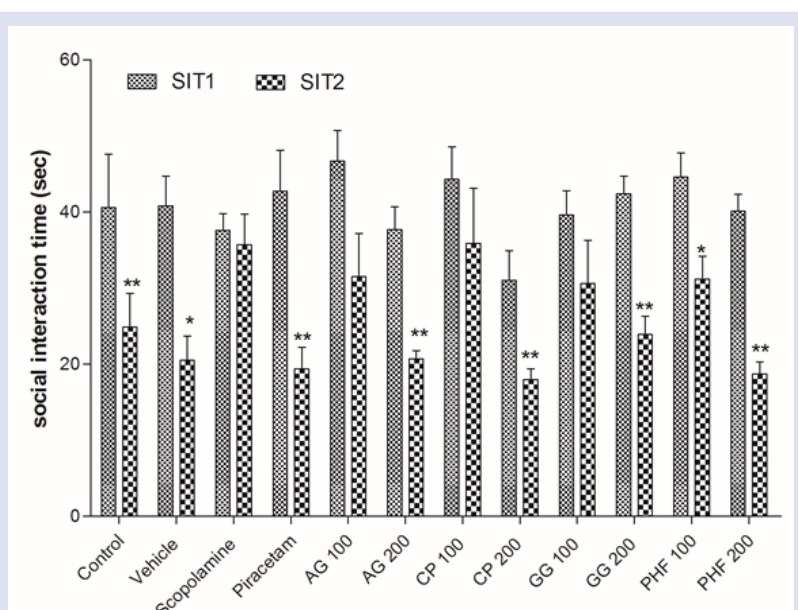


Figure 1: Effect of plant extracts in the social recognition behavioral model. Data are expressed as mean social interaction time (s) \pm S.E.M. *Significant difference (*P<0.05 and **P<0.01) in comparison to trial 1 of the respective group.

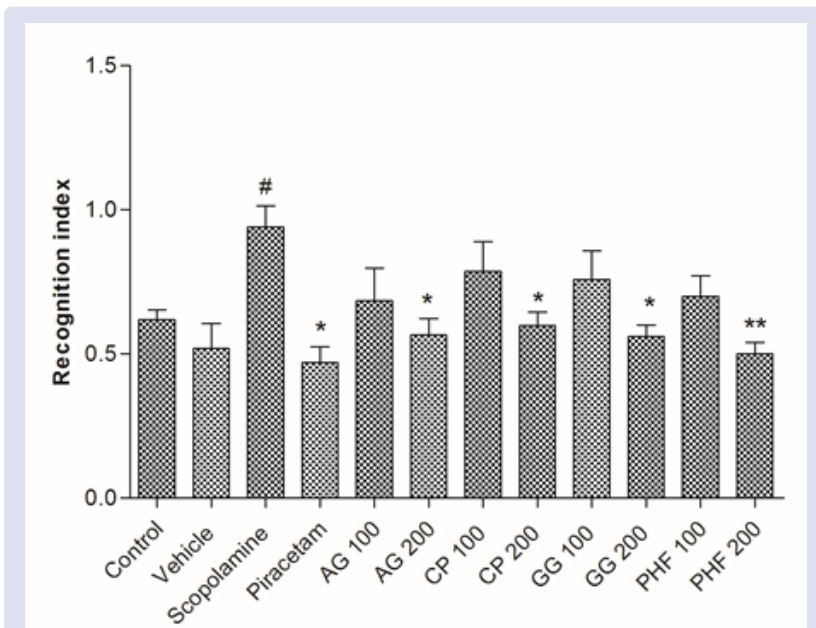


Figure 2: Effect of plant extracts social recognition test behavioral model. Data are expressed as mean recognition index \pm S.E.M. #Significant difference ($\#P < 0.05$) in comparison to the control and vehicle groups and *Significant difference ($*P < 0.05$) in comparison to the scopolamine group.

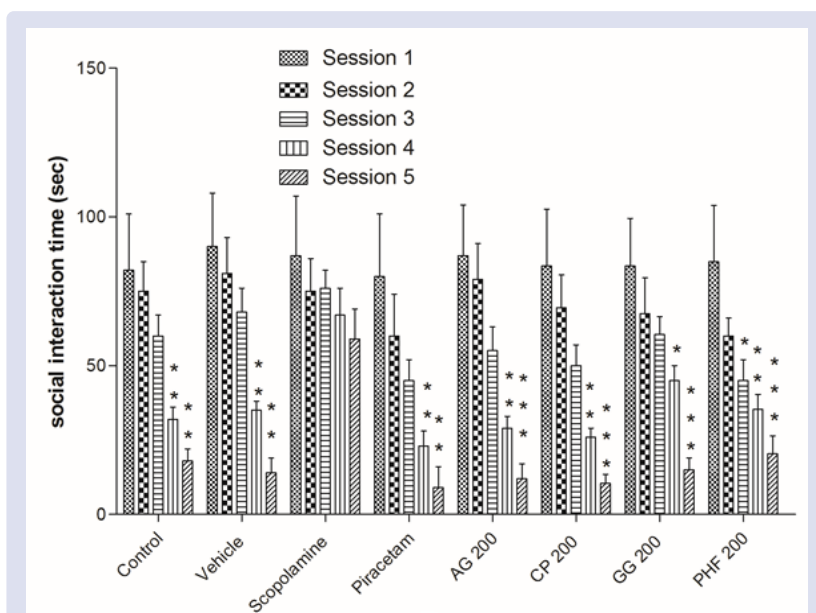


Figure 3: Effect of plant extracts and PHF (200 mg/kg) in the Morris water maze behavioral model. Data are expressed as mean escape latency time (s) \pm S.E.M. *Significant difference ($*P < 0.05$, $**P < 0.01$ and $***P < 0.001$) in comparison to session 1 of the respective groups.

extract raised GSH levels in brain regions. PHF at 200 mg/kg also raised the level of GSH in brain regions (Figure 7).

Effect of PHF on AChE level in scopolamine-induced amnesic rat brain

AChE activity was raised significantly in brain regions in Scopolamine induced memory deficit rats. Preventive treatment with piracetam significantly inhibited AChE activity in brain regions (Figure 8). The administration of plant extract prevented scopolamine-induced elevation of AChE activity. AG, CC, and GG extract significantly decreased AChE activity in animals. PHF at 200 mg/kg also reduced

AChE activity in the brain regions (Figure 8).

DISCUSSION

Plants have been used in the development of the drug as an effective source of novel phytoconstituents in the treatment of several diseases. The phytotherapeutic approach has offered several opportunities to produce new drugs for memory disorders.³⁷ In this study a polyherbal formulation (PHF) of three brain tonic herbs i.e. *Alpinia galanga*; *Glycerrhiza glabra* and *Convolvulus pluricaulis* was evaluated against scopolamine-induced memory loss experimental models. In this cognitive-behavioral study, we found that this formulation was effective

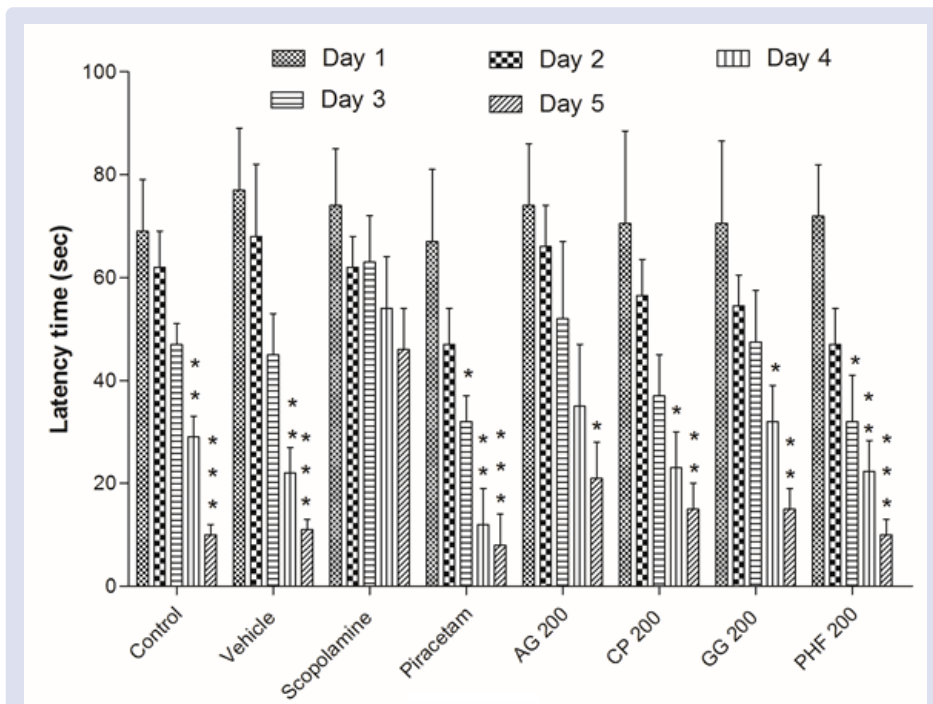


Figure 4: Effect of plant extracts and PHF (200 mg/kg) in pole-climbing behavioral model. Data are expressed as mean latency time (s) ± S.E.M. *Significant difference (*P < 0.05, **P < 0.01 and ***P < 0.001) in comparison to day 1 of the respective group.

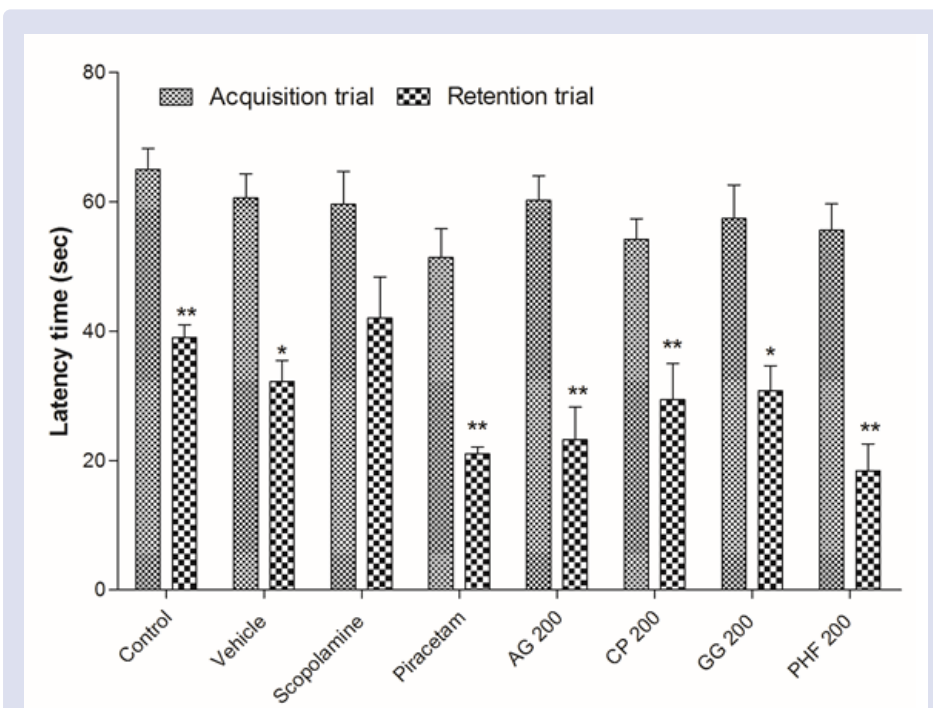


Figure 5: Effect of plant extracts and PHF (200 mg/kg) in elevated plus maze behavioral model. Data are expressed as mean latency time (s) ± S.E.M. *Significant difference (*P < 0.05 and **P < 0.01) in comparison to the retention trial of the respective group.

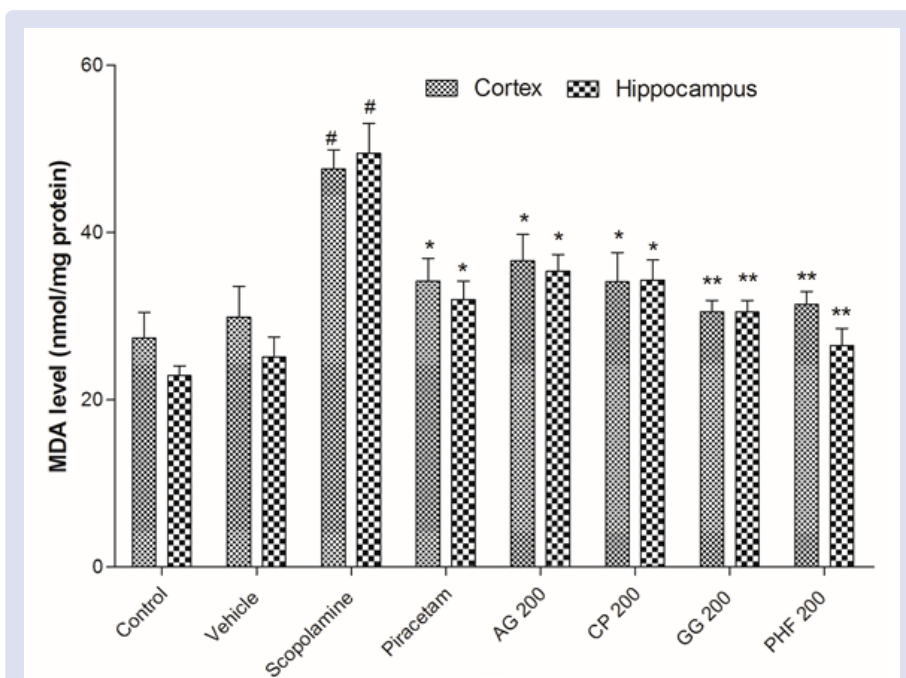


Figure 6: Effect of plant extracts and PHF (200 mg/kg) on the malondialdehyde (MDA) levels. Data are expressed as mean MDA level (nmol/mg protein) \pm S.E.M. [#]Significant increase ($\#P < 0.05$) in comparison to the control and vehicle groups. ^{*}Significant increase ($*P < 0.05$ and $**P < 0.01$) in comparison to the scopolamine group.

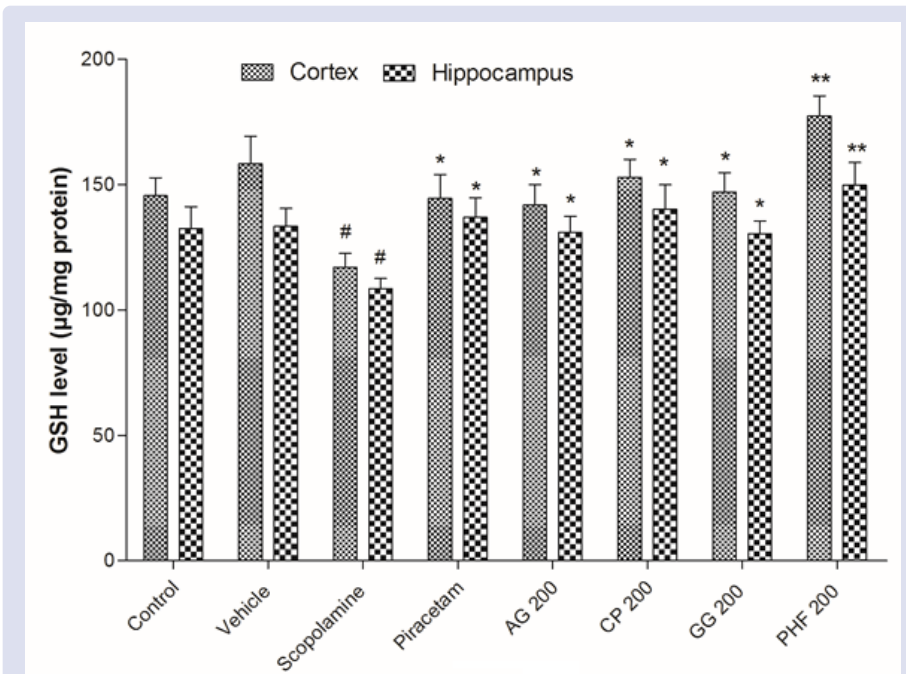
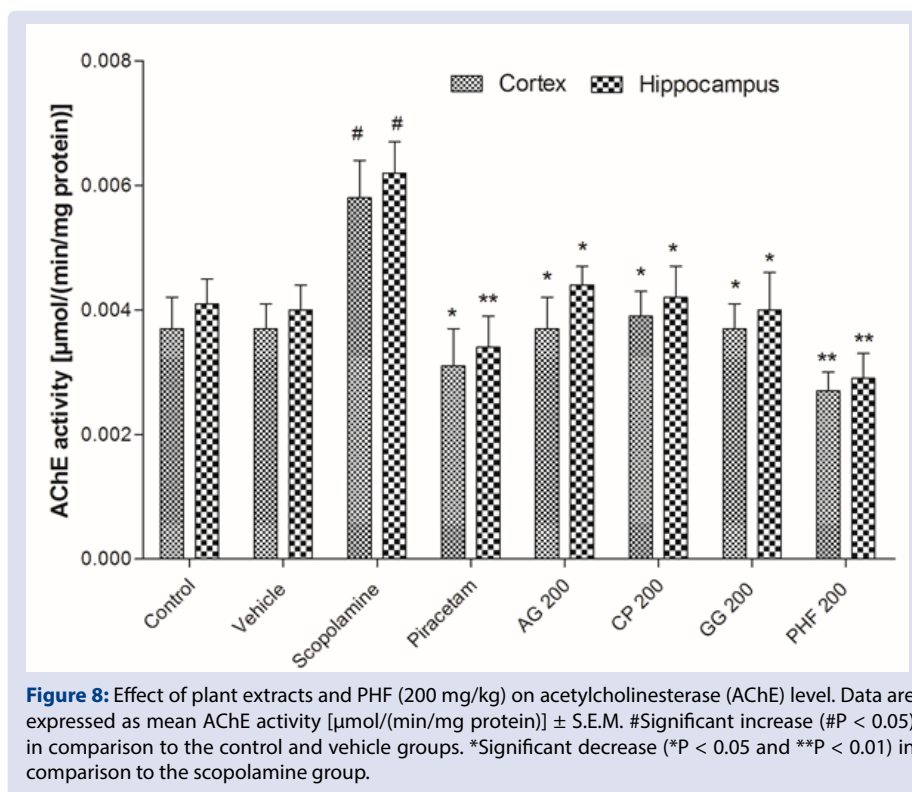


Figure 7: Effect of plant extracts and PHF (200 mg/kg) on the glutathione (GSH) levels. Data are expressed as mean GSH level ($\mu\text{g}/\text{mg}$ protein) \pm S.E.M. [#]Significant decrease ($\#P < 0.05$) in comparison to the control and vehicle groups. ^{*}Significant increase ($*P < 0.05$ and $**P < 0.01$) in comparison to the scopolamine group.



in the learning and memory process of different animal models. These medicinal herbs were selected due to their past India history of traditional uses as a memory enhancer and nervine tonic properties.

It has reported that *Convolvulus pluricaulis* has ameliorated neurotoxicity in the *Drosophila* model and depicted the neuroprotective herb.^[30] In our study, PHF containing CP as one of the herbal drugs attenuated scopolamine-induced amnesia, it is supported by the work of Malik *et al.*³⁸ As scopolamine impairs both short- and long-term memory processes in rodents and humans.³⁹ Researchers also found that CP is an effective neuroprotective herb against the tumor, depression, epilepsy, anxiety and ulcer.^{7,23,27,40,41}

Several behavioral studies have been used neuroprotective drugs against oxidative stress in the brain because the generation of free radicals indicates the progression of cognitive decline.⁴² In this study, PHF reduced AChE activity in both regions of the brain in a dose-dependent manner. Hippocampus consolidates information from short-term to long-term memory and plays a role in forming, organizing and storing memories.⁴³

CONCLUSION

It can be concluded from the study that PHF reduced scopolamine-induced amnesia via cholinergic function improvement, prevention of oxidative damage and behavior enhancement in the brain. Thus, PHF may be a potential candidate to prevent memory deficit in some neurodegenerative diseases.

DECLARATION

The author declares no conflict of interest.

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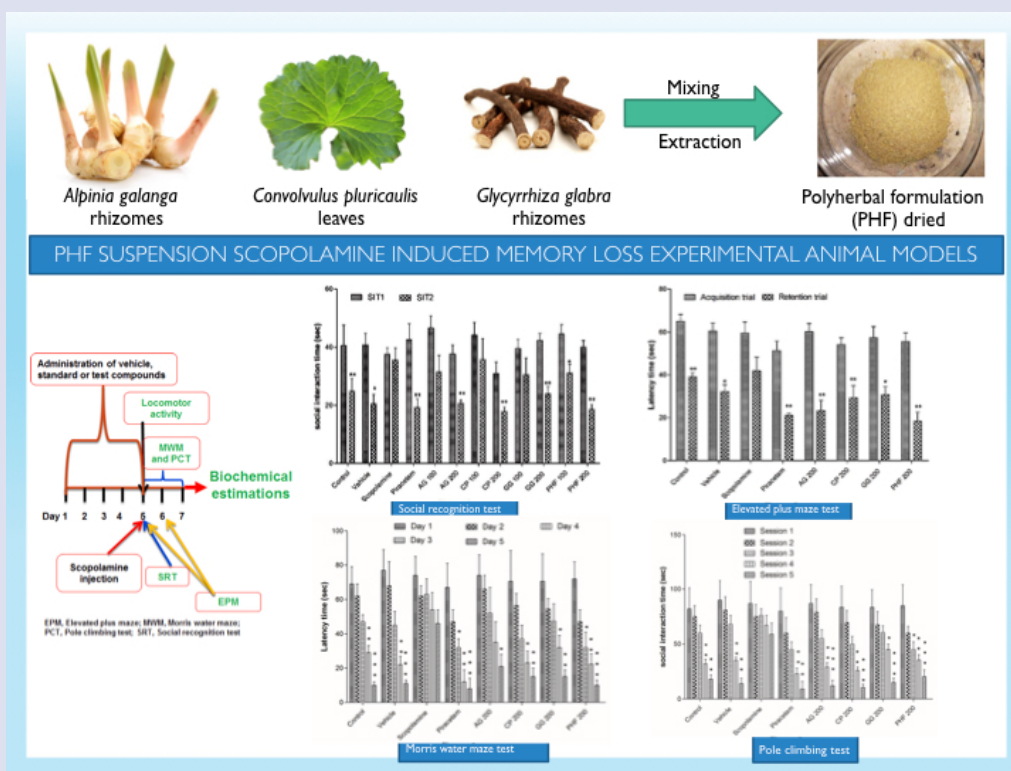
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GRAPHICAL ABSTRACT



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