Research Article

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Evaluation of Anti-Alzheimer's properties of *Bacopa monniera* extract on Morphometric, Behavoural aspects and Cholinergic system in albino mice

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ABSTRACT

The aim of the present study is to assess the anti-Alzheimer's properties of *Bacopa monniera* plant extract **(**BME) on the cholinergic system of AD-induced mice. Male albino mice, *Mus musculus*, of one month old weighing 20 ± 2 grams, obtained from Sri Venkateswara Enterprises, Bangalore were used as the experimental model. They were maintained in the animal house of the Department **(Behringer,1973)** according to the ethical guidelines for animal protection and welfare bearing the Resolution No. 02/(i)/a/CPCSEA/ IAEC/ SVU/ KY-KK/ Dt. 21-03-2011. The mice were fed with standard pellet diet and water *ad libitum*. The mice were divided in to four groups as follows: Group I: Control mice; Group II: mice treated with BME; Group III(AD induced): mice treated with D-Gal & $NaNO_2$; Group IV: AD induced mice simultaneously treated with BME. Changes in Morphometric and Behavioural aspects of four groups of mice were analised by using Morris Water Maze technique. Various constituents of the cholinergic system viz., Acetylcholine (ACh) content and Acetylcholinesterase (AChE) activity were determined in selected regions of all groups of mice at selected time intervals through standard biochemical assay methods. From our results, it was ovious that BME showed positive effects on body weight, learning skills, memory and concentration, whereas D-Gal and $NaNO_2$ caused learning and memory deficits in mice which could be ameliorated by simultaneous administration of BME. Similar protective effectsof BME were noticed on the cholinergic system of mice brain wherein , oral administration of BME could revert both the ACh content and also AChE activity to normal levels in AD induced mice. Form these observations on Morphometric and Behavioural aspects and also on the Cholinergic system it was finally concludd that BME had potential compounds which can prevent the learning and memory deficits effectively and thus confer neuroprotection againt Alzheimers Disease.

Key words: Albino mice, *Bacopa monniera* Plant extract (BME), Alzheimer's disease, Morris Water Maze, Morphometric and Behavioal aspects and Cholinergic system.

INTRODUCTION:

Alzheimer's Disease (AD), also called Senile Dementia of Alzheimer Type (SDAT), is the most common form of dementia. This disease was first described by German Psychiatrist and Neuropathologist, Alois Alzheimer in 1906 and was named after him (Berchtold and Cotman, 1998). AD is becoming a more common cause of death in populations of the United States and other countries since the life span of human beings is increasing. Although other major causes of death in human beings continue to experience significant declines, those from AD have continued to rise. Between 2000 and 2008, deaths attributed to AD increased by 66%, whereas those attributed to heart disease, decreased by 13% (Minino et al., 2010). The incidence of AD rised from 2.8 per 1000 persons in the age group of 65-69 to 56.1 per 1000 persons in the older people of 90 years (Kukull et al., 2002). Alzheimer's disease is primarily characterized by formation of amyloid plaques and neurofibrillary tangles ultimately leading to neuronal death which produces deficits in several neurotransmitter systems. The most prominent changes occur in the loss of cholinergic neurons in the basal forebrain that project to the hippocampus and neocortex, which play an important role in memory and cognitive function. This loss of cholinergic neurons results in upto a 90% reduction in the activity of acetyltransferase, which is needed for the synthesis of acetylcholine (Murphy et al., 1998). Treatment strategies have therefore, focused on elevating the acetylcholine by cholinesterase inhibitors such as Tacrine, Donepezil, Rivastigmine and Galantamine but they have cholinergic side effects such as nausea, anorexia, vomiting, and diarrhea (Akhondzadeh and Noroozian, 2002; Bullock, 2002). Therefore, it is worthwhile to explore the alternative source for a suitable medicine through the ancient Indian Traditional Medical System (Ayurveda) for treatment of various neurological disorders in general and Alzheimers disease in particular.

Since time immemorial, *Bacopa monniera*, a traditional Ayurvedic medicinal plant which belongs to the family Scrophulariaceae is commonly known as Brahmi in Sanskrit and has been used as nervine tonic for promoting mental health and improving memory by Ayurvedic medical practitioners in India. Recent research findings have revealed

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its protective roles as cognition-enhancer (Das et al., 2002), antidepressant, antioxidant in rat frontal cortex, striatum and hippocampus (Bhattacharya et al., 2000; Russo et al., 2003), antiulcerogenic (Sairam et al., 2001), and calcium antagonist (Dar and Channa., 1999). The chemical constituents of *Bacopa monniera* include alkaloids brahmine, herpestine and nicotine, saponin monierin, hersaponin bacoside A1, A2, A3 (Rastogi and Kulshrestha, 1994), and B (Basu et al., 1967) and four saponin bacogenin A1 to A4 (Chandel et al., 1977). Many biological effects of *Bacopa monniera* are documented in traditional as well as in scienti?c literature of which the most important one is bacosides on enhancing the cognition and memory functions (Maher et al., 2002;Russo and Borrelli, 2005).

It was well established that D-Galactose, a physiological nutrient when supplied overdoses will result in abnormal oxidative metabolism eventually leading to production of excess Reactive Oxygen Species, which surpass the ability of the cells to eliminate them, thus finally causing impairment of cell structure and gene expression (Zhang et al., 2003). Similarly, Sodium nitrite can change normal haemoglobin in to methaemoglobin, which reduces the oxygen-carrying capacity of the blood, causing hypoxia, disability of consciousness ultimately depressing the learning and memory ability in mice.

In view of these multiple beneficial properties of Bacopa, in the present study an attempt has been made to assess the protective role of *Bacopa monniera* plant extract on Morphometric and Behavioural aspects and the cholinergic system in AD induced mice by giving a combined chronic intra peritoneal injection of D-Galactose and Sodium nitrite to normal mice (Zhang et al., 2006; Fang and Liu., 2007).

MATERIALS AND METHODS:

Chemicals:

All chemicals used in the present study were Analar Grade (AR) and were obtained from Sigma (St. Louis, MO, USA), Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India) Scientific Companies. For the present investigation, Barnstead Thermoline water purification plant was used for Nano pure water; Hahnvapor Rotary Evaporator HS-2005V for plant exraction; KR 2000T centrifuge for centrifugation of homogenates; Hitachi UV-2800 spectrophotometer, RF 1501 Schimadzu Fluorimeter and other standard equipments for biochemical/physiological analyses.

Maintenance of mice:

Male albino mice, *Mus musculus*, of one month old weighing 20 ± 2 grams, obtained from sri venkateswara enterprises, Bangalore was selected as the experimental model. The mice were maintained in the laboratory conditions according to the instructions of Behringer(1973) and as per the approval of the Institutional Animal Ethical Committee (Resolution No. 02/(i)/a/CPCSEA/ IAEC/ SVU/ KY-KK/ Dt. 21-03-2011).

Collection and preparation of *Bacopa monniera* extract:

Bacopa monniera plant was collected from Talacona forest area which is around 50 Km from Tirupati. The whole plant was dried in shade, powdered and used for extraction by using methanol as solvent. Powdered plant material was soaked in 95% methanol for 2 days at room temperature and the solvent was filtered. This was repeated 3 to 4 times until the extract gave no colouration. The extract was distilled and concentrated under reduced pressure in the Hahnvapor Rotary Evaporator HS-2005V. The resulting methanol crude extract was air-dried and used in the present study.

Induction of Alzheimer's disease in mice:

Until now, several chemical compounds such as Amyloid beta, Aluminium-maltolate, D-Galactose and Sodium nitrite have been used to induce AD in mice. But a combination of the chemicals, D-Galactose and Sodium nitrite together was considered to be quite successful in inducing Alzheimer's disease in mice (Zhang et al., 2006; Fang and Liu., 2007). Hence, in the present study, AD in mice was induced by an intraperitoneal (i.p.) injection of D-Galactose (120mg/kg body weight) and sodium nitrite (90mg/kg body weight) by dissolving in distilled water**.**

Experiment protocol:

GROUPING OF ANIMALS:

After the mice were acclimated to the laboratory conditions for 10 days, they were randomly divided in to four main groups. Each main group was again divided in to 12 sub groups of six each and were housed in separate cages. All the animals in each Group were administered with the following compounds as given below. All doses were given once in a day in the morning hours between 8 to 9 AM, keeping in view the altered activity of mice during the nights.

Group I	Control (C)
Group II	Mice treated with BME (100 mg/kg body weight for 180 days)
Group II (AD induced)	Mice treated with D-Galactose (120 mg/kg body weight) + Sodium nitrite (90 mg/kg body weight) for 60 days
Group IV	AD induced mice simultaneously treated with BME from 10th day up to 180th days, at Doses mentioned above.

Isolation of tissues:

Mice in all groups were sacrificed by cervical dislocation at the selected time periods viz., 15th, 30th, 45th, 60th, 75th, 90th, 105th, 120th, 135th, 150th, 165th and 180th day. Selected regions of mice brain such as Olfactory Lobe(OL), Cerebral Cortex(CC), Hippocampus(Hc), Cerebellum(Cb), Ponsmedulla(Pm) and Spinal cord(Spc) were isolated and immediately homogenized in suitable media for assay of Cholinergic constituents.

PARAMETERS STUDIED:

Morphometric aspects:

The basic Morphometric aspects such as size and total body weight of control and experimental groups have been recorded for every 15 days up to 180th day. The data thus obtained was analyzed and used to correlate with the behavioural aspects and cholinergic system.

BEHAVIOURAL ASPECTS:

Morris Water Maze test:

Learning and memory abilities were determined through Morris water maze technique (Morris, 1984). A great deal of knowledge has been obtained on the neurochemical, neuroanatomical and neurophysiological basis for the behavior associated with this paradigm. The apparatus consisted of a circular tank, 100 cm in diameter and 50 cm in depth. The tank was filled with water (21-26ºC) up to a height of 30cm and the transparent escape platform made of plexiglass (10cm in diameter and 29 cm in height) was hidden at 1.5 cm below the surface of water in a fixed location. The water was made opaque with powdered non-fat milk or non-toxic white coloured dye. The platform was not visible from just above the water level and transfer trials have indicated that escape on to the platform was not achieved by visual or other proximal cues (Morris, 1981). The time spent by the animal to reach the hidden platform was used as the index of memory. Before starting the experiment the mice were acclimatize to the maze environment. The water maze test was conducted for all groups of mice on selected days viz., 15th , 30th , 45th, 60th, 75th, 90th, 105th, 120th, 135th, 150th, 165th and 180th for all six animals in a group separately. For each trial, the time required (in seconds) for individual mouse to find the hidden platform was recorded and the mean data from the tests were used for statistical analysis.

Cholinergic system:

The two constituents of the cholinergic system viz. ACh content and AChE from different regions of all groups of mice brain were assayed according to the method of Metcalf (1957) as given by Augustinsson (1957) and of Ellman et al.(1961) respectively.

Statistical Analysis:

Values of the measured parameters were expressed as Mean ± SEM. Repeated Measures of ANOVA was used to test the significance of difference among four different groups followed by Dunnet's Multiple Range Test (DMRT). Statistical analysis was performed by using Statistical Program of Social Sciences (SPSS) for windows (Version 19; SPSS Inc., Chicago, 1L, USA). The results were presented with the F-value and p-value. In all cases, F-value was found to be significant with p-value less than 0.01**which indicates that the effects of factors were significant.

RESULTS:

Morphometric Aspects: (Table 1; Figure 1)

The total body weights (in grams) of control and all experimental groups of mice were recorded using a digital balance at selected time periods. The results revealed that the control mice showed a gradual increase in their body weights from 15th day (21 grams) to 180th day (43 grams). When compared to the control ones, BME treated mice

Table: 1 Schedule of experiments for analysis of morphometric and behavioural changes and biochemical assays in Control and experimental Groups (BME, D-Gal & NaNO2 and D-Gal & NaNO2 + BME) of mice.

Groups	Age of mice	Duration Of Treatment Days	Time schedule for biochemical analysis Days
Control	30d	15 30 45 60 75 90 105 120 135 150 165 180	45 60 75 90 105 120 135 150 165 180 210 240
BME	30d	15 30 45 60 75 90 105 120 135 150 165 180	45 60 75 90 105 120 135 150 165 180 210 240
D-Gal & $NaNO_2$	30d	15 30 45 60 75 90 105 120 135 150 165 180	45 60 75 90 105 120 135 150 165 180 210 240
D-Gal & $NaNO_2$ + BME	30d	15 30 45 60 75 90 105 120 135 150 165 180	45 60 75 90 105 120 135 150 165 180 210 240

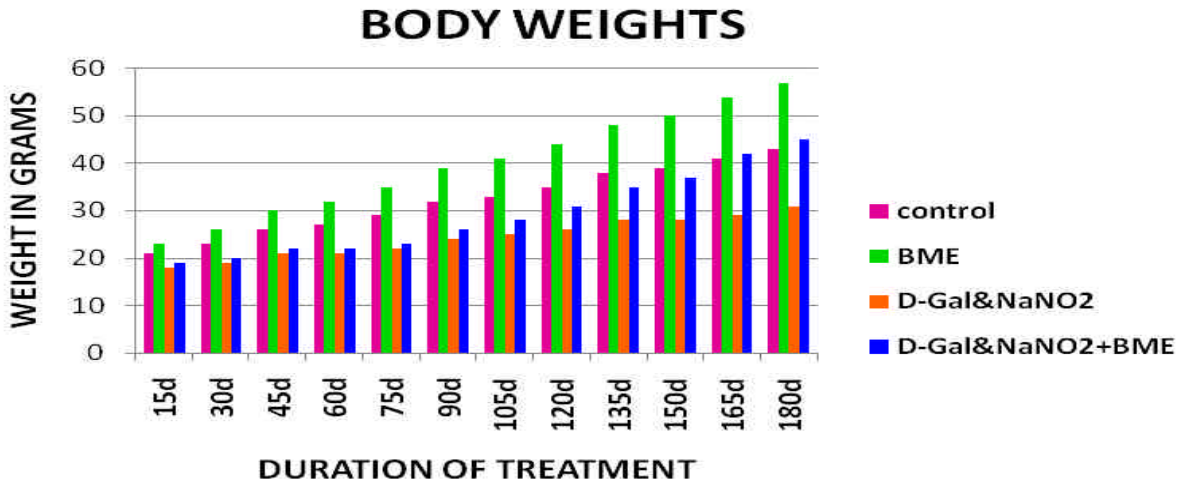


Fig. 1: Graphical representation of differences in the body weights of Control and Experimental groups of mice treated with BME, D-Galactose&NaNO2 and D-Galactose&NaNO2 + BME at selected time intervals.

gained more weight at all time periods from 15th day (23 grams) to 180th day (57.17 grams) whereas the D-Galactose and $NaNO_2$ treated mice gained less weight throughout the period of experiment.(18 grams to 31 grams). Observations on Group IV (D-Galactose and $NaNO_2$, simultaneously treated with BME) revealed that the body weights were lesser than the control mice (19 grams). From 165th day onwards the mice gained more weight (42 grams) against control ones indicating that BME could effectively revert the AD induced changes gradually.

Behavioural Aspects:

Morris water maze test: (Table 2; Figure 2)
Our results on spatial learning and memory abilities in mice assessed through Morris water maze task indicated that, compare to the control ones, escape latency (time taken to reach the hidden platform) was decreased from 150 seconds to 15 seconds in BME treated mice whereas in mice injected with D-Galactose and NaNO2, this escape latency was increased from 190 seconds to 270 seconds throughout the entire tenure of the experiment. One interesting observation on group IV mice treated with D-Galactose and $NaNO_2$ and simultaneously administered with BME was that , eventhough the escape latency was more (185 seconds) than that of control mice at the initial stages, from 90th day onwards the latency time started decreasing and by 165th day, it almost reached normal levels(130 seconds).

Cholinergic system:
The results of the present study clearly indicate that Bacopa monniera extract (BME) has significantly altered the Acetylcholine (ACh) content and the Acetylcholinesterase (AChE) activity in all brain regions of control and all groups of experimental mice.

Acetylcholine (ACh) content: (Figure 3.1 to 3.6)
In control mice, the ACh content was found to be highest in ponsmedulla (5.413) followed by Hippocampus (4.386), Cerebral Cortex (3.275), Cerebellum (2.743), Olfactory lobe (1.982) and Spinal cord (1.651). When compare to the control ones, ACh levels in all the experimental groups showed significant elevation in BME treated mice at all time periods and the percentage of elevation was kept on increasing from 15th day to 180th day. Maximum percent change was noticed in Cerebellum (51.63%) followed by Cerebral cortex (50.98%), Ponsmedulla (49.10%), Spinal cord (48.88%), Olfactory lobe (48.17%) and Hippocampus (45.61%). However, in mice treated with D-Galactose and $NaNO_2$, ACh content showed a significant inhibition in all brain regions at selected time intervals. From 15th day to 180th day, there was a gradual increase in the percentage of inhibition and the maximum percent change was noticed in the Hippocampus (-52.45%) followed by Cerebral cortex (-48.24%), Spinal cord (-47.69%), Olfactory lobe (-47.10%), Ponsmedulla (-44.54%) and Cerebellum (-44.49%).

On comparison with the control group, the mice injected with D-Galactose and $NaNO_2$ simultaneously treated with BME showed a significant inhibition in ACh content from 15th day to 150th day and the maximum inhibition was recorded on 75th day in Hippocampus (-32.57%) followed by Olfactory lobe (-29.39%), Spinal cord (-28.65%). Cerebral Cortex (-27.11%), Cerebellum (-26.47%) and Ponsmedulla (-26.36%). However, from 90th day onwards, this inhibitory trend in ACh content started decreasing from 165th day with respect to the control ones reachin almost control levels by 180th day.

Acetylcholinesterase (AChE) activity: (Figure. 4.1 to 4.6)
The activity of AChE in control mice was recorded highest in Hippocampus (12.43) followed by Ponsmedulla (10.91), Spinal cord (9.86), Cerebral cortex (9.15), Cerebellum (8.64) and Olfactory lobe (7.57). Contrary to the ACh content, the AChE activity was declined significantly in all brain regions of mice treated with BME at selected time intervals. The percent of inhibition was gradually increased from 15th day to 180th day and the maximum percent change was noticed in Cerebellum (-45.79%) followed by Olfactory lobe (-44.73%), Hippocampus (-44.67%), Ponsmedulla (-44.66%), Spinal cord (-42.40%) and Cerebral cortex (-41.90%). When compared with the control group, the activity of AChE in D-Galactose and NaNO2 treated mice increased significantly in all regions of brain from 15th day to 180th day and the maximum percent of elevation was recorded in Ponsmedulla (40.92%) followed by Cerebellum (40.85%) Spinal cord (39.41%), Hippocampus (39.37%), Cerebral cortex (39.18%) and Olfactory lobe (38.61%). The AChE activity in AD induced mice simultaneously treated with BME, though elevated significantly from 15th day to 150th day in all brain

Table 2: Differences in the body weights (Grams) of control and experimental groups treated with BME, D-Gal & $NaNO_2$ and D-Gal & $NaNO_2$ + BME at selected time intervals.

Groups	Days 15th day	30th day	45th day	60th day	75th day	90th day	105th day	120th day	135th day	150th day	165th day	180th day
Control	21	23	26	27	29	32	33	35	38	39	41	43
	±0.68	±0.57	±0.73	±0.73	±0.96	±0.85	±1.31	±1.15	±1.36	±1.18	±1.29	±1.29
BME	23	26	30	32.33	35.33	39	41	44	48.17	50.33	54	57.17
	±0.57	±0.89	±1.06	±1.33	±1.14	±1.18	±1.23	±1.36	±1.66	±1.45	±1.46	±1.51
	-9.52	-13.04	-15.38	-18.51	-20.68	-21.87	-24.24	-25.71	-26.31	-28.2	-31.7	-32.55
D-Gal & $NaNO_2$	18	19.17	21	21	22	24	25	26	28	28.33	29	31.17
	±0.57	±0.54	±0.36	±0.73	±0.68	±0.73	±1.06	±0.81	±1.63	±1.52	±0.73	±1.90
	(-14.28)	(-17.39)	(-19.23)	(-22.22)	(-24.13)	(-25.00)	(-24.24)	(-25.71)	(-26.31)	(-28.20)	(-29.26)	(-32.55)
D-Gal & $NaNO_2$+ BME	19	20.17	22	22	23	26	28	31	35	37	42	45
	±0.57	±1.55	±0.68	±1.23	±0.73	±0.57	±0.93	±0.93	±1.23	±1.46	±1.29	±1.52
	(-9.52)	(-13.04)	(-15.38)	(-18.51)	(-20.68)	(-18.75)	(-15.15)	(-11.42)	(-7.89)	(-5.12)	-2.43	-4.65

Values are Mean ± SEM of six observations each from tissues pooled from 6 mice.Values in parentheses are percent change from control.Values are significantly different from control at p < 0.01

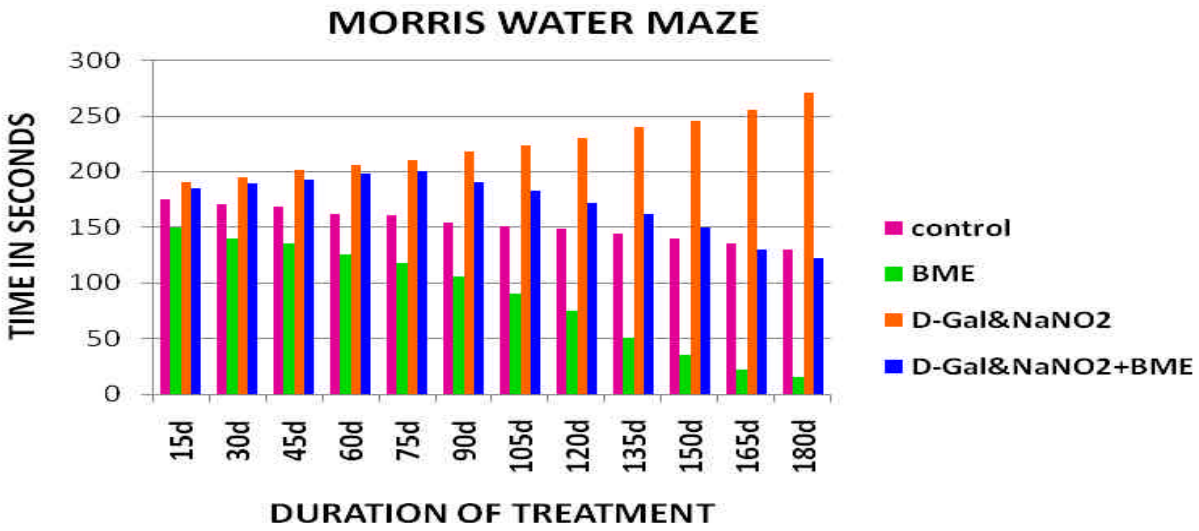


Fig. 2: Graphical representation of Morris Water Maze test results of Control and Experimental groups of mice treated with BME, D-Galactose&$NaNO_2$ and D-Galactose&$NaNO_2$ + BME at selected time intervals

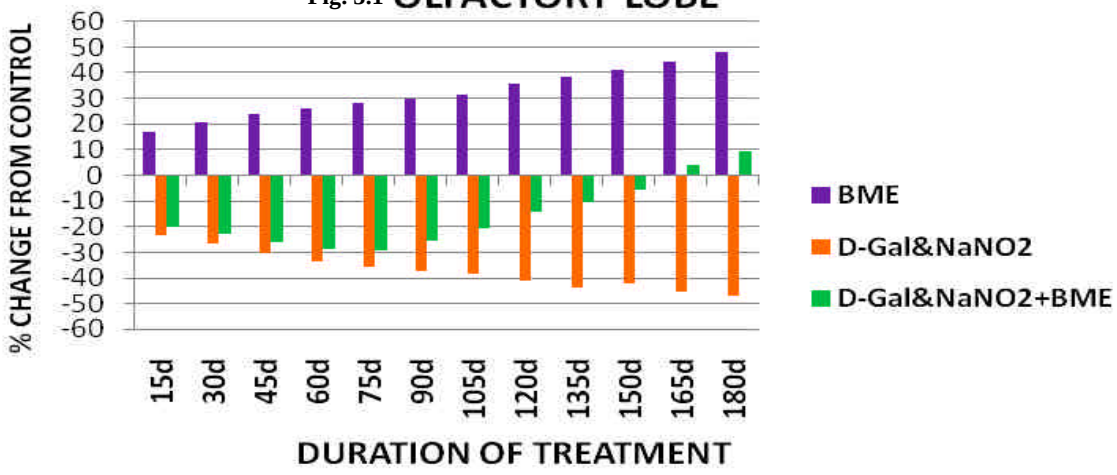
Table 3: Morris Water Maze test results (in seconds) of learning and memory in control and experimental groups of mice treated with BME, D-Gal & $NaNO_2$ and D-Gal & $NaNO_2$ + BME at selected time intervals.

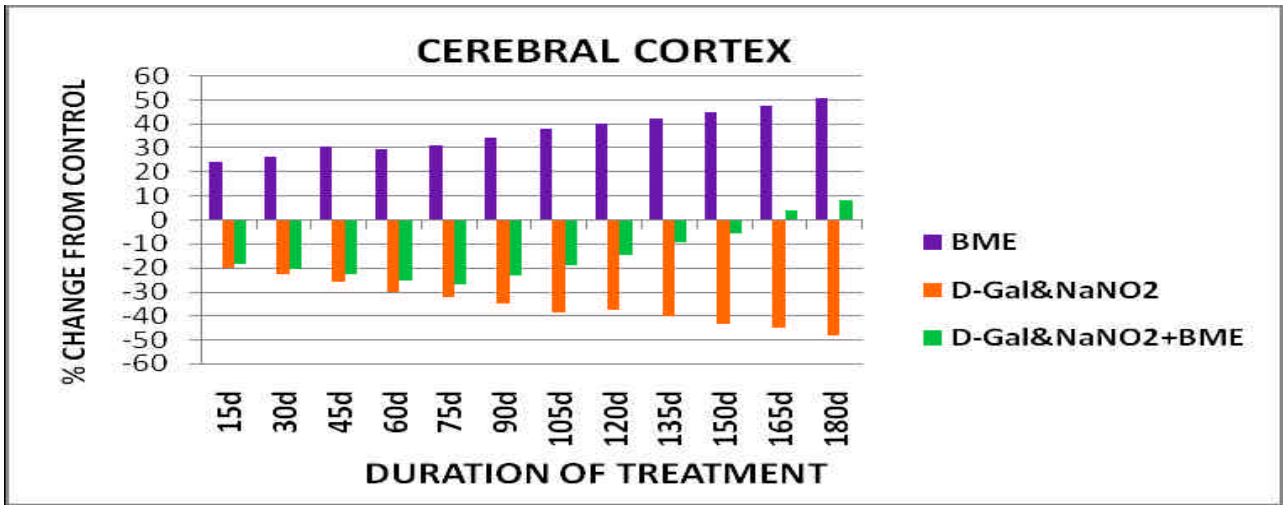
Groups	Days											
	15th day	30th day	45th day	60th day	75th day	90th day	105th day	120th day	135th day	150th day	165th day	180th day
Control	175	170	168.5	162.17	160.5	154	151.17	148	144	140.17	135.67	130.17
	±4.15	±3.53	±3.33	±3.13	±4.49	±4.18	±4.02	±3.37	±3.85	±3.46	±3.36	±3.54
BME	150	140.83	135.17	125	118.17	105.5	90.67	75.67	50.17	35.83	22.17	15
	±3.01	±3.57	±4.08	±4.06	±4.39	±2.93	±3.49	±3.11	±3.37	±3.20	±2.75	±2.28
	(-14.28)	(-17.64)	(-19.42)	(-22.83)	(-26.25)	(-31.81)	(-40.39)	(-49.32)	(-65.27)	(-75.00)	(-83.70)	(-88.46)
D-Gal & $NaNO_2$	190	195	201.33	205.17	210.67	218	223.33	230	240.17	245.17	255.33	270
	±3.50	±3.87	±2.89	±3.94	±2.84	±3.78	±4.12	±2.95	±3.03	±3.97	±3.58	±4.03
	-8.57	-14.7	-19.64	-26.54	-31.25	-41.55	-47.68	-55.4	-66.66	-75	-88.88	-107.69
D-Gal & $NaNO_2$+ BME	185	189	192.17	198	200	190.33	182.17	171.17	162.17	150	130	122.17
	±4.17	±4.58	±3.13	±4.52	±2.74	±3.41	±3.79	±3.47	±2.86	±3.56	±3.23	±3.20
	-5.71	-11.17	-14.28	-22.22	-25	-23.37	-20.52	-15.54	-12.5	-7.14	(-3.70)	(-6.15)

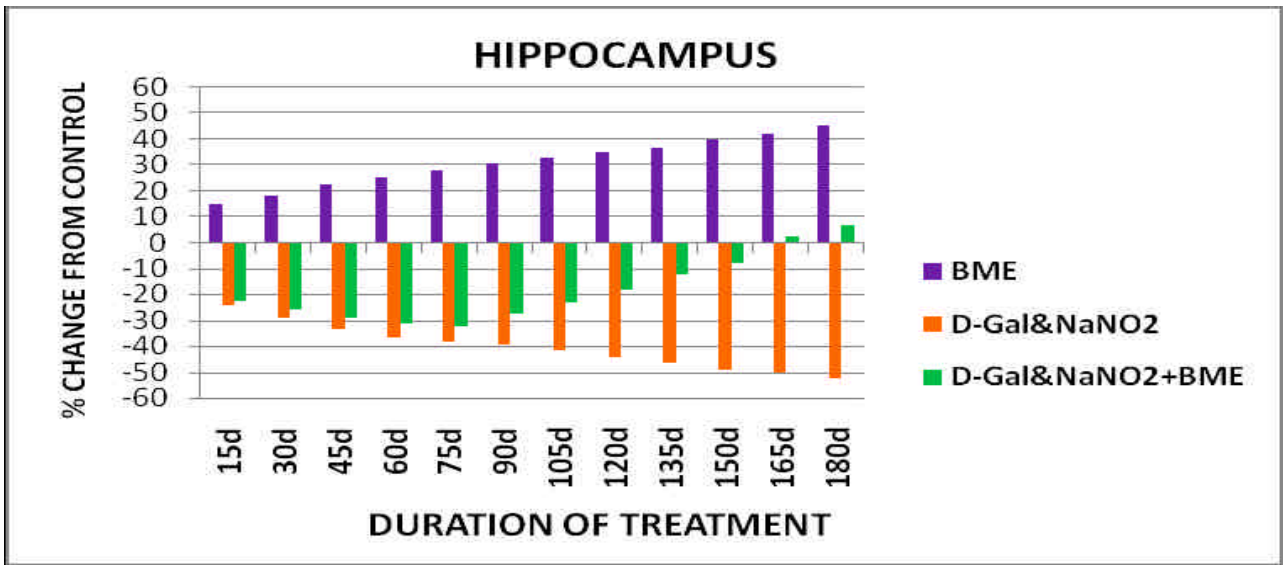
Values are Mean ± SEM of six observations each from tissues pooled from 6 mice.Values in parentheses are percent change from control. Values are significantly different from control at $p < 0.01$

Fig. 3.1-3.6: Graphical representation of percent changes in the content of Acetylcholine (invivo) in Olfactory lobe(OL), Cerebral cortex(CC), Hippocampus(Hc), Cerebellum(Cb), Ponsmedulla(Pm) and Spinal cord(Spc) regions of Experimental groups of mice treated with BME, D-Galactose&$NaNO_2$ and D-Galactose&$NaNO_2$ + BME.

Fig. 3.1 OLFACTORY LOBE




CEREBRAL CORTEX
% CHANGE FROM CONTROL
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
15d
30d
45d
60d
75d
90d
105d
120d
135d
150d
165d
180d
DURATION OF TREATMENT
BME
D-Gal&NaNO2
D-Gal&NaNO2+BME


HIPPOCAMPUS
% CHANGE FROM CONTROL
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
15d
30d
45d
60d
75d
90d
105d
120d
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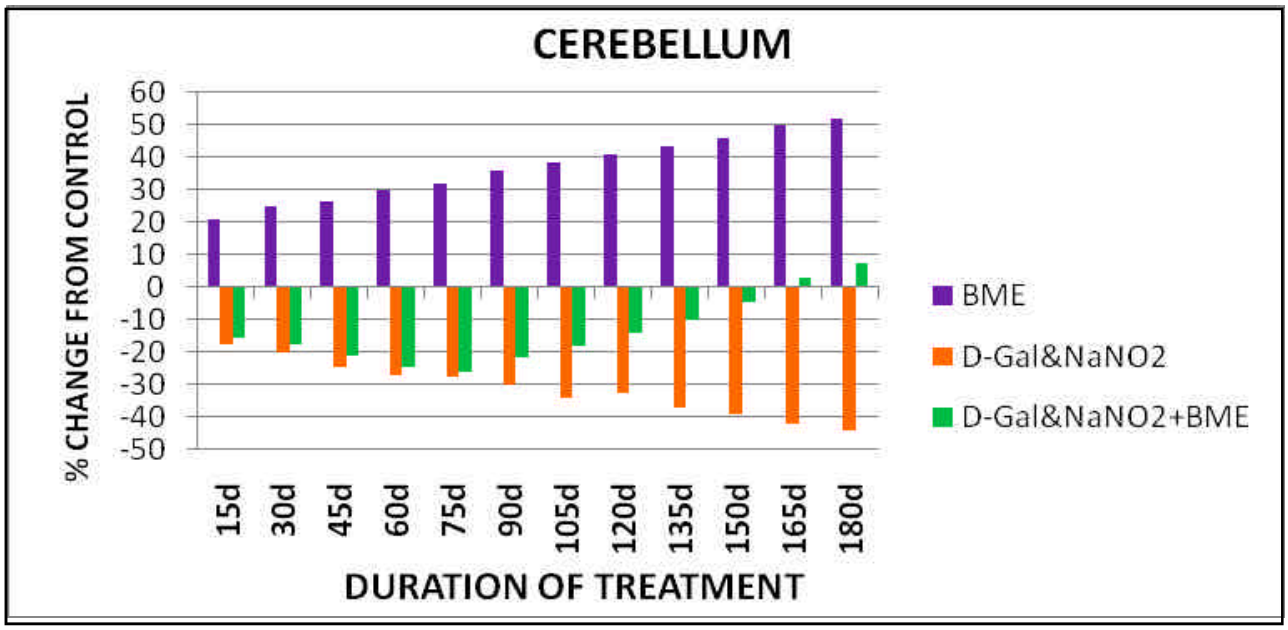
150d
165d
180d
DURATION OF TREATMENT
BME
D-Gal&NaNO2
D-Gal&NaNO2+BME


CEREBELLUM
% CHANGE FROM CONTROL
60
50
40
30
20
10
0
-10
-20
-30
-40
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30d
45d
60d
75d
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180d
DURATION OF TREATMENT
BME
D-Gal&NaNO2
D-Gal&NaNO2+BME

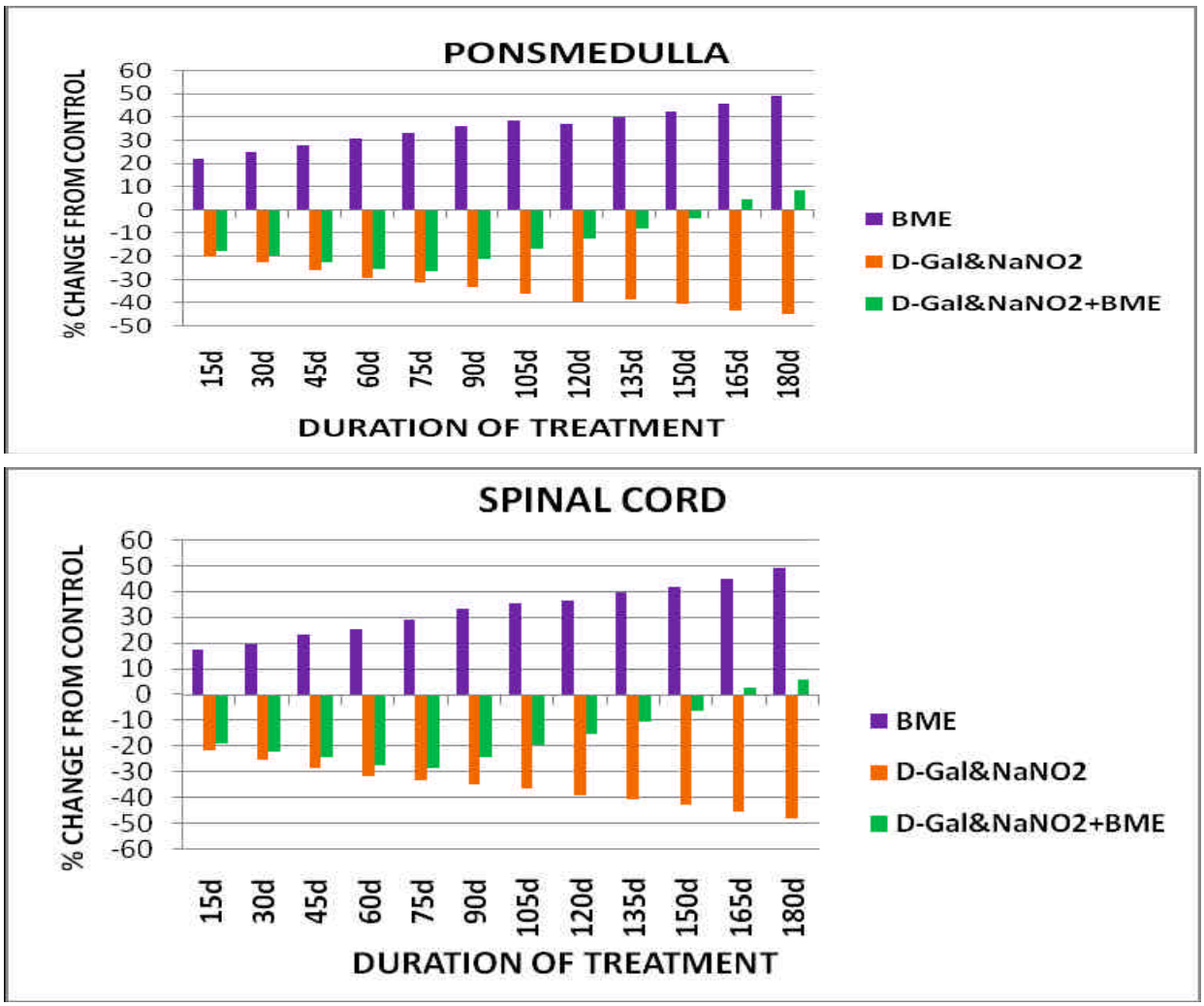

Fig. 4.1 - 4.3: Graphical representation of percent changes in the activity of Acetylcholinesterase (invivo) in Olfactory lobe(OL), Cerebral cortex(CC), Hippocampus(Hc), Cerebellum(Cb), Ponsmedulla(Pm) and Spinal cord(Spc) regions of Experimental groups of mice treated with BME, D-Galactose&$NaNO_2$ and D-Galactose&$NaNO_2$ + BME.

regions with maximum elevation on 75th day in Cerebellum (22.79%); Spinal cord (22.71%); Ponsmedulla (22.28%);Cerebral cortex (22.15%);Olfactory lobe(20.72%); Hippocampus (20.51%), from 90th day onwards, inhibition of AChE activity was noticed from 165th day onwards.

DISCUSSION:

The present findings on morphometric and learning capabilities of control and experimental mice clearly demonstrated that BME showed positive effects on body weight, learning skills, memory and concentration whereas D-Gal and $NaNO_2$ caused learning and memory deficits in mice which could be ameliorated by simultaneous administration of BME. It has been reported that administration of D-Galactose caused a progressive deterioration in learning and memory capacity and increases production of free radicals in the brain (Cui et al., 2006; Wei et al., 2005; Zhang et al., 2005) as measured by open field, avoidance/escape, T-maze, Y-maze and Morris maze in mice (Shen et al., 2002; Xu and Zhang, 2002; Ho et al., 2003). Extensive experimental studies, conducted mostly with standardized extracts of BM, have shown that it facilitates the ion, retention and retrieval of learned tasks in rats (Singh and Dhawan, 1992) and reduced the beta-amyloid deposits in the brain of an Alzheimer's disease animal model (Dhanasekaran et al., 2007). In rodents, spatial learning and memory are closely related to the function of the dorsal hippocampus (Moser et al., 1993), to which cholinergic neurotransmission contributes significantly (Bartus, 2000). Experimental and clinical evidence convincingly indicates that hippocampus formation is critically involved in the memory (Squire, 1992). In AD, atrophy of surviving cholinergic neurons in the cortex and hippocampus occur during normal human ageing (Colom, 2006) and also in aged animals with impaired learning and memory (Muir, 1997).

Similarly, from our observations on the cholinergic system in different brain regions of control and all groups of experimental mice, it was

obvious that Chronic intra peritoneal injection of D-galactose and $NaNO_2$ to the albino mice induced a significant decrease of ACh content and increase of AChE activity in all regions of brain, indicating that there was oxidative damage in the brain. On the other hand Bacopa monniera extract (BME) has significantly elevated the ACh levels and lowered the levels of AChE activity in AD-induced mice indicating the countering action of Bacopa monniera on the cholinergic system.

The first neurotransmitter defect discovered in Alzheimer's disease involved acetylcholine (ACh) since cholinergic function is required for short-term memory function (Francis et al., 1999). Markers for cholinergic neurons such as choline acetyl-transferase and acetylcholinesterase, which are enzymes responsible for synthesis and degradation of ACh, respectively, are decreased in the cortex and hippocampus, areas of the brain involved in cognition and memory (Francis et al., 1999). The earliest loss of neurons occurs in the nucleus basalis and the entorhinal cortex, where cholinergic neurons are preferentially affected. As the illness progresses, up to 90 percent of cholinergic neurons in the nucleus basalis of Mynert may be lost (Wright et al., 1993). Several research findings on various animal models, have demonstrated that loss of cholinergic function in these areas is associated with a decline in learning capacity and memory. The resultant decrease in ACh-dependent neurotransmission is thought to lead to the functional deficits of Alzheimer's disease. Interest on the function of the basal forebrain cholinergic system greatly increased with the neuropathological demonstration that cholinergic markers in the hippocampus and cerebral cortex are changed in AD (Greig et al., 2005; Niewiadomska et al., 2009) which have been linked to its pathology and degree of cognitive impairment (Greig et al., 2005). Therefore, much of research on cognitive decline has been focused on the cholinergic system (Bacciottini et al., 2001).

Aging is a progressive process related to the accumulation of oxidative damage and the brain undergoes morphologic and functional changes resulting in the behavioral retrogression of cognition in the process of normal aging (Joseph et al., 2005). These changes are worsened by neurodegenerative diseases such as Alzheimer's disease which are usually accompanied by a series of abnormal alterations in neuropathology, neurophysiology and behavior. Symptoms linked with this senile dementia are memory impairment, neuronal loss and plaque accumulation (Munoz and Feldman, 2000). Besides the neuropathologic hallmarks of this disease, namely neurofibrillary tangles and neuritic plaques, it is characterized neurochemically by a consistent deficit in cholinergic neurotransmission, particularly affecting cholinergic neurons in the basal forebrain (Fang and Liu, 2007).

Bacopa monniera is well known for its neuropharmacological effects and currently recognized as being effective in the treatment of mental illness, epilepsy and Alzheimer's disease (Russo et al., 2003). Cognition-facilitating effect was due to two active saponins bacosides A and B present in the methanol extract (Singh and Dhawan, 1992). The nootropic activity of the Bacopa monniera plant has been well studied by several researchers and thus inhibition of AChE serves as a strategy for the treatment of Alzheimer's disease (Rahman and Choudhary, 2001) since decrease in AChE activity indicates an increase in the basal level of acetylcholine, which might be helpful in maintaining the learning and memory functions in dementia group.

Our present resuls derive strong support from several resesrch findings (Das et al., 2002; Joshi and Parle, 2006) wherein it has been reported that ehanolic extract of *Bacopa monniera* with 55-60% concentration of Bacosides resulted in anti-cholinesterase and anti-dementia properties in albino mice. The memory enhancing effects have been attributed to the active constituent saponin, as bacosides A and B have been shown to exert facilitatory effects on mental retention and reverse amnesic effects of neurotoxin, scopolamine, electric shock and immobilization stress (Battacharya et al., 2000a).

Previous studies on some well known cognitive enhancers in Indian and Chinese traditional medicine systems viz. *Ginkgo biloba* (Stough et al 2001b), *Mycaria elegans* (Ahmad et al., 2003), *Acorus calamus*, *Epimediumm koreanum* (Oh et al., 2004), *Centella asiatica* (Mohandas rao et al., 2009) also supported the observations in present study wherein these plant extracts and *Bacopa monniera* used in our present study shared similarities with respect to their mechanism of action, neuroprotectors, cognitivers and oxidative stress inhibitors (Bhattacharya et al., 2000; Russo et al., 2003a, b; Holcomb et al., 2006). Several other herbal drugs have also shown promising neuroprotective and antiamnesic effects in rodents (Lee et al., 2003; Chen et al., 2008; Sun et al., 2008; Tian et al., 2008; Zhou et al., 2009 ; Lin et al., 2005; Siripurapu et al., 2005; Wang et al., 2005; Yun et al., 2007; Kim et al., 2008).

As neurologic impairment in D-Galactose treated mice is similar to that in natural aging of mice, D-Galactose treated mice were often used as model of AD animal (Wei et al., 2005; Zhang et al., 2005). Similarly, in the present study i.p. administered D-Galactose and $NaNO_2$ treated mice have been used as AD model for testing the neuro protective role of BME. Mouse aging models induced by Aluminium trichloride and D-Galactose have been widely used for studying mechanism of Alzheimer's disease and for screening the drugs for many years (Song et al., 1999; Shen et al., 2002; Wei et al., 2005; Hua et al., 2008). Although aluminium trichloride and D-Galactose mouse models function their own pathways, they eventually cause similar pathological changes in mouse brains and lead to enhance amyloid-ß peptide (Aß) deposition (Banks et al., 2006; Hua et al., 2008). Accumulation and deposition of Aß (neuritic plaques) in the brain are the key pathological features of AD (Selkoe, 2001; Mattson, 2004; Goedert and Spillantini, 2006).

Previous studies showed that D-Gal and $NaNO_2$ administration significantly increased the brain AChE activity in animal and resulted in the dysfunction of the cholinergic system (Zhong et al., 2009). The present data reiterated these findings wherein intra peritoneal administration of D-Galactose and $NaNO_2$ significantly decreased the ACh content in all regions of mice brain, which reflected the cholinergic

impairment. However, the oral administration of BME increased the ACh levels in the brain regions and reversed the cholinergic impairment. From these observations, it is possible that chronic administration of *Bacopa monniera* for a longer period is able to evoke desirable results in the present study of animal experiments.

It has been shown that i.c.v. administration of colchicines exert a direct cholinotoxic effect, accompanied by reduction in cholinergic markers and induction of cognitive deficits in rats (Emerich and Walsh, 1990) and has been proposed as an experimental model of Alzheimer's disease (Dashieff and Ramirez, 1985). The fact that herbal formulation, Mentat-a nootropic agent reverses the cognitive deficits induced by colchicines indicates its potential for cholinergic properties (Kulkarni and Verma, 1992). *Bacopa monniera* is reported to reverse neurotoxin and colchicines-induced depletion of acetylcholine and suppression of cholinesterase activity and muscarinic receptor binding in frontal cortex and hippocampus (Bhattacharya et al., 2000; Russo and Borrelli, 2005). Moreover, it is documented to inhibit AChE activity in a dose dependent manner (Das et al., 2002) and altered the expression of NRI subunit of NMDA receptor (Hota et al., 2009).

Various experiments have also identified potent antioxidant, antidepressant nd anticholinestirase activity in Bacopa monniera extract (Bhattacharya et al., 2000; Das et al., 2002; Sairam et al., 2002). A detailed research study on rodent comparing the effects of Bacopa and Deprenyl, a drug for Parkinson's disease have showed that BME extended the life span and enhance the antioxidant enzymes in all parts of the brain including hippocampus (Singh and Dhawan, 1982). In epileptic rats *Bacopa monniera* induced the number of seizures per hour which is suggestive for its anticonvulsant property (Reas et al., 2008). More recently, several beneficiary effects of BME have been reported viz. it attenuated scopolamine-induced dementia (Das et al., 2002); exerted antistress activity in both acute and chronic situations (Rai et al., 2003); neuroprotective efficacy against rotenone-induced oxidative stress and neurotoxicity in Drosophila (Hosamani and Muralidhara (2009); neuroprotection against cigarette smoke induced apoptosis (Anbarasi et al., 2005a,b,c. 2006a,b) and Aluminium induced oxidative stress (Jyothi et al., 2007). Further, it was inferred that bacosides BME facilitated anterograde memory and attenuate anterograde experimental amnesia induced by scopolamine and sodium nitrite possibly by improving the acetylcholine level and hypoxic conditions, respectively (Kishore and Singh, 2005). Thus all the above cited research reports have strengthened the contribution of cholinergic system for antiamnesic effect of Bacopa monniera (Das et al., 2002; Kishore and singh, 2005; Nathan et al., 2004) in the present study. Further, evidences showed that other possible effects of *Bacopa monniera* on the cholinergic system include modulation of acetylcholine release and Choline acetylase activity and muscarinic cholinergic receptor binding (Bhattacharya et al., 2000a).

The observations in the present investigation on Morphometric, Behavioural aspects and on the Cholinergic system mice brain following the oral administration of BME have given conclusive evidences on its neuroprotective effect on the nervous system in both normal and AD-induced mice thus confirming that *Bacopa monniera* has potential Anti-alzheimers compounds and can be recommended as a safe and potent drug to treat Alzheimer's disease.

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