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*BACOPA MONNIERA*PLANT EXTRACT (BME) AS COGNITIVE ENHANCER IN ALZHEIMER'S DISEASE (AD) INDUCED ALBINO MICE

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ABSTRACT

Present study investigates the cognitive effects of BME on memory impairment in AD induced mice. Male Albino mice, *Musmusculus* of one month old weighing 20 ± 2 grams, 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 four groups were analyzed by using Morris Water Maze technique. Results revealed that administration of *Bacopamonniera* to mice caused a phenomenal gain in body weight and enhanced the learning skills, memory and concentration whereas in AD induced mice very severe learning and memory deficits were observed. However, these deficiencies in AD induced mice could be ameliorated by simultaneous administration of BME and mice restored normal condition. From these observations, it was finally concluded that BME had potential compounds which can prevent the learning and memory deficits effectively and thus confer neuroprotection against Alzheimer's disease.

Keywords: Bacopamonniera, Alzheimer's disease, Morphometric and Morris Water Maze.

Number of Figures : 2 **Number of Tables**:1**Number of Refernces**:47

INTRODUCTION

Alzheimer's disease (AD) is a common and divesting neurodegenerative disorder and is one of the leading health problems worldwide. Though it is more common among elderly people, it can affect adults of any age. According to World Health Organization (WHO) "it is estimated that there are currently about 18 million people worldwide with Alzheimer's disease," and this figure is expected to nearly double by 2025 to 34 million. The disease is characterized by an insidious decline in cognitive function. 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. Non-cognitive function is the global term used to describe problems such as depression, agitation, personality changes, delusions and hallucinations. 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. Natural products with medicinal value are garnering a lot of attention due to serious side effects often caused by medicines of chemical origin (Zhong*et al*., 2009). Now-a-days several nootropic agents such as Piracetam (Schever*et al*., 1999), Pramiracetam, Aniracetam (Cumin *et al*., 1982) 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 (Rogers *et al*., 1988).Therefore it is worthwhile to explore the application of traditional medicines for the treatment of various cognitive disorders. World Health Organization (WHO) estimates that 80% of the world's population presently uses herbal medicine for some aspects of primary health care (WHO, 2003).

Since time immemorial, *Bacopamonniera*, a traditional Ayurvedic medicinal plant which belongs to the family Scrophulariaceae is commonly known as Brahmi in Sanskrit and has been used as nervine tonic for promoting mental health and improving memory by Ayurvedic medical practitioners in India. Chemical constituents of *Bacopamonniera* include alkaloids brahmine, herpestine and nicotine, saponinmonierin, hersaponin, bacoside A1, A2 (Rastogi and Kulshrestha, 1999), A3 (Rastogi and Kulshrestha, 1994), and B (Basu*et al*., 1967) and four saponinbacogenin A1 to A4 (Chandel*et al*., 1977).Many biological effects of *Bacopamonniera* are documented in traditional as well as in scientific literature of which the most important one is bacosideson enhancing the cognition and memory functions (Russo and Borrelli, 2005). *Bacopamonniera* is rich in pharmacological activities. Recent clinical studies on *Bacopamonniera* had demonstrated that it has memory enhancing properties (Roodenrys*et al*., 2002). Thus the present study was intended to evaluate the effect of *Bacopamonniera* extract on Morphometric, Learning and Memory

deficits in AD mice induced by D-Galactose and $NaNO_2$.

It was well established that D-Galactose, a physiological nutrient when supplied overdoses will result in abnormal oxidative metabolism eventually leading to production of excess Reactive Oxygen Species, which surpass the ability of cells to eliminate them, thus finally causing impairment of cell structure and gene expression (Zhang *et al*., 2003).Similarly, Sodium nitrite can change normal haemoglobin in to methaemoglobin, which reduces the oxygen-carrying capacity of blood, causing hypoxia, disability of consciousness ultimately depressing the learning and memory ability in mice. Combined administration of D-Galactose and $NaNO_2$ to mice presented a series of senescent performances, impairment of learning and memory function, abnormality of several biomarkers in brain, presence of metamorphic and necrotic neurons in cortex and hippocampus etc. (Zhang *et al*., 2006).Thus combined chronic intra peritoneal injection of D-Galactose and Sodium nitrite was found to be a very effective method to induce AD in normal mice.

In view of these multiple beneficial properties of *Bacopa*, in the present study an attempt has been made to assess the protective role of *Bacopamonniera* plant extract on Morphometric and Behavioural aspects in AD induced mice.

MATERIALS AND METHODS

Maintenance of mice: Male albino mice, *Musmusculus*, 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 approval of Institutional Animal Ethical Committee (Resolution No. 02/(i)/a/CPCSEA/ IAEC/ SVU/ KY-KK/ Dt. 21-03-2011).

Collection and preparation of *Bacopamonniera* extract: *Bacopamonniera* plant was collected from Talacona forest area which is around 50 Km from Tirupati. 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 solvent was filtered. This was repeated 3 to 4 times until the extract gave no colouration. The extract was distilled and concentrated under reduced pressure in the Hahnvapor Rotary Evaporator HS-2005V. The resulting methanol crude extract was air-dried and used in the present study.

Induction of Alzheimer's disease in mice: Until now, several chemical compounds such as Amyloid beta, Aluminium-maltolate, D-Galactose and Sodium nitrite have been used to induce AD in mice. But a combination of the chemicals, D-Galactose and Sodium nitrite together was considered to be quite successful in inducing Alzheimer's disease in mice (Fang and Liu.,

2007). Hence in the present study, AD in mice was induced by an intra-peritoneal (i.p.) injection of D-Galactose (120mg/kg body weight) and sodium nitrite (90mg/kg body weight) by dissolving in distilled water

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.

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

Table1

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

Morphometric aspects: Basic Morpho-metric 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 behavioral aspects.

Behavioural aspects (Morris Water Maze test): Morris Water Maze task was performed to evaluate learning and memory efficiencies in rodents (Morris, 1984). This model has several advantages over other models including radial arm maze.

These include:

1) Motivational stimuli such as food and water deprivation, electrical stimulations and buzzer sounds required in active/passive avoidance test, which may interfere the normal process of memory.
2) Animal can be trained in a shorter time (1 week), while the arm maze studies require several weeks of training.
3) Intra maze cues like odor trains are eliminated in the pool.
4) Larger dose-response studies can be conducted in weeks' time.
5) From theoretical point of view, the water maze is an aversively motivated task, while the arm maze is an appetitive-motivated task (Vogel, 2005).
6) This test allows studies on spatial working memory and spatial reference memory (Olton *et al.*, 1979).

In view of these above mentioned advantages, Morris water Maze technique has been extensively used for evaluation of impact of drugs that not only affect spatial memory but also impair loco motor activity (Anand*et al*., 2007).

Morris Water Maze Technique: A great deal of knowledge has been obtained on the neurochemical, neuroanatomical and neurophysiological basis for 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 plexi glass (10cm in diameter and 29 cm in height) was hidden at 1.5 cm below the surface of water in a fixed location. Water was made opaque with powdered non-fat milk or non-toxic white coloured dye. The platform was not visible from just above the water level and transfer trials have indicated that escape on to the platform was not achieved by visual or other proximal cues (Morris, 1981). Time spent by the animal to reach the hidden platform was used as the index of memory. Before starting the experiment mice were acclimatize to the maze environment. 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 mean data from the tests were used for statistical analysis.

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 AND DISCUSSION

Morphometric Aspects: Total body weights (in grams) of control and experimental groups of mice were recorded using a digital balance at selected time periods as given in the materials and methods section. 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 recorded a phenomenal increase in their weights 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, even though 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 over that of control ones indicating that BME could effectively revert the AD induced changes gradually **(Figure 1).**

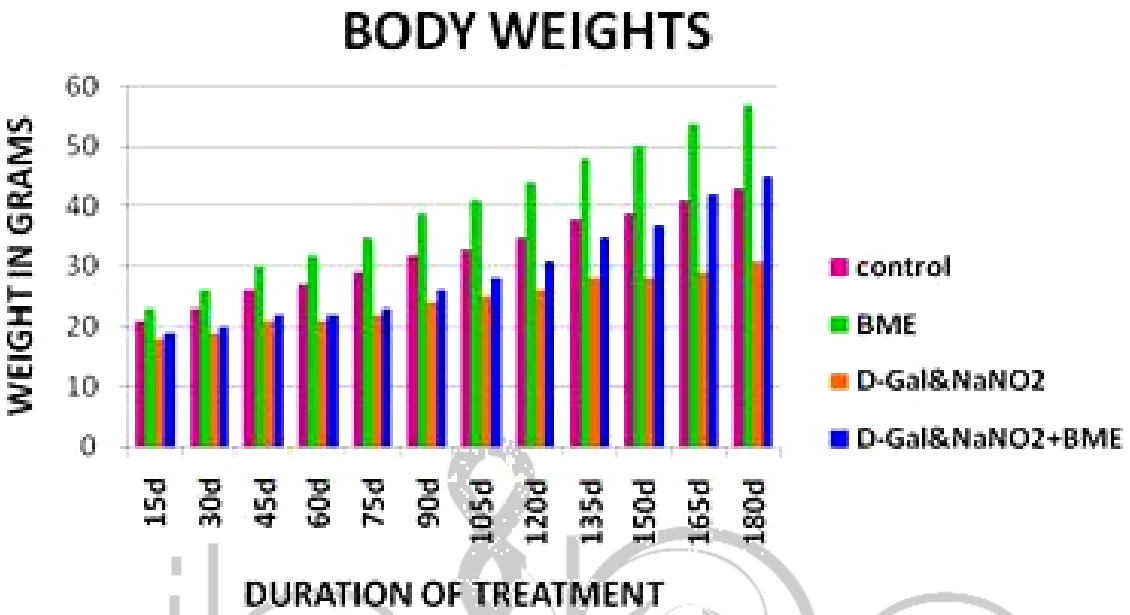

Fig1: *Graphical representation of differences in the body weights of the Control and Experimental groups of mice treat with BME, D-galactose and $NaNO_2$ and D-Galactose and $NaNO_2$ + BME at selected time intervals.*

Behavioral Aspects: 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).**

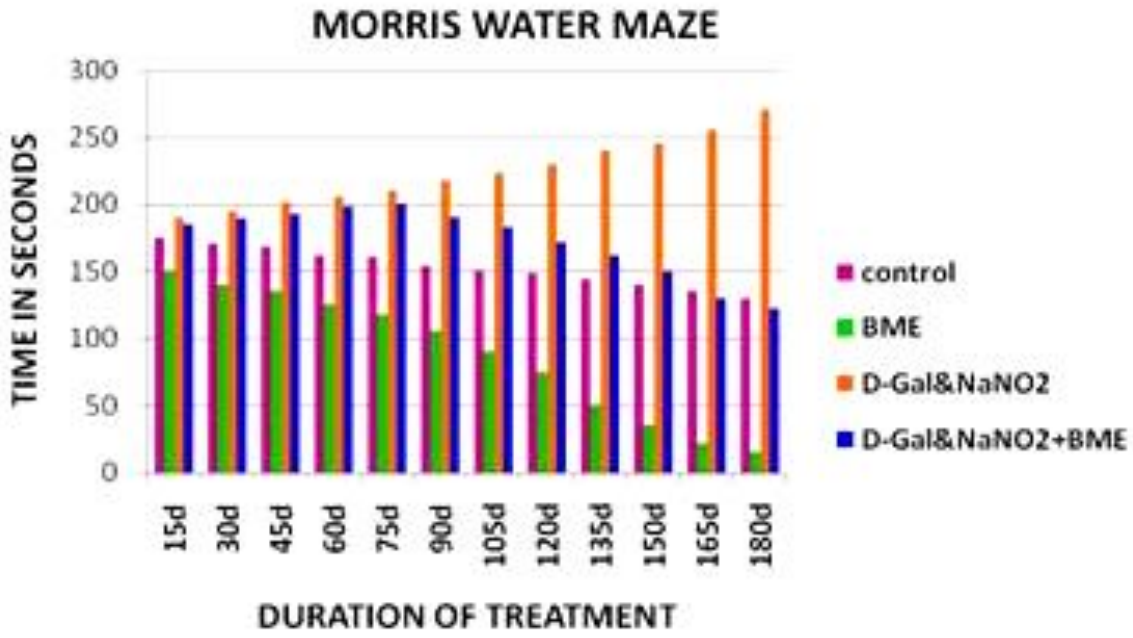

Fig 2: *Graphical representation of Morris Water Maze test results of Control and Experimental groups of mice treat with BME, D-Galactose and $NaNO_2$ and D-Galactose and $NaNO_2$ + BME at selected time intervals.*

Results of the present study clearly demonstrated that administration of BME to control mice caused a phenomenal gain in body weight and simultaneously enhanced the learning skills, memory and concentration, whereas in AD induced mice, 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 (Zelditch*et al*., 2004), evolution (Lieberman, 1998), reconstruction of history and structure of past populations (Gonzalez-Jose *et al*., 2001), sexual dimorphism (Vincent *et al*., 2004), fluctuating asymmetry (Badyaev*et al*., 2000; Willmore*et al*., 2005), ecomorphology (Klingenberg and Ekau, 1996), body condition (Green, 2001), growth (Ackermann, 2005), heritability (Krunk*et al*., 2000) etc. Morphometrics (Marcus, 1990) 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, covariance's between ecological factors and shape, as well as estimating quantitative-genetic parameters of shape.

Learning or acquisition, a highly specialized function of brain, is a process of acquiring knowledge about environment around the organism, while memory is the storage or retention of this learnt knowledge which can be retrieved later (Squire and Schlapfer, 1981). In the process of learning, activation of neurons occurs in specific areas of brain concerned with the processing of specific modality of sensory information (Rolls, 2000). 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 intellectual ability of an individual is dependent on memories to which one is adding constantly.

In the present study, it has been observed that impaired cognitive functions induced by D-Galactose and $NaNO_2$were restored back to almost normally by administering BME which further reiterate 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, neuro degeneration and behavioral impairment (Lu *et al*., 2007). Since these changes are similar to characters of normal aging process, administration of a combined dose of D-Galactoseand $NaNO_2$ 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 (Lu *et al*., 2007).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 (Brandeis *et al*., 1989). In this present study, 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 improve the impaired learning and memory performance in both normal and diseased mice.

Memory is the natural counterpart of learning; it is necessary condition for the behavioral change to be permanent (Vervliet, 2008).*Bacopa*, one such plant with wide medicinal properties is used as a potent drug for treatment of memory-related disorders (Russo and Borrelli, 2005).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 (Singh *et al*., 1988) and reverse amnesic effects of neurotoxin, scopolamine, electric shock and immobilization stress and it improves acquisition, retention and retrieval of learned tasks (Bhattacharya *et al*., 2000a). Bacosides, present in this plant (Sivaramakrishna, 2005), have active principles responsible for improving memory related functions through enhancing the efficiency of transmission of nerve impulses eventually strengthening memory and cognition (Anon, 2004). Brahmighrita,

an Ayurvedic formulation significantly improved latency in elevated plus maze in rats (Achilya*et al*., 2004). 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 (Holcomb *et al*., 2006). It provides protection from phenytoin (an epileptic drug) induced deficit in cognitive function of mice by similar behavioral tasks (Vohora*et al*., 2000) lending versatility to its mechanism of action.

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 **(Song *et al*., 1999).** 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 (Ho *et al*., 2003). The behavioral 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. Our present research observations on Bacopa provide strong evidences that it shares a number of pharmacological properties with other herbal cognitive enhancers such as, *Centellaasiatica*, *Clitoriaternatea*, *Jatamansi* (Rai*et al*., 2003), *Gingko biloba* (Mantle *et al*., 2000)in improving learning and memory. Hence, it is possible that a chronic administration of *Bacopamonniera* extract for a longer period is able to evoke desirable results as reported in the present study. Thus it was finally concluded that, BME can prevent the learning and memory deficits effectively and thus confer neuro protection against Alzheimer's disease.

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