

# Learning and Memory Enhancing Activity of Polyherbal Formulation on Streptozotocin Induced Memory Impairment in Rats *via* Reducing Mitochondria-Targeted Cytochrome

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## ABSTRACT

**Introduction:** Neurodegenerative diseases and/or brain injury may occur due to mild to severe memory disorders in which Alzheimer's disease (AD) is defined as degradation of neurons and there is no effective therapy or cure for the disease. The intention behind this study was to determine memory enhancing effect of an Ayurvedic polyherbal formulation (PHF) in Streptozotocin-induced memory deficit experimental animal models. **Methods:** Polyherbal formulation (PHF) was a suspension of three plant extracts i.e. *Alpinia galanga*, *Glycyrrhiza glabra* and *Convolvulus pluricaulis* along with other excipients. Different behavioral animal models like Social recognition test (SRT), Morris water maze (MWM) test, Pole climbing test (PCT) and Elevated plus maze (EPM) test were used to evaluate efficacy of Polyherbal formulation (PHF) in learning and memory process of animals. Gene expression was performed by RT-PCR and western blot. **Results:** Results indicated that time of social recognition during trial 2 (SIT2) was reduced significantly ( $P < 0.01$ ) due to PHF administration. In MWM test, treatment with PHF significantly ( $P < 0.01$ ) reduced [F (4, 20) = 6.9] latency to reach hidden platform from session 3. Similarly, PHF was also found effective in pole climbing test and EPM test. RT-PCR results showed p53 regulates the Bcl-2 family proteins its expression in the given experiment was appreciably reduced in PHF (200mg/kg). NR1, NR2B and GAP-43 proteins leads to reduction of brain cell damage. **Conclusion:** Thus, PHF may be an effective formulation for learning and memory process against STZ-induced memory impairment.

**Key words:** *Alpinia galanga*, *Glycyrrhiza glabra*, *Convolvulus pluricaulis*, Pole climbing test, Elevated plus maze, Social recognition test.

## INTRODUCTION

Alzheimer's disease (AD) is defined as degradation of neurons and there is no effective therapy or cure for the disease.<sup>1</sup> It has affected nearly 40 million people around the globe and that is expected to increase in the coming years.<sup>2</sup> People with disturbed cognition is in a transition stage between declining due to aging and other clinical syndromes associated with Alzheimer's disease.<sup>3</sup> Neurodegenerative diseases and/or brain injury may occur due to mild to severe memory disorders, all are linked to acquisition, consolidation and retrieval.<sup>4</sup>

A possible step towards standard therapy for the treatment is to slowdown the progression of disease. Thus, research on disease origination and pathophysiology is required along with the testing of new drugs. The medications for treatment of cognitive impairment may cause various side effects and other consequences. To avoid these undesired effects to drugs, the extensive research has been going on medicinal plants and natural supplements due to their wide history of neuroprotective and brain boosting drugs with no side effects. Medicinal plants have been used in various cognitive improving abilities like memory, motivation, attention, intelligence, and concentration.<sup>5</sup> They

are also referred as nootropic drugs that enhance cognition and maintain healthy emotional state, process of learning and motor control.<sup>6</sup>

Ayurveda as an ancient system of medicine of India has gaining importance worldwide due to its disease preventive and health promoting approach.<sup>7</sup> In the present study, an Ayurvedic Polyherbal formulation was prepared by incorporating three traditional herbs i.e. *Alpinia galanga* (AG), *Glycyrrhiza glabra* (GG) and *Convolvulus pluricaulis* (CP). The above-mentioned herbs were selected due to their uses as folk medicine in cognition enhancing and memory boosting effects. *Alpinia galanga*, it is a well-known medicinal plant containing mild spicy fragrance belongs to family Zingiberaceae.<sup>8</sup> It has been used in the Asian countries for the treatment of oxidative stress, stomachache, diarrhea, ulcer, inflammation, diabetes mellitus, spasm and microbial and insecticidal infection.<sup>9</sup> It is a class of edible group and popular nerve tonic herb in India. *Glycyrrhiza glabra* L. is a Leguminosae plant, possess several bioactive compounds like glycyrrhizin, glycyrrhetic acid, octanoic acid etc. that are responsible for neuroprotective, hepatoprotective, immunomodulatory and anticancer activities.<sup>10,11</sup> *Convolvulus pluricaulis* Linn. is a native plant of India used as a neuroprotective and memory enhancer herb since ancient times.<sup>12</sup> It belongs to family

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Convolvulaceae and used in the treatment of diabetes, ulcer, anxiety, liver diseases and oxidative stress.<sup>13,14</sup>

Therefore, in this study, the memory boosting efficacy of Polyherbal formulation (PHF) was evaluated in different behavioral models with a thought to neurodevelopmental origins in memory deficit disorder such as Schizophrenia, Dementia, Alzheimer's and Autism. In addition, to evaluate the biochemical level of Polyherbal formulation (PHF) in brain by estimating enzymatic levels and level of metabolic enzyme acetylcholinesterase (AChE).

## MATERIAL AND METHODS

### Chemicals and reagents

Donepezil, Streptozotocin, Propyl paraben, Methyl paraben, Tween 80 and Sodium carboxy methyl cellulose were purchased from Sigma Aldrich, USA. All other solvents were of analytical grade and distilled water was used throughout the study. Albino Wistar rats were used for evaluating the effect of poly herbal formulation (PHF) in different experiment models.

### Preparation of formulation made up of herbs

The formulation of herbs was prepared by mixing 0.5014 g, 0.3343 g and 0.3343 g of *Alpinia galanga* (AG), *Glycerrhiza glabra* (GG) and *Convolvulus pluricaulis* (CP) extracts, respectively followed by addition of parabens (Propyl paraben, 0.02%, w/v and Methyl paraben, 0.2%, w/v), tween 80 (0.1%, w/v) and a little amount of sodium carboxy methyl cellulose (0.5%, w/v) then uniformly triturated to form a smooth paste. The paste was rinsed with distilled water (100 ml) and vortexed using mechanical stirrer (500 rpm) to get suspension. The formulation was found stable upto 90 days of storage.

### Experimental design and drug administrations

The effect of *Alpinia galanga* (AG), *Glycerrhiza glabra* (GG) and *Convolvulus pluricaulis* (CP) extracts and their formulation was evaluated in experimental models of memory impairment due to streptozotocin (STZ) administration. The test drug along with standard and vehicle were giving for 14 days. Intracerebroventricular (ICV) streptozotocin (STZ) was given prior to test drug administration.

### Streptozotocin (STZ) induced memory impairment and grouping of animals

The test compounds and standard drug were administered for 14 days and behavioral assessments were carried out from 14<sup>th</sup> day onwards. Animals were anesthetized using ether and STZ (3 mg/kg) was given to animals on 1<sup>st</sup> and 3<sup>rd</sup> day through intracerebroventricular (ICV) route. Injections for intracerebroventricular (ICV) route were made with hypodermic needle that was attached to Hamilton microliter syringe. The needle was perpendicularly inserted to the skull of animals to reach into the brain. Streptozotocin (STZ) was prepared freshly by dissolving into cerebrospinal fluid (CSF) (25 mg/ml) solution. The concentration was adjusted in such a way to deliver only required amount of drug (10 µl) through injection.

Animals were divided into seven groups each containing six animals. Group - 1 Control, Vehicle treated, intra-peritoneal (p.o.); Group - 2 Toxic group, STZ (icv); Group - 3 Donepezil (5 mg/kg, i/p); Group - 4, 5 and 6 received extract of AG, GG and CP, respectively in the dose of 200 mg/kg, p.o.; and Group - 7 and 8 received PHF (200 and 400 mg/kg, p.o., respectively).

### Assessment of learning and memory

#### Social recognition test (SRT)

Social Recognition Test (SRT) was performed on male Wistar rats weighing 220-240 g. Animals memory were impaired by administration

of STZ. STZ was administered on 14<sup>th</sup> day to the test animals. The effect of test drugs were determined by introducing unfamiliar juvenile in the cages of animals as social stimuli. The time recorded as T1 that test animal spent with the juvenile for 5 min interval and then juvenile rat was removed. T2 was recorded after 2 h when same juvenile was reintroduced into their cages. SRT of test animal was determined by significant reduction of in interaction time in trial 2 (T2) as compared to T1. The recognition index (RI) was calculated using following formula:

$$RI = \frac{T_2}{T_1}$$

#### Morris water maze (MWM) test

Morris Water Maze (MWM) test was performed on male Wistar rats weighing 220-240 g. Animals memory were impaired by administration of STZ on 14<sup>th</sup> day of treatment<sup>15</sup>. The arrangement was made with this test by placing the rats in a circular pool with dimensions of 45 × 26 × 20 cm which was filled with water. The pool was marked with four different starting points i.e. N-E-ES-WN. The animals were allowed to stay for 30 s if they were unable to escape themselves from the pool in 120 s. The prior trials were given to each animal; daily four trials were given for five days before placing them into the pool. The effect of test drugs was determined by noting the escape latency time of the animals.

#### Pole climbing test (PCT)

Cook's pole climbing test apparatus was used to study the effect of test compounds on STZ induced memory impaired animals. The apparatus consists of a 25 cm × 25 cm × 40 cm chamber along with dim light and sound box. The electric shock was given to animals by grid floor of the chamber. The animals avoided electric stimulus by jumping onto the pole. This jumping was noted as an escape time of the animals to avoid foot shock. However, jumping on to the pole before shock due to buzzer sound was considered as avoidance. 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. A significant reduction in escape latency time was considered as successful retention of avoidance memory.<sup>16</sup>

#### Elevated plus maze (EPM) test

Elevated Plus Maze (EPM) test apparatus was used to study the effect of test compounds on STZ induced memory impairment animal model. The apparatus consists of two open and two closed arms with dimension of 16 × 5 cm and 16 × 5 × 12 cm, respectively. The apparatus height is such that it is 25 cm above from the floor. The animals were placed on the open arm of the EPM and noted the time taken by animals to move in different arms and the time consume by the animals was termed as transfer latency (TL). TL was assigned 90 s if one of the two arms was pushed gently. The animals were allowed to stay on the EPM for 10 s and then returned to the cages. After the completion of first day trial the memory retention of animals was examined.<sup>17</sup>

### Biochemical estimation

#### Malondialdehyde (MDA) estimation

Malondialdehyde was estimated according to the method of Colado *et al.*, 1997. Briefly, TCA and HCl (5N) were mixed with brain homogenate in which 2% TBA was added. The mixture was then heated at 900°C for 15 min followed by centrifugation for 10 min at 12000 × g. The supernatant was isolated and measured at 532 nm using ELISA plate reader (Biotek, USA). The determined MDA in supernatant was expressed as nmoles/mg of protein. It was calculated using standard curve of Tetra ethoxy propane (TEP).

#### Glutathione (GSH) estimation

Gultathione level in animals were determined according to the method of Sharm and Gupta, 2001. Briefly, trichloroacetic acid was mixed with

brain the homogenate in the ratio of 1:1 followed by centrifugation for 10 min at 12000 × g. The supernatant was separated and mixed (0.01 ml) a mixture containing 5,5-dithiobis (2-nitrobenzoic acid (0.05ml) and phosphate buffer (0.2 ml, pH 8.5) with distilled water (0.04 ml). The mixture was measure at 412 nm after vortexing using ELISA plate reader (Biotek, USA). The determined GSH in supernatant was expressed as µg/mg of protein.

#### Acetylcholine (AChE) activity assay

Acetylcholine (AChE) activity in animals brain was determined according to the method of Das *et al.*, 2001. The activity of AChE was measured at 412 nm using ELISA plate reader (Biotek, USA) and expressed in µmol/min/mg protein.

#### RT-PCR (Reverse Transcriptional Polymerase Chain Reaction) analysis of mRNA Expression

TRIzol reagent was used to extract total RNA from the hippocampal tissue of the animals and cDNA Reverse Transcription Kit was used for reverse transcription. qPCR Master Mix (Thermo Scientific Luminaris) was used for performance of quantitative RT-PCR. The housekeeping gene for mRNA-specific primers were Bcl-2, Bak, Bax and β-actin. Mastercycler ep realplex was used for quantitative RT-PCR reactions for data analysis. The expression of gene after data analysis was calculated according to the method of Livak and Schmittgen.<sup>18</sup>

#### Western Blot Analysis of protein expression

Western Blot Analysis was performed on brain tissues obtained from the hippocampus region of the animals after 24 h of treatment. The tissues were homogenized with the buffer containing NaCl (50 nM/L), EDTA (1 mM/L), SDS (0.5%), Triton X-100 (1%), Tris HCl (20 mM/L) and sodium deoxycholate (0.5%). It was centrifuged for 20 min at 15,000 × g. The samples (50 µg) were ran on polyacrylamide gel and then transferred to PVDF membrane. It was blocked by milk solution (5%) for 2 h. Incubation of membrane was performed at 4°C along with the specific antibodies *i.e.* mouse monoclonal anti-β-actin (1:10000; dilution), NR2B (1:1000, dilution), NR1 (1:1000, dilution), and GAP-43 (1:1000, dilution). The membrane was then incubated with conjugated anti-rabbit IgG (1:10000; dilution) horseradish peroxidase-conjugated goat anti-mouse IgG for a duration of 2 hours at a dilution of 1:2000 (at room temperature) anti mouse at room temperature for 1 h after washing with TBST. Detection was carried out by an enhanced chemiluminescence method and photographs were taken by Biospectrum Gel Imaging System. The data were normalized with the help of GAPDH (objective protein IOD Vs GAPDH protein IOD).<sup>19</sup>

#### Statistical analysis

Results are expressed as mean ± S.E.M. Statistical analysis was carried out by One-way ANOVA and after that dunnett's multiple comparison assay was done. The results were significantly different at \**p*<0.05, \*\**p*<0.01 and \*\*\**p*<0.001 in comparison to control group.

## RESULTS

### Effect of PHF using SRT on memory animals

The effect of PHF was determined on 14<sup>th</sup> day after memory impairment by STZ injection. Results showed that SRT for trial 2 was decreased as compared to trial 1 SRT in treated animals as compared to control group. The standard drug donepezil also prevent STZ induced memory impairment in animals as shown in Figure 1. Treatment with AG, CP and GG extracts at 200 mg/kg each have shown significant reduction in SIT2 (*P*<0.01) in comparison to SIT1 indicating prevention of STZ induced memory impairment. PHF was more effective than individual plant extracts in preventing STZ induced memory impairment. Further,

difference in the recognition index of control, vehicle and STZ group, confirming impairment of memory (Figure 2). The recognition index was significantly lower in donepezil, plant extracts and PHF treated groups in comparison to memory impaired group.

### Memory enhancement effect of PHF using MWM test

A significant decrease in latency time during the 4<sup>th</sup> and 5<sup>th</sup> sessions was observed in control and vehicle groups in comparison to the session 1 (Figure 3). ICV injection of STZ caused memory impairment in rats as shown by no significant reduction in latency time through session 5. Treatment with the standard drug donepezil prevented memory impairment as indicated by a significant reduction in latency time from third session onwards in comparison to the first session. At a dose of 200 mg/kg, plant extracts caused amelioration of STZ induced memory impairment in rats. AG and GG significantly decreased the latency time during the 4<sup>th</sup> and 5<sup>th</sup> session while CP significantly reduced latency time during 5<sup>th</sup> session in comparison to session 1. No significant change was observed between the latency times of session 1 (*P* > 0.05) and session 2 (*P* > 0.05) of all groups (Figure – 3). Results showed that PHF decreased latency for animals to reach the platform from session 3 onwards.

### Effect of PHF on STZ induced memory impairment in pole climbing test

Results showed that there was a significant reduction in the latency time of treated animals on days 4 and 5, whereas in the STZ group, no significant reduction was seen throughout all days. The standard drug donepezil caused significant reduction in the latency time from day 3 onwards indicating prevention of STZ induced memory impairment. Treatment with AG, CP and GG extracts caused amelioration of STZ induced memory impairment as indicated by reduced latency time on day 4 and 5. However, no change was seen between the latency time of day 1 (*P* > 0.05) of all groups (Figure 4). The PHF also significantly reduced latency time from day 3 onwards in STZ-injected rats.

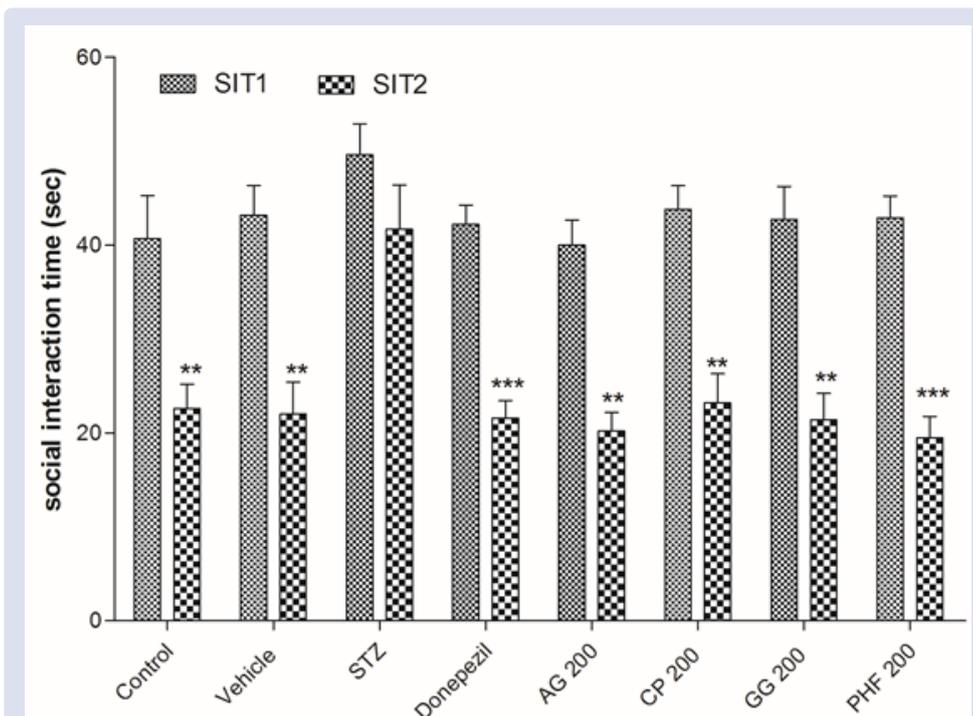
### Effect of PHF on STZ induced memory impairment in EPM test

Memory enhancement effect of PHF was determined using elevated plus maze (EPM) on days 14 and 15. Acquisition trial was given on 14<sup>th</sup> day and retention was studied 24 h after acquisition. As shown in Figure 5, the transfer latency time in retention trial was significantly (*P*<0.01) lower that acquisition trial in control and vehicle groups. However, STZ group showed no significant change in retention latencies in comparison to acquisition trial, indicating impairment in learning and memory. Donepezil at 5 mg/kg significantly reduced latency time during retention trial in comparison to acquisition trial indicating prevention of memory impairment. Treatment with AG, CP and GG extracts caused amelioration of STZ induced memory impairment as indicated by significantly lower latency time in retention trial (Figure 5). The PHF also significantly reduced latency time during retention test in STZ-injected rats.

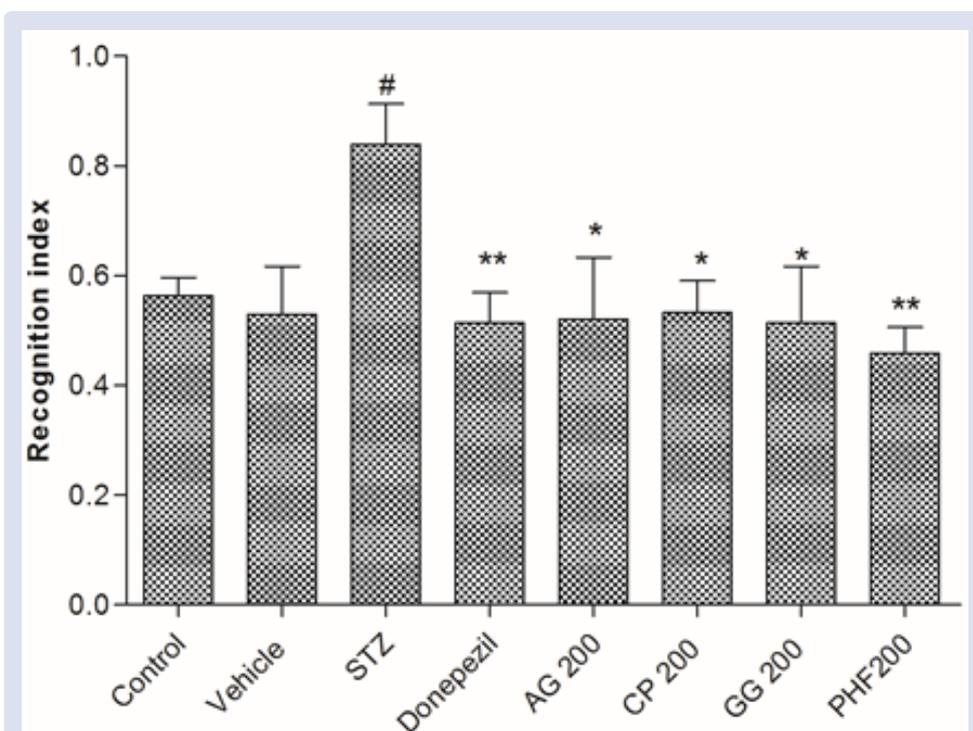
### Biochemical estimations

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

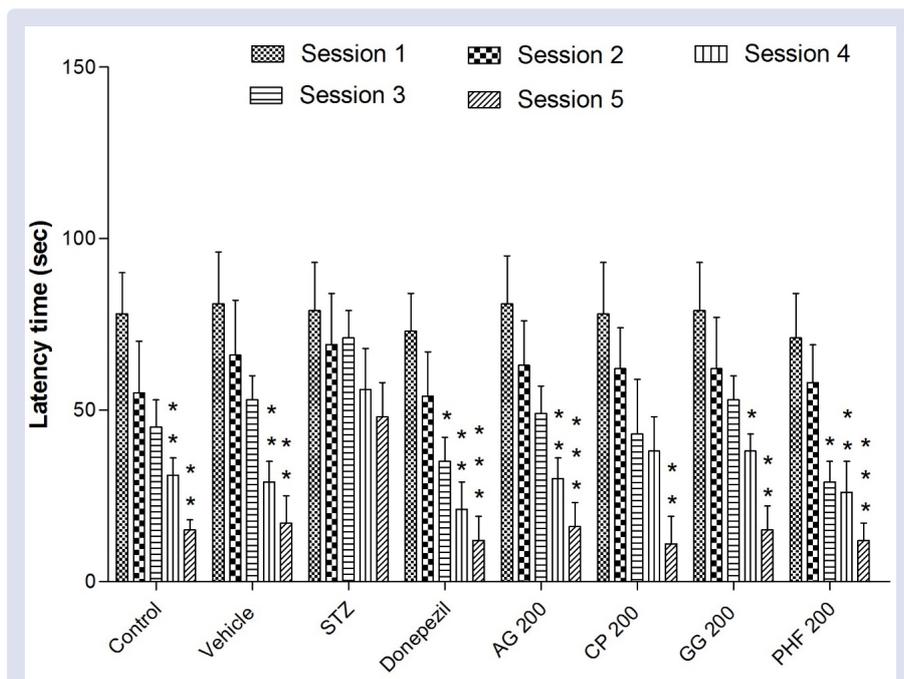
The MDA level was increased in the cortex and hippocampus regions of the memory impaired rats significantly as compared to the control and vehicle groups. Treatment with donepezil reduced MDA levels in both brain regions significantly as compared to STZ group. Administration of 200 mg/kg of plant extracts significantly decreased MDA levels in cortex and hippocampus of STZ-injected rats. PHF treatment also caused significant decrease in MDA level in cortex and hippocampus of STZ injected rat brain (Figure 6).



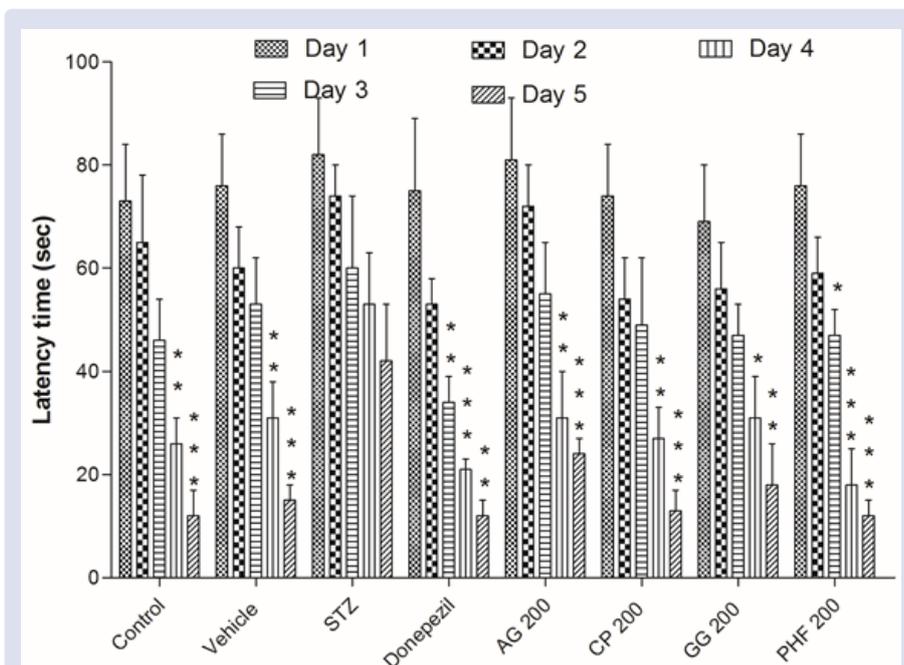
**Figure 1:** Effect of plant extracts and PHF on cognition impaired rats using the social recognition test. Results were presented as mean  $\pm$  S.E.M., significantly different at  $**P<0.01$  and  $***P<0.001$  as compared to trial 1 of the respective group.



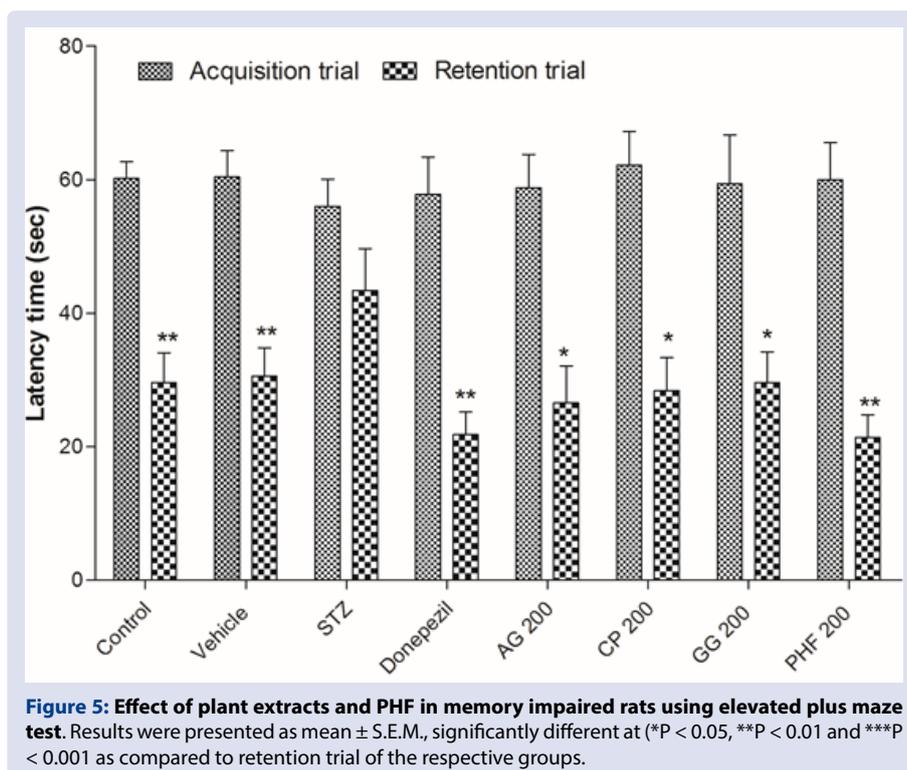
**Figure 2:** Effect of plant extracts and PHF on recognition index of STZ treated animals. Results were presented as mean  $\pm$  S.E.M., significantly different at  $\#P<0.05$  as compared to control and vehicle groups and  $*P<0.05$  and  $**P<0.01$  as compared to the STZ group.



**Figure 3:** Effect of plant extracts and PHF in memory impaired rats using Morris water maze test. Results were presented as mean  $\pm$  S.E.M., significantly different at (\*P < 0.05, \*\*P < 0.01 and \*\*\*p < 0.001 as compared to session 1 of the respective groups.



**Figure 4:** Effect of plant extracts and PHF in memory impaired rats using pole climbing test. Results were presented as mean  $\pm$  S.E.M., significantly different at (\*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001 as compared to day 1 of the respective groups.



#### Effect of PHF on GSH level in memory impaired rats

Effect of PHF was calculated by estimating the level of GSH in brain regions of rat using the calibration curve obtained with different concentration of glutathione. STZ administration caused reduction in GSH levels in brain regions as compared to control and vehicle groups. As shown in Figure 7, donepezil prevented reduction in GSH level in cortex and hippocampus regions significantly (P<0.01). Administration of plant extract prevented STZ induced reduction in GSH levels. AG, CC and GG extracts increased GSH levels significantly in STZ treated rat brain regions.

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

Results exhibited that administration of STZ caused increase in the AChE activity in both cortex and hippocampus regions of treated animals. Preventive treatment with donepezil significantly inhibited AChE activity in cortex (P<0.05) and hippocampus (P<0.01) of STZ injected rats (Figure 8). Administration of plant extract prevented elevation of AChE activity. AG, CC and GG extracts significantly decreased AChE activity in impaired memory rats. There was significant decrease in the AChE activity in brain regions of the rats treated with 200 mg/kg PHF (Figure 8).

#### Effect of PHF on m RNA expression and protein expression

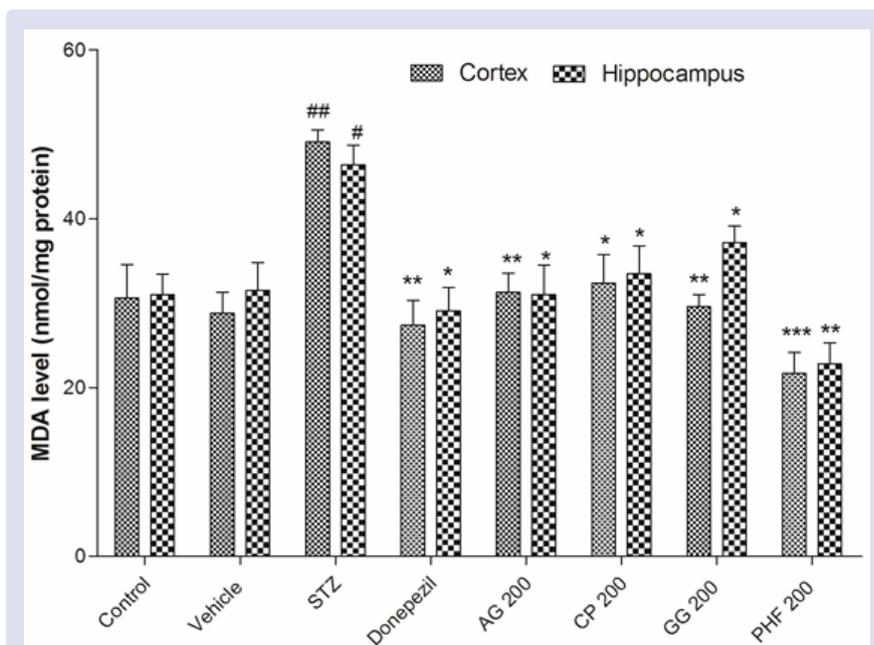
Expressions of Bcl-2, Bak, and Bax mRNA are demonstrated in for excluding the variations due amount and nature of RNA, the results recorded were adjusted according to the expression of GAPDH. Hippocampal tissue in STZ group was indicated by considerably amplified the levels Bak & Bax and significantly declined the levels of Bcl-2. Their levels were appreciably inverted in PHF (200mg/kg). PHF 200 group showed best results which were almost comparable to STZ group. In comparison to STZ group, PHF (200mg/kg) demonstrated more significant effect in up-regulation of Bcl-2 protein and down-regulation of p53 protein. In mitochondrial pathway cell apoptosis chiefly involves

the Bcl-2 g. Bcl-2 and Bax (both are belong toBcl family) controls the secretion of proapoptotic factors from mitochondria. In the present study, Bax and Bak mRNA (proapoptotic) were down-regulated, while Bcl-2 mRNA and protein which are antiapoptotic were up-regulated in PHF (200mg/kg) group opposite to STZ group. Also, p53 regulates the Bcl-2 family proteins its expression in the given experiment was appreciably reduced in PHF (200mg/kg) group opposite effect was recorded in STZ group. These findings indicated that PHF could exert its memory enhancement by interacting with these proteins (Figure 9).

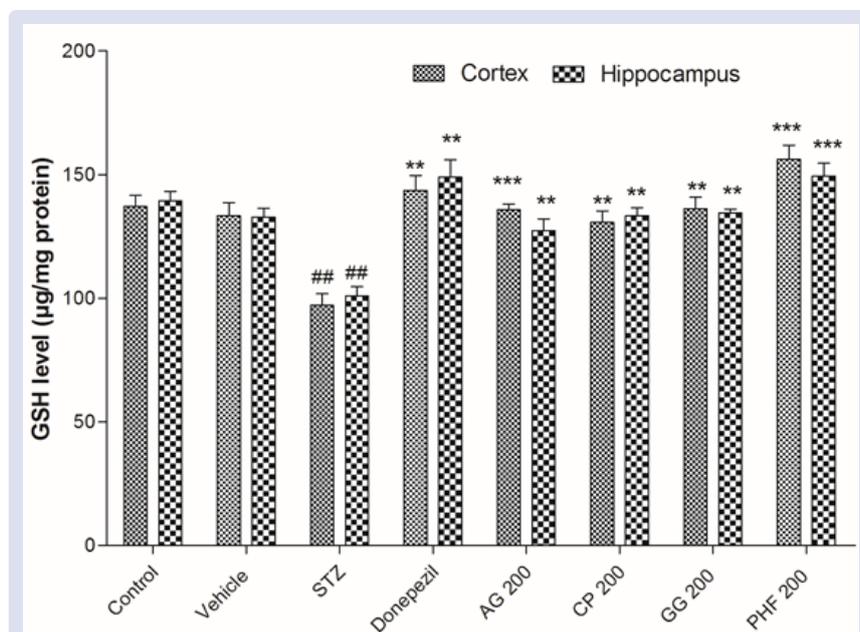
Western blot analysis was carried out for recording the memory enhancer activity of PHF (200 mg/kg). NR1, NR2B, and GAP-43 levels in hippocampus of amnesic rats were significantly lower as compared to control group. Conversely, PHF groups showed significantly higher NR1, NR2B and GAP-43 protein levels as compared to STZ treated group. As shown in Figure 10 protein expressions of NR1 and NR2B were augmented by almost two times and five times in the hippocampus of mice of STZ group as compared to the mice of control group, whereas their expressions were appreciably reduced in PHF mice. NR1 expression was amplified by almost six-folds in sSTZ treated mice while PHF treated mice partially prevented this effect. Majorly two pathways are involved in cell apoptosis (death receptor pathway and mitochondrial pathways). Reported findings say that, interaction of death receptor and its ligand e.g., interaction of NR1/NR2B, are important for initiating apoptosis (extrinsic pathway). Prior studies have shown that by suppressing NR1,NR2B and GAP-43 proteins leads to reduction of brain cell damage. PHF 200mg/kg showed a significant effect by shielding against STZ induced brain damage.

## DISCUSSION

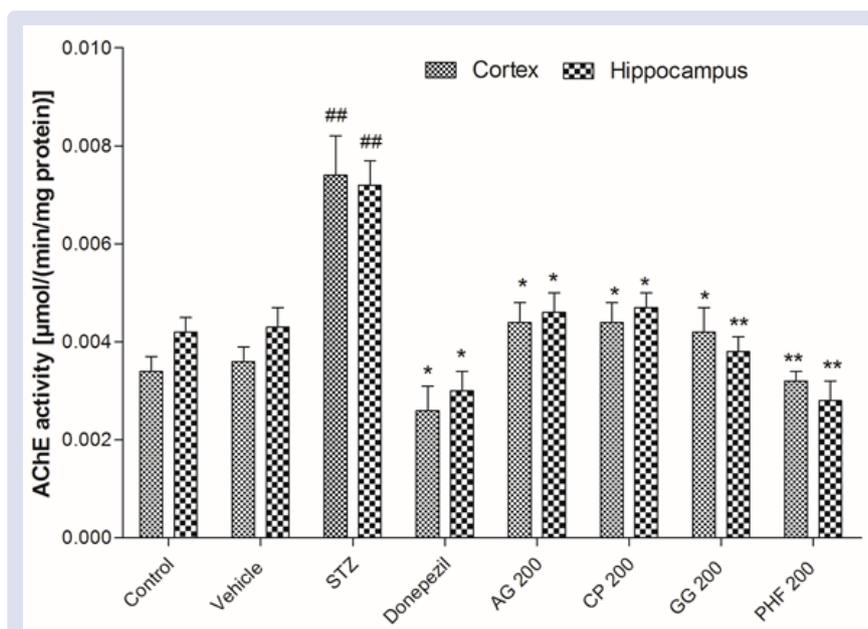
Pathogenesis of cognition impairment starts from temporal lobe or dysfunction of prefrontal part of brain. It can be ranging from mild cognitive impairment to dementia.<sup>20</sup> Learning and memory processes are based on electro-chemical signaling, acts as a network within the brain. The signaling may occur through biogenic monoamines, acetylcholine, amino acid, neuropeptides and other gene families.<sup>21</sup> Memory formation in the brain can be divided into different consecutive



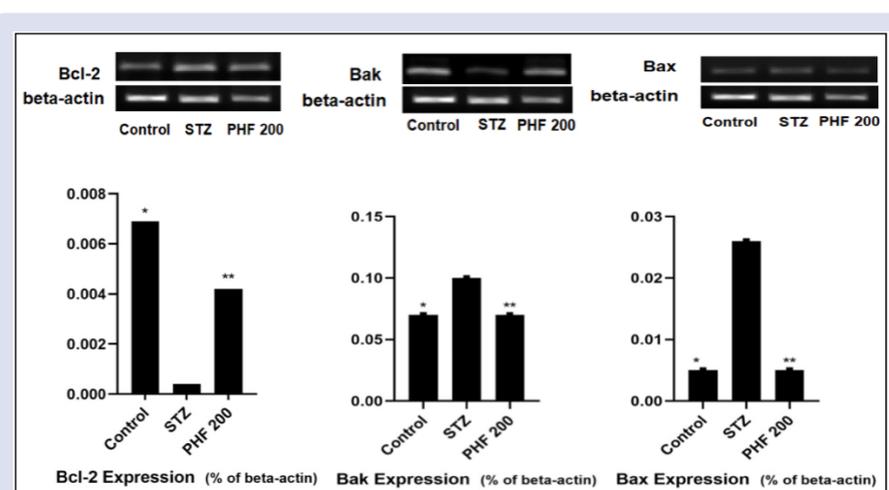
**Figure 6: Effect of plant extracts and PHF (200 mg/kg) on the malondialdehyde (MDA) levels in animals.** Results were presented as mean  $\pm$  S.E.M., #Significant difference ( $\#P < 0.05$  and  $\#\#P < 0.01$ ) in comparison to the respective brain region of control and vehicle groups and \*Significant difference ( $*P < 0.05$ ,  $**P < 0.01$  and  $***P < 0.001$ ) in comparison to the respective brain region of STZ group.



**Figure 7: Effect of plant extracts and PHF on the glutathione (GSH) levels in animals.** Results were presented as mean  $\pm$  S.E.M., significantly different at  $\#P < 0.05$  and  $\#\#P < 0.01$  as compared to control and vehicle groups and significantly different at  $*P < 0.05$ ,  $**P < 0.01$  and  $***P < 0.001$  as compared to STZ administered group.



**Figure 8: Effect of plant extracts and PHF on acetylcholinesterase activity in animals.** Results were presented as mean  $\pm$  S.E.M., significantly different at #P < 0.05 as compared to control and vehicle groups and significantly different at \*P < 0.05 and \*\*P < 0.01 as compared to STZ administered group.

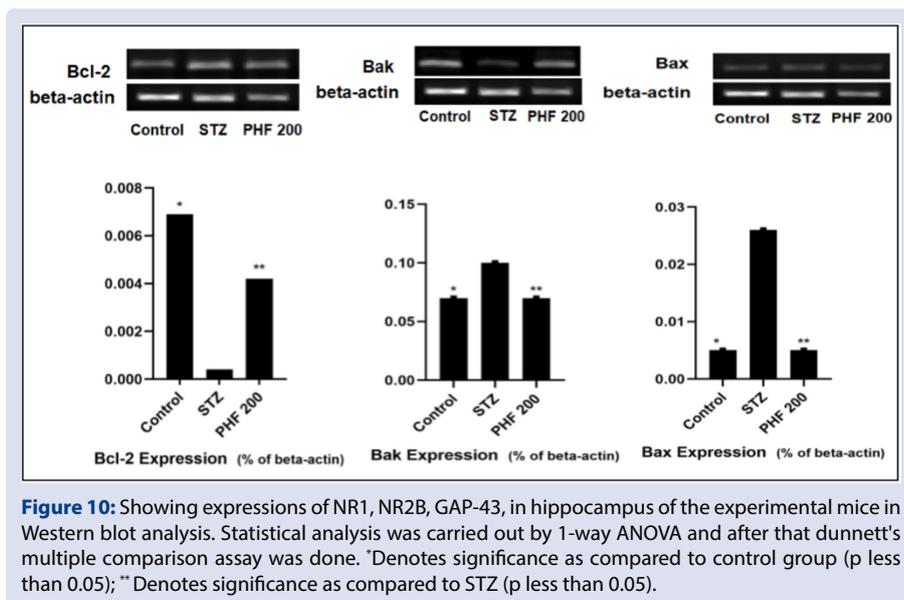


**Figure 9:** Showing protein expressions of Bcl-2, Bak, and Bax in hippocampus of the experimental mice in RT-PCR analysis. Statistical analysis was carried out by 1-way ANOVA and after that dunnett's multiple comparison assay was done. \*Denotes significance as compared to control group (p less than 0.05); \*\* Denotes significance as compared to STZ (p less than 0.05).

phases such as acquisition, consolidation, retrieval and in some condition, reconsolidation or extinction phases.<sup>22</sup> The acquisition phase in the learning of new behavior, consolidation phase is the stabilization or storage of earned memory, retrieval phase is accessing the stored memory, reconsolidation is re-evaluation of prior gained memory and extinction phase is inhibition of stored memory.<sup>23</sup> Serial acquisition of memory is required for learning new information, however, memories are also acquired from past knowledge. Generalization and interference are the memory slots that are produced due to interaction of two different memory traces from behavioral point of view.<sup>24</sup>

The Ayurvedic Polyherbal formulation (PHF) was used for memory enhancement in experimental behavioral animal models due to wide utilization of selected folk medicines and phytotherapy as well. The protocol of the study was designed to evaluate efficacy of these herbs

(*A. galanga*, *G. glabra* and *C. pluricaulis*) in the form of PHF as brain tonic against mental illnesses with a thought to be an effective herbal supplement for neurological disorders. Herbal approach has offered several opportunities to produce new drugs for memory disorders<sup>25</sup> In this cognitive behavioral study, the PHF was found effective in boosting learning and memory process of animals in different behavioral models i.e. SRT, MWM, PCT and EPM. Results exhibited that STZ losses animal memory while testing in different behavioral models. It has reported that STZ mimics biochemical parameters in animals.<sup>26</sup> PHF was found effective in reducing STZ induced toxicity (learning and memory deficit) in animal models along with restoring biochemical enzymatic levels of MDA and GSH. PHF was also found effective in reducing metabolic enzyme activity of acetylcholinesterase. The test drug enhances the level of acetylcholine in the animal brain. In several



behavioral studies the neuroprotective drugs aimed at preventing the production of free radicals in the brain. Production of free oxidative radicals indicate progression of cognitive decline.<sup>27</sup> PHF reduced AChE activity at both cortex and hippocampus regions of the brain in a dose-dependent manner. Our findings were matched with the work of *Parfitt et al., 2012*, that hippocampus consolidate information from short-term memory to long-term memory and plays role in forming, organizing and storing memories.<sup>28</sup>

Bcl2, Bax and Bcl-2 mRNA expression in PHF treated group was significant as compared control and STZ groups. Pro-apoptotic function in cells is showed by Bax and Bak<sup>18</sup>, however, Bcl-2 is anti-apoptotic in function. Bcl-2 expression in inversely proportional to apoptosis rate that causes cell apoptosis. Present study showed expression of Bak, Bax and Bcl-2 in brain, however the mechanism of expression is yet to be investigated. The western blot technique was used to evaluate NR1 and NR2B expression. Results showed that PHF increased NR1 and NR2B expression. It has reported that NMDA receptor plays immense role in plasticity of synapse as seen in learning and memory. GAP-43 plays role in regulation of growth, neurite outgrowth and synaptic plasticity. The reduction of GAP-43 level in brain is seen due to STZ administration. It was raised by PHF and it may be attributed to recovery of synaptic plasticity in the brain.

## CONCLUSION

It can be concluded from the study that PHF alleviated amnesia in STZ treated rats by improving cholinergic function, reduction of oxidative stress and enhancement of behavior. Thus, PHF may be an alternative compound for the treatment of memory loss memory deficit cases.

## DECLARATION

Author declare no conflict of interest.

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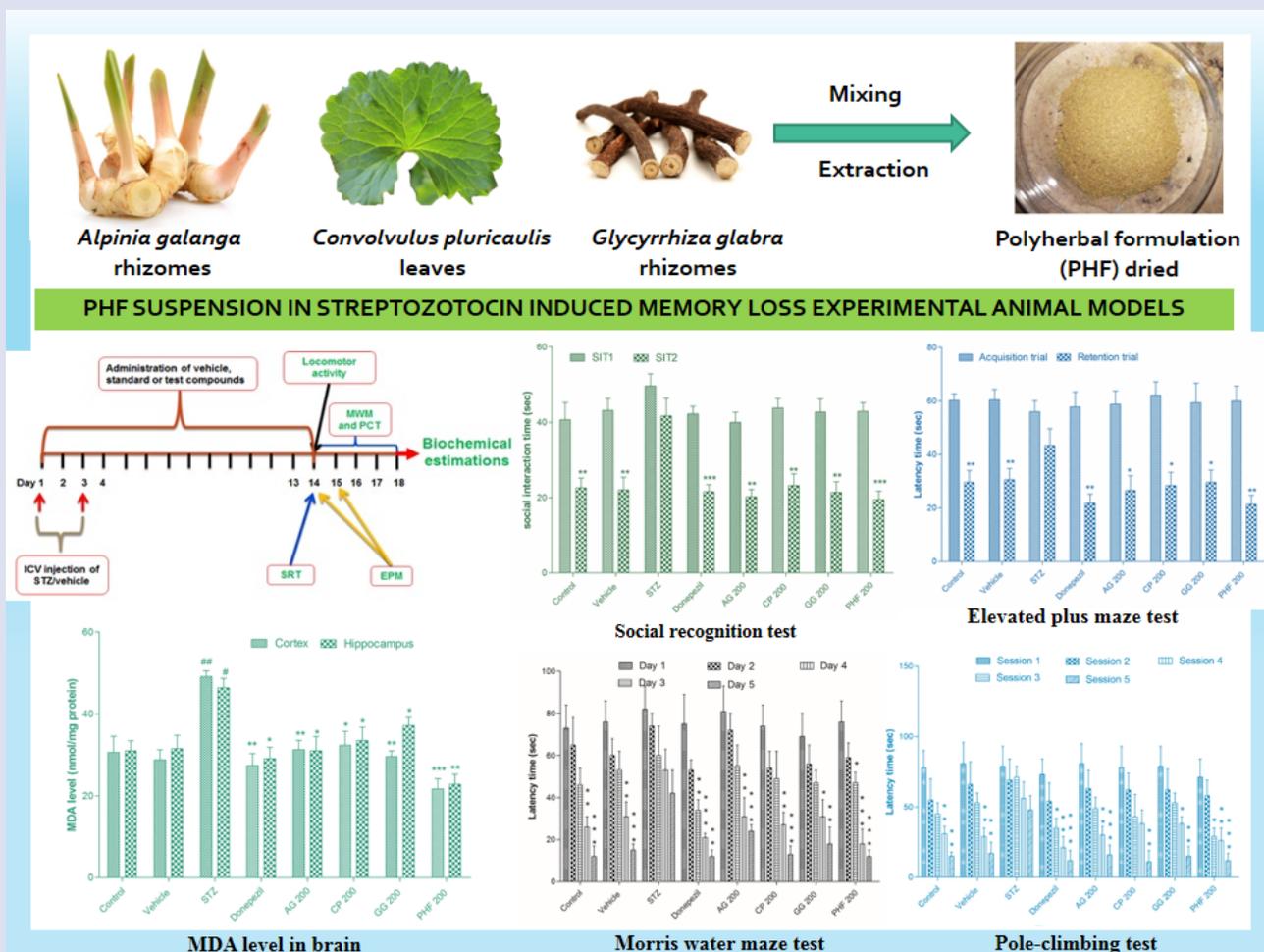
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## GRAPHICAL ABSTRACT



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