AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Antiamnesic and *In Vitro* Antioxidant Effect of Ethanolic Extract of *Bacopa Caroliniana*

Kakarparthy Ravishankar[1], Garlanka Venkata Kiranmayi[1], Medapati Sridevi[1*]
1. Department of pharmacology, Sri SaiAditya Institute of Pharmaceutical Sciences and Research, ADB Road, Surampalem, 533 437, East Godavari District (India).

ABSTRACT

Bacopa is the genus in which the Indian medical herb Brahmi *(Bacopa monnieri)* is included, which have been reported to have memory enhancing effect. *Bacopa monnieri* and *Bacopa caroliniana* are orthologous in nature. In the present study the effects of ethanolic extract of *Bacopa caroliniana* on behavioural changes of Albino rats in normal and stress induced rats was investigated. The animals were divided into 3 group's control, low dose (75mg/kg) and high dose (150mg/kg).Rats in each of these groups were sub divided into 2 groups i.e., with stress and without stress. The animals in stress induced group were forced to swim in a cylindrical vessel containing water at room temperature (28˚c) i.e., chronic mild stress. After the treatment period, the rats of each group were trained on Cooks pole climbing apparatus, Elevated plus maze and on Stair case to assess cognitive improving activities. The results showed improvement in learning performance and enhanced memory retention in rats treated with *Bacopa caroliniana* extract when compared with control group. The *in vitro* antioxidant activity was carried out to correlate its protective effect against stress, significant inhibition against DPPH & Nitric oxide radicals were observed with extract in dose dependent manner and the results were compared with that of standard Gallic acid.

Keywords: *Bacopa caroliniana*, Oxidative stress, Cognition.

*Corresponding Author Email: Sridevi.medapati07@gmail.com
Received 14 August 2014, Accepted 12 September 2014

Please cite this article in press as: Sridevi M *et al*., Antiamnesic and In-vitro Antioxidant Effect of Ethanolic Extract of Bacopa Caroliniana. American Journal of PharmTech Research 2014.

INTRODUCTION

The brain is the central part that controls physiological and cognitive functions in our body and memory is important function of brain. Normal brain functioning, including memory is impaired when connections in brain are lost. Oxidative stress is one of the factors causing the death of neurons. In today's stressful and competitive world poor memory, lower retention and slow recall are common problems. Age, stress and emotion are the conditions that may lead to amnesia (loss of memory), anxiety, high blood pressure, dementia; to more ominous threat like schizophrenia and Alzheimer's disease .Alzheimer's disease is a neurodegenerative disorder whose worldwide prevalence is estimated to be more than 30 million people. Alzheimer's disease is the most common cause of progressive loss of memory and dementia in the elderly. Literature also indicates the role of free radicals in the pathogenesis of Alzheimer's disease, aging, diabetes etc. The compounds having capacity to scavenge these radicals have great potential in mitigation of these disorders. *Bacopa caroliniana* Linn belongs to the family Scrophulariaceae, is an immersed plant (an aquatic plant that rises above the surface of the water) commonly found in fresh and brackish waters. This mat forming, herbaceous species with opposite, sessile leaves up to 2.5 cm long and 2cmwide is distinguished by the lemony scent of these leaves when they are crushed. The blue flowers also distinguish *B. caroliniana* from similar species in this genus. It is native to the southern U.S.

Figure 1: Image of *Bacopa caroliniana.*

Pharmacological studies showed that *Bacopa monnieri* can be used as a memory enhancing agent[3,10,11]. A mixture of 2 saponins designated as Bacosides A& B are responsible for the facilitatory effect of *bacopa monnieri* on learning schedule. The bacosides also attenuated the retrograde amnesia produced by immobilisation induced stress, Electro-convulsive shock and scopolamine and enhanced protein kinase activity and increased the protein content on

hippocampus[12,13,14].The preliminary phytochemical screening of the ethanolic extract of *Bacopa caroliniana* revealed the presence of Protein, Saponins, Tannins, Flavanoids. So far no phytochemical and pharmacological work is reported on this plant, hence a sincere attempt was made to explore for its therapeutic efficacy against stress induced amnesia.

MATERIALS AND METHODS:

Collection and extraction of plant material:

Bacopa caroliniana was collected from an aquatic nursery AQUA SHOPPE PVT LTD Coimbatore, India was authenticated by taxonomist T. Raghu Ram, Maharani College of peddapuram. The aerial parts of the plant were separated from roots, washed; air dried under shade and coarsely powdered. The powdered material was extracted with 95% ethanol in soxhlet apparatus for 8 hrs. Extract was concentrated by distilling of the solvent to obtain the crude extract.

Experimental animals:

Albino rats (75-80g) of either six were used in the study. Animals were housed in colony cages at ambient temperature of 25±2˚c, 12h light/dark cycle and 50±5% relative humidity with free access to food and water *ad libitum*. Food but not water was deprived overnight and during the experiment. All the experiments were carried out during the light period (9:00-16:00h). Each group of 9 animals were taken and after learning each particular group was divided into two sub group of 3 animals each (one sub group without stress and one sub group with stress).

Experimental Design:

Stress procedure:

The animals were subjected to chronic mild stress using following protocol. The rats were forced to swim in a cylindrical vessel of height 60cm and diameter 45cm containing water at room temperature (28˚c). Water depth was maintained at 40cm. The swim stress was conducted for about 15 min and 4 times a day about a week. The animals are placed on small platform (3cm height and 3.5cm diameter) fixed at center chamber and surrounded by water 2cm depth at 22˚c for 24hrs deprived of food and water during night, following 3hrs access to restricted food and 2hrs access to an empty water bottle in alternate days about a week and in the night time rats were subjected to illumination during those alternative days.

Nootropic Activity:

Conditioned avoidance response (CAR) using cooks pole climbing apparatus:

Using the cooks pole climbing apparatus the nootropic activity of ethanolic extract of *B.caroliniana* in normal and stress induced rats was evaluated. Initially Rats were divided into 3

groups' each containing 3 animals. Group I served as control were as group II and III were administered *B.caroliniana* extract 75 and 150mg/kg. Depending on the body weight the extract was administered orally. After 60 minutes of drug administration, all the animals were given training by placing inside the chamber of the apparatus. After 5mins of accustomed period, a buzzer was given which was followed by a shock. The rat has to jump on the pole to avoid foot shock through grid floor. Jumping of the rat on to the pole terminates the shock and this was noted as an "Escape" while jumping prior to the onset of the shock by listening to the buzzer sound was noted as "Avoidance".30 trails was performed every day with an interval of 20-30 seconds given for each trail, until all groups reach 95 to 99 avoidance. After completion of training the groups were again divided in to two sub groups containing 2 animals each, sub group I with stress and sub group II without stress (left normal). The stress was induced to sub group I for about week and another subgroup II was left normal, the drug was usually given to both groups. Again on 7th day the retention of memory was checked in both normal and stress induced groups. The days taken to reach 95 to 99 avoidance was noted again. Daily dose of extract was continued until all groups returned to normal level from stress induced amnesia.

Transfer latency using elevated plus maze test:

The elevated plus maze made of wood and fabricated locally was used for the study. The plus maze apparatus consists of two open arms (50×10 cm) and two closed arms (50×10×30 cm) with an open roof. The entire maze was elevated to a height of 60 cm from the floor. Rats were initially divided into 3 groups each containing 3 animals. Group I served as control were as group II and III were administered *B.caroliniana* extract 75 and 150mg/kg. Depending on the body weight the extract was administered orally. After 60 minutes of drug administration on 1st day rats in each group were placed individually at the end of an open arm facing away from the central platform. The time taken for each rat to move from open arm to either of the closed arms (transfer latency, TL) was recorded. After measurement of Transfer latency the rats were allowed to explore the maze for about 5 min. On the 2nd day again the rats were placed on the elevated plus maze and Transfer latency was noted again. After 2nd day each particular group was divided into two sub groups as sub group I with stress and sub group II without stress (left normal).Then subgroup I was subjected to stress for about 7 days and subgroup II was left normal. On 9th day again transfer latency was noted for all groups. Then inflexion ratio was calculated.

Staircase test:

The staircase was made of wood and consisted of five identical steps 2.5cm high, 10cm wide, 7.5cm deep surrounded by walls, the height of which (10cm) was constant along the whole length

of the staircase. Rats were initially divided served as control, were as Group II and III were administered B.*caroliniana* extract orally 75 and 150mg/kg body weight respectively. Different groups of mice were administered 60min prior to the experiment. The mouse was gently placed on the floor of the box with its back to the staircase. During a 3min period, the number of steps climbed and the number of rearings made were recorded. A step was considered climbed when all four paws were placed on the step[18,19]. After learning on the 2nd day each particular group was divided into two groups as normal and stress groups. Then each subgroup of particular group subjected to stress from 2nd day to 7th for 4 days. On 7th day again the rats of each group were placed on the floor of the box and the no. of steps climbed and no. of rearings made were recorded.

***In Vitro* Antioxidant Activity:**

Chemicals and reagents:

All the chemicals Gallic acid (gifted sample), DPPH (purchased from research-labfine chem industries, Mumbai). Methanol, Trichloroacetic acid, Phosphate buffer, Ammonium molybdate, Sodium phosphate, DMSO, Sodium nitro prusside was of analytical grade.

DPPH (1, 1-diphenyl -2-picryl hydrazyl) Free Radical Scavenging Activity:

The radical scavenging activity (RSA) of different extracts was determined using DPPH assay; it was measured by decrease in absorbance at 517nm of methanolic solution of DPPH after addition of antioxidant (plant extract).Gallic acid was taken as reference standard. Different concentrations of test samples (100µg/ml, 200µg/ml &400µg/ml) and standard (1.0µg/ml, 2.5µg/ml, and 5.0µg/ml) were prepared using methanol. 0.1mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of tests and standard separately. These solutions were kept in dark for about 30min and the absorbance's were measured at 517nm, methanol (3ml) in the place of test samples was used as the blank. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH Scavenged (\%)} = \mathbf{\{(A_0-A_1)/A_0\} \times 100}$$

Where A_0 is the absorbance of the blank (containing all reagents except the test sample), and A_1 is the absorbance of test sample. The antioxidant activity of test sample was expressed in IC_{50} values. The IC_{50} value is defined as concentration in (µg/ml) of test sample that scavenges Free radicals by 50%.

Nitric oxide generation & assay of nitric oxide scavenging:

Nitric oxide was generated from sodium nitro prusside & measured by the Greiss reaction. Sodium nitro prusside (5mM) in phosphate buffered saline was mixed with different concentrations of test samples(100µg/ml,200µg/ml,& 400µg/ml) dissolved in DMSO & incubated at 25°Cfor

150min.The samples above were reacted with Greiss reagent(1% sulphanilamide,2% ortho phosphoric acid & 0.1% naphthyl ethylenediaminedihydrochloride).The absorbance of the chromophore formed during the naphthylethylenediamine was read at 546nm & referred to the absorbance of standard solutions of ascorbic acid was treated in the same way with Greiss reagent. All the tests were performed in triplicate & the results were averaged. Gallic acid was used as reference compound. The % decrease in absorbance was calculated.

$$\text{Nitric oxide Scavenged (\%)} = \mathbf{\{(A_0\text{-}A_1)/A_0\} \times 100}$$

Where A_0 is the absorbance of the blank (containing all reagents except the test sample), and A_1 is the absorbance of test sample. Gallic acid was used as reference standard.

Phosphomolybdenum Assay:

Different concentrations of test samples (100µg/ml, 200µg/ml & 400µg/ml) and standard (1.0µg/ml, 2.5µg/ml, and 5.0µg/ml) Gallic acid were prepared using suitable solvent (DMSO for test substances). 0.3 ml of each concentration of test sample and standard were mixed with 3.0ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate).The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution after cooling to room temperature, was measured at 695 nm using UV Spectrophotometer. Distilled water (0.3 ml) was used as blank.

Reducing power method:

Different concentrations of test samples (100µg/ml, 200µg/ml & 400µg/ml) and standard (1.0µg/ml, 2.5µg/ml,and 5.0µg/ml) Gallic acid were prepared. 1ml of each concentration of test and standard were mixed in to phosphate buffer (2.5ml, 0.2M, pH6.6) and potassium ferricyanide (2.5ml, 1%). Then the mixtures were incubated at 50˚c for 20mins. 2.5ml of trichloroacetic acid (10%) was added to all the mixtures, which was then centrifuged at 3000rpm for 10 mins. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and $Fecl_3$ (0.5ml, 0.1%) and the absorbance was measured at 700nm.

RESULTS AND DISCUSSION

Conditioned avoidance response (CAR) using cooks pole climbing apparatus:

As shown in figure 2 and 3 the Conditioned avoidance response of rats in group III administered with the ethanolic extract of *Bacopa caroliniana (*150mg/kg) increased gradually to 90-99% within 7 days, group II (75 mg/kg) in 9 days and group III in 12 days. The percent avoidance was always higher in the group II (98.33% on 9^{th} day) and group III (99.16% on 7^{th} day) compared to vehicle treated control group (97.5% on 12^{th} day) and the results were found to be dose dependent. After

induction of stress, reduction in CAR is a clear indication of stress induced amnesia compared to normal sub groups (which were not subjected to stress). However, continued treatment of *Bacopa caroliniana* produced better retention and recovery in a dose dependent manner than the vehicle treated animals in stress induced groups. There was a less fall in mean percentage of CAR in extract treated stress induced subgroups, group III (85.83%) and group II (75.00%) compared to vehicle treated stress control group (53.33%). But in normal sub groups (which were not subjected to stress) there is no significant reduction in CAR. Animals treated with higher dose (150mg/kg) took less time to achieve 95%CAR and fewer fall in mean percentage of CAR and recovery in stress induced subgroup compared to low dose and control treated stress group and the percentage of avoidance was always higher in higher dose (150mg/kg) and lower dose (75mg/kg) compared to control group.

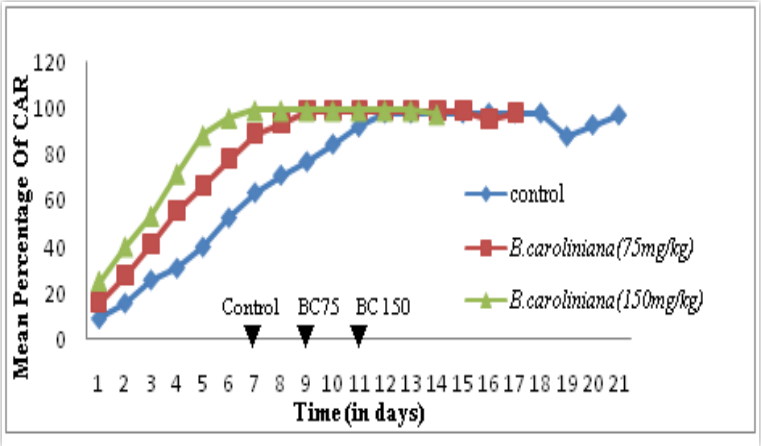

Figure 2: Effect of Ethanolic Extract of *Bacopa caroliniana* on Mean Percentage of Conditioned Avoidance Response in Normal Rats.

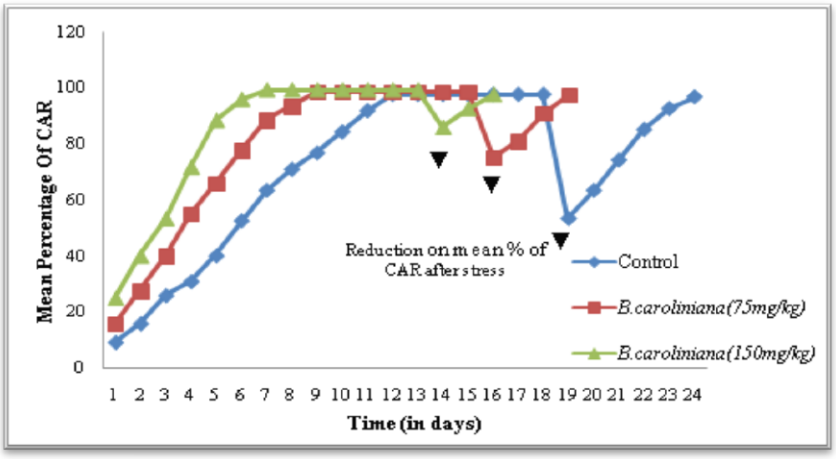

Figure 3: Effect of Ethanolic Extract of *Bacopa caroliniana* on Mean Percentage of Conditioned Avoidance Response Rats after stress.

Transfer latency using elevated plus maze:

As shown in table 1, 2 and figure 4 in Normal control group there is a significant decrease in transfer latency (27.5±1.55) and increase in inflexion ratio (1.66) on day 9, in stress induced sub groups (control), on 9th day there was an increase in the transfer latency (43.5±1.19) compared to day 2 and decrease in inflexion ratio (0.68) which clearly indicates stress induced amnesia. In normal group II (75mg/kg) there is a significant decrease in transfer latency (18.75±0.75) and increase in inflexion ratio (2.57) on day 9, in stress induced sub groups (low dose), on 9th day there was an increase in the transfer latency (37±1.47) compared to day 2 and decrease in inflexion ratio (0.81). In normal group III (150 mg/kg) treated group the decrease in transfer latency (20.75±0.47) and increase in inflexion ratio (2.90) on day 9, in stress induced sub groups (low dose), on 9th day there was an increase in the transfer latency (41.75±0.85) compared to day 2 and decrease in inflexion ratio (0.94). Pretreatment with *Bacopa caroliniana* extract (group II and group III) significantly decreased the transfer latency to enter in to closed arms from open arm. In stress induced sub groups, the extract (75 and 150mg/kg) treated groups exhibited significant decrease in transfer latency and increase in inflexion ratio compared to vehicle treated stress subgroup.

Table1: Effect of ethanolic extract of *Bacopa caroliniana* on Transfer Latency (in secs) on 2nd and 9th day in normal rats using elevated plus maze.

Treatment	Transfer latency(in secs)			Inflexion ratio	
	Day1	Day2	Day9	Day2	Day9
Vehicle	73.25±1.88	41.75±1.2$^{@**}$	27.5±1.55$^{@**}$	0.75	1.66
B.caroliniana (75mg/kg)	67±3.69	29.75±2.0$^{@*}$	18.75±0.7$^{@*}$	1.25	2.57
B.caroliniana (150mg/kg)	81±5.80	35.25±1.7$^{**\#}$	20.75±0.4$^{**\#}$	1.29	2.90

Values are mean ± SEM, n=4 in each group; @P=0.037, **P=0.09compared to day1 (one- way ANOVA); *P=0.032, #P= 0.073 compared to control (one- way ANOVA).

Table2: Effect of ethanolic extract of *Bacopa caroliniana* on Transfer Latency (in secs) on 2nd and 9th day in stress rats using elevated plus maze.

Treatment	Transfer latency(in secs)			Inflexion ratio	
	Day1	Day2	Day9	Day2	Day9
Vehicle	73.25±1.88	41.75±1.2$^{@**}$	27.5±1.55$^{@**}$	0.75	1.66
B.caroliniana (75mg/kg)	67±3.69	29.75±2.0$^{@*}$	37±1.47$^{@*}$	1.25	0.81
B.caroliniana	81±5.80	35.25±1.70$^{\#**}$	41.75±0.85$^{\#**}$	1.29	0.94

Values are mean ± SEM, n=4 in each group; @P=0.055, # P=0.10 compared to day1 (one- way ANOVA);*P=0.048, **P= 0.98 compared to control (one- way ANOVA).

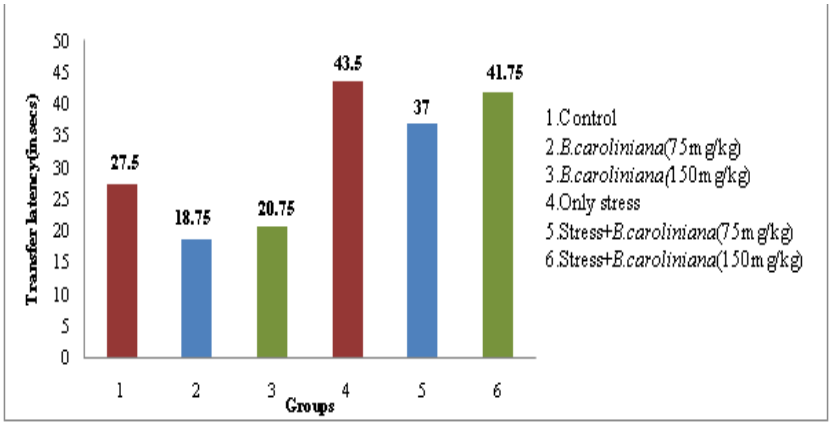

Figure4: Effect of ethanolic extract of *Bacopa caroliniana* on transfer latency on elevated plus maze on 9th day in normal and stress induced rats.

Stair case test:

As shown in table 3 and 4 and figure 5 in Normal control group (which were not subjected to stress) there was no significant decrease in no. of steps climbed (24.5±0.95) and no. of rearings (16±0.81) on day 7 compared to day 1 and 2. In normal group II (75mg/kg extract treated) there is a significant decrease in no. of steps climbed (18.75±1.25) and no. of rearings (10.5±1.32) on day 7 compared to control. In normal group III (150mg/kg) there is a significant decrease in no. of steps climbed (10.25±1.31) and no. of rearings (5.25±1.10) on day 7 compared to low dose. In stress induced control group, on 7th day there was an increase in no. of steps climbed and no. of rearings which clearly indicates stress induced amnesia and depression. In stress induced sub groups, the extract (75 and 150mg/kg) treated groups exhibited significant decrease in no. of steps climbed and no. of rearings on day 7 compared to control treated stress subgroup. The stair case test is considered a simple, rapid and sensitive test and clinically Neuroleptics and antidepressants produced a progressive and simultaneous reduction of both the steps climbed and rearings. The *Bacopa caroliniana* extract treated groups there was a significant reduction in the number of steps climbed and number of rearings compared to control treated in both stress induced and normal groups.

Table: 3 Effect of ethanolic extract of *Bacopa caroliniana* on no. of steps climbed and rearings in normal rats using stair case.

Treatment	Day1		Day 2		Day7	
	No. of steps climbed	No. of rearings	No. of steps climbed	No. of rearings	No. of steps climbed	No. of rearings
Control	26.5±1.03	15.5±1.19	23.75±0.85$^{@\#}$	17.25±0.47$^{@\#}$	24.5±0.95$^{@\#}$	16±0.81$^{@\#}$
B.*caroliniana* (75mg/kg)	22.5±0.86	16.75±0.75	22.5±0.95$^{@*}$	12.25±0.62$^{@*}$	18.75±1.25$^{@*}$	10.5±1.32$^{@*}$

B.caroliniana (150mg/kg)	15.5±1.19	10.5±0.64	15.75±0.85$^{\#**}$	6.5±0.64$^{\#**}$	10.25±1.31$^{\#**}$	5.25±1.10$^{\#**}$

Values are mean ± SEM, n=4 in each group @P=0.43, #P=0.45 No. of steps climbed compared to day1; *****P=0.11, **P=0.02 No. of steps climbed compared to control group (one- way ANOVA).@P=0.10, #P=0.05 No. of rearings compared to day1; *P=0.01, **P=0.0006 No. of rearings compared to control group (one- way ANOVA).

Table: 4 Effect of ethanolic extract of *Bacopa caroliniana* on no. of steps climbed and rearings in stress rats using stair case.

Treatment	Day1		Day 2		Day7	
	No. of steps climbed	**No. of rearings**	**No. of steps climbed**	**No. of rearings**	**No. of steps climbed**	**No. of rearings**
Control+ stress	26.5±1.03	15.5±1.19	23.75±0.85$^{@\#}$	17.25±0.47$^{@\#}$	30.25±0.6$^{@\#}$	18.75±0.75$^{@\#}$
B.*caroliniana* (75mg/kg)+ stress	22.5±0.86	16.75±0.75	22.5±0.95$^{@*}$	12.25±0.62$^{@*}$	25±1.08$^{@*}$	15.75±0.85$^{@*}$
B.caroliniana (150mg/kg)+ stress	15.5±1.19	10.5±0.64	15.75±0.85$^{\#**}$	6.5±0.64$^{\#**}$	19.25±1.10$^{\#**}$	8.75±0.47$^{\#**}$

Values are mean ± SEM, n=4 in each group; @P=0.26, #P=0.20 No. of steps climbed compared to day1; *****P=0.09, **P=0.009 no. of steps climbed compared to control group (one- way ANOVA).@P=0.16, #P=0.07 No. of rearings compared to day1; *P=0.02, **P=0.0006 No. of rearings compared to control group (one- way ANOVA).

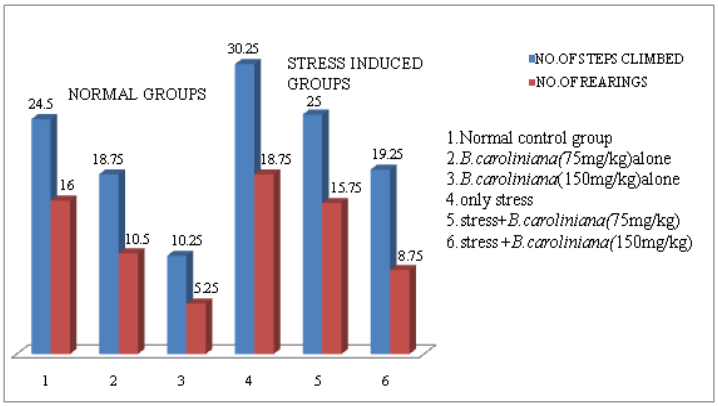

Figure 5: Effect of ethanolic extract of *Bacopa caroliniana* on the no. of steps climbed and rearings on 7th day in Normal and stress induced rats using stair case.

Antioxidant activity:

The results of antioxidant values expressed as IC_{50} with different antioxidant markers. Results were tabulated in Table 5 and 6. Loss of memory is the main symptom of brain damage and for a variety of disorders including Alzheimer's disease. Stressful conditions are often associated with

Table 5: DPPH, Nitric Oxide Methods of Test Samples and Gallic Acid.

Sample	Concentration (µg/ml)	DPPH scavenging assay		Nitric oxide scavenging assay	
		% inhibition	IC_{50}(µg/ml)	%inhibition	IC_{50}(µg/ml)
Gallic acid	1	81.81±0.001		36.12±0.01	
	2.5	88.71±0.002	0.061	51.67±0.008	2.117
	5	91.22±0.001		67.72±0.004	
Extract	100	52.35±0.002		77.59±0.006	
B.caroliniana	200	60.81±0.002	101.9	85.28±0.004	21.18
	400	85.89±0.002		91.47±0.002	

Values are expressed as mean ±SEM

Table 6: Phosphomolybdenum and Reducing power Methods of Test Samples and Gallic Acid

Reducing power method			Phospomolybdenum method		
Sample	Concentration (µg/ml)	Absorbance(nm)	Sample	Concentration (µg/ml)	Absorbance(nm)
Gallic acid	1	0.079±0.001	Gallic acid	1	0.098±0.004
	2.5	0.142±0.003		2.5	0.017±0.004
	5	0.191±0.001		5	0.033±0.003
Extract	100	0.313±0.001	Extract	100	0.092±0.004
B.Caroliniana	200	0.446±0.002	*B.Caroliniana*	200	0.192±0.004
	400	0.534±0.002		400	0.269±0.004

Values are expressed as mean ±SEM.

loss of memory and other cognitive functions. The brain is especially sensitive to oxidative damage because of its high content of readily oxidized fatty acids, high use of oxygen and low levels of antioxidants. The anti oxidant and anti stress activity were correlated with the nootropic activity since the role of stress and free radicals have been implicated in the loss of memory, concentration. Several research studies have identified the natural compounds that serve as nootropic agents. *Bacopa monniera* is one of such plant used for treating memory related disorders. A mixture of 2 saponins Bacosides A and B of *Bacopa monnieri* can able to induce membrane dephosphorylation with an increase in protein and RNA turn over in specific brain areas. These saponins can also reverse the depletion of acetylcholine and decreases the muscarnic receptor binding in forntal cortex and hippocampus. *Bacopa monnieri* and *Bacopa caroliniana* are orthologous in nature. Other species of Bacopa (i.e., *B.eiseni,B.repens*) are originated after the splitting of gens but *B. caroliniana* and *B. monnieri* are formed after the speciation and the cultivation of *Bacopa caroliniana* is easier when compared to other species. The Phytochemical screening and antioxidant activity of *Bacopa caroliniana* was studied. The test for saponins was positive and the extract has significant antioxidant activity compared to standard Gallic acid. Memory performance depends upon the type of difficulty and the nature of task, hence new drug

evaluation is usually carried out using multiple models. These results clearly indicate that oral administration of the *Bacopa caroliniana* extract improved learning and memory in rats. Therefore the protective effect of ethanolic extract of *Bacopa caroliniana* against stress induced amnesia may be due to cognitive enhancement via its modulatory effect on cholinergic system and anti oxidant activity.

CONCLUSION:

The present study demonstrates scientific support for the protective effect of ethanolic (stem and leaves) extract of *Bacopa caroliniana* to combat stress induced amnesia and lends some credence to traditional claims of its therapeutic benefits in stress and stress related disorders.

ACKNOWLEDMEMENT:

The authors are very thankful to N.Sathish reddy, Vice Chairman of Aditya Educational Institutions, Surampalem for providing necessary facilities to carry out this research work.

REFERENCES:

1. Rama Krishna s, Abhinav k, Jogendhar M, Madhusudana K, GangaModiNaidu V, Yogendra Kumar G. Anti amnesic activity of *Vitex negundo* in scopolamine induced amnesia in rats. Pharmacol Pharm 2010;1(1):1-8.
2. Maier SF, Watkins LR. Cytokines for psychologists: Implications for bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. Psychol Rev 1998 JAN;105(1):83-107.
3. VenkataRamana V, Subramanya U, Satheesha N. Effect of Bacopa monniera Linn. (brahmi) extract on learning and memory in rats:A behavioral study. J Vet Behav 2010;5:69-74.
4. Sunil N, Shirsagar K. Nootropic Activity of dried seed kernels of *Caesalpinia crista* Linn against Scopolamine induced amnesia in mice. IJPRIF 2011;3(1):104-9.
5. Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganquli M et al. Global prevalence of Dementia: a Delphi consensus study. Alzheimer's Disease international Lancet Publishers 2005(Dec 17);366:2112-7.
6. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. Oxfork: Clarendon Press 1995;279-313.
7. Goverdhan P, Sravanthi A, Mamatha T. Neuroprotective Effects of Meloxicam and Selegiline in Scopolamine-Induced Cognitive Impairment and Oxidative Stress. Int J Alzheimer's Dis 2012; http://dx.doi.org/10.1155/2012/974013.

8. Brycemillar MD, Walter Robins BL. Plant of the Month issue. Tropical fish magazine 2010 May.
9. http://www.floridaplants.com/Eflora/bacopa_caroliniana.htm.
10. Zhou Y, Shen YH, Zhang C, Zhang WD. Chemical constituents of *Bacopa monnieri*. Chemistry of Natural products 2007;43(3):355-6.
11. Singh HK, Dhawan BN. Neuropsychopharmacological effects of the Ayurvedic nootropic *Bacopa monniera* Linn. (Brahmi). Indian J Pharmacol 1997;29:359-65.
12. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of Medicinal Plants and Natural Products. Indian J Pharmacol 2000;32:81-118.
13. Keyvan D, Damien Dorman HJ, Heikki V, Raimo H. Plants as Potential Sources for Drug Development against Alzheimer's disease. Jaime A. Teixeira da Silva(ed) International Journal of Biomedical and Pharmaceutical sciences: Global book sciences 2007 Japan Availableonline: www.globalsceincebooks.info
14. Prakash O, Singh GN, Singh RM, Mathur SC, Bajpai M, Yadav S. Determination of bacoside A by HPTLC in *Bacopa monnieri* extract. Int J Green Pharm [serial online] 2008[cited 2014Aug20];2:173-5. Availableonline: http://www.greenpharmacy.info/text.asp?2008/2/3/173/42738.
15. Gerhard vogel H(ed). Drug discovery and evaluation: Pharmacology assays, 2nd edition. New York, Springer-Verlag Berlin Heidelberg 2002;626-797.
16. Cook L, Weidley e., behavioural affects of some psychopharmacol agents. Ann N Y Acad Sci 1957;66:740-52.
17. Vyawahare NS, Ambikar D.B. Evaluation of Neuropharmacological Activity of Hydroalcoholic extract of fruits of trapabispinosa in laboratory animals. Int J Pharma Pharma Sci 2010;2(2):32-5.
18. Simiand J, Keane PE, More M. The staircase test in the mice: a simple and efficient procedure for primary screening of anxiolytic agents. Psychopharmacol Ser 1984;84(1):48-53.
19. Steru L, Thierry B, Chermat R, Millet B, Siamon P, Porsolt RD. Comparing benzodiazepoines using the Stair case test in mice. Psychopharmacol Ser 1987;92:106-9.
20. Sharma A, Bharadwaj S, S.Maan A, Jain A, D. Kharya M. Screening of Antioxidant activity: An overview. Pharmacog Rev 2007;1(2):232-8.
21. Kornal Kumar J, DeviPrasad AG, AustinRichard S. In vitro antioxidant activity of and phytochemical analysis of Medicallegumes. J Pharm Res 2012;5(6):3059-62.

22. Huda AW, Munira MW, Fitriya SD,Salmah M. Antioxidant activity of *Aquilaria malaccensis* leaves. Pharmacognosy Res 2009;1(5):270-3.
23. Pushpalatha B, Venumadhav N, Swathi M, Butchiraju AK. Neuroprotective effect of resveratrol against scopolamine induced cognitive impairment and oxidative stress in rats. Arch Biol Sci Belgrade 2013;65(4):1381-6.
24. Ehab E, Tuppo DO, Ms Lloyd, J Forman. Free radical oxidative damage and Alzheimer's disease. J Am Osteopath Assoc 2001;101(12):11-5.
25. Singh HK, Rastogi RP, Srimal RC, Dhawan BN. Effect of bacosides A and B on avoidance responses in rats. Phytother Res 1988 JAN 11;2(2):70-5.
26. Ashokan KV, Mundaganaur DS, Mundaganar YD. Sequence variability of *Bacopa* sp NADH dehydrogenase sub unit-F and relationship with physico-chemical characteristics of proteins. Ijphc [internet]. 2011;3(1):[about 1p.]. Available from: http://www.rspublication.com/ijphc/index.html.