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Anti-Alzheimer's Activity of Bacopa Monniera Extract on Monoamines in AD Induced Albino Mice

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ABSTRACT: The aim of the study is to investigate the anti-Alzheimer's activity of Bacopa monniera extract(BME) on Monoamines (Dopamine, Norepinephrine and Epinephrine) in AD induced mice with reference to Morphometric and Behavioural aspects. Male Albino mice(Mus musculus) of one month old weighing 20±2 grams, used as experimental model and were maintained according to ethical guidelines for animal protection and welfare. Mice were divided into 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 behavioural aspects of four groups of mice were analyzed by using Morris Water Maze technique. Dopamine(DA), Norepinephrine(NE) and Epinephrine(EP) contents were determined in brain regions of all groups of mice at selected time intervals through standard biochemical assay techniques. From the results, it was obvious 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. Similarly, protective effects of BME were noticed on Dopamine, Norepinephrine and Epinephrine 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, Morris Water Maze, Dopamine, Norepinephrine, Epinephrine.

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurologic disease of the brain leading to the irreversible loss of neurons and loss of intellectual abilities, including memory and reasoning. This disease was first described by German Psychiatrist and Neuropathologist, Alois Alzheimer in 1906 and was named after him [1]. AD is becoming a more common cause of death in the populations of the United States and other countries since the life span of human beings is increasing. Although other major causes of death 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 the number one cause of death, heart disease, decreased by 13% [2]. An estimated 26.6 million people worldwide had Alzheimer's disease in the year 2006, and this number may quadruple by 2050. The incidence of AD raised from 2.8 per 1000 people in the age group of 65-69 to 56.1 per 1000 people in older age of 90 years [3]. There are both neurochemical and neurohistologic alterations in the brains of AD patients contributing to the clinical manifestations. The term "Behavioural and Psychological Symptoms in Dementia" (BPSD) includes a heterogeneous group of non-cognitive symptoms and behaviours occurring in patients with dementia. Non-cognitive function is the global term used to describe problems such as depression, agitation, personality changes, delusions and hallucinations. A cholinergic-monoaminergic imbalance has been hypothesized in the pathogenesis of mood disorders. It has been suggested that depression results from a cholinergic predominance and mania from an adrenergic predominance. 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 [4]. Now-a-days several nootropic agents such as Piracetam [5], Pramiracetam, Aniracetam [6] and Cholinesterase inhibitors are being primarily used to improve memory, mood, behavior and for some neurological disorders viz., Alzheimer's disease, but the resulting adverse effects associated with these agents made their use limited [7]. 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. The World Health Organization (WHO) estimates that 80% of the world's population presently uses herbal medicine for some aspects of primary health care [8].

Bacopa monniera (Brahmi) is a well-known Nootropic plant with wide medicinal properties that is being used for treatment of memory-related disorders [9]. 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 [10] for treatment of epilepsy, insomnia [11] and anxiety and as a mild sedative and memory enhancer [11]. Besides, Bacopa monniera displays antistress [12] and anxiolytic [13] activities too in animals. It has also been shown to exert antioxidant effects through the chelating of metal ions, breaking oxidative chain reaction [11], improving activities of antioxidative defense enzymes [14] and scavenging the free radicals [15]. 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 [10].

The plant has many active moieties, but its major bioactive putative component is Bacoside A, which is mainly responsible for its neuropharmacological effects [16]. Bacoside A is a mixture of major components, Bacoside A3 ($C_{47}H_{76}O_{18}$), Bacoside II ($C_{47}H_{76}O_{18}$) and Bacosaponin C ($C_{46}H_{74}O_{17}$) and isomer of Bacosaponin C.

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 the Dopamine(DA), Norepinephrine (NE), and Epinephrine(EP) system in the brain of normal and AD induced mice with particular reference to Morphometric and Behavioural aspects.

2. 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, were used for biochemical analyses, Kubota KR 2000T centrifuge for homogenates centrifugation, Hitachi UV-2800 spectrophotometer, RF 1501 Schimadzu Fluorimeter and other standard equipments were used 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 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 plant extract: Bacopa monniera plant was collected from Talacona region 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.

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 quite successful in inducing Alzheimer's disease in mice. Thus, in the present study, memory impairment 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 [17, 18].

Experiment protocol: After the mice were acclimated to the laboratory conditions for 10 days, the mice randomly divided into four main groups. Each main group was again divided into 12 sub-groups of six each 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 daytime.

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.

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 biochemical assays.

Behavioural aspects:

Morris Water Maze test: Learning and memory ability was detected by using the Morris water maze test [19] 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 [20]. The time spent by the animal reaching the hidden platform was used as the index of memory. Before starting the experiment, the mice were acclimatizing 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 trial, the time required (in seconds) for individual mice to find the hidden platform was recorded and the mean data from the tests were used for statistical analysis.

Dopamine (DA), Norepinephrine (NE) and Epinephrine (EP): The brain samples from all the four groups were analyzed for determination of the Dopamine (DA), Norepinephrine (NE) and Epinephrine (EP) according to the method of Kari et al.,1978 [21].

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

Morphometric Aspects:

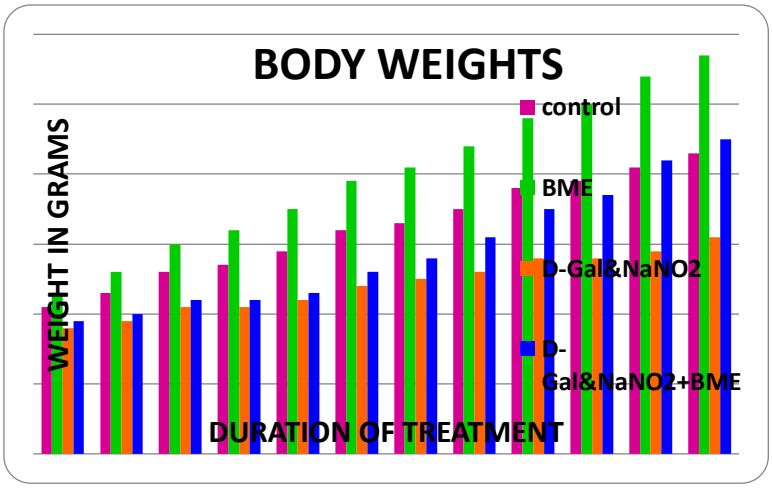


Fig. 1: Graphical representation of differences in the body weights of Control and Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME at selected time intervals.

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.

Behavioural Aspects:

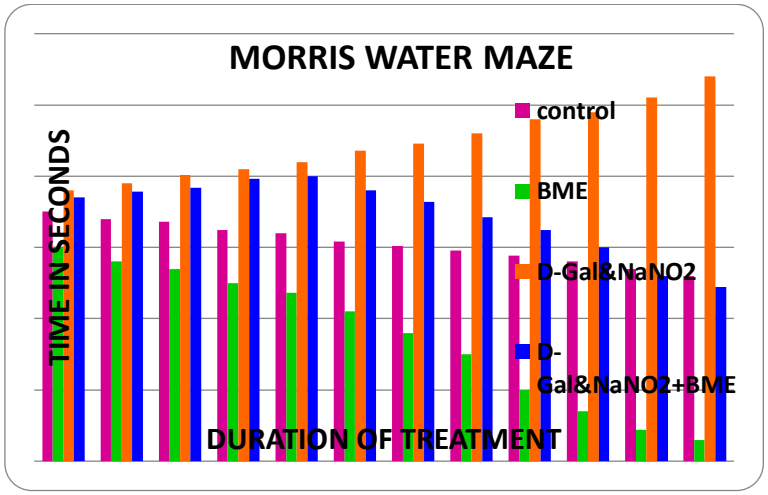
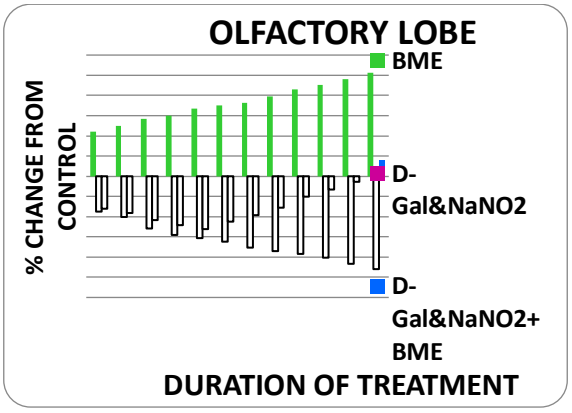
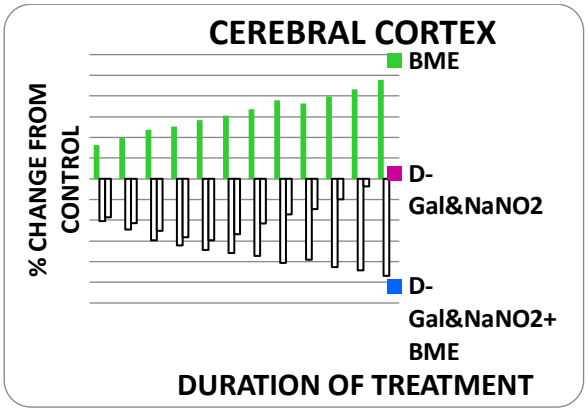


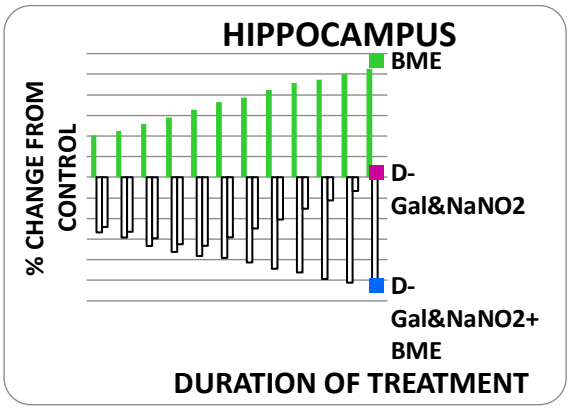
Fig. 2: Graphical representation of Morris Water Maze test results of Control and Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME at selected time intervals.

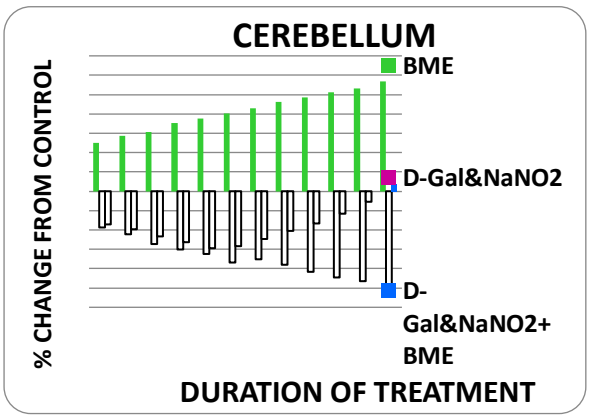
Morris water maze test: In the present study, the Morris water maze task was used to assess spatial learning and memory ability in mice. The results indicated that, compare to the control ones, escape latency (time taken to reach the hidden platform) was decreased from 15th day (150 seconds) to 180th 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 the normal levels. From 165th day (130 seconds), the mice took less time to reach the hidden platform from that of controls.

Monoamines:









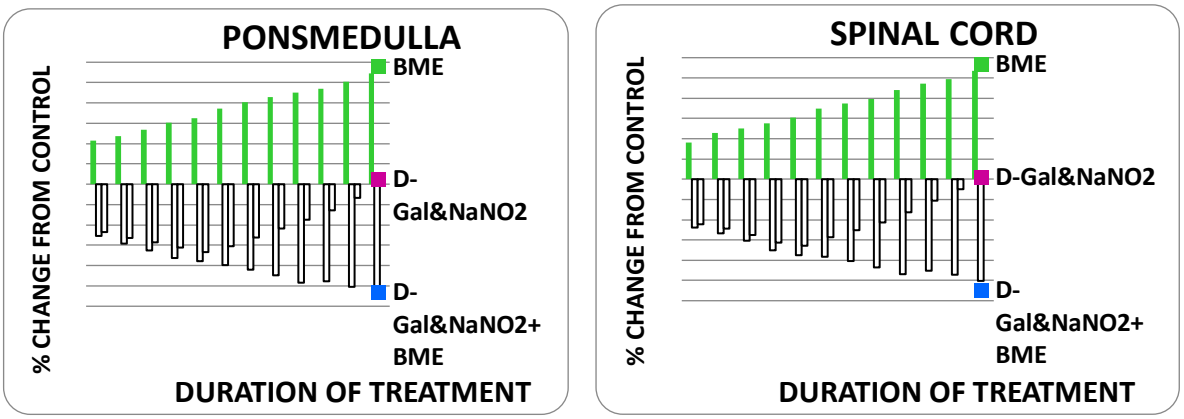
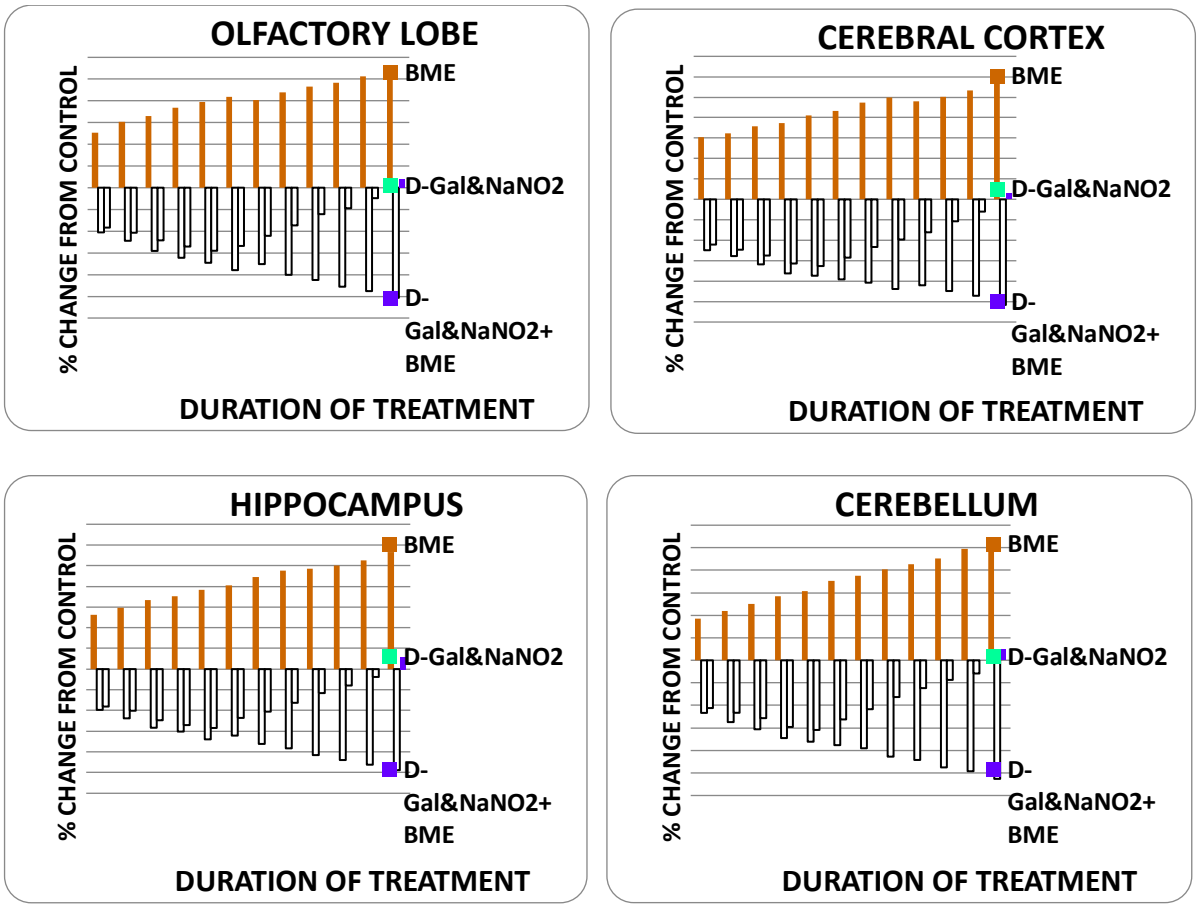
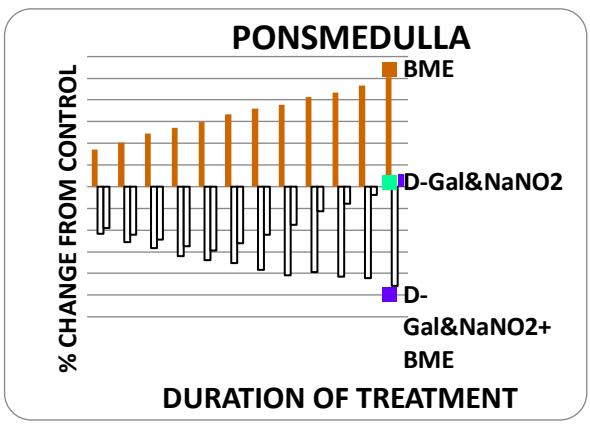


Fig. 3.1-3.6: Graphical representation of percent changes in the content of Dopamine (invivo) in Olfactory Lobe(OL), Cerebral Cortex(CC), Hippocampus(Hc), Cerebellum(Cb), Ponsmedulla(Pm) and Spinal cord(Spc) regions of Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose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





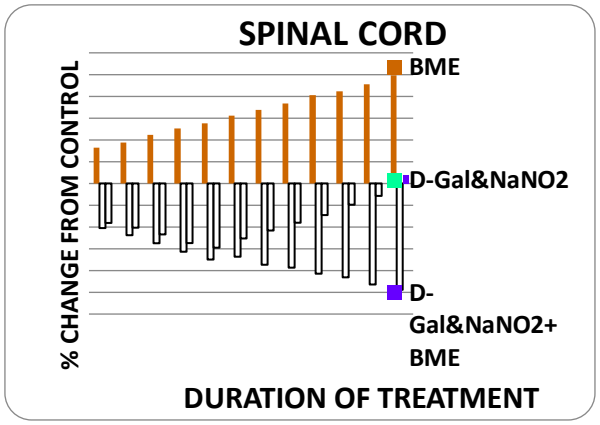
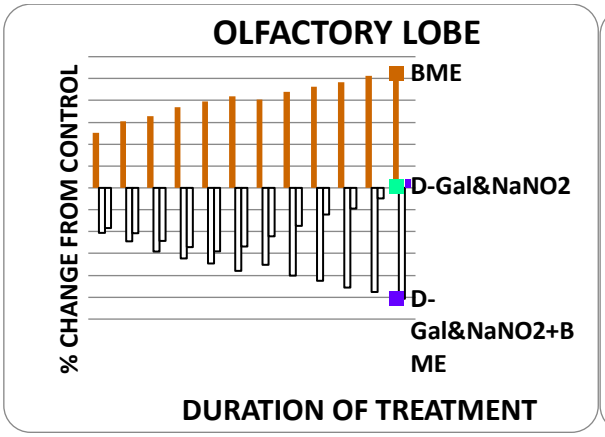
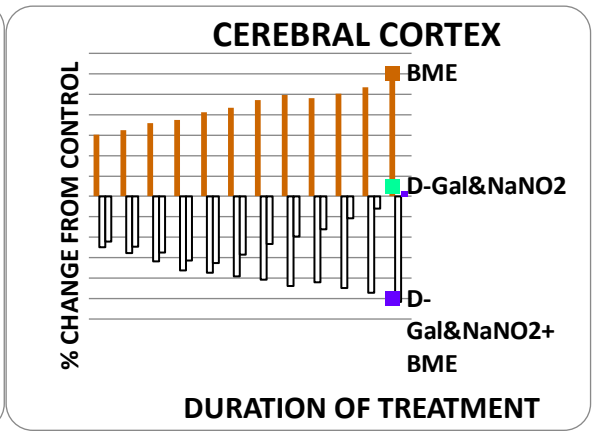
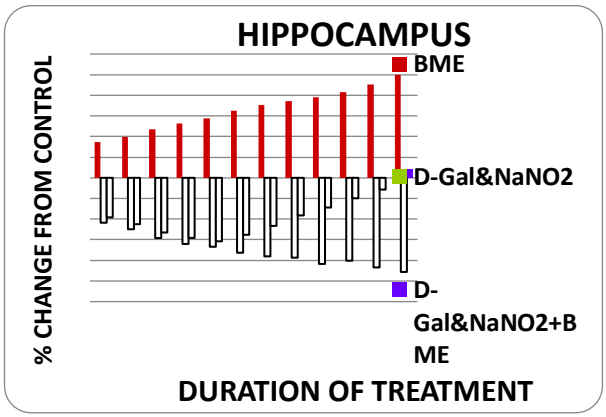
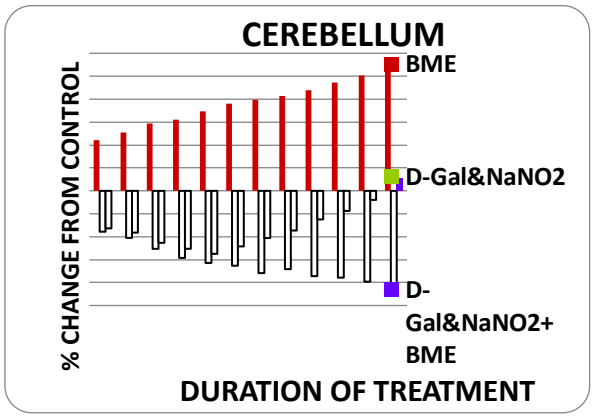


Fig. 4.1-4.6: Graphical representation of percent changes in the content of Norepinephrine (invivo) in Olfactory Lobe(OL), Cerebral Cortex(CC), Hippocampus(Hc), Cerebellum(Cb), Ponsmedulla(Pm) and Spinal cord(Spc) regions of Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME.









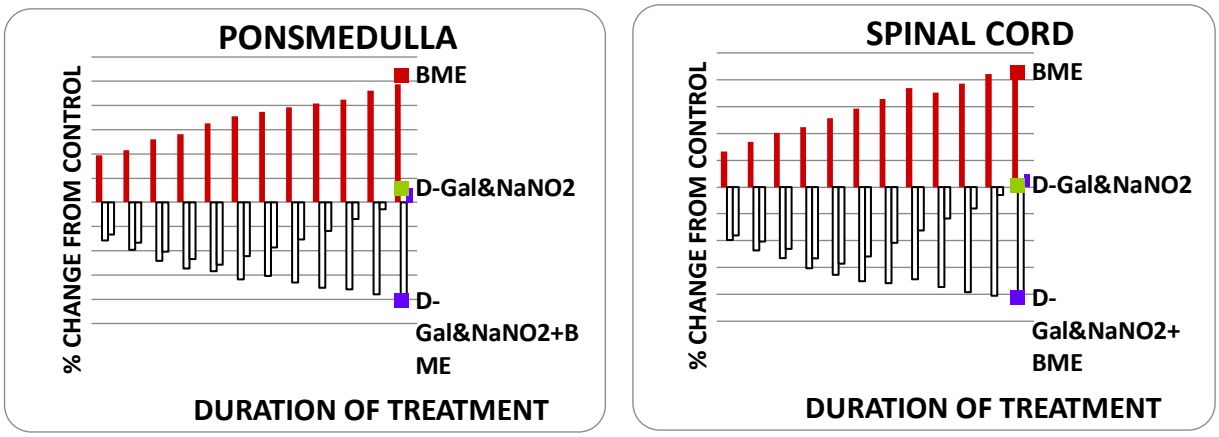


Fig. 5.1-5.6: Graphical representation of percent changes in the content of Epinephrine (invivo) in Olfactorylobe(OL), Cerebral cortex(CC), Hippocampus(Hc), Cerebellum(Cb), Ponsmedulla(Pm) and Spinal cord(Spc) regions of Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME.

The activity levels of Monoamines (Dopamine (DA), Norepinephrine (NE) and Epinephrine (EP) were estimated in different brain regions of all four groups of mice at selected time intervals.

In control mice, the distribution of these monoamines was not uniform in different regions of mice brain. Dopamine was highest in the Hippocampus (HC) and lowest in Spinal cord (Spc). Regarding Norepinephrine and Epinephrine maximum activity was noticed in Ponsmedulla (Pm) and minimum in Spinal cord (Spc), Cerebellum (Cb) respectively.

On comparison with the control ones, the Monoamines were elevated significantly in all brain regions of BME treated mice at selected time intervals. The percentage of elevation kept on increasing from 15th day to 180th day and the maximum percent change was recorded in different regions are mentioned below in Table-1.

Table 1. Maximum Percent change of Monoamines in different brain regions of BME treated mice

DA	Cb 56.66%	>	Pm 54.52%	>	SpC 53.58%	>	Hc 52.77%	>	OL 51.18%	>	CC 47.90%
NE	CC 57.73%	>	Hc 56.54%	>	OL 53.70%	>	Cb 51.80%	>	Pm 50.44%	>	Spc 49.67%
EP	Cb 52.60%	>	CC 49.87%	>	Hc 49.83%	>	Pm 48.63%	>	Spc 45.33%	>	OL 42.97%

From the above order, it was observed that Dopamine and Epinephrine contents recorded highest percent change in Cerebellum (Cb) and lowest percent change in Cerebral Cortex (CC) and Olfactory Lobe (OL), whereas Norepinephrine exhibited more percent change in Cerebral Cortex (CC) and lesser percent change in Spinal cord (Spc) region.

Contrary to the BME treated mice, the Monoamines in AD-induced mice were inhibited significantly in all regions of mice brain at all selected time periods. The percent of inhibition increased continuously from 15th day to 180th day and the maximum percent change was recorded in different regions are mentioned below in Table -2.

Table 2. Maximum Percent change of Monoamines in different brain regions of AD-induced mice

DA	Hc -53.79%	>	Pm -52.68%	>	Spc -50.21%	>	Cb -49.73%	>	CC -46.89%	>	OL -45.97%
NE	Cb -52.62%	>	CC -51.64%	>	OL -50.71%	>	Spc -42.96%	>	Hc -48.85%	>	Pm -45.74%
EP	Hc -45.63%	>	OL -43.81%	>	Cb -43.00%	>	Spc -42.96%	>	CC -41.83%	>	Pm -41.40%

As seen from the above trend, it was evident that among the six regions of mice brain, the maximum percent change of Dopamine and Epinephrine contents were noticed in Hippocampus (Hc) whereas Norepinephrine content was recorded in

Cerebellum (Cb). Ponsmedulla (Pm) has recorded lowest percent change in Norepinephrine and Epinephrine contents, whereas Olfactory Lobe (OL) the least percent change in Dopamine content.

However, in the case of AD-induced mice simultaneously administered with BME, even though all Monoamines were inhibited significantly in all regions of mice brain from 15th day to 165th day with respect to the control ones, maximum inhibition was noticed on 75th day in the order as mentioned below in Table-3, from 90th day onwards this inhibitory trend in Monoamines started decreasing and finally reached approximately the control levels by 180th day.

Table 3. Maximum Percent change of Monoamines in different brain regions of AD-induced mice simultaneously administered with BME.

DA	Pm -33.38%	>	Hc -33.28%	>	Spc -32.87%	>	CC -29.68%	>	Cb -29.45%	>	OL -26.16%
NE	CC -32.62%	>	Cb -30.90%	>	Pm -29.71%	>	Spc -29.53%	>	OL -29.04%	>	HC -28.45%
EP	Hc -30.77%	>	OL -28.77%	>	Spc -28.54%	>	Cb -27.47%	>	Pm -25.73%	>	CC -23.16%

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. Morphometric data is an important source of information to understand many biological phenomena such as phylogenetic relationships [22], evolution [23], reconstruction of history and structure of past populations [24], sexual dimorphism [25], fluctuating asymmetry [26], ecomorphology [27], body condition [28], growth [29], heritability [30] etc. Morphometrics [31] 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 [32]. 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 [33]. Physiologically, memories are caused by changes in the capacity of synapses to transmit activity from one neuron to another in a neural circuit because of previous neural activity. These changes in turn establish new pathways which, called memory traces, are important because once established, they can be activated by thinking process to reproduce memories whenever required. The intellectual ability of an individual is dependent on memories to which one is adding constantly. 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 [34]. In rodents, spatial learning and memory are closely related to the function of the dorsal hippocampus [35], to which cholinergic neurotransmission contributes significantly [36]. Although especially prominent in AD, cholinergic deficits in the cortex and hippocampus occur during normal human ageing [37] 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 [38].

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-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 [39]. 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]. 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 [40]. 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 AD induced mice which further proved that long treatment of BME effectively improves the impaired learning and memory performance in both normal and diseased mice.

Anti-Alzheimer's Activity of Bacopa Monniera Extract on Monoamines in AD Induced Albino Mice

Memory is the natural counterpart of learning; it is necessary condition for the behavioural change to be permanent [41] Bacopa, one such plant with wide medicinal properties, is used as a potent drug for treatment of memory-related disorders [9]. 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 [42] and reverse amnesic effects of neurotoxin, scopolamine, electric shock and immobilization stress and it improves acquisition, retention and retrieval of learned tasks [14]. The bacosides, present in this plant [43, 44] have active principles responsible for improving memory related functions through enhancing the efficiency of transmission of nerve impulses eventually strengthening memory and cognition [45]. Brahmi ghrita, an Ayurvedic formulation significantly improved latency in elevated plus maze in rats [46]. BME also reverses y-maze performance and open field hyper location behavioural changes and reduces the level of amyloid especially Abeta 1-40 and 1-42 [47]. It provides protection from phenytoin (an epileptic drug) induced deficit in cognitive function of mice by similar behavioural tasks [48] lending versatility to its mechanism of action.

Several reports demonstrated that mice, continuously exposed to high dosage of D-Galactose showed an increase in levels of cleaved caspase-3, apoptosis and karyopknosis in hippocampal neurons and a decline in spatial learning and memory [39]. The D-Galactose treated rats had a decreased density of synapse on the catecholaminergic region and a forebrain cholinergic neuronal loss was also reported [49]. In addition, long-term injection of D-Galactose in mice impaired neurogenesis in the dentate gyrus and induced newly formed neurons death in the granular cell layer [4]. The D-Galactose lesioned rodents have been used for brain aging studies, as D-Galactose induced behavioural and neurochemical changes can mimic many characters of the natural brain aging process [39,49]. Chronic systemic D-Galactose exposure induces memory impairments, neurodegeneration and oxidative damage in mice [50]. It has been reported that rodents injected with D-Galactose caused a progressive deterioration of learning and memory capacity and increases production of free radicals in the brain [51]. 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 [52,53,54]. The behavioural trials showed that learning and memory performance in water maze tasks were severely impaired in rats treated with D-Galactose and $NaNO_2$. The results of the present study agree 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.

Similarly, from our observations, it was obvious that, when compared to the control mice, the levels of Monoamines (Dopamine, Norepinephrine and Epinephrine) were significantly elevated in BME treated mice whereas in AD-induced mice, their levels were inhibited at all selected time periods. Finally, restoration of normal levels of all monoamines as observed during the subsequent period of treatment of AD induced mice with BME indicated the neuroprotective role on neurotransmitter systems.

Neurotransmitters, such as Acetylcholine, Norepinephrine, Epinephrine, Dopamine etc. are important for memory, learning and for overall behavior of animals. The brain contains several hundred types of chemical messengers that act as communication agents between different brain cells. Previous studies demonstrated that the numeric working could be modulated by Dopamine, Norepinephrine and other main neurotransmitters [55] and during spatial memory task, both Acetylcholine and serotonin in hippocampus were simultaneously activated [56]. It has been suggested that the behavioural effects of cholinergic degeneration can be changed by a reduction in noradrenergic function [57]. Bacopa monniera is known to increase norepinephrine and 5- hydroxyl tryptamine levels in the hippocampus, hypothalamus and cerebral cortex [58]. Observations in the present study coincide well with the above research reports where the methanol extract of Bacopa monniera elevated the Dopamine, Norepinephrine and Epinephrine levels in hippocampus, cerebellum, cerebral cortex along with olfactory lobe, ponsmedulla and spinal cord.

Locus Coeruleus (LC) is the major nucleus of origin of noradrenergic fibres in the mammalian brain. Anatomical and physiological properties of noradrenergic (NA) neurons of LC have led to the formulation of hypotheses on the role of this brainstem nucleus that is probably involved in sleep, attention, memory and vigilance [59]. Unfortunately, the exact role of Nor Adrenaline system in behavioural symptoms associated with AD is unclear. Several studies have found greater loss of LC neurons in depressed subjects with AD [60]. Furthermore, preservation of NA nerve fibres and increase in α2 receptors in the cerebellum have been correlated with aggressive behavior in AD [61].

Dopamine (DA) originating from nerve cell bodies located in mid brain is present in forebrain and particularly in basal ganglia. It is involved in motor control and in modulating activity of limbic centers. At the cortical level, DA is more abundant in the frontal than in posterior lobes [62]. Its anatomical distribution itself suggests that DA is in a position in the forebrain to "tune" the activity of these different cortico-cortical loops and thus contribute to the drive of action and continuous adaptation of behavioural concerning perceptual changes of the environment. Dopamine agonists have psycho stimulant effect and inactivation of D2 receptors in deficient mice, induces behavioural changes related to limbic system activity. Levels of DA are decreased in discrete areas of the brains of AD patients and aggressive behavior is likely related, like psychosis, to dopaminergic system

dysfunction. In the present study, the concentration of Dopamine along with Norepinephrine and Epinephrine also decreased in brain regions of AD induced mice and consecutive treatment of Bacopa monniera significantly attenuated these alterations and restored back their levels approximately close to the control mice, indicating the neuroprotective role of Bacopa monniera against impairments induced by D-Galactose and $NaNO_2$.

Bacopa monniera is a wonderful plant with wide medicinal properties that is being used for treatment for memory-related disorders [9]. The bacosides are the memory chemicals in brahmi [43,44], which are active principles responsible for improving memory related functions, are attributed with the capability to enhance the efficiency of transmission of nerve impulses, thereby strengthening memory and cognition [45]. Memory is the natural counterpart of learning; it is necessary condition for the behavioural change to be permanent. Alcoholic extract of Bacopa monniera increases the learning performance of rats and the activity is attributed to a saponin mixture consisting of bacosides A, B and other saponins [42]. The triterpenoid saponins and their bacosides are responsible for Bacopa's ability to enhance nerve impulse transmission. The bacosides aid in repair of damaged neurons by enhancing kinase activity, neuronal synthesis, restoration of synaptic activity and ultimately nerve impulse transmission [58]. The plant has been found to be safe and well tolerated in humans as herbal products are commercially available for enhancing memory in old age patients [63]. Bacopa monniera has been reported to have a protective effect against morphine toxicity at various organs level including liver, kidneys and brain [64]. The plant has prominent action on the central nervous system, where it improves understanding, memory, intellect and speech and correct aberrations of emotions, mood and personality in an individual. Various experiments have identified potent antioxidant activity in BM [11,65]. According to scientists at the CDRI, several compounds have been identified in Bacopa monniera including bacosides A and B, two chemicals that improve the transmission of impulses between nerve cells in the brain. These Bacosides regenerate synapses and repair damaged neurons, making it easier to learn and remember new information.

Observations in the present study clearly suggest the protective effect of Bacopa monniera against AD-induced mice. In support of this, several other herbal drugs have also shown the promising neuroprotective effect in rodents [66,67,68,69]. Some of them have shown both antiamnesic effects and neuroprotective effects [66]. Similar to the Bacopa monniera, cognitive enhancement is an important addition to neuroprotective properties of Ginkgo biloba. The neuroprotective property of Ginkgo biloba leaf has been shown to be because of ginkgolide, a terpene fraction but its flavonoid fraction containing free radical scavengers is also important [70]. According to Sheikh et al.,2007 [71] Bacopa monniera also normalizes the level of stress-induced alteration in the monoamine level in the cortex and hippocampus.

Bacopa monniera has also adaptogenic properties [72] which are helpful in attaining the general homeostatic response under various physiological conditions. Initial hypothesis of depression was formulated about 40 years ago, proposing that the main symptoms of depression are due to functional deficiency of cerebral monoaminergic transmitters such as Norepinephrine, Serotonin and Dopamine located at synapses [73]. Previous studies also shown the adaptogenic effect of Bacopa monniera and Panax quniquefolium extracts via normalization of the various stress parameters and monoaminergic levels which may provide a clue that these extracts are bringing their possible antidepressant effect through restoration of normal monoaminergic neurotransmission [72]. Bacopa monniera is also an antioxidant and it decreases reactive oxidative species(ROS) levels by enhancing antioxidant enzymes, for example Super oxide dismutase, glutathione peroxidase and catalase in frontal cortex, striatum and hippocampus of rats [74].

5. CONCLUSION

The observations in the present investigation on Morphometric, Behavioural aspects and on the Monoamines (Dopamine, Norepinephrine and Epinephrine) 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.

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