JOURNAL OF INTERNATIONAL ACADEMIC RESEARCH FOR MULTIDISCIPLINARY
Impact Factor 1.393, ISSN: 2320-5083, Volume 2, Issue 1, February 2014

# NEUROPROTECTIVE ROLE OF BACOPA MONNIERA EXTRACT (BME) ON MONOAMINE OXIDASE (MAO) IN AD INDUCED MICE WITH PARTICULAR REFERENCE TO MORPHOMETRIC AND BEHAVIOURAL ASPECTS

**DR. KALYANI BAI KUNTE***
**PROF. YELLAMMA KUNA****

*Dept. of Zoology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India
**Chairperson, Board of Studies (BOS), Dept. of Zoology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India

## ABSTRACT

Current study aspire is to investigate the neuroprotective role of Bacopa monniera on MAO in AD induced mice with particular reference to Morphometric and Behavioural aspects. Mus musculus of one month old weighing 20±2gms, were used as experimental model and were maintained according to ethical guidelines for animal protection and welfare. 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 mice and MAO activity were analyzed through standard techniques. Results revealed 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 effects of BME were noticed on MAO of mice brain wherein, oral administration of BME in AD induced mice could revert the changes to normal levels. From these observations, it was inferred that BME had potential compounds which can prevent learning and memory deficits effectively and thus confer neuroprotection against Alzheimer's Disease.

**KEYWORDS:** Alzheimer's disease, Bacopa monniera, Morphometric, Morris water Maze, Monoamine Oxidase

## 1. INTRODUCTION

Alzheimer's disease(AD) is a progressive neurodegenerative disease characterized by neuropsychological, neuropsychiatric and neurologic manifestations. There are both neurochemical and neurohistologic alterations in the brains of AD patients contributing to the clinical manifestations. Classically, short and long-term memory is impaired while language skills, concentration and attention are often affected. This results in impaired ability to learn and retain new skills as well as the loss of existing ones. Currently available treatments can modulate the disease course and ameliorate some symptoms but no proven effective

therapeutic cure for Alzheimer's has been identified to date. Therefore, natural products with medicinal value are garnering a lot of attention due to serious side effects often caused by medicines of chemical origin [1]. Bacopa monniera (Brahmi) is a well known plant with wide medicinal properties that is being used for treatment of memory-related disorders [2]. Many biological effects of Bacopa monniera are documented in traditional as well as in scientific literature of which, the most important one is bacosides on enhancing the cognition and memory functions [2]. It has also been shown to exert antioxidant effects through the chelating of metal ions, breaking oxidative chain reaction improving activities of antioxidative defense enzymes and scavenging the free radicals. It also exhibits anti-stress activity in rats, repairing the damaged neurons by enhanced kinase activity, neuronal synthesis coupled with restoration of synaptic activity and nerve impulse transmission. In view of beneficial properties of Bacopa, hence an attempt has been made in the present study to explore the protective effects of Bacopa monniera extract on the MAO in the brain of normal and AD induced mice with particular reference to Morphometric and Behavioural aspects.

## 2. MATERIALS AND METHODS:

### 2.1 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, were used for biochemical analyses, Kubota KR 2000T centrifuge for homogenates centrifugation, Hitachi UV-2800 spectrophotometer and other standard equipments were used for biochemical/physiological analyses.

### 2.2 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 the approval of the Institutional Animal Ethical Committee (Resolution No. 02/(i)/a/CPCSEA/ IAEC/ SVU/ KY-KK/ Dt. 21-03-2011.

### 2.3 Collection and preparation of Bacopa monniera plant extract:

Bacopa monniera plant was collected from Talacona and identified by the Botanist, Department of Botany, S.V. University, Tirupati, India. The whole plant was dried in shade, powdered and used for extraction by using 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.

**2.4 Induction of Alzheimer's disease in mice:**

Until now, a combination of chemicals, D-Galactose (which is a physiological nutrient causes impairment of cell structure and gene expression)[3] and Sodium nitrite (which reduces the oxygen-carrying capacity of blood, causing hypoxia, disability of consciousness ultimately depressing the learning and memory ability in mice) together was considered to be quite successful in inducing Alzheimer's disease in mice [4]. 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**.**

**2.5 Experiment protocol:**

After the mice were acclimated to the laboratory conditions for 10 days, the mice randomly divided in to four main groups. Each main group was again divided in to 12 sub groups of six each were housed in separate cages. Group I mice were treated as control group; Group II mice were orally administered with 100 mg/kg body weight of Bacopa monniera plant extract for 180 days; Group III and Group IV mice were intraperitoneally injected with D-Galactose ( 120 mg/kg body weight ) and Sodium nitrite (90 mg/kg body weight ) once daily for 60 days. From $10^{th}$ day onwards the Group IV mice were orally administered with Bacopa monniera plant extract (100 mg/kg body weight) up to $180^{th}$ day. All doses were given once in the morning hours between 8 to 9 AM, keeping in view the altered activity of mice during the nights compared to the day time.

**2.6 Isolation of tissues:**

The animals were sacrificed by cervical dislocation at the selected time periods viz., $15^{th}$, $30^{th}$, $45^{th}$, $60^{th}$, $75^{th}$, $90^{th}$, $105^{th}$, $120^{th}$, $135^{th}$, $150^{th}$, $165^{th}$ and $180^{th}$ 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 biochemical assays.

**2.7 Parameters studied:**

**2.7.1 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 180$^{th}$ day. The data thus obtained was analyzed and used to correlate with the behavioural aspects and biochemical assays.

**2.7.2 Behavioural Aspects - Morris Water Maze test:**

Learning and memory ability was detected by using the Morris water maze test [5] which was originally designed to test the learning and memory ability in rodents. 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 (21-26$^{o}$C) up to a height of 30cm and the transparent escape platform made of plexiglass, 10cm in diameter and 29 cm in height was hidden 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 [6]. 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 time periods viz., 15th , 30th , 45th, 60th, 75th, 90th, 105th, 120th, 135th, 150th, 165th and 180th days for all six animals in a group separately. For each trail, 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.

**2.7.3 Monoamine Oxide:**

The brain samples from normal and AD induced mice were analyzed for determination of the levels of Monoamine Oxidase (MAO) by the method of Green Haughton, 1961[7].

**2.8 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**. This indicates that the effects of factors are significant.

## 3. RESULTS:

### 3.1 Morphometric Aspects:

The total body weights (in grams) of control and 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 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 from 15th day (18 grams) to 180th day (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 from 15th day (19 grams) to 150th day (37 grams). From 165th day (42 grams) onwards the mice gained more weight to that of control ones indicating that BME could effectively revert the AD induced changes gradually. **(Figure 1)**

### 3.2 Behavioural Aspects - Morris water maze test:

In the present study, the Morris water maze task was used to assess the spatial learning and memory ability in mice. The results indicated that, compare to the control ones, escape latency (time taken to reach the hidden platform) was decreased from 15th day (150 seconds) to 180th day (15 seconds) in BME treated mice whereas in mice injected with D-Galactose and NaNO2, this escape latency was increased from 15th day (190 seconds) to 180th day (270 seconds). When observed the group IV mice treated with D-Galactose and $NaNO_2$ and simultaneously administered with BME, the escape latency was more than that of control mice from 15th day (185 seconds) to 150th day (150 seconds) and the maximum escape latency was noticed on 75th day (200 seconds). From 90th day (190.33 seconds) onwards the time taken to reach the hidden platform started decreasing and reached to the normal levels. From 165th day (130 seconds), the mice took less time to reach the hidden platform from that of controls. **(Figure 2)**

### 3.3 Monoamine oxidase:

The enzyme activity of MAO levels were inhibited significantly in BME treated mice whereas it was elevated in AD- induced mice, in all regions of brain at selected time intervals. The percent of inhibition in BME treated mice and elevation in AD induced mice was increased gradually from $15^{th}$ day to $180^{th}$ day.

Among the six regions of mice brain treated with BME, the higher percent change was noticed in Spinal cord (-49.52%) followed by Olfactory lobe (-48.57%), Ponsmedulla (-

47.66%), Hippocampus (-47.60%), Cerebellum (-42.07%) and Cerebral Cortex (-41.27%) whereas in AD induced mice, the percent change was more in Cerebellum (45.54%) followed by cerebral cortex (44.95%), Ponsmedulla (42.78%), Hippocampus (42.53%) Spinal cord (41.37%) and Olfactory Lobe (40.93%).

Observations on the AD induced mice simultaneously treated with BME, revealed that MAO activity levels were elevated significantly from $15^{th}$ day to $165^{th}$ day in all brain regions at selected time intervals. But the maximum percent change of elevation was noticed on $75^{th}$ day in Cerebral Cortex followed by Hippocampus, Spinal Cord, Ponsmedulla, Cerebellum and Olfactory lobe. From $90^{th}$ day onwards, elevation of MAO activity started decreased and reached to the normal levels, on $180^{th}$ day, the inhibition of MAO levels was observed as against the control mice. **(Figures 3.1 to 3.6)**

## 4. 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. Morphometrics [8] refers to the quantitative analysis of form, a concept that encompasses size and shape which are commonly performed on organisms and are useful in analyzing their fossil record, the impact of mutants on shape, developmental changes in form, covariances between ecological factors and shape, as well as estimating quantitative-genetic parameters of shape.

Learning or acquisition, a highly specialized function of the brain, is a process of acquiring knowledge about the environment around the organism, while memory is the storage or retention of this learnt knowledge which can be retrieved later [9]. In the present study, it has been observed that the impaired cognitive functions induced by D-Galactose and $NaNO_2$ were restored back to almost normally by administering BME which further reiterates that BME has anti-Alzheimer's properties. It has been reported that long-term injection of D-Galactose inhibited antioxidant enzyme activity leading to decline of immune response, neurodegeneration and behavioural impairment [10]. Since these changes are similar to characters of normal aging process, administration of a combined dose of D-Galactose and $NaNO_2$ has become the most effective technique to induce AD in experimental animals which served as ideal aging animal model for Physiological, Behavivoural and Pharmacology studies recently [10]. Similarly, it has been well established that water maze performance

abilities decline with aging and thus it is a very sensitive method for assessing the impairment of spatial learning and memory [11]. In this present study, the impaired spatial learning and memory abilities caused by D-Galactose and $NaNO_2$ treatment were reverted back to normalcy by simultaneous administration of BME to AD induced mice which further proved that long treatment of BME effectively improve the impaired learning and memory performance in both normal and diseased mice. The memory enhancing properties of Bacopa have been attributed to the active constituent saponin, as bacosides A and B which have been shown to exert facilitatory effects on mental retention in avoidance response in rats[12] and reverse amnesic effects of neurotoxin, scopolamine, electric shock and immobilization stress and it improves acquisition, retention and retrieval of learned tasks [13].The bacosides present in this plant [14] have active principles responsible for improving memory related functions through enhancing the efficiency of transmission of nerve impulses eventually strengthening memory and cognition[15]. Further it was reported that a low dose of D-Galactose caused mental retardation and cognitive dysfunction as measured by open field, avoidance/escape, T-maze, Y-maze and Morris maze in mice [16]. The behavioural trials showed that learning and memory performance in water maze task was severely impaired in rats treated with D-Galactose and $NaNO_2$. The results of the present study are in agreement with these findings that chronic administration of D-Galactose and $NaNO_2$ impaired the performance of mice in a water maze task whereas BME treated mice showed better cognitive parameters as compared to the control and D-Galactose and $NaNO_2$ group.

Observations on Monoamine oxidase activity showed a significant decline in all brain regions of mice treated with BME whereas the D-Galactose and $NaNO_2$ treated mice showed a significant elevation in all regions of mice brain on any selected day. Finally, restoration of normal levels was observed during the subsequent period of treatment of AD induced mice with BME indicated the neuroprotective role against Alzheimer's disease. Monoamine oxidases (MAO) are the major intracellular enzymes localized in the outer mitochondrial membrane and the central nervous system (CNS) that catalyze the degradation of neuroactive and vasoactive amines [17]. Products of MAO-Catalyzed reaction, such as aldehydes and $H_2O_2$ are compelling inductors of lipid peroxidation. MAO dysfunction (too much or too little MAO activity) is thought to be responsible for a number of psychiatric and neurological disorders. For example, unusually high or low levels of MAOs in the body have been associated with depression [18], schizophrenia [19,20], substance abuse, attention deficit disorder, migraines, and irregular sexual maturation. Monoamine oxidase inhibitors are one

of the major classes of drug prescribed for the treatment of depression, although they are often last-line treatment due to risk of the drug's interaction with diet or other drugs. It is assumed that activation of monoamine oxidase is associated with age-related disturbances of the homeostasis and generation of free radicals in involution of nervous tissue [21]**.** Brain tissues from AD induced mice showed increased activities of MAO. However, the administration of Bacopa monniera extract could significantly prevent these effects thus eventually leading to improvement in memory performance of AD induced mice.

The observations in the present investigation on Morphometric, Behavioural aspects and on the MAO activity of 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-Alzheimer's compounds and can be recommended as a safe and potent drug to treat Alzheimer's disease.

## GRAPHS

**Fig. 1:** Graphical representation of differences in the body weights of Control and Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME at selected time intervals.

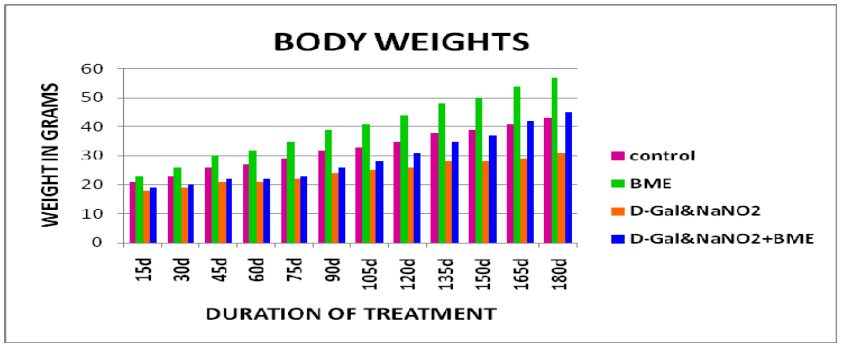



**Fig. 2:** Graphical representation of **Morris Water Maze** test results of Control and Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME at selected time intervals.

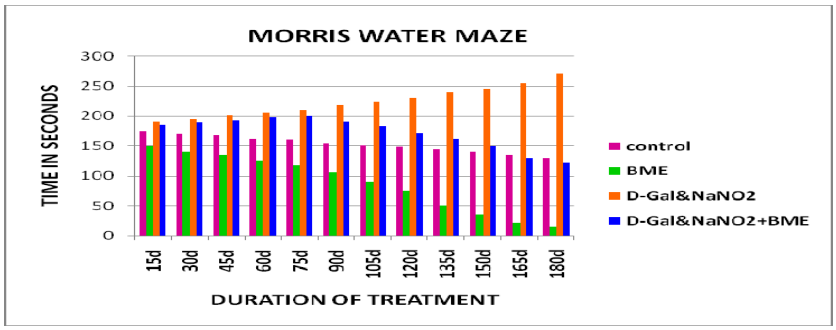


**Fig. 3.1 - 3.3:** Graphical representation of percent changes in the activity of **MAO** (invivo) in Olfactory lobe(OL), Cerebral cortex(CC) and Hippocampus(Hc) regions of Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME.

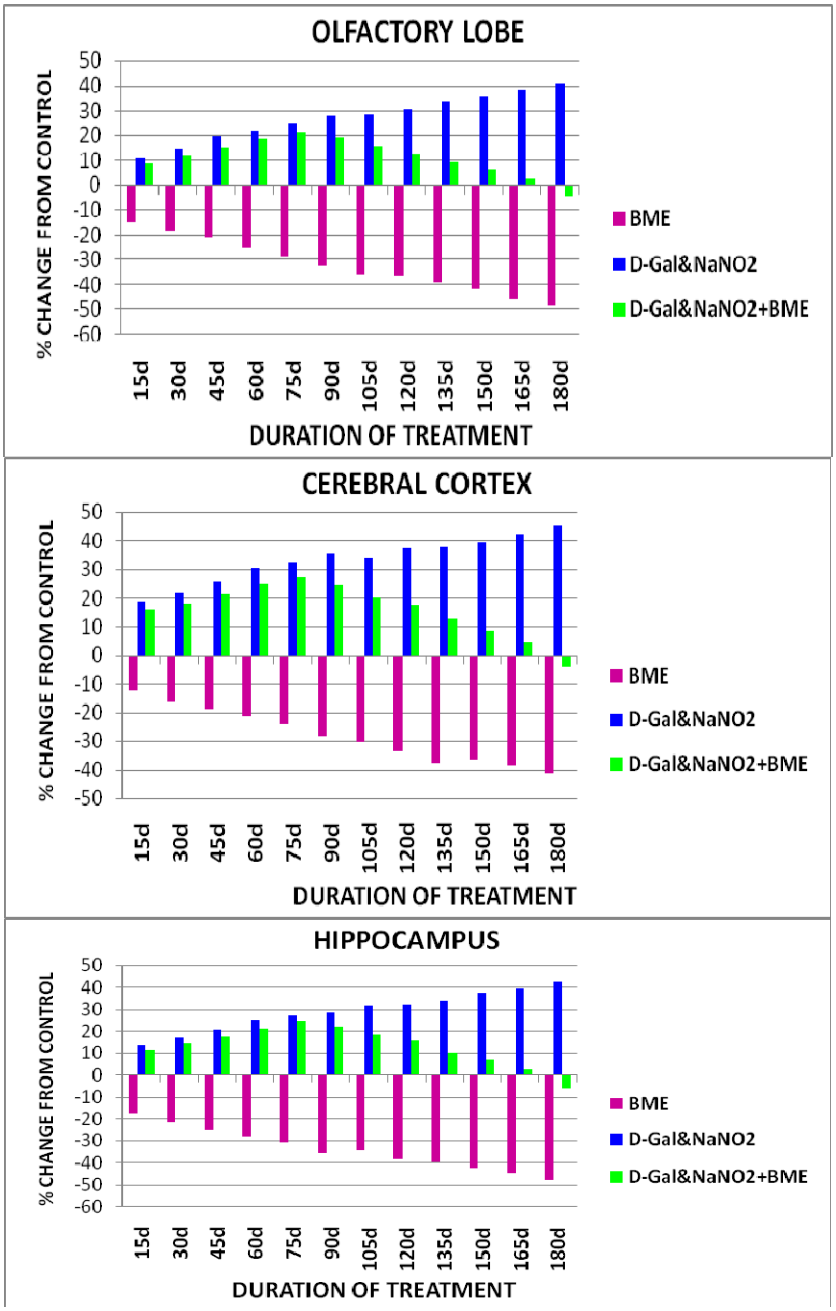
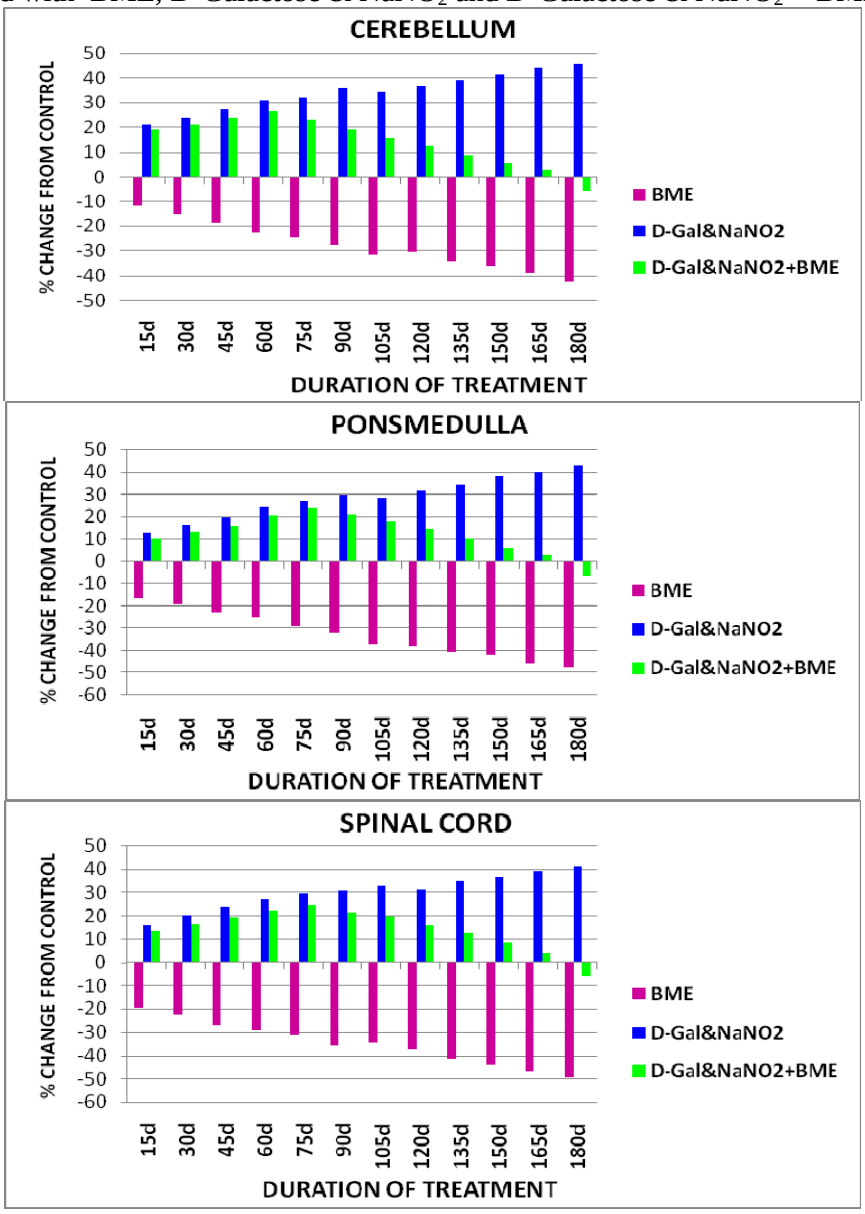


**Fig. 3.4 - 3.6:** Graphical representation of percent changes in the activity of **MAO** (invivo) in Cerebellum(Cb), Ponsmedulla(Pm) and Spinal cord(Spc) regions of Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME.



## REFERENCES

1. Zhong S Z, Ge Q H, Qu R, Li Q and Ma S P. Paeonol attenuates neurotoxicity and ameliorates cognitive induced by D-Galactose in ICR mice. J Neurol Sci 2009; 277: 58-64.
2. Russo A and Borrelli F. Bacopa monniera, a reputed nootropic plant: An overview, Phytomedicine 2005; 12: 305-317.
3. Zhang C, Wang S Z, Zuo P P, Cui X and Cai J. Effect of tetramethylpyrazine on learning, memory and cholinergic system in D-Galactose-lesioned mice. Acta Acad Med Sin 2003; 25: 553-556.
4. Fang F and Liu G. A novel cyclic squamosamide analogue compound FLZ improves memory improvement in artificial senescence mice induced by chronic injection of D-Gal and $NaNO_2$. Basic Clin Pharmacol Toxicol 2007; 101: 447-454.
5. Morris R. Developments of a water maze procedure for studying spatial learning in the rat. Neurosci Methods 1984;11: 47-64.
6. Morris R G M. Spatial location does not require the presence of local cues. Learn Motiv 1981; 12: 239-260.
7. Green A L and Haughton T M. A colorometric method for the estimation of monoamine oxidase. Biochem J 1961; 78: 172-5.
8. Marcus L F. Chapter 4. Traditional morphometrics. In proceedings of the Michigan Morphometric Workshop. Special publication No.2F.J.Rohlf and FL Bookstein. Ann Arbor Ml. the University of Michigan Museum of zoology 1990; 77-122.
9. Squire L R and Schlafer W T. Handbook of Biological Psychiatry, Vanpraag HM, Lader MH, Rafaelsen OJ, Sachar EJ (Eds). Raven Press: Newyork, USA: 1981; 249.
10. Lu J, Zheng Y, Wu D, Luo L, sun D and Shan Q. Urosolic acid ameliorates cognition deficits and attenuates oxidative damage in the brain of senescent mice induced by D-Galactose. Biochemical Pharmacology 2007; 74: 1078-1090.
11. Brandeis R, Brandys Y, Yehuda S. The use of Morris water maze in the study of memory and learning. The international Journal of Neuroscience 1989; 48 (1-2): 29-69.
12. Singh H K, Rastogi R P, Sriman R C, Dhawan B N. Effect of Bacoside A and B on avoidance response in rats. Phytother Res 1988; 2: 70-75.
13. Bhattacharya S K, Kumar A, Ghosal S. Effect of Bacopa monniera on animal models of Alzheimer's disease and perturbed central cholinergic markers of cognition in rats. In: Siva Sankar DV (Ed) Molecular aspects of asian medicines. PJD publications, Newyork 2000a; (in press).
14. Sivaramakrishna. Triterprnoid glycosides from Bacopa monniera. Phytochemistry 2005; 66: 2719-2728.
15. Anon. Bacopa monnieri Monograph. Altern Med Rev 2004; 9: 79-85.
16. Ho S C, Liu J H and Wu R Y. Establishment of the mimetic aging effect in mice caused by D-Galactose. Biogerontology 2003; 4: 15-18.
17. Bonnet U. Moclobemide: therapeutic use and clinical studies. CNS drug reviews 2003; 9(1): 97-140.
18. Meyer J H, Ginovart N, Boovariwala A, Sagrati S, Hussey D, Garcia A, Young T, Praschak-Rieder N, Wilson A A and Houle S. Elevated monoamine oxidase a levels in the brain: an explanation for the monoamine imbalance of major depression. Arch. Gen. Psychiatry 2006; 63 (11): 1209–16.
19. Domino E F and Khanna S S. Decreased blood platelet MAO activity in unmedicated chronic schizophrenic patients. Am J Psychiatry 1976; 133 (3): 323–6.
20. Schildkraut J J, Herzog J M, Orsulak P J, Edelman S E, Shein H M and Frazier S H. Reduced platelet monoamine oxidase activity in a subgroup of schizophrenic patients. Am J Psychiatry 1976; 133 (4): 438–40. PMID 1267046.
21. Shemyakov S E. Monoamine oxidase activity, lipid peroxidation and morphological changes in human hypothalamus during aging. Bulletin of Experimental Biology and Medicine 2001; 131(6): 586-588.