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Research Article

MEMORY ENHANCING ACTIVITY OF POLYHERBAL FORMULATION MEDHYA RASAYANA IN SCOPOLAMINE INDUCED AMNESIA IN RAT

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ABSTRACT

Objective: Memory enhancing activity of polyherbal formulation Medhya Rasayana in scopolamine induced amnesia in rats using morris water maze and pole climbing models. **Material and Methods:** About 48 male rats (200-300gm) were randomly divided into six groups and each group contains four rats. the six groups are: **Control group**, **Diseased group** (scopolamine 0.7 mg/kg i.p. treated group), **Positive group** (piracetam 200mg/kg p.o. treated group), **Scopolamine plus piracetam group** (Scopolamine 0.7 mg/kg i.p. plus piracetam 200 mg/kg p.o. group), **Test dose 1 group** (scopolamine plus Medhya Rasayana 100 mg/kg treated group), **Test dose 2 group** (scopolamine plus piracetam 200 mg/kg treated group 200 mg/kg). **Results:** The result of the effect of administration of Medhya Rasayana on scopolamine induced memory deficits in the morris water maze and pole climbing model were evaluated in rats. The effect of control treated, scopolamine treated (0.7 mg/kg i.p.), piracetam (200 mg/kg p.o.) and Medhya Rasayana at doses 100 mg/kg p.o. and 200 mg/kg p.o. body weight, were evaluated at regular 5 days of treatment. The transfer latency on the acquisition as well as on the retention days was found increased at 123±2.8099 sec on 5th day. The latency time of control group is 39.25±2.8099 sec on 1st day and 32±2.1602 sec on 5th day. The effect of Medhya Rasayana after continuously 5 days administration was observed and compared with scopolamine treated group and piracetam treated groups. The decrease in latency period in compared to scopolamine treated group was observed at dose of 200 mg/kg as compare to 100 mg/kg dose of Medhya Rasayana. **Conclusion:** Our data suggested that Medhya Rasayana of 200 mg/kg shows better result in comparison to Medhya Rasayana 100 mg/kg.

Keywords: Medhya Rasayana, scopolamine, piracetam, morris water maze, pole climbing model.

INTRODUCTION

In today's life of stress and strain, there is a dire need for agents having neuroprotective and neurophamacological activity enhancing learning and memory function of the brain. Importantly stress is also known to interfere with cognitive functions, tending to retard the memory rather than the acquisition of learning (Thakur et al 2005). Formation of memory is the most complex process and involves multiple neuronal pathways and neurotransmitters. It is well known that the cholinergic neuronal system plays an important role in learning and memory in humans as well as animals (Kulkarni et al 2010). Based on experimental and clinical evidences, Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions. Besides the neuro pathological hallmark of the Alzheimer's disease, neurofibrillary tangles and neuritic plaques, Alzheimer disease is

characterized by a consistent deficit in cholinergic neurotransmission particularly in basal forebrain. The therapeutic strategies to combat miseries of cognitive disorder have been aimed to improve acetylcholine activity. Therefore, the cholinergic receptors agonists (muscarinic and nicotinic) and enhancers of endogenous level of acetylcholine (synthesis promoters and inhibitors of its metabolizing enzymes) have been tried to treat senile dementia of Alzheimer's type. Cholinesterase inhibitors are the only class of compounds consistently proven to be efficacious in treating the cognitive and functional symptoms of Alzheimer's disease. Blockade of central muscarinic acetylcholine receptors disrupts learning and memory functions in animals as well as human beings. Anti- cholinergic drugs (muscarinic blockers) such as scopolamine have been in use as potent amnesic agents. Scopolamine induced cognitive deficit in young volunteers is similar to that occurring in senile, demented subjects when tested on same clinical battery. These studies highlight the importance of administration of drug in relation to stages of memory process. There are three basic activities associated with memory process – acquisition, consolidation and recall (Rahul et al 2009). The decrease in neurotransmitter formation and increased oxidative stress that comes with aging effect brain functions such as memory, especially recognition, and causes dementia. Acetylcholine is the first substance to be proven to be neurotransmitter and has an important role in the enhancement of sensory perception when we wake up and in sustaining attention. Acetyl cholinesterase is an enzyme that degrades the neurotransmitter acetylcholine, producing acetate and choline group. An overall decrease of acetyl cholinesterase in the brain of people with Alzheimer's disease has been observed. Thus, natural anti dementia material that can alter the disease progression, has been of great interest to people (Kim et al 2010). Recent studies indicate that nitric oxide modulates synaptic transmission in both central and peripheral nervous system by acting as a retro gate messenger. Further, we also examined the role of nitric oxide in the ameliorative effects of Neurosteroids on the aging induced cognitive dysfunction in aged rats (Reddy et al 1998). Effect of stress on hippocampal cholinergic system varies with the strain of rat. Inconsistent and varied changes have been reported in the level of brain acetylcholine, uptake of choline, receptor activity, activity of choline acetyl transferase and acetyl cholinesterase, following stress. Central cholinergic system particularly in hippocampus plays an undisputed key role in regulation of learning and memory, which are the key constituent of cognitive behavior. Acetyl cholinesterase enzyme enjoys a unique status, as its inhibition is the most important therapeutic means to achieve cognitive improvement in the patients of dementia at present. Outcome of certain behavioral responses depends upon the type of stress applied (Dasi et al 2005). The administration of neurotropic factors can prevent neuronal cell death during degeneration and enhanced repair mechanism after the damage (Turner et al 1996). The hippocampal formation is a key cortical structure involved in a number of normal physiological processes, including learning and memory. Glutamate is the primary excitatory neurotransmitter in the hippocampus and glutamate receptors have been implicated in the formation and retrieval of memory. Previously, we reported that cyclothiazide acts at the positive allosteric modulatory site on AMPA receptors to selectively potentiate AMPA induced release of [^{3}H] nor epinephrine from rat hippocampal slices. This effect of cyclothiazide is likely mediated by inhibition of AMPA receptor desensitization (Desai M.P. et al 1995). The mosy fiber-CA3 pyramidal system which is one of the 3 major synapsis in the hippocampal formation plays a role in learning. For example, lesions in the CA3 region have been reported to impair special memory acquisition Ishihara Kumatoshi et al 1997). The Indian system of medicine is replete with medicinal plants claimed to promote learning, memory and intelligence. The plants like *Acorus calamus, Bacopa monniera, Tinospora cordifolia* and *Withania somnifera* have been investigated for their effect on cognitive function of the brain. These plants have been grouped under the general class of Medhya rasayanas that is substance/agents that counter the degenerative changes associated with aging and are beneficial in promoting intellect (Sharma et al 1981).

Ayurvedic System of Medicine has mentioned several naturally occurring medicinal preparations under the category 'Medhya'. By virtue of inducing mental upliftment as major influence several medicinal plants mentioned as 'Rasayana drugs' in Ayurveda are primarily claimed as 'Medhya'. Further there is a special class of some Rasayana drugs called 'Medhya Rasayana' which is supposed to be having specific influence on higher brain functions.

Medhya Rasayana is a group of medicinal plants described in Ayurveda (Indian system of medicine) with multi-fold benefits, specifically

to improve memory and intellect by Prabhava (specific action). Medha means intellect and/or retention and Rasayana means therapeutic procedure or preparation that on regular practice will boost nourishment, health, memory, intellect, immunity and hence longevity. Medhya Rasayana is a group of 4 medicinal plants that can be used singly or in combinations.

They are Mandukaparni (*Centella asiatica* Linn.), Yastimadhu (*Glycirrhiza glabra*), Guduchi (*Tinosporacordifolia* and Shankhapushpi *(Convolvulus pleuricaulis*), specially mentioned with wide range of applications on different systems. Yet in practice few more handful drugs used with same aim are mentioned elsewhere in the Ayurveda classical textbooks. They are Aindri *(Bacopa monniera),* Jyothishmati *(Celastrus panniculata),* Kushmanda *(Benincasa hispida),* Vacha *(Acorus calamus)* and Jatamamsi *(Nardostachys jatamamsi).* Medhya Rasayana is used either in polyherbal preparations or alone. This paper is an attempt to present update on these drugs. Evidences used are mostly facts from researches on animal model or on bioactive principles with some of preclinical works on human system

METHODOLOGY

Collection of Medhya Rasayana

The sample of **Medhya Rasayana** were collected from local market.

Animals

The Nootropic activity was carried out on Wistar albino rats of either sex (180-220 g), were purchased from animal house of Central Drug Research Institute, Lucknow. They were maintained in 24 h light/dark cycle at 25±2 ^{o}C. They were allowed to standard pellet diet and water, ad libitum. The study was approved by Institutional Animal Ethics Committee (IAEC) according to the regulation of Committee for the purpose of Control and Supervision of Experimental Animal (CPCSEA) and ethical norms was strictly followed during all experimental procedure.

Drugs/Chemicals

Scopolamine hydrobromide (Sigma Aldrich,USA) and piracetam (Nootropil® , UCB India Ltd, Vapi, Gujrat) were diluted in normal saline and administered peritoneally. Volume of administration was 1ml/100gm/body weight. All the drugs were administered orally in the morning session i.e. 8 AM-9 AM on each day.

PHARMACOLOGICAL EVALUATIONS

The pharmacological evaluation of nootropic activity was done by using various extroceptive behavioral models.

Morris water maze Test (Russo *et al* 2005)

The nootropic activity of the drug in this model was assessed by its ability to use as potent nootropic agent and protect against Scopolamine induced amnesia.

Animals were first weighed and then divided into six groups of 4 rats in each groups.

The rats were trained on the standard Morris navigation task in a black water tank. The rats were started from 4 different, randomly chosen start positions and trained to find an invisible platform (Diameter 11 cm) that was at a fixed position in the water tank 1 cm below the surface of the water. The temperature of the water was 20-22 ^{o}C. A trial lasted until a rat had found the platform. If a rat did not find the platform within 300 seconds it was placed on the platform for 30 seconds and then removed from the water tank. On the first to fifth day rats were given 2 trials per day on consecutive days. Before administering the Scopolamine, standard (piracetam), and test drug total 10 trials were given to rats in 5 days of training.

Pole climbing method Test (Joshi *et al* 2006)

Passive avoidance behavior based on negative reinforcement was used to examine the long term memory. The apparatus consists of a box (27cm× 27cm × 27cm) having three wall of wood and one wall of Plexiglas, featuring a grid floor (made up of 3mm stainless steel rod set 8 mm apart), with a wooden platform (10cm × 7cm × 1.7cm) in the center of grid floor. The box was illuminated with a 15 watt bulb during the experimental period. Electric shock (20 volt A.C.) was delivered to the grid floor. Training was carried out in 2 similar sessions. Each mouse was gently place on the wooden platform set in the center of the grid floor. When the mouse stepped down, placing all its paws on the grid floor, shocks were delivered for 15 second and the step down latency, which was defined as the time (in seconds) taken by the mouse to step down from the wooden platform to grid floor with all its paws on the grid floor was recorded. Animals showing step down latency in range of 2 to 15 seconds during the first test were used for the second session and the retention test. The second session was carried out 90 minute after the first test. During second session, if the animals stepped down before 60 seconds electric shock was delivered for 15 seconds. During the second test, animals were removed from the shock

free zone, if they did not step down for a period of 60 seconds and was subjected to retention test. Retention was tested after 24 hours in a similar manner, except that the electric shock was not applied to the grid floor observing an upper cut off time of 300 seconds. Significant increase in step down latency period value indicates improvement in the memory.

The experiment was performed in different steps-

1. **Familiarization:** The animal was placed on the platform, after 10 second of exploration; it is return to the home cage.
2. **Learning (acquisition):** An unavoidable foot shock is applied once (foot shock; 50 Hz; 1.5 mA) and the animal was allowed to jump on the pole, and then returned to the home cage.
3. **Retention test:** 24 hours after the learning trials the animal was again placed on the platform and the time taken by the animal to jump on the pole (latency) was measured.

The nootropic activity of the drug in this model was assessed by its ability to use as potent nootropic agent and protect against Scopolamine induced amnesia.
Animals were first weighed and then divided into six groups of 4 rats in each groups.
Group 1: Control Group: It represented the control group (n=4). This group received normal diet and vehicle.
Group 2: Disease control Group: It represented the positive control group (n=4). This group received Scopolamine (0.7 mg/kg i.p.) from 6th to 10th day and latency time was noted after 45 min. administration of Scopolamine.
Group 3: Positive control Group: It represented the standard group (n=4). This group received Piracetam 200 mg/kg (p.o.) from 6th to 10th day and latency time was noted after 45 min. administration of Piracetam.
Group 4: Scopolamine + Piracetam Group: It represented the standard group (n=4). This group received Scopolamine (0.7 mg/kg i.p.) and piracetam (200 mg/kg p.o.) from 6th to 10th day and latency time was noted after 45 min. administration of Scopolamine and Piracetam.
Group 5: Test Dose 1 Group: It represented the Medhya Rasayana group (n=4). This group received Scopolamine (0.7 mg/kg i.p.) and Medhya Rasayana (100 mg/kg p.o.) from 6th to 10th day and latency time was noted after 45 min. administration of Scopolamine and Medhya Rasayana.
Group 6: Test Dose 2 Group: It represented the Medhya Rasayana group (n=4). This group received Scopolamine (0.7 mg/kg i.p.) and Medhya Rasayana (200 mg/kg p.o.) from 6th to 10th day and latency time was noted after 45 min. administration of Scopolamine and Medhya Rasayana.

BIOCHEMICAL ESTIMATION

1. Acetyl cholinesterase estimation (Mani Vasudevan *et al* 2007)
The pharmacological estimations was done by estimating the acetylcholine level in the brain by quantifying cholinesterase inhibition. After accessing the learning and memory paradigm in scopolamine induced amnesia, rats from each group were euthanized by cervical decapitation. The whole brain was immediately removed and chilled in ice cold phosphate buffer. After washing in ice cold phosphate buffer the brain were homogenized in 5 ml of phosphate buffer in glass Teflon homogenizer. The brain homogenate was than evaluated for enzyme activity using augistinssons method of analysis.

2. MDA Estimation (Dhir Ashish *et al* 2007)
The method is also called as lipid peroxidation. The quantitative measurement of lipid peroxidation in brain was performed according to the method of wills. The amount of malondialdehyde (MDA) an indicator of lipid peroxidation was measured by reaction with thiobarbituric acid at 532 nm, using spectrophotometer.

3. GSH or Reduced Glutathione Estimation (Dhir Ashish *et al* 2007)
Reduced glutathione (GSH) in brain was estimated according to method described by Ellman. A 1 mL supernatant was precipitated with 1 mL of 4% sulfosalicylic acid and cold digested at 4 ^{o}C for 1 hrs. The samples was centrifuged at 12000g for 15 minutes at 4 ^{o}C. To 1 mL of this supernatant, 2.7 mL of phosphate buffer (0.1 M, pH 8) and 0.2mLof 5, 5, Dithio-Bis- (2-Nitro Benzoic acid) (DTNB) were added the yellow color was developed is read immediately at 412 nm at spectrophotometer.

4. Protein estimation (Lowry *et al* 1951)
Protein concentration as estimated by the method of Lowry's. Different dilutions of Bovine serum albumin (BSA) solutions was prepared by mixing stock BSA solution (1 mg/ ml) and water in the test tube. The final volume in each of the test tubes was 5 ml. The

BSA range is 0.05 to 1 mg/ ml. From these different dilutions, 0.2 ml protein solution was pippeted out to different test tubes and 2 ml of alkaline copper sulphate reagent was added (analytical reagent). The solutions were mixed well. This solution was incubated at room temperature for 10 mins. Then 0.2 ml of reagent Folin solution (reagent solutions) was added to each tube and incubated for 30 min. Set the colorimeter with blank with zero and the optical density (measure the absorbance) was taken at 660 nm.

RESULTS

Table 1: Estimation of Latency Time (in second) morris water maze

	Day 1	Day 2	Day 3	Day 4	Day 5
Control	39.25±2.809	39.25±2.926	35.5±1.848	34.5±2.101	32±2.160
Scopolamine 0.7 mg/kg i.p.	132.5±8.539	136.5±6.849	134±6.480	126.25±5.297	123±5.066
Piracetam 200 mg/kg p.o.	42.5±2.783	37.5±2.629	33.5±1.848*	29.25±1.493$^{\alpha}$	24±1.354$^{\#}$
Scopolamine + Piracetam 200 mg/kg p.o.	104.5±6.980	89.5±3.068	77±2.857$^{\alpha}$	63.25±4.534$^{\#}$	46.25±1.750$^{\#}$
Scopolamine + MR 100 mg/kg p.o.	118±4.546	113.75±5.7	102.5±3.796	87.5±3.227$^{\#}$	75.5±2.101$^{\#}$
Scopolamine + MR 200 mg/kg p.o.	85.5±4.272	75±3.000	62.25±4.905$^{\alpha}$	53.5±4.051$^{\#}$	43.75±1.750$^{\#}$

All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test) Where, *p < 0.05; $^{\alpha}$ p<0.001; $^{\#}p$ < 0.0001.

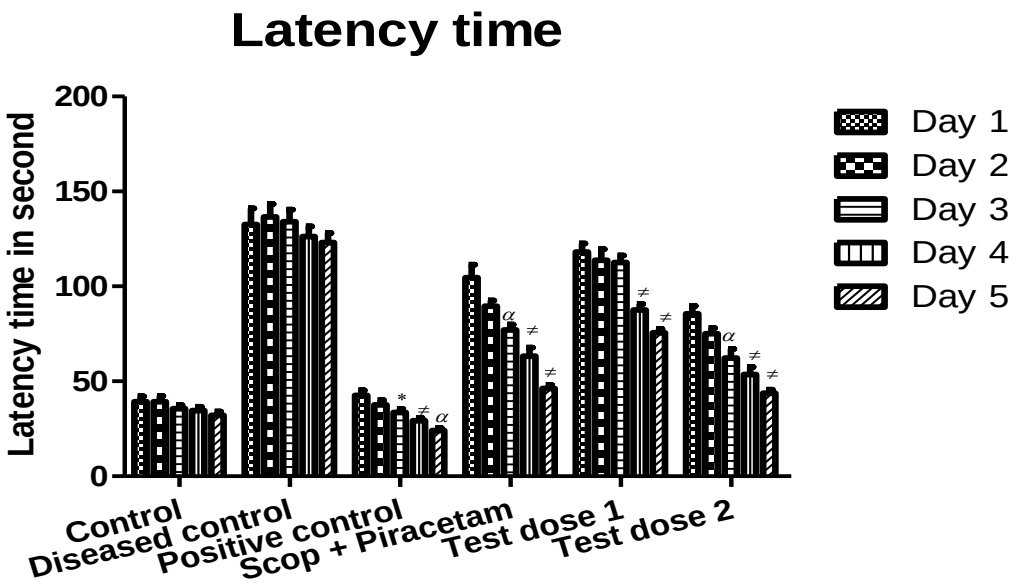

Fig. 1: Estimation of Latency time all Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test Where, *p < 0.05; $^{\alpha}p$<0.001; #p < 0.0001.

Estimation of Protein

Table 2: Effect of Medhya Rasayana on Protein in morris water maze model

GROUP	PROTEIN (Moles/mg)
Control	1.183±0.005
Scopolamine 0.7 mg/kg i.p.	2.279±0.074***
Piracetam 200 mg/kg p.o.	1.36±0.033
Scopolamine 0.7 mg/kg i.p.+ Piracetam 200 mg/kg p.o.	1.935±0.049***
Scopolamine 0.7 mg/kg i.p.+ M.R. 100 mg/kg p.o.	1.541±0.141*
Scopolamine 0.7 mg/kg i.p.+ M.R. 200 mg/kg p.o.	1.355±0.078**

All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test
Where, *p < 0.05; ** p<0.001; ***p < 0.0001.

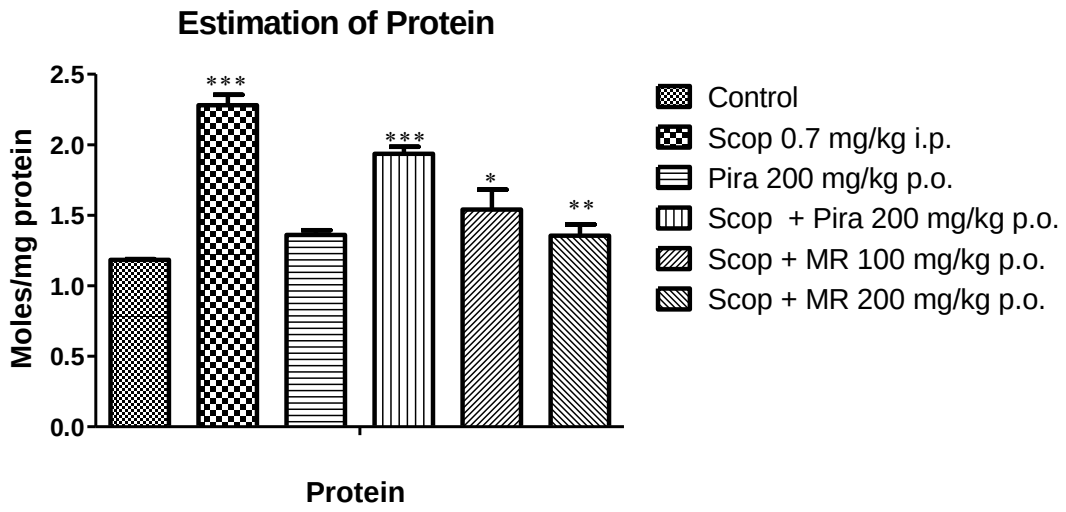

Fig. 1: Estimation of Protein: All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test Where, *p < 0.05; ** p<0.001; ***p < 0.0001.

Estimation of MDA

Table 2: Effect of Medhya Rasayana on MDA in morris water maze model

GROUP	MDA (nmoles/mg protein)
Control	0.248±0.015
Scopolamine 0.7 mg/kg i.p.	0.526±0.047***
Piracetam 200 mg/kg p.o.	0.255±0.033
Scopolamine 0.7 mg/kg i.p.+ Piracetam 200 mg/kg p.o.	0.353±0.016*
Scopolamine 0.7 mg/kg i.p.+ M.R. 100 mg/kg p.o.	0.438±0.011***
Scopolamine 0.7 mg/kg i.p.+ M.R. 200 mg/kg p.o.	0.417±0.002**

All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test
Where, *p < 0.05; ** p<0.001; ***p < 0.0001.

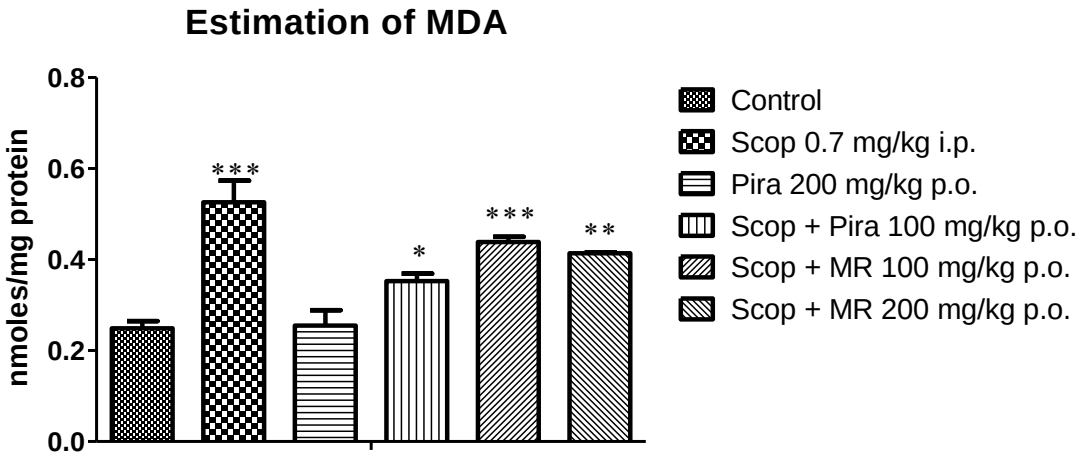

Fig. 2: Estimation of MDA: All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test Where, *p < 0.05; ** p<0.001; ***p < 0.0001.

Estimation of GSH

Table 3: Effect of Medhya Rasayana on GSH in morris water maze

GROUP	GSH (nmoles/mg protein)
Control	0.539±0.017
Scopolamine 0.7 mg/kg i.p.	0.247±0.021***
Piracetam 200 mg/kg p.o.	0.522±0.009
Scopolamine 0.7 mg/kg i.p.+ Piracetam 200 mg/kg p.o.	0.441±0.004
Scopolamine 0.7 mg/kg i.p.+ M.R. 100 mg/kg p.o.	0.419±0.004***
Scopolamine 0.7 mg/kg i.p.+ M.R. 200 mg/kg p.o.	0.432±0.015***

All Values are expressed as Mean + S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test
Where, *p < 0.05; ** p<0.001; ***p < 0.0001.

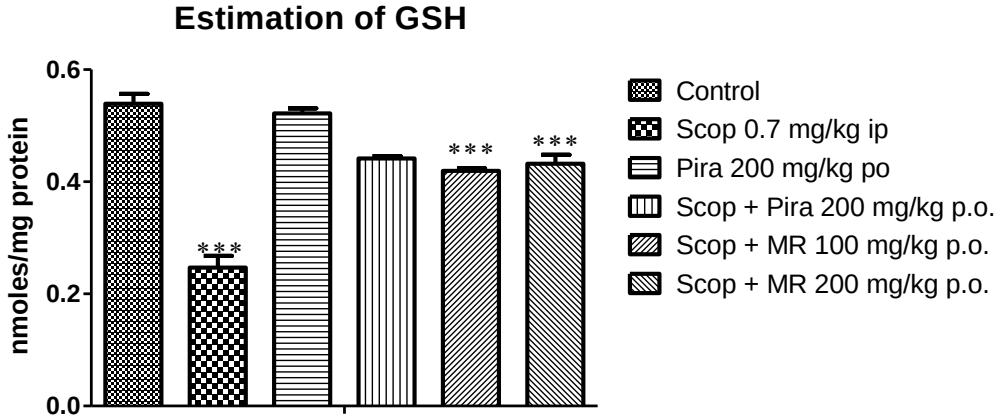

Fig. 3: Estimation of GSH: All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test Where, *p < 0.05; **p<0.001; ***p < 0.0001.

Estimation of AChE

Table 4: Effect of Medhya Rasayana on AChE in morris water maze

GROUP	AChE (nmoles/mg protein)
Control	1.183±0.005
Scopolamine 0.7 mg/kg i.p.	2.279±0.074***
Piracetam 200 mg/kg p.o.	1.36±0.033
Scopolamine 0.7 mg/kg i.p.+ Piracetam 200 mg/kg p.o.	1.935±0.049***
Scopolamine 0.7 mg/kg i.p.+ M.R. 100 mg/kg p.o.	1.541±0.141*
Scopolamine 0.7 mg/kg i.p.+ M.R. 200 mg/kg p.o.	1.355±0.078

All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test
Where, *p < 0.05; ** p<0.001; ***p < 0.0001.

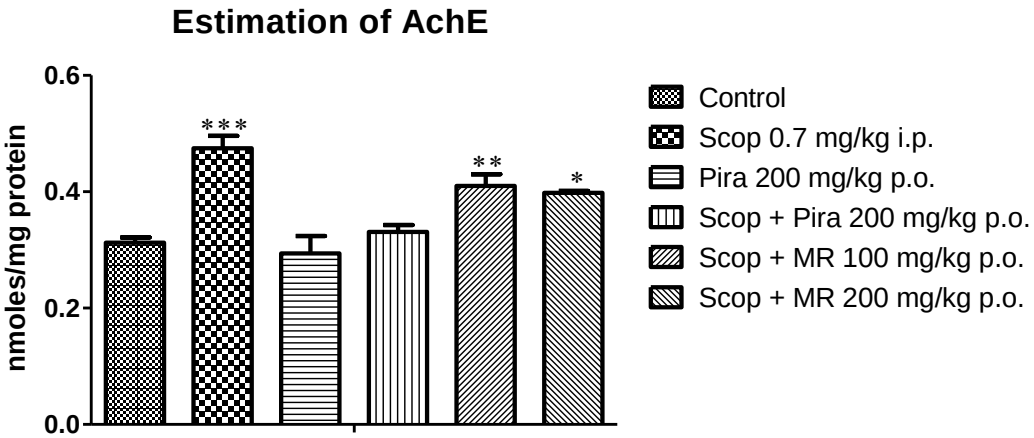

Fig. 4: Estimation of AChE: All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test Where, *p < 0.05; ** p<0.001; ***p < 0.0001.

Table 5: Effect of Medhya Rasayana in latency time (in second) in pole climbing model

	Day 1	Day 2	Day 3	Day 4	Day 5
Control	55.75±9.294	52.5±10.103	52.25±10.491	48±8.205	41.25±6.574
Scopolamine 0.7 mg/kg i.p.	131.25±4.269	137.75±6.060	126.25±4.732	123.25±4.497	116.25±3.145
Piracetam 200 mg/kg p.o.	35.5±3.329	33±2.380	31.75±2.780	28.25±2.358	25.25±2.056*
Scopolamine + Piracetam 200 mg/kg p.o.	112.5±12.5	103±12.069	85±6.454	68.75±4.269*	57±5.066α
Scopolamine + MR 100 mg/kg p.o.	120±3.535	111.25±4.269	102.5±4.787*	97.5±5.204α	99.75±3.300*
Scopolamine + MR 200 mg/kg p.o.	120.75±6.101	105±4.564	93.75±6.25α	88±3.135#	82.25±2.625#

All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test Where, *$p < 0.05$; $^{\alpha}p<0.001$; $^{\#}p < 0.0001$.

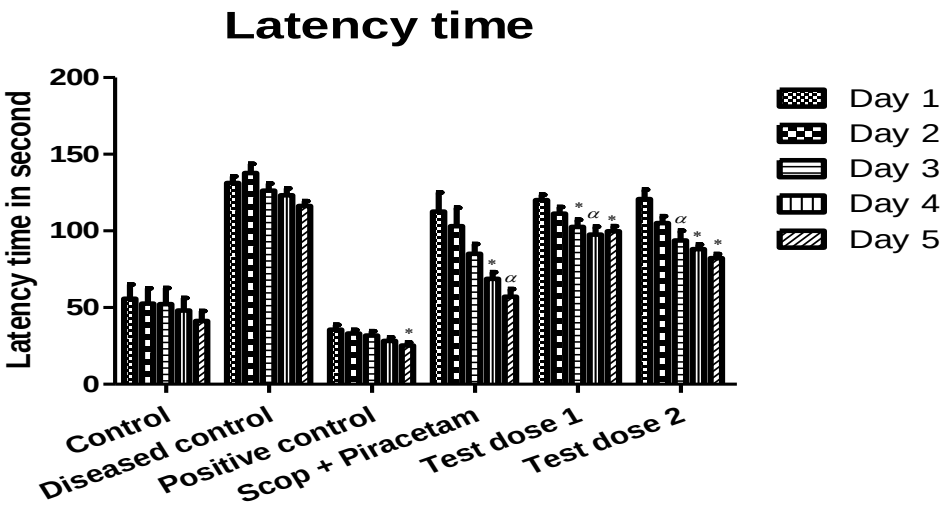

Fig. 5: Estimation of Latency time all Values are expressed as Mean + S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test Where, *$p < 0.05$; $^{\alpha}p<0.001$; $^{\#}p < 0.0001$.

Estimation of Protein

Table 6: Effect of Medhya Rasayana on Protein in pole climbing model

GROUP	PROTEIN (moles/mg)
Control	1.2272±0.0989
Scopolamine 0.7 mg/kg i.p.	2.3237±0.0634***
Piracetam 200 mg/kg p.o.	1.1077±0.0651
Scopolamine 0.7 mg/kg + Piracetam 200 mg/kg p.o.	1.6632±0.1798
Scopolamine 0.7 mg/kg + M.R. 100 mg/kg p.o.	2.0452±0.1121***
Scopolamine 0.7 mg/kg + M.R. 200 mg/kg p.o.	1.815±0.0347*

All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test Where, *$p < 0.05$; ** $p<0.001$; ***$p < 0.0001$.

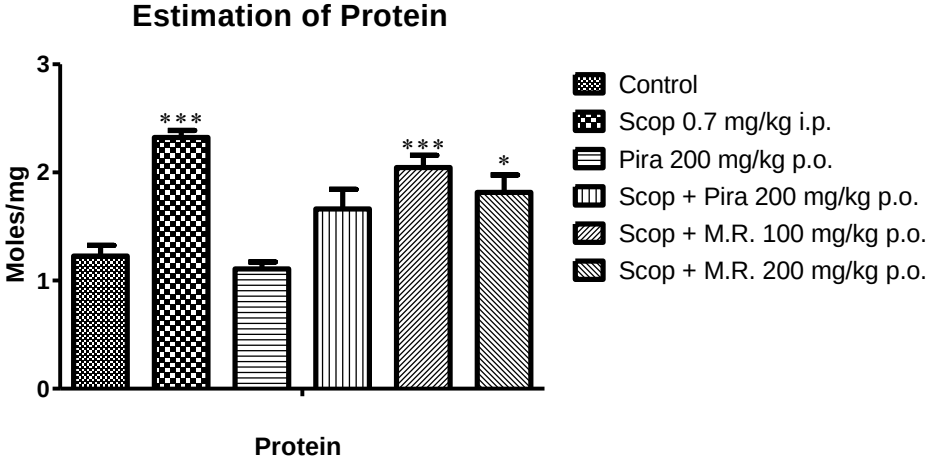

Fig. 6: Estimation of Protein: All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test Where, *$p < 0.05$; **$p<0.001$; ***$p < 0.0001$.

Estimation of MDA

Table 7: Effect of Medhya Rasayana on MDA in pole climbing model

GROUP	MDA (nmoles/mg protein)
Control	0.243±0.008
Scopolamine 0.7 mg/kg i.p.	0.588±0.027***
Piracetam 200 mg/kg p.o.	0.240±0.016
Scopolamine 0.7 mg/kg i.p.+ Piracetam 200 mg/kg p.o.	0.324±0.009*
Scopolamine 0.7 mg/kg i.p. + M.R. 100 mg/kg p.o.	0.569±0.027***
Scopolamine 0.7 mg/kg i.p.+ M.R. 200 mg/kg p.o.	0.454±0.022***

All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test
Where, *p < 0.05; ** p<0.001; ***p < 0.0001.

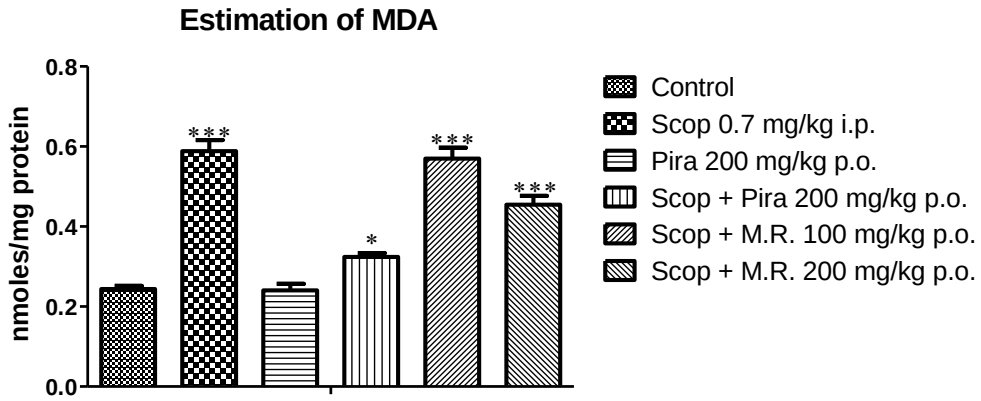

Fig. 7: Estimation of MDA: All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test Where, *p < 0.05; **p<0.001; ***p < 0.0001.

Estimation of GSH

Table 8: Effect of Medhya Rasayana on GSH in pole climbing model

GROUP	GSH (nmoles/mg protein)
Control	0.529±0.013
Scopolamine 0.7 mg/kg i.p.	0.219±0.024***
Piracetam 200 mg/kg p.o.	0.563±0.019
Scopolamine 0.7 mg/kg i.p. + Piracetam 200 mg/kg p.o.	0.537±0.022
Scopolamine 0.7 mg/kg i.p. + M.R. 100 mg/kg p.o.	0.330±0.022***
Scopolamine 0.7 mg/kg i.p. + M.R. 200 mg/kg p.o.	0.410±0.005**

All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test
Where, *p < 0.05; ** p<0.001; ***p < 0.0001.

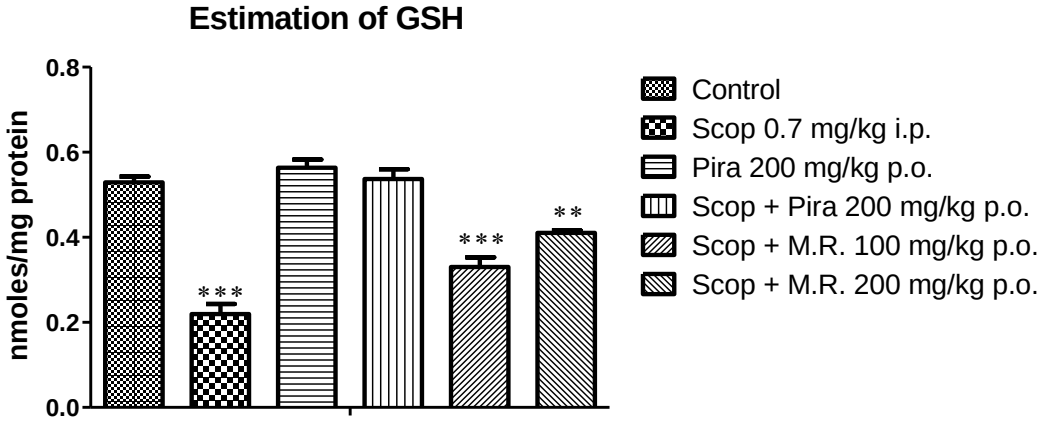

Fig. 8: Estimation of GSH: All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test Where, *p < 0.05; **p<0.001; ***p < 0.0001.

Estimation of AChE

Table 9: Effect of Medhya Rasayana on AChE in pole climbing model

GROUP	AChE (nmoles/mg protein)
Control	0.185±0.020
Scopolamine 0.7 mg/kg i.p.	0.483±0.026***
Piracetam 200 mg/kg p.o.	0.186±0.016
Scopolamine 0.7 mg/kg i.p.+ Piracetam 200 mg/kg p.o.	0.383±0.016
Scopolamine 0.7 mg/kg i.p.+ M.R. 100 mg/kg p.o.	0.383±0.030***
Scopolamine 0.7 mg/kg i.p.+ M.R. 200 mg/kg p.o.	0.285±0.009*

All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test
Where, *p < 0.05; ** p<0.001; ***p < 0.0001.

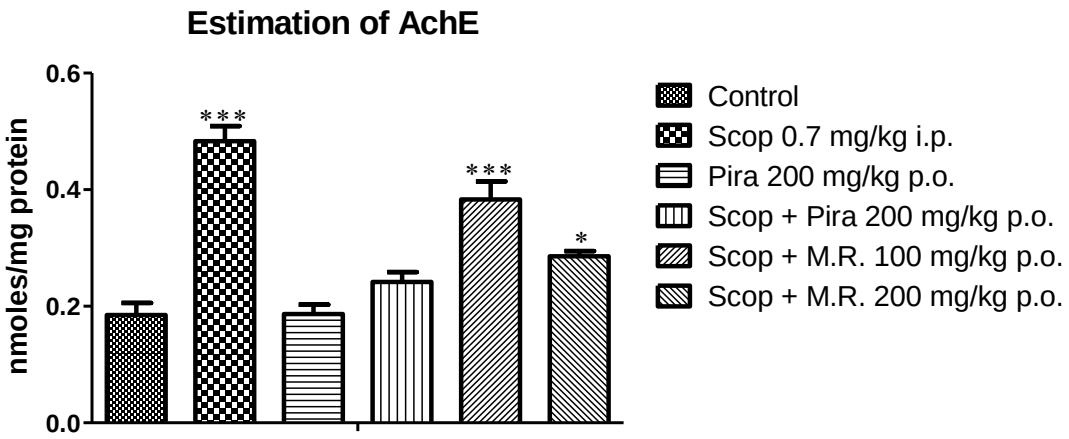

Fig. 9: Estimation of AChE: All Values are expressed as Mean ± S.E.M (n=4) analyzed by One Way ANOVA followed by Dunnett test Where, *p < 0.05; **p<0.001; ***p < 0.0001.

DISCUSSION

Memory enhancing drugs are thought to work by increasing the brain's supply of neurochemicals (neurotransmitters, enzymes and hormones), improving brain's oxygen supply or by stimulating nerve growth. Nootropics agents such as piracetam, aniracetam and choline esterase inhibitors like donepezil are being used for improving memory, mood and behavior but not used generally because of more side effects associated with these agents have made their applicability limited. In the present study, we have focused upon exploring the potential of Ayurvedic poly-herbal formulation, Medhya Rasayana for its efficacy in reversing the memory deficits, improving acquisition and memory retention in experimental animals using passive avoidance and morris water maze model. In the present study, Medhya Rasayana administered orally improved learning and memory of rats significantly in both models. Furthermore, pre-treatment with Medhya Rasayana (100, 200 mg/kg) protected the animals from learning and memory impairment produced by scopolamine. These findings suggested the possible neuroprotective role for Medhya Rasayana.

The scopolamine (0.7 mg/kg i.p) is a muscarinic receptor antagonist act by prolonging transfer latency. Medhya Rasayana (100, 200 mg/kg, p.o.) have reversed amnesia induced by scopolamine indicating nootropic activity which is statistically significant as compare to standard.

The polyherbal formulation Medhya Rasayana and piracetam when given along with scopolamine, significantly reversed scopolamine-induced amnesia, protective effect was observed with all parameters tested at a dose of 100 and 200 mg/kg, p.o of Medhya Rasayana. In morris water maze acquisition (learning) can be considered as transfer latency on first day trials and the retention/ consolidation (memory) is examined 24 h later. The animal shows significant decrease in transfer latency as compared with the standard group. The dose of 200 mg/kg p.o of Medhya Rasayana shows significantly better response in comparison to 100 mg/kg p.o dose of Medhya Rasayana. All data's are analyzed by one way ANOVA followed by Dunnett test to compare all groups with control group using Graph Pad Prism 5 software.

The passive avoidance is a classical model for the assessment of cognitive performance after

brain lesions or pharmacological manipulation. In this the avoidance cage consists of single box without any compartment that prevent inter compartment transfers. A sequence of stimuli is presented during which the animal has the freedom to transfer in the same compartment. Failure to transfer or the latency to transfer to a shock free area indicates the passive behavior which is used as short term memory task.

Morris water maze is employed to test spatial memory, which is affected in Alzheimer's disease.

Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions. According to the cholinergic hypothesis, memory impairments in patients with senile dementia are due to the selective and irreversible deficiency in the cholinergic functions in the brain. The cognitive dysfunction has been shown to be associated with impaired cholinergic function. The selective loss of cholinergic neurons and decrease in cholinacetyltransferase activity was reported to be a characteristics of the Alzheimer disease.

In the present study, MDA, GSH, and Acetylcholinesterase activity were estimated on the 5th day. Scopolamine treatment showed an increase in MDA and acetylcholinesterase activity and a decrease in GSH level increased the generation of free radicals.

The treatment of Medhya Rasayana in rats produced a significant fall in MDA levels in comparison to that of diseased control rat. There was a significant increase in the level of GSH in the brain.

REFERENCES

1. Agrawal Rahul, Tyagi Ethika, Saxena Gunjan and Nath Chandeshwar. Cholinergic influence on memory stages. A study on scopolamine amnesia mice, Indian journal of pharmacology. 2009;41(4):192-196
2. Dasi Amitava, Rai Deepak, Dikshit Madhu, Palit Gautam and Nath Chandishwar. Nature of stress: Differential effects on brain acetylcholinesterase activity and memory in rats, Life Sciences. 2005;77:2299–2311.
3. Desai MP, Valli JM, Monn AJ, Schoepp, AJ 1-BCP. A Memory-enhancing Agent, Selectively Potentiates AMPA-induced [3H] Norepinephrine Release in Rat Hippocampal Slices. Neuropharmacology. 1995;34(2):141-147.
4. Desai S, Korgaonkar, Gadre P and Ghogare A. Effect of bacopamine, a polyherbal formulation on learning and memory. Journal of Herbal Medicine and Toxicology. 2009;3:31-36.
5. Dhir Ashish et al. Neuroprotective effect of nimesulide, a preferential COX-2 inhibitor, against pentylenetetrazol (PTZ)-induced chemical kindling and associated biochemical parameters in mice. Seizure. 2007;16:691—697.
6. Glownisky J and Iversen LL. Regional Studies of Catecholamines in the rat Brain. The deposition of 3H-Norepinephrine, 3H-Dopamine and 3H-DOPA in various regions of rat Brain. J Neurochem. 1966;13:655-669.
7. Joshi Hanumanthachar and Parle milind . Nootropic activity of Calyces of *Hibiscuss sabdariffa* Linn. IJPT. 2006;5:15-20.
8. Joshi Hanumanthachar and Parlemilind. Nootropic activity of Calyces of *Hibiscuss sabdariffa* Linn. IJPT. 2006;5:15-20.
9. Kim Ik. Dae, Lee Hyen-Sung, Hong Heon- Joo, Lillehoj Soon Hyun, Park Ju Hong, Rhie Gyo Seung and Lee Soon Gun. The butanol fraction of *Eclipta prostrate* (Linn.) increases the formation of brain acetycholine and decreases oxidative stress in brain and serum of cesarean derived rats. Nutrition research. 2010;30:579-584.
10. Kulkarni S. Kirti, Kasture BS and Mengi SA. Effficacy study of *Prunus amygladus* (Almond Nut) in scopolamine induced amnesia in rats. Indian Journal of Pharmacology. 2010;42(3):168-173.
11. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with the Folins-phenol Reagent. J boil Chem. 1951;193:265-275.
12. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with the Folins-phenol Reagent. J boil Chem. 1951;193:265-275.
13. Mani Vasudevan and Milind Parle. Memory enhancing activity of Anwala churna (*Emblica officinalis* Gaertn.): An Ayurvedic preparation, Physiology & Behavior. 2007;91:43-73.
14. Mani Vasudevan and Milind Parle. Memory enhancing activity of Anwala churna (*Emblica officinalis* Gaertn.):

An Ayurvedic preparation, Physiology & Behavio. ,2007;91:46-54.
15. Okhawa H, Qohishi N and Yagi K. Assay for lipid peroxides in animal tissues by Thiobarbituric acid reaction. Annals of Biochemistry. 1979;95:351-358.
16. Reddy S. Doodipala and Kulkarni K. Shrinivas. Possible role of nitric oxide in the Nootropic and antiamnesic effect of neurosteroid on aging and Dizoclipine induced learning impairment. Brain research. 1998;799:215-229.
17. Russo. A and Borrelli F. *Bacopa monniera*, a reputed nootropic plant: an overview. Phytomed. 2005;12(4):305-317.
18. Sharma P. Charaka saamhita, agniveha treatise, Sutrasthana Varanasi, Chokambha, Sanskrit sansthan. 1981;5:342- 345.
19. Thakur VD and Mengi SA. Neuropharmacological profile of *Eclipta alba* (Linn.) Hassk. Journal of Ethnopharmacology. 2005;102:23-31.
20. Turner Francis Liliana and Valouskova Vera. Nerve growth factor and nootropic drug cerebrolysin but not fibrinoblast growth factor can reduce spatial memory impairment, elicited by fimbria-forenix transaction, short term study. Neuroscience letter. 1996;202:193-196.