International Invention of Scientific Journal
ISSN: 2457-0958

Research Paper

Available Online at http://iisj.in/index.php/iisj June, 2018|Volume 02|Issue 06|

Nootropic Activity of Ajuga Bracteosa Wall on Scopolamine Induced Memory Deficits in Swiss Albino Mice

[1]Raghunath Singh Khatri, [2*]Vrish Dhwaj Ashwlayan, [3]Divya Sharma

[1]Research Fellow (visiting), Monash University Malaysia, Ph.D. Research Scholar; University Institute of Pharmaceutical Sciences Panjab University, Chandigarh INDIA

[2]Associate Professor, Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, Meerut, Uttar Pradesh; INDIA.

[3]Lecturer & Head of Department, Department of Computer Science, Deva Nagri Degree College, Meerut, U. P. INDIA

***Correspondence Author:** *vrish.ashwlayan@miet.ac.in*

Accepted 2018-06-27, Published 2018-06-30

Abstract:

Objective: Nootropic activity of Ajuga bracteosa herb was investigated using scopolamine induced amnesia (memory deficits), elevated plus-maze (EPM) and Morris water maze (MWM) experimental models in Swiss albino mice.

Materials and Methods: Successive maceration of the plant was made using n-hexane followed by methanol solvent to extract out active principles according to their solubility. Methanolic herbal extract of Ajuga bracteosa (ABE) was prepared using maceration. Neuroprotective effect of ABE in Swiss albino mouse was recorded in transfer latency time (TLT) as inflation-ratio in EPM, escape latency time (ELT) and time spent in target quadrant (TSTQ) in MWM model using scopolamine induced amnesia. Drug induced lipid per-oxidation was measured by estimation of the content of acetyl cholinesterase (AChE), glutathione (GSH), malondialdehyde (MDA) and total protein in brain blood sample of the mouse.

Results: ABE (500 and 750 mg/kg, p.o.) increased the TLT, ELT and TSTQ. Scopolamine markedly decreased the TLT over 3 minutes, ELT, TSTQ over 90 sec and consecutively impaired learning and memory. Higher levels of brain AChE and MDA but lower levels of brain GSH and total protein were significantly attenuated by chronic administration of ABE herb in scopolamine treated mice at higher doses. The herb improves learning and memory of scopolamine-induced amnesia in mice.

Conclusion: Reversal of scopolamine induced amnesia by ABE may be mediated through the inhibition of oxidative stress and due to presence of withanolides containing anti-cholinesterase activity. ABE may be beneficial in management of memory deficits with normal life and clinical dementia associated with ageing and neurodegenerative states.

Key Words: Ajuga bracteosa, acetylcholine, Nootropic, memory deficits, Dementia, Scopolamine

Introduction:

A specialized cell designed to transmit information to other nerve cells, muscle or gland cells, and the neuron is the basic functional unit of the brain [1]. The brain is a single organ that controls all body activities, ranging from heart rate and sexual function to emotion, thinking, feeling, wanting, perceiving, learning and memory curiosity and behavior. Central cholinergic system plays an important role in the process of learning and memory. Its hypo-function may induce aspect of dementia such as memory loss and disorientation in Alzheimer's disease [2]. Dementia is taken from Latin, originally meaning "madness", from *de-* "without" + *ment*, the root of *mens* "mind". It is a serious loss of cognitive ability in a previously unimpaired person, beyond what might be expected from normal ageing. It affects memory, thinking, orientation and comprehension, calculation, learning

capacity, language and judgment. Alzheimer disease (A. D.) is the most common cause of dementia in elder persons accounting for over 60% of cases of late-life cognitive dysfunction. The prevalence of A.D. increases exponentially with age, affecting approximately 7% of individuals aged 65 to 74 years, 53% aged 75 to 84, and 40% of persons aged 85 years and older. Loss of cortical cholinergic neurons containing acetylcholine (ACh) is a prominent feature of dementia of AD [3]. Worldwide, 35.6 million people have dementia with just over half (58%) living in low and middle–income countries. Every year, there are 7.7 million new cases. The estimated proportion of the general population aged 60 and over with dementia at a given time is between 2 to 8 per 100 people. The total number of people with dementia is projected almost double every 20 years, to 65.7million in 2030 and 115.4 million in 2050 [4]. The disease usually becomes clinically apparent as insidious impairment of higher intellectual function, with alteration in mood and behaviour. Later, progressive disorientation, memory loss, and aphasia indicate severe cortical dysfunction, and over the next 5 to 10 years, the patient becomes profoundly disabled, mute, and immobile. Memory is a fundamental mental process and without memory one is capable of nothing but simple reflexes and stereotyped behaviours. Thus, learning and memory is one of the most intensively studied subjects. Nootropic represents psycotropic agents such as piracetam [5] and cholinesterase inhibitors like donepezil [6] with selective facilitatory effect on intellectual performance, learning capability and memory in both senile and vascular dementia especially in the early and moderate stages [7]. Donepezil has recently been approved as acetyl cholinesterase (AChE) inhibitor and neuroprotective drug to be used clinically for memory impairment of A. D. The miosis, salivation, hypothermia and tremor side effects [8] have made their applicability limited [9]. In order to improve quality of life like academic performance, mental health, relief from stress and anxiety, to avoid ageing, nootropic herbs may be good health supplement to overcome diseases associated with memory impairment. The word nootropic was coined in 1972 by a Romanian psychologist and chemist, Corneliu E. Giurgea, from the Greek words noos or "mind", and tropein meaning to bend or turn [10]. Nootropic drugs belong to the category of psychotropic agents with selective facilitatory effect on intellectual performance, learning and memory. Nootropics work by altering the availability of the brain's supply of neuro-chemicals (neurotransmitters, enzymes and hormones) by improving the brain's oxygen supply or by stimulating nerve growth factor. A number of drugs including piracetam have now been introduced in therapy to ameliorate cognitive deficits. *Ajuga bracteosa* Wall (Neelkanthi) herb belonging to Labiatae is found in sub-Himalayan tract, plains of Punjab and the high altitude (1200-5100m) areas of Uttrakhand, India. Plant is found in sandy, loamy and clay soils and requires well drained, moist soil. It is an evergreen, erect, ascending, perennial, hairy herb, with woody base. Leaves are sub-entire, obtuse and toothed. Flowers are in axillary spike, pink in colour and flowering time is May-August. Leaves are simple, margin dentate to incised, rarely sub-entire. This perennial herb has been used in north area of India as traditional medicine a remedy for fever, neuro diseases, toothache, dysentery, malaria, diabetes, gastrointestinal disorders and also used as anthelmintic, diuretic, antimycobacterial antibacterial [11], antiestrogenic, antifungal, anti-inflammatory, antihypertensive, antileukemic, antioxidant, cardiotonic, cytotoxic, and vasorelaxing agent. A large number of compounds have been isolated from the *Ajuga* plants, including phytoecdysteroids, *neo*-clerodane-diterpenes and diterpenoids, triterpenes, sterols, anthocyanidin-glucosides and iridoid glycosides, withanolides, flavonoids, triglycerides and essential oils. Withanolides are the phyto-constituent isolated from *Ajuga bracteosa* herb and are found to have acetylcholinestrase inhibitory property [12] and its neuroprotective effect may be beneficial in treatment of disease associated with cholinergic system such as Alzheimer's disease. Therefore, the present study was carried out to investigate neuroprotective activity of *Ajuga bracteosa* Wall (Labiatae) using Introspective (scopolamine induced amnesia) and exetroceptive (Morris water maze and elevated plus maze) behavioural models and provide scientific basis for the same.

Material and Methods:

Experimental Animals:

Swiss albino mice of either sex (20–30g) were obtained from Central Drug Research Institute (CDRI), Lucknow and were randomly distributed into different experimental groups. The mice were kept in the departmental animal house provided with 12 hours light and 12 hours dark cycles at an ambient temperature 25±2°C and had free access to water and standard laboratory diet (Ashirwad Industries, Chandigarh, India). The experimental protocol was approved by institutional animal ethics committee (No. 28/1279/ac/SU/IAEC) and experiment was

carried out according to the guidelines of Committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi on the use and care of experimental animals. Experiments were carried out between 09:00 and 17:00 hours. Efforts were made to minimize animal suffering and number of animals used.

Drugs and Chemicals:
Piracetam, donepazil, scopolamine, analytical reagents were purchased from Sigma Aldrich. Chemicals of analytical reagent grade (n-haxane & methanol) were used in the study.

Plant Material:
Whole plant of *Ajuga bracteosa* was collected from Garhwal (Chamoli) area of Uttrakhand and authenticated by Mrs. Sayyada Khatoon (Scientist) Pharmacognosy and Ethanopharmacology Division, National Botanical Research Institute, Council of Scientific & Industrial Research (CSIR), Lucknow, Uttar Pradesh, India.

Extraction:
Ajuga bracteosa Wall (Labiatae) herb was grinded to coarse powder and the powder was divided into three different batches of 20g, each to standardize the extraction procedure in three consequent steps using percolator. Each batch was first extracted with n-haxane for defatting of crude drug. Finally, each batch was extracted with methanol of HPLC grade by maceration at room temperature for seven days with intermittent shaking. This was repeated thrice with fresh solvent each time. The extracts from all the three washes were pooled and concentrated using rotatory vacuum evaporator (Perfit) under reduced pressure at $\leq$ 50 °C temperature to obtain dark viscous mass. The extract obtained with each solvent was weighed, and its percentage was calculated regarding air dried weight of the plant drug. The percentage yields of n-haxane and methanol extract was found to be 0.624 and 20.65 respectively. The residue was then dried at room temperature. The extract was subjected to phyto-chemical analysis as per protocols [13]. Preliminary phyto-chemical screening revealed that saponins and fatty acids were present in n-haxane extract while antharaquinone, cardiac and cynogenetic glycosides, carbohydrates, tannins, tri-terpinoids and steroids were present in methanolic extract. The extract was freshly prepared by suspending the semisolid extract into 1% w/v carboxy methyl cellulose (CMC) solution on the day of experiment.

Pharmacological Screening Techniques to Evaluate Neuroprotective Activity of *Ajuga Bracteosa*:

Introspective Behavioural Model (Scopolamine Induced Amnesia): Anti-cholinergic drugs such as scopolamine (3mg/kg, i.p.) produce amnesia in rodents [14]. Scopolamine, centrally acting anti-muscaranic drug impairs learning and memory both in mouse and human beings, particularly the process of learning (acquisition) and short-term memory [15, 16].

Exteroceptive Behavioral Models:

A. Morris Water Maze (Mwm) Test

The MWM as an exteroceptive behavioral model has been used extensively to investigate spatial learning and memory in rodents [17]. The MWM consisted of the circular pool (diameter 70 cm, height 31 cm) containing water at 25±1 ˚C to a depth 30 cm and rendered opaque by the addition of powder. A circular platform (diameter 10 cm) was hidden 1 cm below the surface of water and placed in a constant position.

Acquisition Trials: Each mouse was placed in the pool for six consecutive trials on each day with an interval of five minutes, allowed 90 s to find the hidden platform, and permitted to stay there for 10 s (modified from Morris, 1984). During training and testing sessions, escape latency time (ELT), the time to find the hidden platform was recorded [18]. Extensive pre-training is not required in this model because animals learn rapidly to locate the hidden platform. The starting position on each day to conduct four acquisition trials was changed.

Retrieval Trial: On the next day, platform was removed, and each rat was allowed to explore the pool for 90 seconds. Mean time spent by the mouse in each of four quadrants was noted. The mean time spent by the mouse in target quadrant (Q1) for searching the hidden platform was noted as an index of retrieval conducted on day 7.

B. Elevated Plus Maze (EPM) Model

The EPM served as the exteroceptive behavioural model to evaluate learning and memory in mice. An elevated plus maze consisting of two open arms (16×5 cm) and two enclosed arms (16×5×12 cm) was used. The arms extended from a central platform (5cm x 5cm) and the maze was elevated to the height of 25 cm from the floor. Mice were placed individually at the end of an open arm facing away from the central platform and the time they took to move from the end of open arm to either of the closed arms (transfer latency time, TLT) was recorded [19]. On the first day, mice were allowed to explore the plus maze for 3 min after the measurement of TLT. The TLT was

expressed as retention after 24 h by calculating the ''inflation-ratio (IR)'' in mouse using the formula described earlier [20]:

Inflation-ratio (IR) =L_1 - L_0/L_0

Where, L_0 = initial transfer latency in seconds and L_1 = transfer latency after 24 h.

Experimental Protocol: Ten groups, each comprising of six mice were employed in the present study (shown in Table 1).

Table 1: Experimental protocol

Groups	Treatment	Dose
I Control	1% w/v CMC solution	10 ml/kg,i.p.
II Negative Control	Scopolamine	3 mg/kg, i.p.
III Standard Drug	Donepezil	0.1 mg/kg, i.p.
IV Donepezil + SCOP	Donepezil+ Scopolamine	0.1mg/kg,i.p.+ 3 mg/kg, i.p.
V ABE Per-se	Ajuga bracteosa Methanoloc extract	250 mg/kg, p.o.
VI ABE Per-se	Ajuga bracteosa Methanoloc extract	500 mg/kg, p.o.
VII ABE Per-se	Ajuga bracteosa Methanoloc extract	750 mg/kg, p.o.
VIII ABE + SCOP	Ajuga bracteosa Methanoloc extract + Scopolamine	250mg/kg, p. o+3 mg/kg,i.p.
IX ABE + SCOP	Ajuga bracteosa Methanoloc extract + Scopolamine	500mg/kg, p. o+3 mg/kg,i.p.
X ABE + SCOP	Ajuga bracteosa Methanoloc extract + Scopolamine	750mg/kg,p.o+ 3 mg/kg,i.p.

Control: I; Negative control: II; Protective: III; Curative: V, VI and VII groups.

Transfer Latency Time as Inflation-Ratio was Estimated on Day 6 And 7 After 45 Minutes Of Dosing in all above Groups before Exposure to MWM.

Group I- control group: Mice were treated with vehicle, 1% w/v CMC solution (10 ml/kg, i.p.) 45 min before the first acquisition trial for 6 consecutive days and 45 min before the retrieval trial on 7th day.

Group II- Scopolamine treated group: Mice, were administered scopolamine (3mg/kg i.p.) dissolved in vehicle, daily for 6 days and then subjected to Morris water maze test. The administration of scopolamine (administered 45 min before) was also continued during acquisition trials conducted from day 1 to day 6. On day 7, these mice were administered vehicle only, and then subjected to retrieval test after 45 min.

Group III- Donepezil treated group: Mice, were administered donepezil (0.1 mg/kg i.p.) dissolved in vehicle, daily for 6 days and then subjected to Morris water maze test. The administration of Donepezil (administered 30 min before) was also continued during acquisition trials conducted from day 1 to day 6. On day 7, these mice were administered vehicle only, and then subjected to retrieval test after 30 min.

Group IV- Donepezil+scopolamine treated group: Mice, were administered donepezil followed after 15 min by scopolamine daily for 6 days and then subjected to Morris water maze test. The administration of donepezil and scopolamine (administered 60 min and 45 min, respectively before) was also continued during acquisition trials conducted from day 1 to day 6. On day 7, these mice were administered vehicles only, and then subjected to retrieval test.

Group V, VI, VII- *Ajuga bracteosa* herbal extract (ABE) 250,500,750 mg/kg: Mice, were administered methanolic herbal extract of ABE (250,500,750 mg/kg, p.o.) daily for 6 days and then subjected to Morris water maze test. The administration of methanolic extract of ABE (administered 45 min before) was also continued during acquisition trials conducted from day 1 to day 6. On day 7, these mice were administered vehicle only, and then subjected to retrieval test after 45 min.

Group VIII, IX, X – (ABE 250, 500, 750 mg/kg +scopolamine): mice were administered methanolic herbal extract of ABE (250,500,750 mg/kg, p.o.) 45 min before scopolamine, daily for 6 days and then subjected to Morris water maze test. The treatment was continued during acquisition trials conducted from day 1 to day 6. On day 7, these mice were administered vehicle only, and then subjected to retrieval test after.

Estimation of Oxidative Stress Biomarkers

Drug induced lipid per-oxidation was measured by estimation of the content of acetyl cholinesterase

(AChE), glutathione (GSH), malondialdehyde (MDA) and total protein in brain blood sample of the mouse. The mice were decapitated by light ether anaesthesia. A 10% (w/v) homogenate of brain samples (0.03 M sodium phosphate buffer, pH 7.4) was prepared by using an Ultra-Turrax T25 (USA) homogenizer at a speed of 9500 rpm. The determination was done by precipitating the protein substance using trichloroacetic acid (10% w/v), the protein free sample used for estimation of lipid per-oxidation parameters as follows.

Protocol for the Estimation of Acetyl Cholinesterase (Ache) Activity in Brain Homogenate:

The whole brain AChE activity of mouse was measured [21] with slight modifications [22]. 0.5 ml of supernatant liquid of the brain homogenate was pipetted out into 25 ml volumetric flask and dilution was made with a freshly prepared DTNB {5,5'-dithiobis (2-nitro benzoic acid)} solution (10 mg DTNB in 100 ml of sorenson phosphate buffer, pH 8.0). From the volumetric flask, two 4 ml portions were pipetted out into two test tubes. Into one of the test tube, 2 drops of Donepezil solution were added. 1 ml of substrate solution (75 mg of acetylcholine iodide per 50 ml of distilled water) was pipetted out into both of the test tubes and incubated for 10 min at 30 °C. The solution containing Eserine solution was used for zeroing the colorimeter and change in Absorbance per min of the sample was read spectrophotometrically with colorimetry at 420 nm. AChE activity was calculated using the following formula:

$$\mathbf{R = äOD \times Volume\ of\ Assay\ (3\ ml)/E \times mg\ of\ protein}$$

Where, **R**= rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed/minute/mg protein; äO.D. = change in Absorbance/minute; E**=** extinction coefficient=13,600/M/cm.

Protocol for Measurement of Malondialdehyde (MDA) in Brain Homogenate:

MDA, which is a measure of lipid peroxidation, was estimated spectrophotometrically [23] using 1, 1, 3, 3-tetraethoxypropane as the standard. MDA was expressed as µM per mg protein. The reagents acetic acid 1.5 ml (20%) pH 3.5, 1.5 ml thiobarbituric acid (0.8%) and 0.2 ml sodium dodecyl sulfate (8.1%) were added to 0.1 ml of processed tissue sample. The mixture was then heated at 100 °C for 60 min. The mixture was cooled under tap water and 5 ml of n-butanol: pyridine (15:1% v/v), 1 ml of distilled water was added. The mixture was shaken vigorously on vortex. After centrifugation at 4000 rpm for 10 min, the organic layer was withdrawn, and Absorbance was measured at 532 nm using spectrophotometer.

Protocol for the Estimation of Reduced GSH Level in Brain Homogenate:

The reduced GSH level in mouse brain was estimated [24] with slight modifications. Tissue homogenate was taken, and the proteins were precipitated with 10% w/v chilled trichloroacetic acid. Samples were kept in ice bath and were centrifuged after 30 min at 1000 ×g for 10 min at 4°C. GSH levels were measured in the supernatant. 0.5 ml supernatant was mixed with 2.0 ml of 0.3 M disodium hydrogen phosphate solution and 0.25 ml of freshly prepared DTNB {5,5'-dithiobis (2-nitro benzoic acid)} solution (40 mg/100 ml in 1% w/v sodium citrate) was added just before measuring the absorbance spectrophotometrically at 412 nm. Different concentration of GSH standard was also processed similarly to prepare a standard curve (5–50 µg) simultaneously. Results were expressed as µM of GSH/mg of protein.

Protocol for Estimation of Total Protein in Brain Homogenate:

For the estimation of total protein in brain, method of Lowry *et al*., 1951 with slight modifications was used [25]. 150 µl of supernatant was taken in a test tube, volume was made up to 1 ml with distilled water then 5 ml of Lowry's reagent (freshly prepared mixture of 1% w/v copper sulphate, 2% w/v sodium potassium tartrate and 2% w/v sodium carbonate in 0.1 N NaOH in the ratio of 1:1:98 respectively), was added and mixed thoroughly. Mixture was allowed to stand for 15 min at room temperature and then 0.5 ml of 1:1 v/v diluted Folin–Phenol reagent was added. Contents were vortexed and incubated at 37 °C for 30 min. Then Absorbance was determined spectrophotometrically at 750 nm against suitably prepared blank. A standard curve using 25–200 mg of BSA was plotted. The amount of total protein was expressed in mg.

Statistical Analysis

All the results were expressed as mean ± standard error mean (SEM). The data were analyzed with one-way ANOVA followed by Tukey's multiple comparison tests using Graph Pad Prism. $p \leq 0.05$ was considered statistically significant.

Results:

Methanolic Extract of *Ajuga Bracteosa* (ABE) Herbal Drug Improved Behavioral Alteration in Scopolamine Treated Mice:

Methanolic extract of *Ajuga bracteosa* (ABE) herbal drug was investigated for its neuroprotective effect on scopolamine induced amnesia using Morris water maze and elevated plus maze Models. The effect of ABE herbal drug at dose of 250, 500, 750 mg/kg, p.o. were observed using scopolamine induced amnesia in mice. After the intraperitoneal injection (i.p.) of scopolamine (3 mg/kg) amnestic drug, mice showed impairment of memory compared to that of control group in which there was little change in the escape latency time to find the hidden platform. ELT was recorded for 6 consecutive days using MWM Model (Fig. 1) Donepezil, standard drug was given in dose of 0.1mg/kg, i.p.

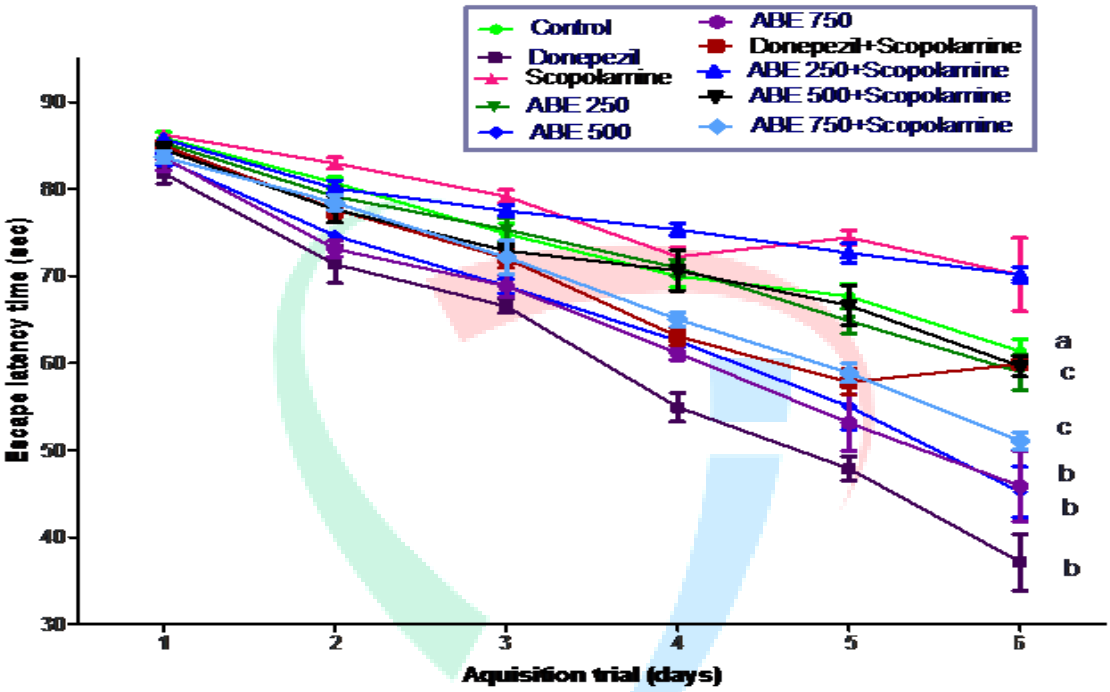

Figure 1: Effect of methanolic extract of *Ajuga bracteosa* (ABE) herbal drug on scopolamine induced changes in ELT during acquisition trials.

The ELT of methanolic extract of *Ajuga bracteosa* (250, 500mg/kg and 750 mg/kg, p.o.) herbal drug, conducted on six consecutive days are shown. It is noted that the scopolamine has a significantly increasing effect on ELT. ABE (methanolic extract of *Ajuga bracteosa*) herbal drug and donepezil (0.1 mg/kg, i.p.) have a decreasing effect. Each value of ELT is a mean value of six consecutive acquisition trials conducted from day 1 to day 6. Each group (n=6) represents mean ± S.E.M. a=p≤ 0.05 Vs ELT on day 1, b= p ≤0.05 ELT in control group on respective day and c=p≤0.05Vs ELT in scopolamine treated group. One-way ANOVA test was employed.

Effect of Methanolic Extract of *Ajuga Bracteosa* (ABE) Herbal Drug on Scopolamine Induced Changes in TSTQ During Retrieval Trial:

The effect of methanolic extract of *Ajuga bracteosa* (ABE) herbal drug at doses of 250, 500, 750 mg/kg, p.o. were observed using scopolamine induced amnesia in MWM. The TSTQ was assessed to evaluate the retrieval of memory during retrieval trial conducted on day 7. Like donepezil, methanolic herbal extract of *Ajuga bracteosa* (ABE) herbal drug 750 mg/kg, p.o. showed significant protection against scopolamine induced amnesia in mice (Fig. 2).

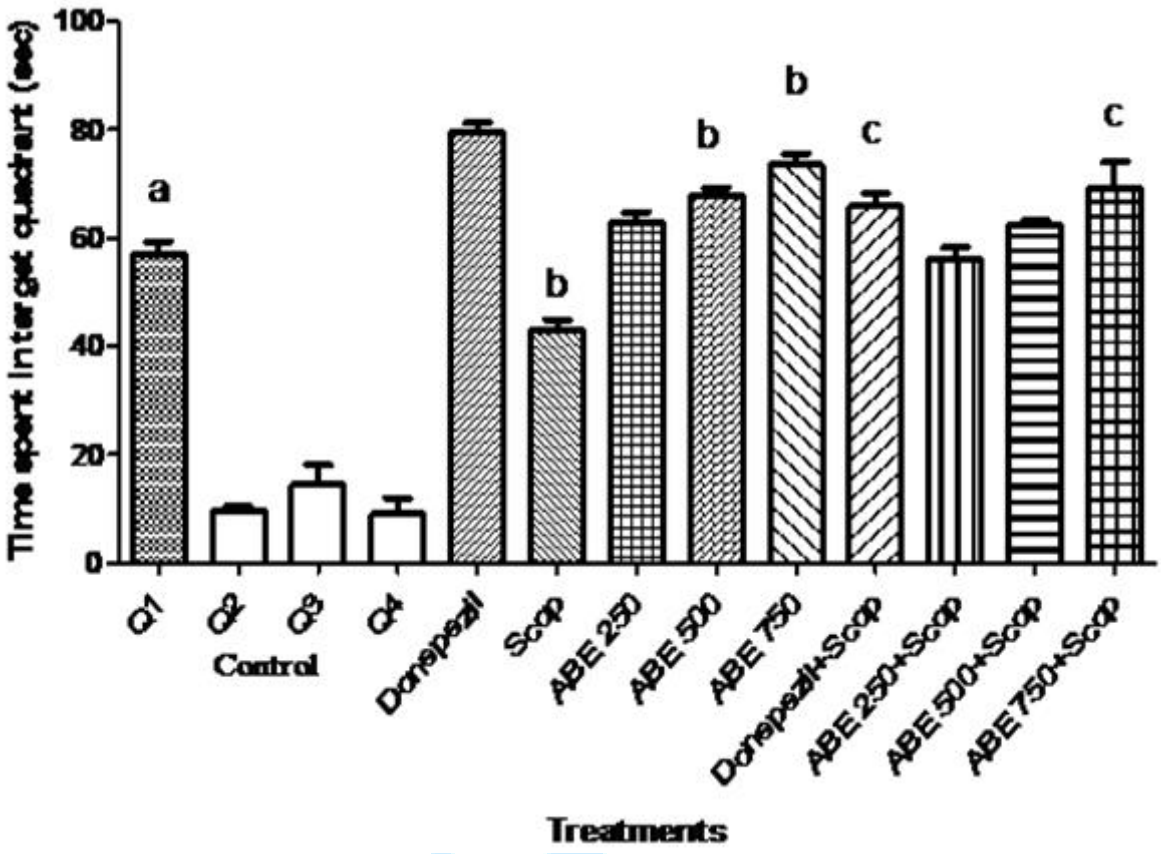

Figure 2: Effect of methanolic extract of *Ajuga bracteosa* (ABE) herbal drug on scopolamine using decrease in TSTQ

Standard drug (Donepezil 0.1 mg/kg, i.p.), ABE (methanolic extract of *Ajuga bracteosa*) herbal drug at doses of 250, 500, 750 mg/kg, p.o. treated groups increased time spent in Q1 target quadrant and showed retrieval. Scopolamine treated mouse decreased time spent in target quadrant as compared to Q1 target quadrant of control group. Results were expressed as mean ± S.E.M with n=6 in each group. a=p≤0.05 versus time spent in other quadrants (Q2, Q3 and Q4), b=p≤0.05 versus time spent in target quadrant (Q1) of control group. One-way ANOVA test was employed.

Effect of Methanolic Extract of *Ajuga Bracteosa* Herbal Drug on Scopolamine Induced Changes in Transfer Latency Time (TLT) as Inflation-Ratio:

The effect of methanolic extract of *Ajuga bracteosa* herbal drug on TLT at doses of 250, 500 and 750 mg/kg, p.o. was observed against scopolamine induced amnesia in mice using Elevated plus maze test. As compared to control group, significant decrease in transfer latency time (TLT) as inflation-ratio was noted in case of scopolamine treated mice. Like donepezil, methanolic extract of *Ajuga bracteosa* (ABE) herbal drug was significantly and dose dependently found to increase in TLT as inflation-ratio and restore memory function ($p \leq 0.05$) at higher dose (Fig, 3).

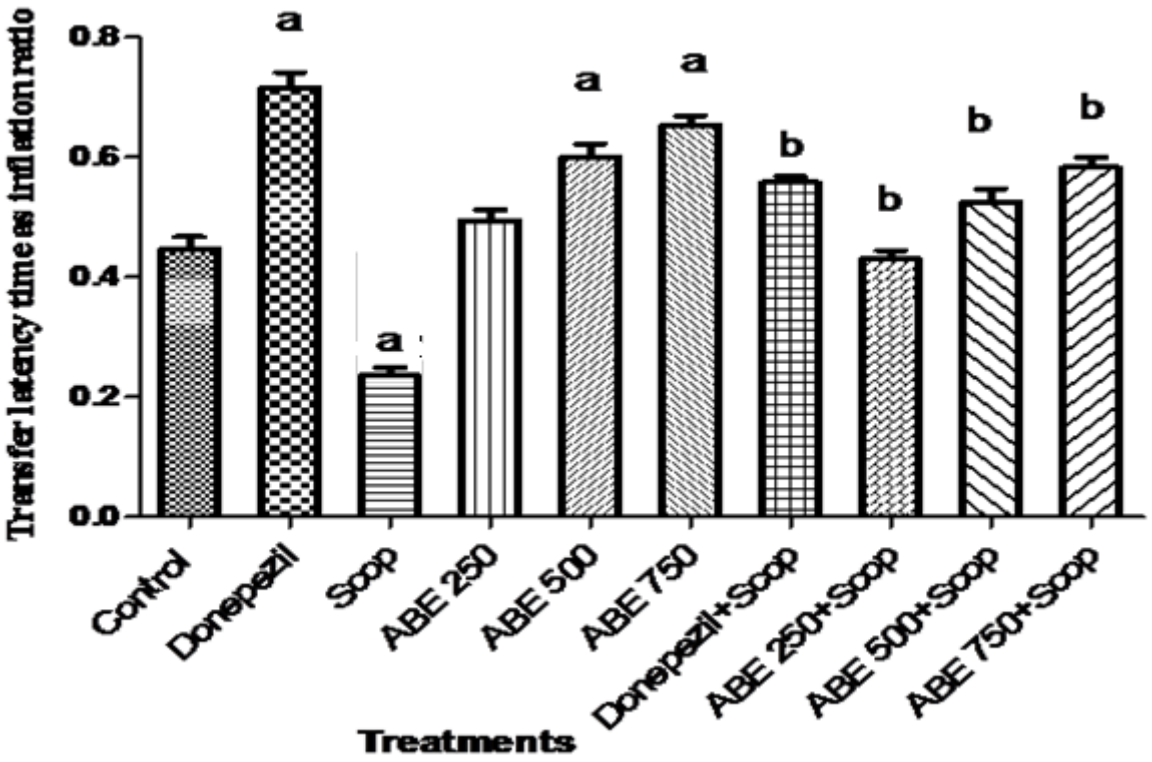

Figure 3: Effects of methanolic extract of *Ajuga bracteosa* (ABE) herbal drug on TLT as inflation-ratio in scopolamine induced amnesia

The effect of methanolic extract of *Ajuga bracteosa* (250, 500 and 750 mg/kg, p.o.) herbal drug on TLT as IR was observed in mouse using EPM. TLT as inflation-ratio (IR) conducted on day 7 i.e. after 24 hrs of acquisition trials indicates memory retention. It is noted that the scopolamine has a significant decreasing effect on memory retention. ABE (methanolic extract of *Ajuga bracteosa*) herbal drug and donepezil have an increasing effect on memory retention. Each group (n=6) represents mean ± S.E.M. a=$p \leq 0.05$ Vs TLT as IR in control group. One-way ANOVA test was employed.

Antioxidant Effect of Methanolic Extract of *Ajuga bracteosa* Herbal Drug in Scopolamine Treated Mice:

First, a decrease in ACh, a cholinergic neurotransmitter essential for the process of normal learning, memory and attention as well as a decrease in level of natural antioxidants in the brain by activating microglia, a source of reactive oxygen species, has been reported [26]. As compared to control group, scopolamine caused significant rise in levels of brain AChE**,** MDA and depletion of GSH and total protein as compared to CMC solution. Chronic administration of methanolic extract of *Ajuga bracteosa* (ABE) herbal drug treatment at 250, 500 and 750 mg/ kg, p.o. significantly ($p \leq 0.05$) and dose dependently attenuated the increase in levels of AChE (Figure 4), MDA (Figure 5) and restored decrease in levels of GSH (Figure 6) and total protein (Figure 7) as compared to scopolamine treated group. Like donepezil, ABE herbal drug reversed amnesia induced by scopolamine.

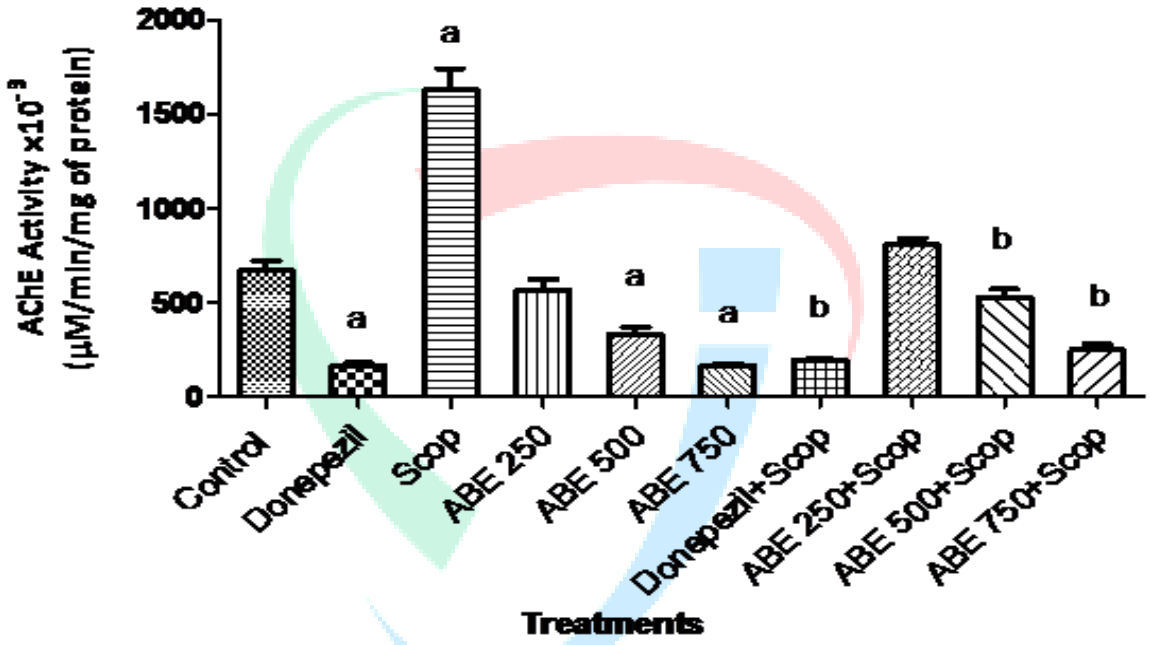

Figure 4: Effects of methanolic extract of *Ajuga bracteosa* (ABE) herbal drug on brain AChE activity

Bar graph represents the effect of methanolic extract of *Ajuga bracteosa* (ABE) herbal drug on brain AChE activity. Each value represents mean AChE level ± S.E.M. with n=6 in each group. The AChE level for control (1% w/v carboxy methyl cellulose i.e. CMC solution), standard drug (Donepezil), amnestic agent (scopolamine) and ABE (methanolic extract of *Ajuga bracteosa*) herbal drug are shown. Results were expressed as mean ± S.E.M. with n=6 in each group. As compared to control group, significant increase in AChE level in scopolamine treated group was noted. It is noted that CMC vehicle, donepezil and methanolic extract of *Ajuga bracteosa* (250, 500 and 750 mg/kg, p.o.) herbal drug treated group decreased AChE level, while the amnestic agent has a significantly increasing effect on AChE level. a = $p \leq 0.05$ Vs AChE level in control group; b = $p \leq 0.05$ Vs AChE level in scopolamine treated group. AChE activity was expressed in µM/min/mg of protein.

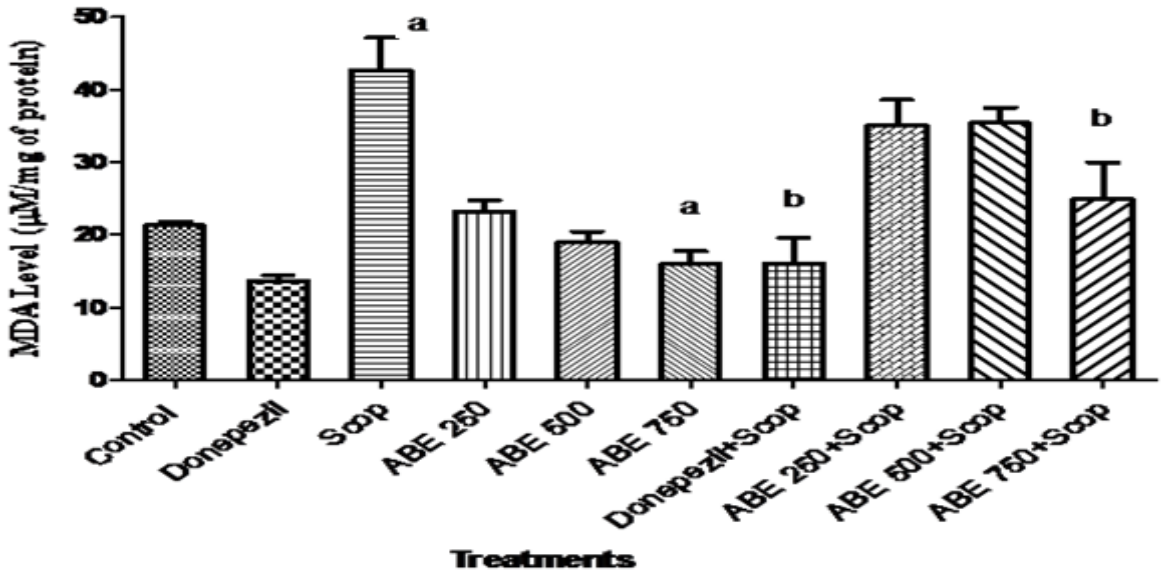

Figure 5: Effects of methanolic extract of *Ajuga bracteosa* (ABE) herbal drug on brain MDA level

Bar graph represents effect of methanolic extract of *Ajuga bracteosa* (ABE) herbal drug on brain MDA level. Each value represents mean MDA level ± S.E.M. with n=6 in each group. The MDA level for control (1% w/v Carboxy Methyl Cellulose i.e. CMC solution), standard drug (donepezil), amnestic agent (scopolamine) and ABE (methanolic extract of *Ajuga bracteosa*) herbal drug are shown. Results were expressed as mean ± S.E.M. with n=6 in each group. As compared to control group, significant increase in MDA level in scopolamine treated group was noted. It is noted that CMC vehicle, donepezil and methanolic extract of *Ajuga bracteosa* (750 mg/kg, p.o.) herbal drug treated group significantly decreased MDA level, while the amnestic agent has a significantly increasing effect on MDA level. a = $p \leq 0.05$ Vs MDA level in control group; b = $p \leq 0.05$ Vs MDA level in scopolamine treated group. MDA level was expressed as µM /mg of protein.

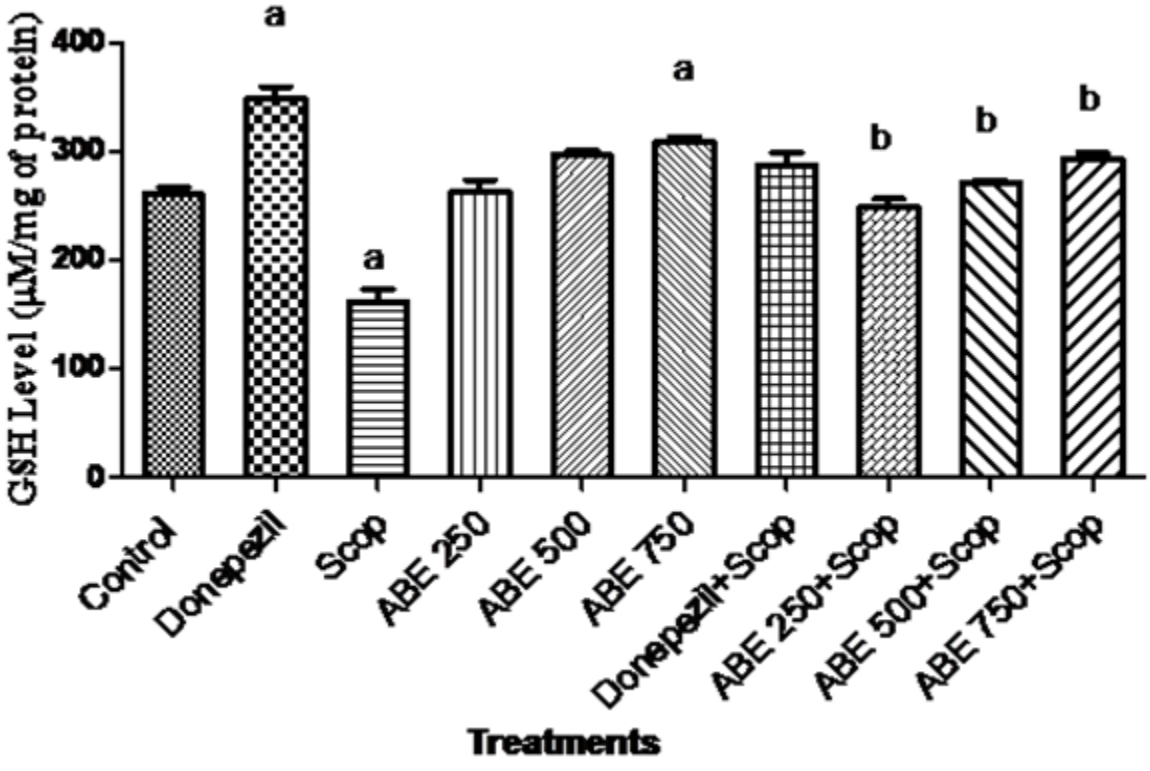

Figure 6: Effect of methanolic extract of *Ajuga bracteosa* (ABE) herbal drug on brain GSH level

Bar graph represents effect of methanolic extract of *Ajuga bracteosa* (ABE) herbal drug on brain GSH level. Each value represents mean GSH level ± S.E.M. with n=6 in each group. The GSH level for control (1% w/v carboxy methyl cellulose i.e. CMC solution), standard drug (donepezil), amnestic agent (scopolamine) and ABE (methanolic extract of *Ajuga bracteosa*) herbal drug are shown. As compared to control group, significant decrease in GSH level in scopolamine treated group was noted. It is noted that CMC vehicle, donepezil and methanolic extract of *Ajuga bracteosa* (250, 500 and 750 mg/kg, p.o.) herbal drug treated group increased GSH level, while the amnestic agent has a significantly decreasing effect on GSH level. a = $p \leq 0.05$ Vs GSH level in control group; b = $p \leq 0.05$ Vs GSH level in scopolamine treated group. GSH level was expressed as µM /mg of protein.

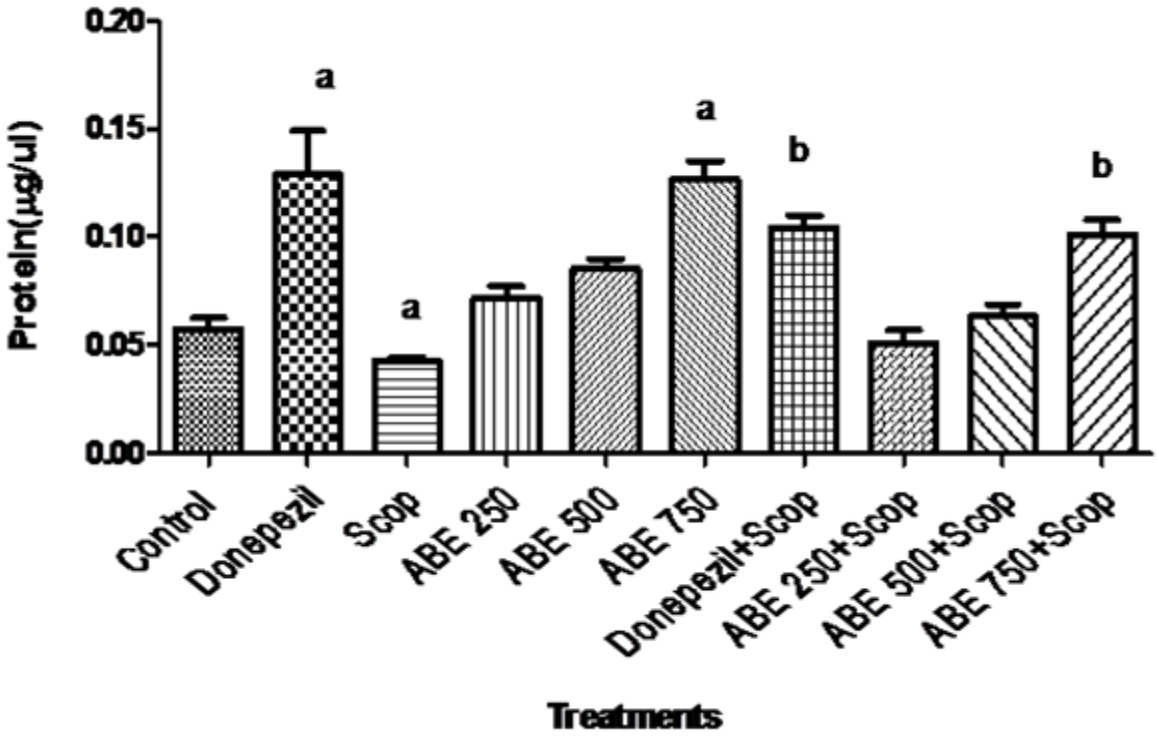

Figure 7: Effect of methanolic extract of *Ajuga bracteosa* (ABE) herbal drug on brain total protein level

Bar graph represents effect of methanolic extract of *Ajuga bracteosa* (ABE) herbal drug on brain total protein level. Each value represents mean total protein level ± S.E.M. The total protein level for control (1% w/v carboxy methyl cellulose i.e. CMC solution), standard drug (Donepezil), amnestic agent (scopolamine) and herbal drug (ABE) are shown. Results were expressed as mean ± S.E.M. with n=6 in each group. As compared to control group, significant decrease in total protein level in scopolamine treated group was noted. It is noted that CMC vehicle, donepezil and methanolic extract of *Ajuga bracteosa* (750 mg/kg, p.o.) herbal drug treated group significantly increased total protein level, while the amnestic agent has a significantly decreasing effect on total protein level. a = $p \leq 0.05$ Vs total protein level in control group; b = $p \leq 0.05$ Vs total protein level in scopolamine treated group. Total protein level was expressed as µg/µg of protein.

Discussion:

A marked decrease in escape latency time (ELT) in control group animals during ongoing acquisition trials denotes normal acquisition of memory and an increase in TSTQ (Q_1) in comparison with other quadrants (Q_2, Q_3, Q_4) search of missing platform during retrieval trial indicates retrieval of memory. Further estimation of TLT as inflation-ratio was done with help of elevated plus maze in scopolamine induced amnesia in mice. TLT as inflation-ratio was measured on day 6 and day7 of study. ABE herbal drug was significantly found to increase the TLT as inflation-ratio and restore the memory function at higher dose. The increase in the inflation-ratio by methanolic extract of *Ajuga bracteosa* (ABE) herbal drug per-se has proved that the herbal drug possessed nootropic activity at 500 and 750 mg/kg dose

dependently. No effects of vehicles employed in the present study to prepare various solutions of drugs have been noted on acquisition and retrieval of memory. Thus, the ABE meets major criteria for nootropic activity, namely, improvement of memory in absence of cognitive deficit [27]. This observation has been strengthened by the finding that ABE has increased the transfer latency time as IR in the elevated plus maze model indicating improvement in memory, which is in accordance with the hypothesis of Itoh *et al*., 1990. Therefore, the effect of pharmacological intervention on acquisition and retrieval of memory is due to herbal drug and not because of its vehicle. Scopolamine, anti-cholinergic drug produces impairment of acquisition and retrieval of memory as reflected by significant increase in ELT on day 6 and decrease in time spent in Q1 target quadrant on day 7 respectively. Moreover, there was an enhancement of brain acetyl cholinesterase (AChE) activity and increase in oxidative stress as reflected by reduction in GSH and total protein levels and increase in MDA level shows the higher lipid peroxidation. Scopolamine significantly increases AChE and MDA levels in the cortex and hippocampus respectively [16, 28]. Cholinomimetic drugs have been reported to improve memory deficits [29, 30]. Therefore, the noted reversal of scopolamine induced amnesia by ABE may be due to its AChE inhibitory and neuroprotective action. The *in-vivo* study of *Ajuga bracteosa* was further supported by estimation of biochemical parameters like brain AChE, MDA, GSH and total protein estimations. The action path of the herbal drug was determined by its neuroprotective action that decreased brain AChE activity, raised brain GSH and total protein levels and reduced MDA level in mice which are the markers of oxidative stress. Enhanced levels of beta-amyloid in the brain have been reported to induce memory dysfunction and dementia due to increase in oxidative stress and AChE activity [31, 32]. ABE herbal drug contains withanolides, which possessed anti-cholinesterase activity in view of its facilitatory effect on retention of acquired learning in mice. Drug that inhibits AChE enzyme protects acetylcholine from hydrolysis and may increase the level of acetylcholine in the brain and has nootropic activity [33]. Therefore, the observed ameliorative effect of ABE herbal drug on scopolamine induced amnesia in mice may be attributed to its potential anti-oxidative [34] and anti-cholinesterase activities.

Conclusion:

Methanolic extract of the *Ajuga bracteosa* (ABE) herbal drug was investigated for its nootropic effect on scopolamine induced amnesia. Morris water maze's result revealed that the chronic administration of methanolic herbal extract of *Ajuga bracteosa* to mice significantly reversed the amnesia induced by scopolamine. The dose dependently increases in the inflation-ratio by ABE (500 and 750 mg/kg, p.o.) has proved that the herbal drug possessed significant ($p \geq 0.05$) nootropic activity in normal and scopolamine treated mice. As compared to scopolamine treated group, increase in brain AChE activity and MDA level was attenuated but decrease in the brain glutathione and total protein levels was significantly reversed by the methanolic herbal extract. Thus, the herbal drug was found to have neuroprotective effect. Methanolic extract of *Ajuga bracteosa* herbal drug also improves learning and memory of scopolamine-induced amnesia in mice by its potent cholinomimetic and antioxidant actions. Therefore, *Ajuga bracteosa* herbal drug can be useful in management of memory deficit and clinical dementia.

Acknowledgement:

The authors are thankful to Prof (Dr.) Ranjit Singh, Pro-Vice Chancellor, Shobhit University, Gangoh, Saharanpur, (Former Director School of Pharmaceutical Sciences, Shobhit University, Meerut) Uttar Pradesh, India for rendering the necessary requirements in this work.

"This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors".

References:

1) J. Carey, Brain facts: a primer on the brain and nervous system, The society for neuroscience, printed by Everbest Printing Company China (2002) 4-6.
2) D. Dhingra, M. Parle, and S.K. Kulkarni, Effect of combination of insulin with dextrose, d (-) fructose and diet on learning and memory in mice (2003), Indian J Pharmacol; no. 35(15):1-6.
3) S.H. Lu et al, The discovery of potential acetyl cholinesterase inhibitors: a combination of pharmacophore modelling, virtual screening and molecular docking studies, J Biomed Sci (2011), no. 18(1) pp. 1-13.
4) World Health organization: Dementia, Fact sheet N 362, 2012.
5) R. Cumin, Effects of the novel compound aniracetam (Ro-13-5057) upon impaired learning and memory in rodents, Psychopharmacol (1982), no. 78: 104-111.
6) R. Bullock, Future directions in the treatment of Alzheimer's disease, Drugs; no.13: 303, 2004.

7) M. Weinstock, Selectivity of cholinesterase inhibition: Clinical implication for the treatment of Alzheimer's disease, J Neurol Neurosurg Psychiatry (1999); no. 12(1), pp. 303-307.
8) S. Yoshida, and N. Suzuki, Antiamnesic and cholinomimetic side-effects of the cholinesterase inhibitors, physostigmine, tacrine and NIK-247 in mouse, Eur. J. Pharmacol; (1993); no. 250 (1):117-124.
9) P. Scartezzini, and E. Speroni, Review on some plants of Indian traditional medicine with antioxidant activity, J Ethnopharmacol 2000; no. (1-2): 23-43.
10) P.I. Sibi, and P.G. Delphia, Nootropic studies of ethanolic extract of Mimosa pudica Linn in albino wistar rats, AJPCT (2014); no. 1: 266-275.
11) A. Vohra, and H. Kaur, Chemical investigation of medicinal plant Ajuga bracteosa", J Nat Prod Plant Resour (2011); no. 1 (1):37-45.
12) M.I. Chaudhary, S.A. Nawaz, Z.U. Haq, M.A. Lodhi, M.N. Ghayur, S. Jalil, and N. Riaz, Withanolides: a new class of natural cholinesterase inhibitors with calcium antagonistic properties, Biochem Biophys Res C (2005); no.334: 276-287.
13) P.K. Lala, Lab Manuals of Pharmacognosy 5th edition, CSI Publishers and Distributors, Calcutta, India 1993.
14) J. Itoh, T. Nabeshima, and T. Kameyzma, Utility of an elevated plus maze for the evolution of memory in mice: effects of nootropics, scopolamine and electro convulsive shock, Psychopharmacol (1990); no. 101: 27-32.
15) R.W. Jones, K.A. Wesnes, and J. Kirby, Effects of NMDA modulation in scopolamine dementia, Ann N Y Acad Sci (1991); no. 640:241-244.
16) R.W. Jeong, K.A. Wesnes, and J. Kirby, Cognitive enhancing and antioxidant activities of iridoid glycosides from Scrophularia buergeriana in scopolamine treated mice, Eur J Pharmacol (2008); no. 288:78-84.
17) R.G.M. Morris, Developments of a water maze producer for studying spatial learning in the rats, J Neurosci Methods (1984), no.11:47-60.
18) W. Nakdooka, T. Niwat, O. Kornkanok, P. Ingkaninanb, and Khongsombata, The effects of Tabernaemontana divaricata root extract on amyloid β-peptide 25-35 peptides induced cognitive deficits in mice, J Ethnopharmacol (2010); no.130:122-126.
19) T. Ogasawara, Y. Itoh, M. Tamura, and T. Mushiroi, Involvement of cholinergic and GABAergic systems in the reversal of memory disruption by NS-105, a cognition enhancer, Pharmacol. Biochem. Behav. (1999); no. 64, (1): 41-52.
20) A.K. Jaiswal, and S.K. Bhattacharya, Effects of shilajit on memory, anxiety and brain monoamines in rats, Indian J. Pharmacol (1992), no. 24:12-17.
21) G.L. Ellman, K.D. Courtney, J.V. Andres, and R.M. Feather-Stone, a new and rapid colorimetric determination of acetylcholinesterase activity, Biochem Pharmacol (1961); no.7: 88-95.
22) G. Voss, and K. Sachsse, Red cell and plasma cholinesterase activities in microsamples of human and animal blood determined simultaneously by a modified acetylthiocholine / DTNB procedure, Toxicol Appl Pharmacol (1970); no. 16: 764-772.
23) H. Ohkawa, N. Ohishi, and K. Yagi, Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction, Anal Biochem (1979); no. 95 (2):351-358.
24) E. Beutler, O. Duron and B. Kelly, Reduced glutathione estimation, J. Lab Clin Med., 1963 no. 61:82.
25) O.H. Lowry, N.J. Rosebrough, A.I. Farr and R.J. Randall, Protein measurement with the folin phenol reagent. J Biol Chem (1951); no. 193:265-75.
26) Z. Pei, H. Pang, L. Qian, S. Yang, T. Wang, W. Zhang, et al., MAC1 mediates LPS-induced production of superoxide by microglia: The role of pattern recognition receptors in dopaminergic neurotoxicity, Wiley online library (2007) no. 55 (13):1362-1373.
27) L.P. De Vreese et al., Memory training and drug therapy act differently on memory and meta-memory functioning: evidence from a pilot study, Arch Gerontol Geriatr (1996); no. 22, 1: 9-22.
28) T. Sakurai, T. Kato, K. Mori, E. Takano, S. Watab, and T. Nabshima, Nefiracetam elevates extracellular acetylcholine level in the frontal cortex of rats with cerebral cholinergic dysfunctions: an in-vivo micro-dialysis study, Neurosci Lett (1998); no. 246: 69-72.
29) A. Madhukar, and V.D. Ashwlayan: Antidementic activity of Brasicca olarecae L. var. Italica (Brassicaceae) on memory deficits in young male rats, PPIJ (2017); no. 5, 3:1-9.
30) V.D. Ashwlayan, and R. Singh: Evaluation of memory enhancing effect of a compound isolated from Emblica officinalis fruit, Biomedicine (2017); no. 37 (1): 058-068.
31) D.K. Song, M.H. Won, J.S. Jung, J.C. Lee, T.C. Kang, and H.W. Suh: Behavioural and neuropathological changes induced by central

injection of carboxyl-terminal fragment of beta-amyloid precursor protein in mice, J Neurochem (l998); 71:875-8.

32) S.H. Choi, C.H. Park, J.W. Koo, J.H. Seo, H.S. Kim, and S.J. Jeong: Memory impairment and cholinergic dysfunction by centrally administered amyloid beta and carboxyl terminal fragment of Alzheimer's APP in Mice, FASEB J (2001); no. 15:1816-1818.

33) S. Bhidwaria, and V.D. Ashwlayan: Neuroprotective effect of Brasicca oleraca L. Var. Botrytis (Brassicaceae) flowers extract on memory deficit in aged & young rats, GJPPS (2017), no. 3 (2) 001-009.

34) A. Vohra, and H. Kaur: Chemical investigation of medicinal plant Ajuga bracteosa, J Nat Prod Plant Resour (2011); no. 1(1):37-45.