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Neuroprotective effect of *Bacopa monniera* on memory deficits and ATPase system in Alzheimer's disease (AD) induced mice

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Abstract

Theme of the present study is to evaluate neuroprotective properties of *Bacopa monniera* (Brahmi) extract (BME) on memory deficits and biochemical changes in ATPases system of AD induced mice. *Mus musculus*, of one month old weighing 20 ± 2 grams, were used as experimental model and were maintained in Animal House 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 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 and NaNO2; Group IV: AD induced mice simultaneously treated with BME. Changes in Morphometric and Behavioural aspects of four groups were analyzed by using Morris Water Maze technique. Various constituents of ATPase system were determined in selected regions of mice brain through standard biochemical assay methods. Results revealed that BME showed positive effects on body weight, learning skills, memory and concentration, whereas D-Gal and NaNO2 caused learning and memory deficits in mice which could be ameliorated by simultaneous administration of BME. Similar, protective effects of BME were noticed on the ATPase system which could revert all the constituents of ATPase system to normal levels in AD induced mice. From these observations, it was concluded that BME had potential compounds which can prevent the learning and memory deficits effectively; and to maintain ion gradients across biological membranes, thus confer significant neuroprotection against AD by stabilizing the structural and functional integrity of the membrane.

Keywords: Albino mice, *Bacopa monniera* (Brahmi), Alzheimer's disease (AD), Morphometric and Behavioral aspects, ATPase system

Introduction

Brain aging is a risk factor of neurodegenerative diseases such as Alzheimer's disease (AD), the most common cause of dementia which accounts 70% of dementia causes in the most industrialized countries and is characterized by cell atrophy and extensive neuronal loss. It is a complex and heterogeneous disorder particularly prevalent in those over the 60 years of age. The incidence of AD rises from 2.8 per 1000 person years in the 65-69 year age group to 56.1 per 1000 person years in the older age group beyond 90 years.[1] According to the World Health Organisation (WHO), it is estimated that there are currently about 18 million people worldwide with Alzheimer's disease. This figure is expected to nearly double by 2025 to 34 million. Much of this increase will be in the developing countries, and will be due to the ageing population.

In Alzheimer's disease, there is a progressive degeneration of basal forebrain cholinergic neurons innervating the hippocampus and the cortex. Although other neurotransmitters decline during Alzheimer's-associated neurodegeneration, the degree of brain Acetylcholine (ACh) reduction directly correlates with deterioration of cognition and of daily activity in AD patients.[2] Since deficits in cholinergic function contribute to the pathology of Alzheimer's disease, attempts to delay the progression of the illness and improve patients' daily activities are based on pharmacological strategies to increase ACh levels by means of anti-cholinesterasic agents.[3] But, some anticholinesterasic drugs have serious side effects on patients because they not only act specifically on the acetylcholinesterases, but also affect other ion channels such as potassium channels.[4] Disturbances in the ionic equilibrium of the cells as a result of inactivation of ATPases are believed to be the major factors in the pathogenesis of various neurological disorders.[5] Neuronal membrane damage was evident from the decreased activities of membrane bound enzymes such as Na^+/K^+, Mg^{2+} and Ca^{2+} -ATPases. Treatment strategies have been investigated to cure AD, and the developed anti-Alzheimer's drugs showed positive results, but with relevant side effects. Therefore it is worthwhile to choose the application of alternative traditional medical system for treatment of Alzheimer's disease. Many natural herbal medicines for treatments of Alzheimer's disease have been touted to extend desirable and promising positive effects beyond that of modern allopathy drugs.

Bacopa monniera (Brahmi) is a well known plant with wide medicinal properties that is being used for treatment of memory-related disorders.[6] In Ayurveda, *Bacopa monniera* has been classified under medicinal plants for rejuvenating intellect and memory. The medicinal efficacy of *Bacopa monniera* is extensively reported in Indian Traditional literature such as Athar-Ved, Carak Samhita, Susrutu Samhita[7] for treatment of epilepsy, insomnia,[8] anxiety and as a mild sedative and memory enhancer.[8] Besides, *Bacopa monniera* displays antistress[9] and anxiolytic[10] activities too in animals. It has also been shown to exert antioxidant effects through the chelating of metal ions, breaking oxidative chain reaction8 improving activities of antioxidative defense enzymes[11] and scavenging the free radicals.[12] It also exhibits antistress activity in rats, repairing the damaged neurons by enhanced kinase activity, neuronal synthesis coupled with restoration of synaptic activity and nerve impulse transmission.[7] In view of the above mentioned multiple beneficial qualities of bacopa, an attempt has been made in the present study to explore the protective effects of *Bacopa monniera* extract on membrane bound enzymes viz. Na^+/K^+, Mg^{2+} and Ca^{2+} -ATPases in the brain of normal and AD induces mice with particular reference to Morphometric and Behavioural aspects.

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 Animals

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, a combination of the chemicals, D-Galactose and Sodium nitrite together was considered to be quite successful in inducing Alzheimer's disease in mice.[13,14] 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 was 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.

Table 1: Grouping of animals

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 10^{th} day up to 180^{th} 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 the ATPase system.

Behavioural aspects: Morris Water Maze test: Learning and memory abilities were determined through Morris water maze technique.[15] 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^{0}C) up to a height of 30 cm and the transparent escape platform made of plexiglass (10 cm 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 colored 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.[16] 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.

Biochemical analysis: Tissue samples from different regions of control and AD induced mice were analyzed for Na^+/K^+ -ATPase and Mg^{2+} -ATPase activity levels according to the method of Tirri et al., 1973.[17] Ca^{2+} -ATPase was assayed according to the method of Fritz and Hamrick (1966)[18] as supported by Desaiah and Ho (1979) and Inorganic phosphates was estimated by the method of Fiske and Subba Row (1925).[19]

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.

Results

Morphometric Aspects

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 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 $_{NaNO2}$, 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 (Table 1; Figure 1).

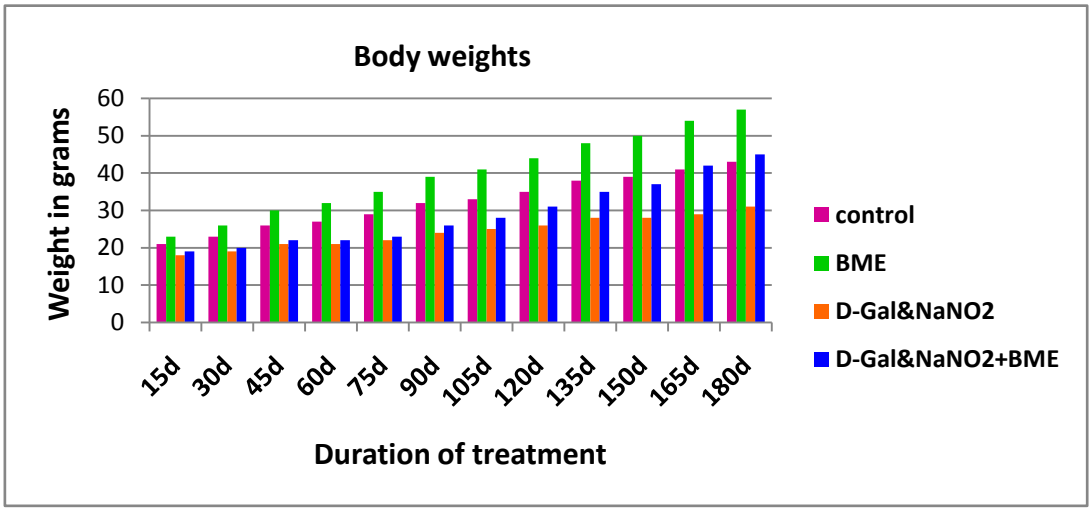


Figure 1: Graphical representation of differences in the body weights of Control and Experimental groups of mice treated by BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME at selected time intervals.

Behavioural Aspects

Morris water maze test: 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 $NaNO_2$, 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, even though 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) (Figure 2).

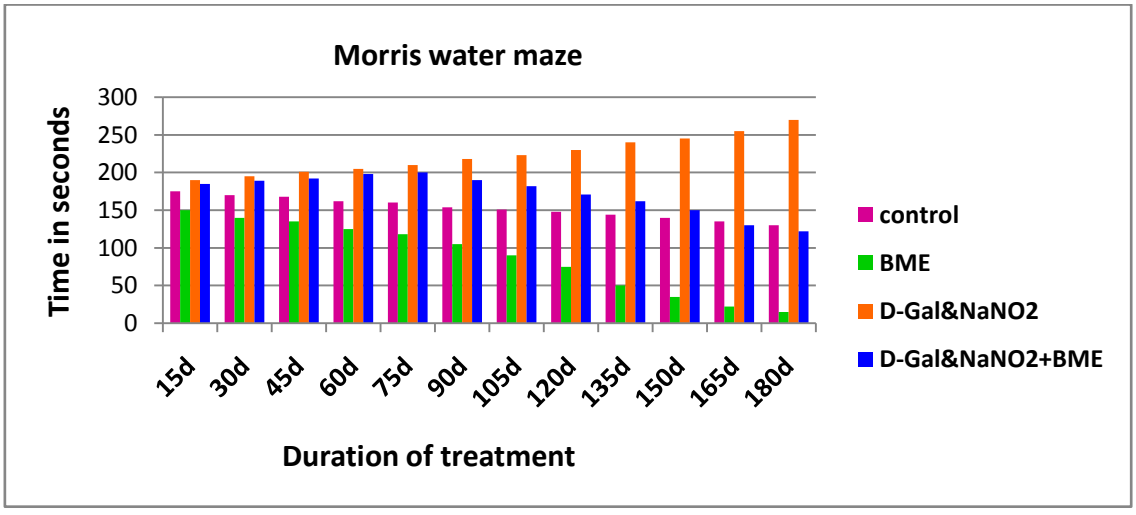


Figure 2: Graphical representation of Morris Water Maze test results of Control and Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME at selected time intervals.

ATPases system

The levels of various parameters related to energy metabolism, viz., activity of enzymes (Na^+ /K^+ -ATPase, Mg^{2+} -ATPase and Ca^{2+} -ATPase were estimated in different regions of brain in control and experimental groups of mice.

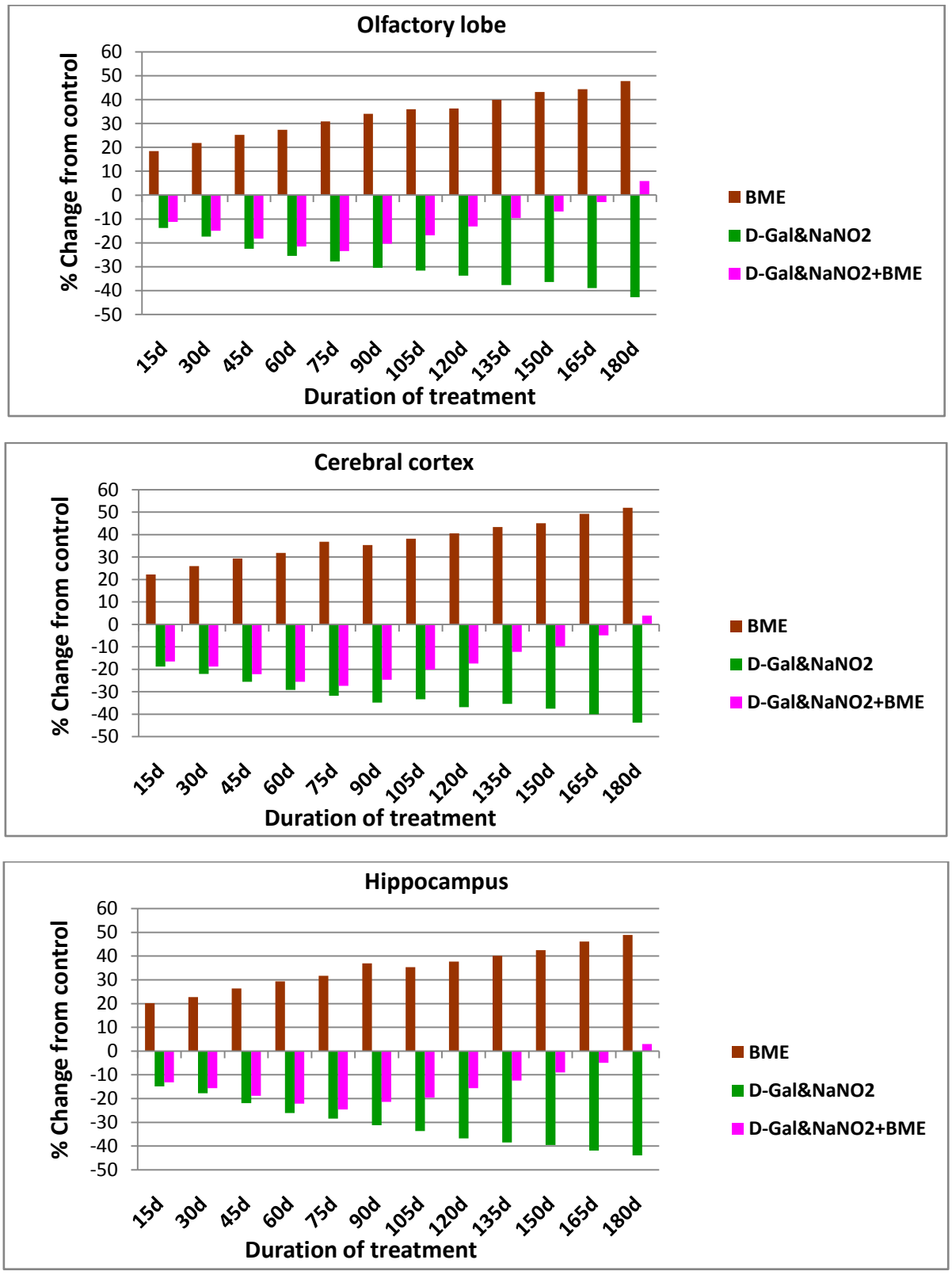


Figure 3.1 - 3.3: Graphical representation of percent changes in the activity of Na^+, K^+ -ATPase (*in-vivo*) in Olfactory lobe(OL), Cerebral cortex(CC) and Hippocampus(Hc) regions of Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME.

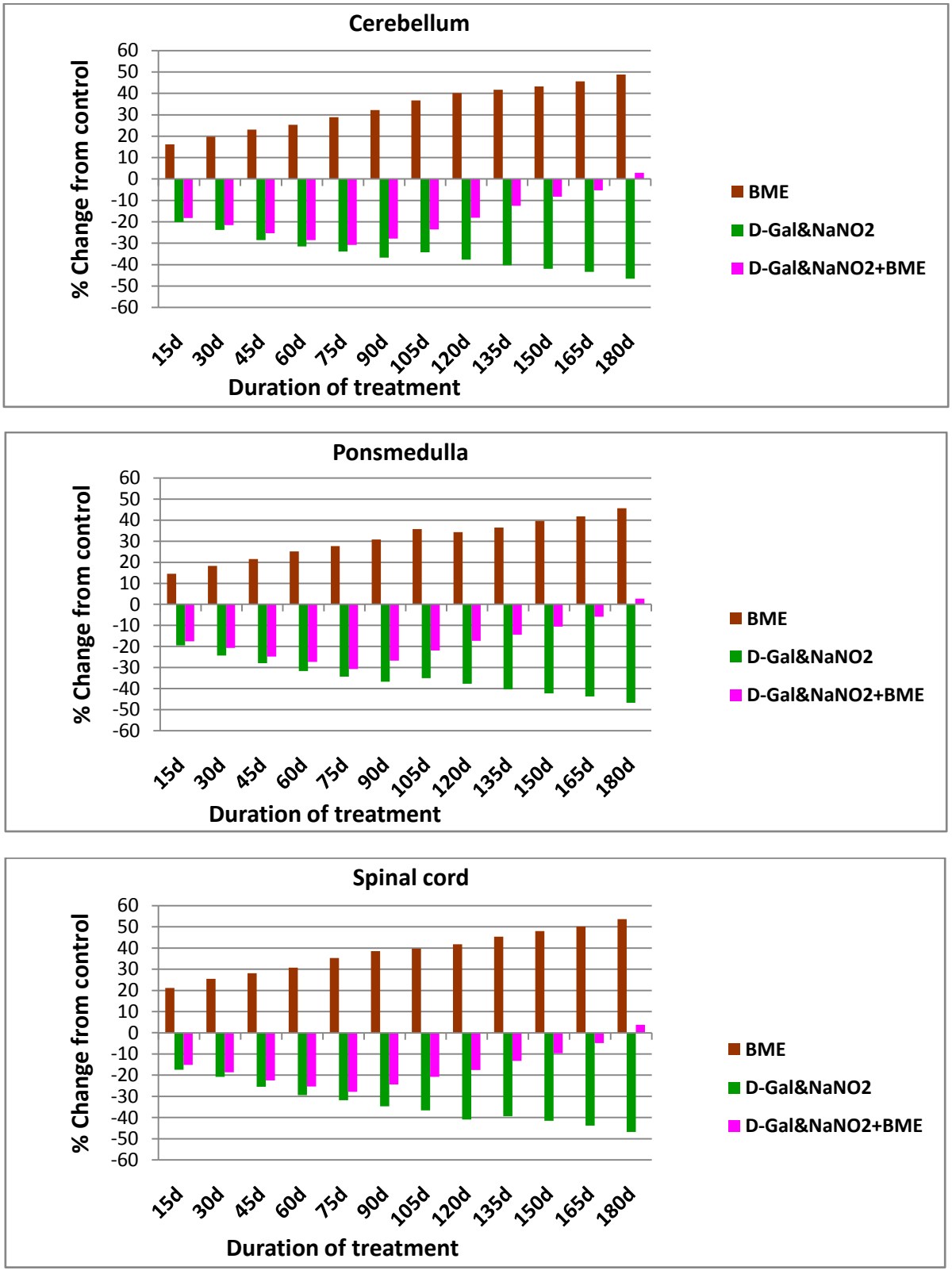


Figure 3.4 - 3.6: Graphical representation of percent changes in the activity of Na^+, K^+ -ATPase (*in-vivo*) in Cerebellum(Cb), Ponsmedulla(Pm) and Spinal cord(Spc) regions of Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME.

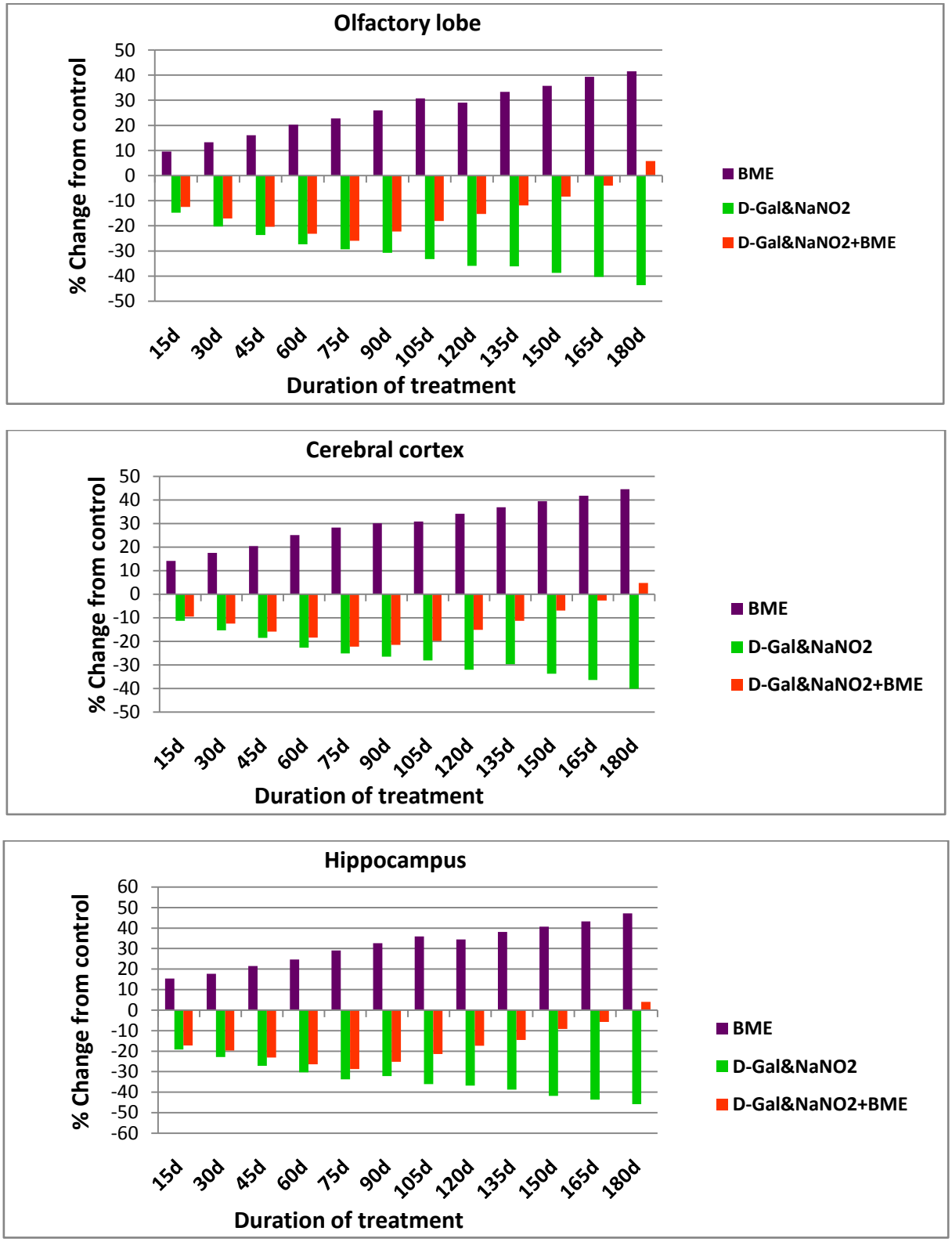
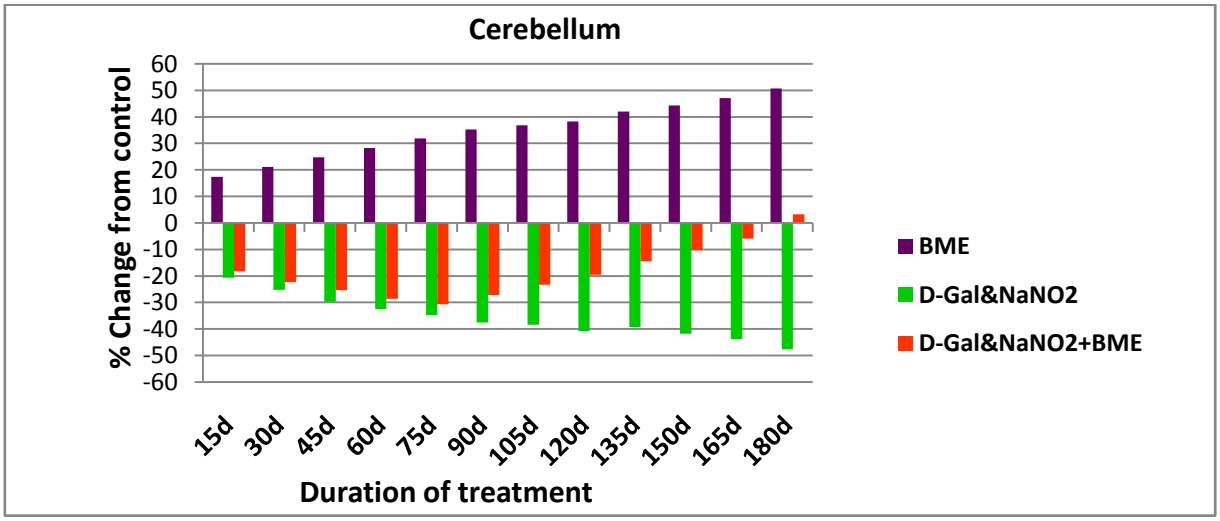
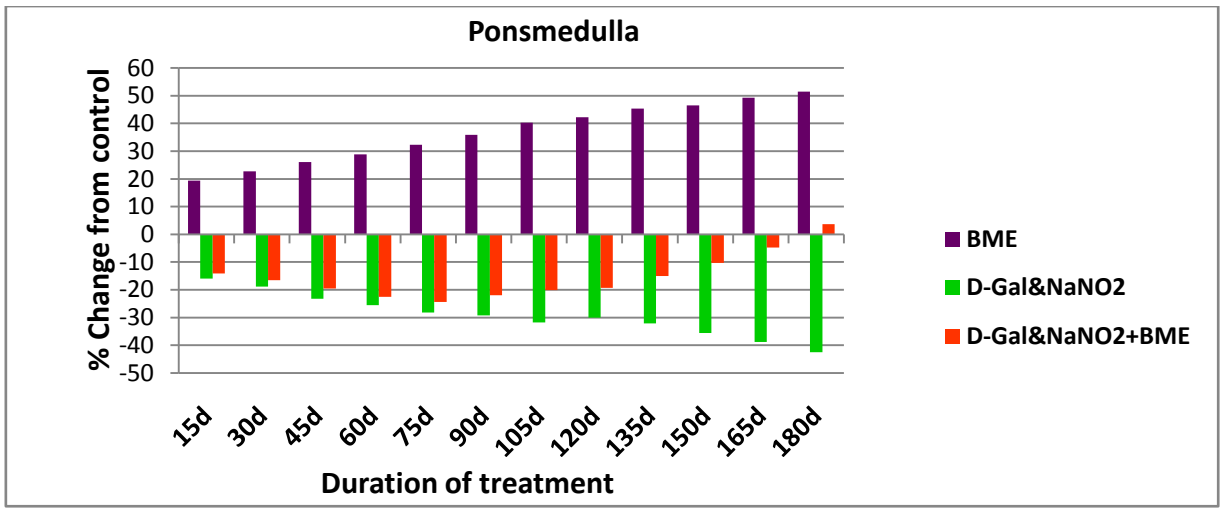


Figure 4.1 - 4.3: Graphical representation of percent changes in the activity of Mg^{2+} -ATPase (*in-vivo*) in Olfactory lobe(OL), Cerebral cortex(CC) and Hippocampus(Hc) regions of Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME.





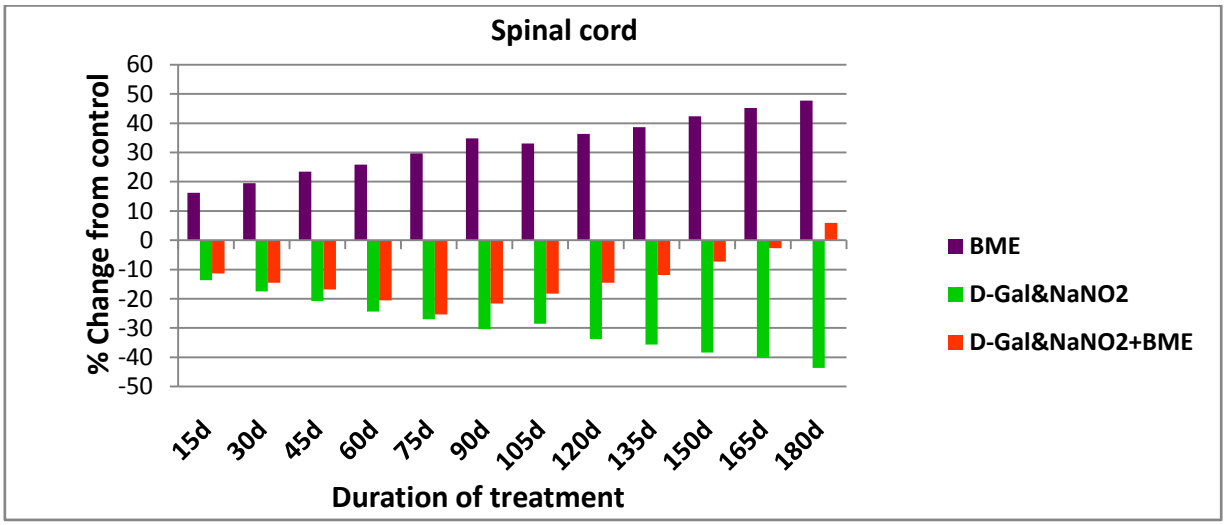
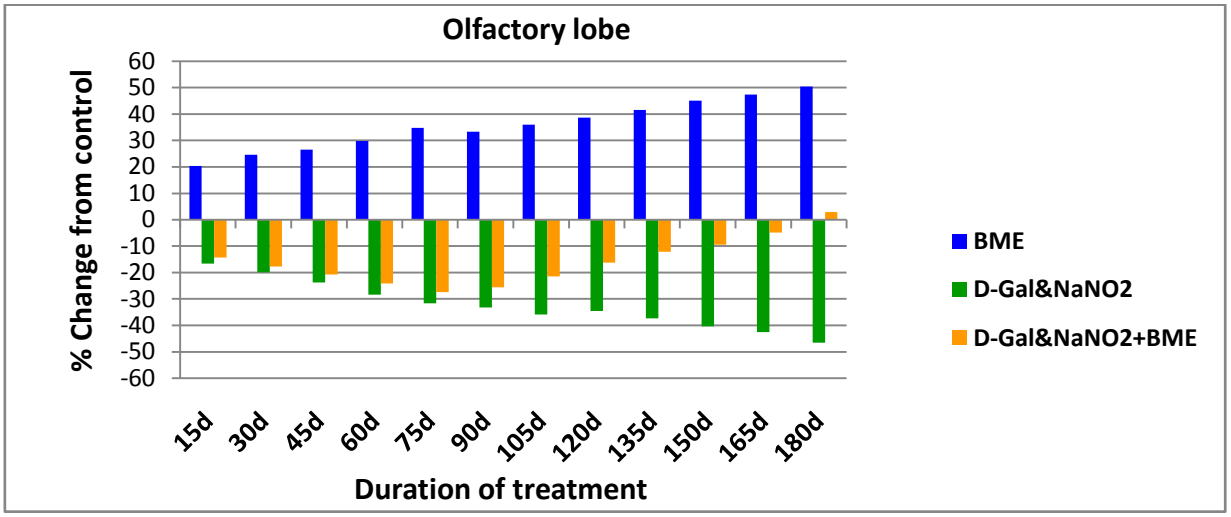
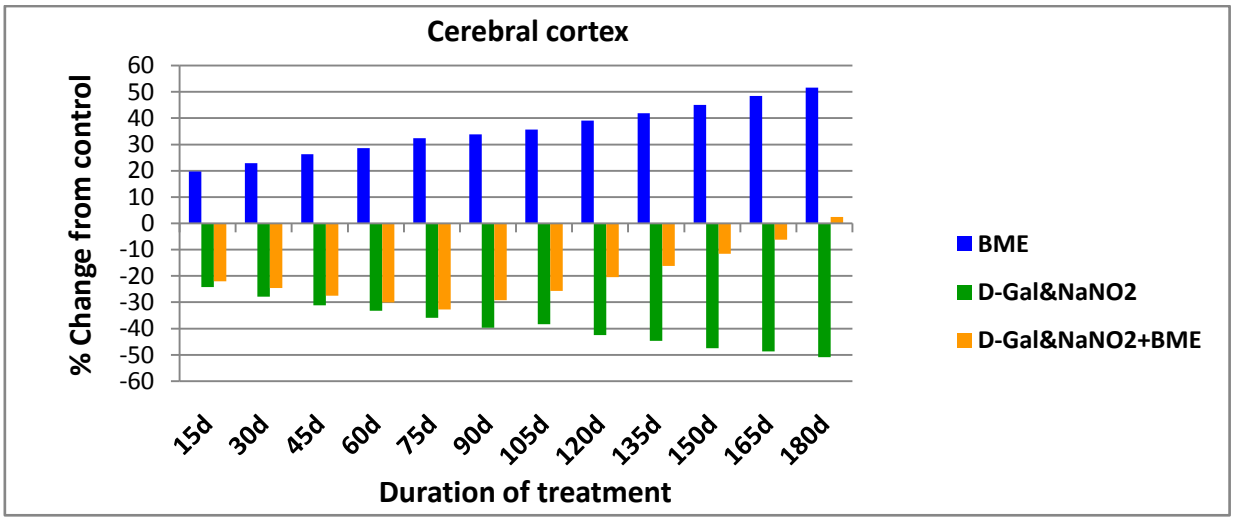


Figure 4.4 - 4.6: Graphical representation of percent changes in the activity of Mg^{2+} -ATPase (*in-vivo*) in Cerebellum(Cb), Ponsmedulla(Pm) and Spinal cord(Spc) regions of Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME.





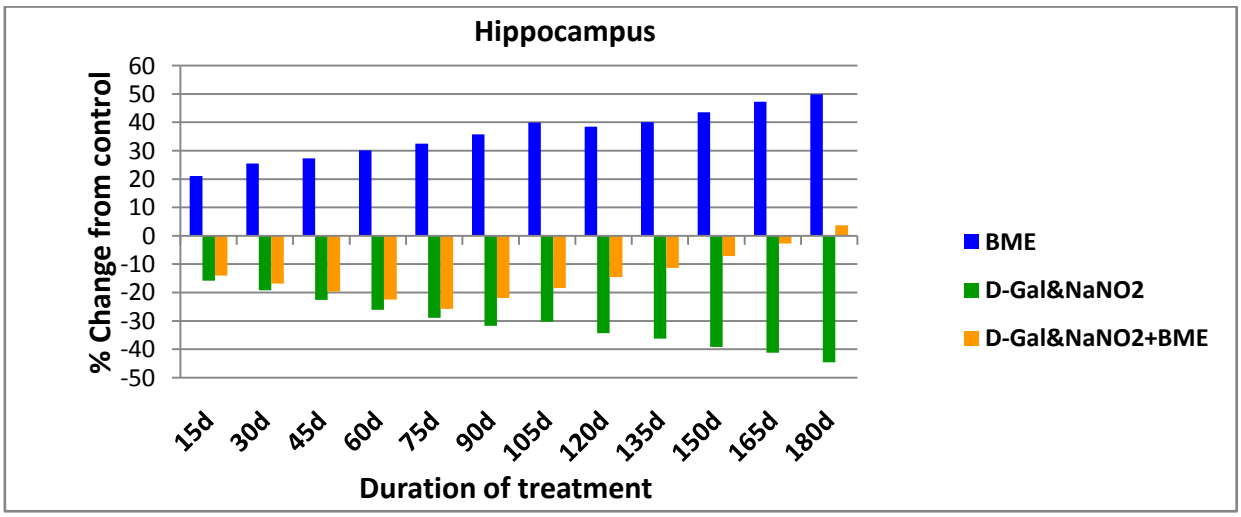


Figure 5.1 - 5.3: Graphical representation of percent changes in the activity of Ca^{2+} -ATPase (*in-vivo*) in Olfactory lobe(OL), Cerebral cortex(CC) and Hippocampus(Hc) regions of Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME.

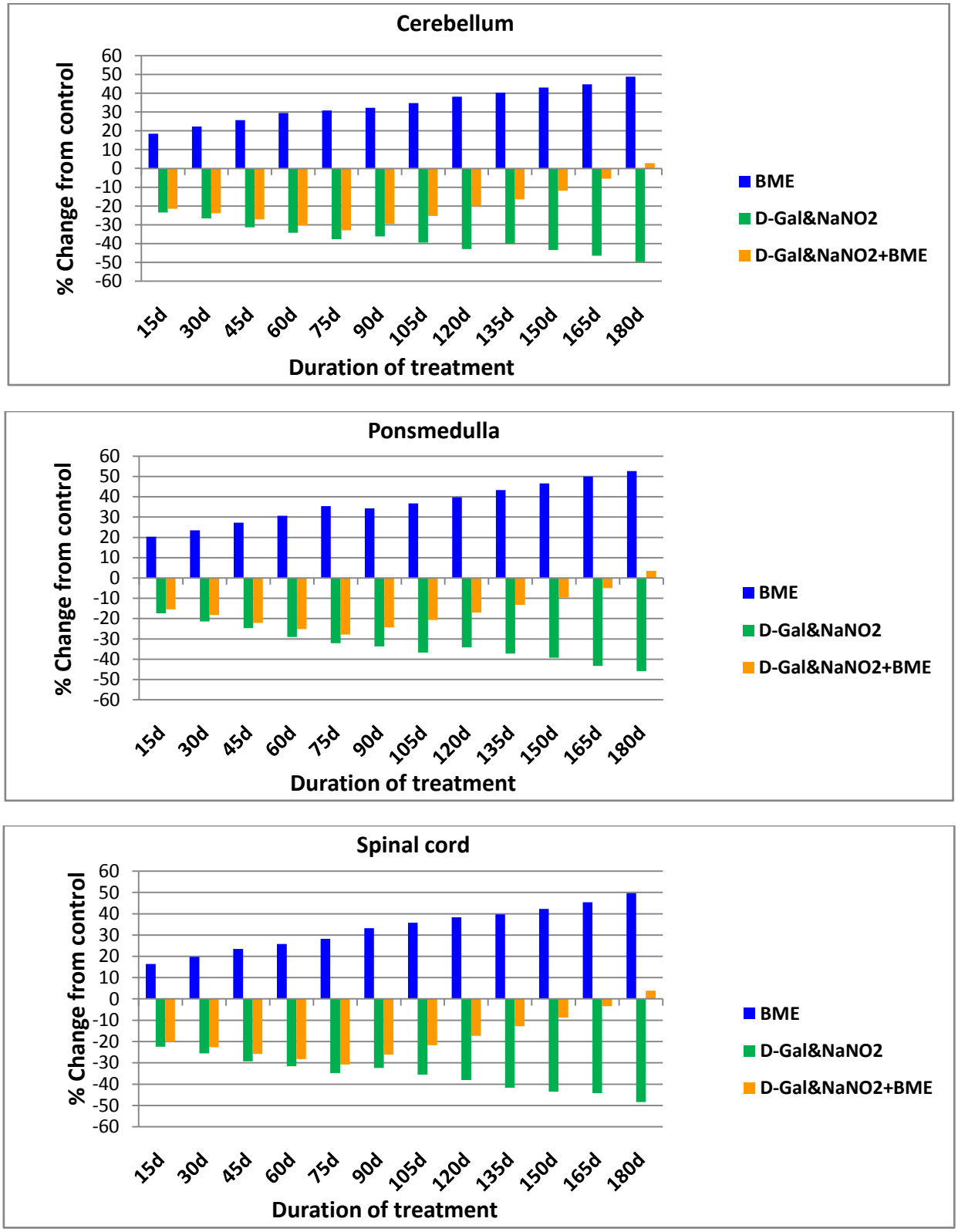


Figure 5.4 - 5.6: Graphical representation of percent changes in the activity of Ca^{2+} -ATPase (*in-vivo*) in Cerebellum(Cb), Ponsmedulla(Pm) and Spinal cord(Spc) regions of Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME.

The activity levels of Na^+/K^+, Mg^{2+} and Ca^{2+} -ATPases in selected brain regions of control albino mice was found to be highest Cerebral Cortex (CC), Hippocampus (Hc), Ponsmedulla (Pm), Spinal cord (Spc), Cerebellum (Cb) and Olfactory Lobe (OL).When compared to the control mice, the Na^+/K^+, Mg^{2+} and Ca^{2+} -ATPases in BME treated

mice were elevated significantly in all brain regions at selected time intervals. The percentage of elevation kept on increasing from 15th day to 180th day. On observing the trend in six regions of mice brain, it was obvious that elevation of Na^+/K^+ -ATPase was more in Spinal cord (53.62%) and less in Ponsmedulla (45.64%) whereas Mg2+ -and Ca2+ -ATPase were recorded highest in Ponsmedulla (51.43%) and lower in Olfactory Lobe (41.49%) and Cerebellum (48.81%) regions respectively.

Contrary to the BME treated mice, the Na^+/K^+, Mg^{2+} and Ca^{2+} -ATPases in AD induced mice were inhibited significantly and the percentage of inhibition was continuously increased from 15th day to 180th day in all regions of brain at all time periods. On comparing the six regions higher level of inhibition in Na^+/K^+, Mg^{2+} and Ca^{2+} -ATPases of AD induced mice were found in Spinal Cord (-46.87%), Cerebellum (-47.68%) and Cerebral Cortex (-50.87%) whereas lower levels were noticed in Olfactory Lobe (-42.74%), Cerebral Cortex (-40.14%) and Hippocampus(-44.55%) respectively.

In case of AD induced mice which was simultaneously treated with BME, even though the activity levels of all ATPases were inhibited in all regions of brain up to 75 days, surprisingly, from 95th day onwards all the constituents of the ATPase system started showing recovery trend from AD induced effect and by 180th day most of them reached approximately near control levels thus restoring normal condition in AD induced mice with respect to ATPase system (Figures- 3.1 to 3.6; 4.1 to 4.6; 5.1 to 5.6).

Discussion

The results of the present study clearly demonstrated that administration of *Bacopa monniera* extract (BME) to control mice caused a phenomenal gain in body weight and simultaneousy enhanced the learning skills, memory and concentration, whereas in AD induced mice, the learning and memory deficits were reversed back to near normal conditions indicating that BME had potential compounds for preventing learning and memory deficits.

Morphometric data is an important sources of information to understand many biological phenomena such as phylogenetic relationships,[20] evolution,[21] reconstruction of history and structure of past populations,[22] sexual dimorphism,[23] fluctuating asymmetry,[24,25] ecomorphology,[26] body condition,[27] growth,[28] heritability.[29]

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.[30] In the process of learning, activation of neurons occurs in specific areas of the brain concerned with the processing of the specific modality of sensory information.[31] Physiologically, memories are caused by changes in the capacity of synapses to transmit activity from one neuron to another in a neural circuit as a result of previous neural activity. These changes in turn establish new pathways which, called memory traces which are important because once established, they can be activated by thinking process to reproduce memories whenever required. The hippocampus and amygdale are concerned with the storage of recent memory and emotional behavior. The structural organization of these areas has been reported to be highly plastic, particularly in hippocampus.[32,33] In rodents, spatial learning and memory are closely related to the function of the dorsal hippocampus[34] to which cholinergic neurotransmission contributes significantly.[35] Although especially prominent in AD, cholinergic deficits in the cortex and hippocampus occur during normal human ageing[36] and smaller numbers of neurons and atropy of surviving cholinergic neurons in the basal forebrain were shown in aged animals with impaired learning and memory.[37]

Brain aging is a risk factor of neurodegenerative diseases such as Alzheimer's disease. 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-Alzheimers 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.[38-42] 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, Behavioural and Pharmacology studies recently.[39,40,42] 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.[43]

Memory is the natural counterpart of learning; it is necessary condition for the behavioural change to be permanent.[44] Bacopa, one such plant with wide medicinal

properties is used as a potent drug for treatment of memory-related disorders.[6] 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[45] and reverse amnesic effects of neurotoxin, scopolamine, electric shock and immobilization stress and it improves acquisition, retention and retrieval of learned tasks.[11] The bacosides, present in this plant[46,47] have active principles responsible for improving memory related functions through enhancing the efficiency of transmission of nerve impulses eventually strengthening memory and cognition.[48] Brahmi ghrita, an Ayurvedic formulation significantly improved latency in elevated plus maze in rats.[49] BME also reverses y-maze performance and open field hyperlocation behavioural changes and reduces the level of amyloid especially Abeta 1-40 and 1-42.[50] It provides protection from phenytoin (an epileptic drug) induced deficit in cognitive function of mice by similar behavioural tasks[51] lending versatility to its mechanism of action.

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.[52-54] 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. The present study also showed that the simultaneous administration of BME could attenuate the impairment of memory and improve behavior performance in the D-Galactose and $NaNO_2$ induced AD mice, indicating that BME had potential to prevent the learning and memory deficits effectively thus paving a way for discovery of novel anti-Alzheimer's drugs in future.

D-Galactose, a normal sugar in the mammalian body, at a higher level, however, may lead to the formation of reactive oxygen species by galactose oxidase.[54] An increasing evidences indicates that long-term systemic exposure of D-Galactose to rodents causes progressive decline in cognitive function and mimics aging progress, such as hippocampal-dependent cognitive dysfunction,[40] neurodegeneration[39] and impairments in antioxidant capacity.[55] Thus in the present study, a combined i.p. administration of D-Galactose and $NaNO_2$ was used to establish an AD induced mice model.

Our observations on ATPases viz, Na^+/K^+, Mg^{2+} and Ca^{2+} - ATPases in different brain regions of control and experimental groups of mice at selected time intervals clearly indicated that oral administration of BME significantly elevated the levels of all ATPases, whereas in AD induced mice the ATPase activity in all brain regions was inhibited significantly, which could be reverted back to normal level by the consecutive treatment with BME.

The membrane-bound ATPases are integral proteins responsible for the maintenance of ion homeostasis through active transport and control of delicate chemical gradient that is necessary for the optimal function of the central nervous system.[56] Any alteration in the membrane lipid components of brain results in the inactivation of these membrane-bound enzymes.[57] Loss of activity of ATPases is known to be involved in the development of a number of disorders such as neurological diseases, hypertension, diabetes, coronary artery diseases, stroke and tumor.[58] The cation transport across the neuronal membrane mediated by the ATPases plays a significant role in many biological functions such as electron transport chain, biological oxidation in the mitochondria, synaptic transmission, and antioxidant enzyme functions. Disturbances in the ionic equilibrium of the cells as a result of inactivation of ATPases are believed to be the major factors in the pathogenesis of various neurological disorders.[59]

The energy needed for the active transport of these ions is provided by the hydrolysis of ATP. In brain, ~40% of the energy released by mitochondrial respiration is utilized by ATPases to maintain the ionic gradients across the cell membranes compared with 5% in other tissues.[60] Mitochondria represent potential hot spots for free radical-induced damage and the resultant mitochondrial dysfunction leads to a decline in the efficiency of ATP synthesis.[61] Studies of mitochondria isolated from brains of rodents of different ages have provided evidence that the ability of mitochondria to generate ATP is compromised with advancing age and those mitochondria from old brain cells exhibit increased free radical-mediated damage.[62] Impaired cellular energy metabolism may render neurons vulnerable to excitotoxic damage,[63] particularly when neurons are subjected to additional stresses of Aβ and tau accumulations.[64] Oxidative stress may promote Aβ production[65] by increasing APP cleavage by both β- and γ-secretases.[66] Elevated intracellular Ca2+ levels resulting

from age related increases in oxidative stress and Aβ toxicity may contribute to the increased amyloidogenic processing of APP in Alzheimer's disease.[67]

The reversal effects of BME in AD induced mice with respect to ATPase system as noticed in our present investigation further reiterate that BME has potential compounds necessary to maintain ion gradients across biological membranes and thus confer significant protection to the brain by stabilizing the structural and functional integrity of the membrane. *Bacopa monniera* is used in the indigenous systems of medicine for the treatment of various nervous system ailments such as Alzheimer's disease, insomnia, anxiety, epilepsy and hysteria.[68] In the past preclinical and clinical studies on mental retention in avoidance response of rats[45] have demonstrated[69] the memory enhancing effects of BME which have been attributed to the active constituent saponin, as bacosides A and B. It is widely accepted that neuronal damage can be significantly minimized by free radical scavengers. *Bacopa monniera* showed significant antioxidant effect per se and in stressed animals.[70,71] It has also been shown that *Bacopa monniera* protected morphine induced rat brain mitochondrial damage that could have favored the efficiency of ATP production.[72] This energy-promoting action may be responsible for the improved mitochondrial function and maintenance of ATPases in the present study, upon administration of *Bacopa monniera* extract. Maintenance of these ionic pumps helped in the maintenance of associated ionic homeostasis.

Brain oxidative metabolism is very active, mostly required to maintain cellular Na^+/K^+ gradients for keeping nerve impulse propagation, neurotransmitter release and cation homeostasis. Na^+, K^+ -ATPase activity decreased age-dependently in rat brain and was detected as a consequence of oxidative damage.[73,74] It has been proposed that alterations in Na^+/K^+ -ATPase activity may represent an important neurotoxic mechanism for neurons[75] and inhibition of Mg^{2+} enzyme may produce abnormalities related to the modulation of Mg^{2+} dependent enzyme activities.[76] In the present study, as expected, the decline of brain Na^+/K^+ and Mg^{2+} -ATPase activity observed in AD-induced mice was prevented by long-term supplement with BME.

Ca^{2+}-ATPase regulates Ca^{2+} pump activity which acts as a second messenger in the control of cellular processes that plays a central role in mediating neurosecretion[77] and Inhibition of Ca^{2+} ATPase activity can in turn increase intracellular concentration of Ca^{2+} and alter the signal transduction pathways and cellular fluidity and eventually results in cell death.[78] In the present study, administration of BME might have attenuated the influx of Ca^{2+} and favored its sequestration in the stores, thus maintaining the D-Galactose and $NaNO_2$ induced alteration in calcium ion homeostasis through its calcium antagonistic property. Our present observations derive strong support from earlier research findings wherein it was suggested that administration of bacosides could be a useful therapeutic strategy in ameliorating hypobaric hypoxia-induced cognitive dysfunctions[79] and other related neurological disorders viz., Alzheimer's disease possibly mediated by inhibition of calcium-ion influx into cell membranes.

Conclusion

The observations in the present investigation on Morphometric and Behavioural aspects mice following the oral administration of BME have given conclusive evidences on its neuroprotective effect on the nervous system in AD-induced mice. Further the reversal effects of BME on the changes caused in AD induced mice with respect to ATPase system reiterate that BME has potential compounds necessary to maintain ion gradients across biological membranes and thus confer significant protection to the brain by stabilizing the structural and functional integrity of the membrane.

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