Neuroprotective role of *Bacopa monniera* on Serotonin/5-Hydroxytryptamine (5-HT) in AD induced mice

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Abstract

The current study is to investigate the neuroprotective role of *Bacopa monniera* on Serotonin or 5-Hydroxytryptamine(5-HT) in AD induced mice with particular reference to Morpometric 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 in to four groups as follows: Group I: Control mice; Group II: mice treated with BME; Group III (AD induced): mice treated with D-Gal & $NaNO_2$; Group IV: AD induced mice simultaneously treated with BME. Changes in Morphometric and Behavioural aspects of mice and Serotonin(5-HT) activity were analyzed through standard techniques. Results revealed that BME showed positive effects on body weight, learning skills, memory and concentration, whereas D-Gal and $NaNO_2$ caused learning and memory deficits in mice which could be ameliorated by simultaneous administration of BME. Similar, protective effects of BME were noticed on Serotonin(5-HT) 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.

KEY WORDS: Alzheimer's disease, *Bacopa monniera*, Morphometric, Morris Water Maze, Serotonin.

1. INTRODUCTION

Dementia is a loss of brain function that occurs with certain diseases. Alzheimer's disease is the most common cause of dementia, or loss of intellectual function, among people aged 65 and older. It is a progressive, degenerative disorder that attacks the brain's nerve cells, or neurons, resulting in loss of memory, thinking and language skills, and behavioral changes. AD is becoming a more common cause of death in populations of the United States and other countries since the life span of human beings is increasing. Although other major causes of death in human beings continue to experience significant declines, those from AD have continued to rise. 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. Since time immemorial, *Bacopa monniera*, a traditional Ayurvedic medicinal plant which

belongs to the family Scrophulariaceae is commonly known as Brahmi in Sanskrit and has been used as nervine tonic for promoting mental health and improving memory by Ayurvedic medical practitioners in India. Recent research findings have revealed its protective roles as cognition-enhancer (1) antidepressant, antioxidant in rat frontal cortex, striatum and hippocampus (2) antiulcerogenic (3) and calcium antagonist (4). The chemical constituents of *Bacopa monniera* include alkaloids brahmine, herpestine and nicotine, saponin monierin, hersaponin bacoside A1, A2, A3 (5) and B (6) and four saponin bacogenin A1 to A4(7). Many biological effects of *Bacopa monniera* are documented in traditional as well as in scientific literature of which the most important one is bacosides on enhancing the cognition and memory functions (8).

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 Serotonin in the brain of normal and AD induced mice with particular reference to Morphometric and Behavioural aspects.

2. MATERIALS AND METHODS

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

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

2.3 Collection and preparation of *Bacopa monniera* plant extract: *Bacopa monniera* plant was collected from Talacona and identified by the Botanist, Department of Botany, S.V. University, Tirupati, India. The whole plant was dried in shade, powdered and used for extraction by using solvent. Powdered plant material was soaked in 95% methanol for 2 days at room temperature and the solvent was filtered. This was repeated 3 to 4 times until the extract gave no colouration. The extract was distilled and concentrated under reduced pressure in the Hahnvapor Rotary Evaporator HS-2005V. The resulting methanol crude extract was air dried and used in the present study.

2.4 Induction of Alzheimer's disease in mice: 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) (9) by dissolving in distilled water**.**

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

2.6 Isolation of tissues: The animals were sacrificed by cervical dislocation at the selected time periods viz., 15^{th}, 30^{th}, 45^{th}, 60^{th}, 75^{th}, 90^{th}, 105^{th}, 120^{th}, 135^{th}, 150^{th}, 165^{th} and 180^{th} day. Selected regions of mice brain such as Olfactory Lobe(OL), Cerebral Cortex(CC), Hippocampus(Hc), Cerebellum(Cb), Ponsmedulla (Pm) and Spinal cord(Spc) were isolated and immediately homogenized in suitable media for biochemical assays.

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

3. RESULTS

3.1 Morphometric Aspects: The total body weights (in grams) of control and experimental groups of mice were recorded using a digital balance at selected time periods then analyzed and used to correlate with the behavioural aspects and biochemical assays. The results revealed that the control mice showed a gradual increase in their body weights from 15th day (21 grams) to 180th day (43 grams). When compared to the control ones, BME treated mice gained more weight at all time periods from 15th day (23 grams) to 180th day (57.17 grams) whereas the D-Galactose and $NaNO_2$ treated mice gained less weight throughout the period of experiment from 15th day (18 grams) to 180th day (31 grams).Observations on Group IV (D-Galactose and $NaNO_2$, simultaneously treated with BME) revealed that the body weights were lesser than the control mice from 15th day (19 grams) to 150th day (37 grams). From 165th day (42 grams) onwards the mice gained more weight to that of control ones indicating that BME could effectively revert the AD induced changes gradually. **(Figure 1)**

3.2 Behavioural Aspects (Morris water maze test): In the present study, the Morris water maze (10) task was used to assess the spatial learning and memory ability in mice. This test was conducted for all groups of mice on selected time periods for all six animals in a group separately. For each trail, the time required (in seconds) for individual mouse to find the hidden platform was recorded and the mean data from the tests were used for

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

3.3 Serotonin: The changes in the levels of Serotonin content in mice brain was estimated by the method of (11). The results of the present study clearly indicate that *Bacopa monniera* extract (BME) has significantly altered the Serotonin (5-HT) content in all brain regions of control and all groups of experimental mice. In control mice, the serotonin content was found to be highest in Ponsmedulla (1.083) followed by Cerebral Cortex (0.954), Hippocampus (0.848), Cerebellum (0.771), Spinal cord (0.694) and Olfactory lobe (0.646). When compare to the control ones, Serotonin levels showed significant elevation in BME treated mice at all time periods and the percentage of elevation was kept on increasing from 15th day to 180th day. Maximum percent change was noticed in Cerebral cortex (56.39%) followed by Cerebellum (52.91%). Hippocampus(52.24%). Spinal cord (50.86%), Ponsmedulla (48.48%), Olfactory lobe (43.80%) and However, in mice treated with D-Galactose and NaNO2, Serotonin content showed a significant inhibition in all brain regions at selected time intervals. From 15th day to 180th day, there was a gradual increase in the percentage of inhibition and the maximum percent change was noticed in the Hippocampus (-48.82%) followed by Ponsmedulla (-45.76%), Spinal cord (-44.52%), Cerebral cortex (-42.97%), Olfactory lobe (-42.87%), and Cerebellum (-40.85%).

On comparison with the control group, the mice injected with D-Galactose and NaNO2 simultaneously treated with BME showed a significant inhibition in Serotonin content from 15th day to 150th day and the maximum inhibition was recorded on 75th day in Hippocampus (- 33.41%) followed by Ponsmedulla (- 29.04%), Olfactory lobe (- 28.60%), Spinal cord (-26.88%). Cerebral Cortex (-25.34%), Cerebellum (-24.29%) and However, from 90^{th} day onwards this inhibitory trend in Serotonin started decreasing and finally reached approximately to the control levels by 180^{th} day. **(Figure 3.1 to 3.6)**

4. DISCUSSION

The present findings on morphometric and learning capabilities of control and experimental mice clearly demonstrated that BME showed positive effects on body weight, learning skills, memory and concentration whereas D-Gal and $NaNO_2$ caused learning and memory deficits in mice which could be ameliorated by simultaneous administration of BME. Morphometrics (12) 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 (13). *Bacopa*, one such plant with wide medicinal properties is used as a potent drug for treatment of memory-related disorders (8).

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(14,15). Since these changes are similar to characters of normal aging process, administration of a combined dose of D-Galactose and $NaNO_2$ has become the most effective technique to induce AD in experimental animals which served as ideal aging animal model for Physiological, Behavivoural and Pharmacology studies recently(15).

It has been reported that administration of D-Galactose caused a progressive deterioration in learning and memory capacity and increases production of free radicals in the brain (16) as measured by open field, avoidance/ escape, T-maze, Y-maze and Morris maze in mice (17). Extensive experimental studies, conducted mostly with standardized extracts of BM, have shown that it facilitates the ion, retention and retrieval of learned tasks in rats (18) and reduced the beta-amyloid deposits in the brain of an Alzheimer's disease animal model (19). 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.

Similarly, from our observations on serotonin, it was obvious that, when compared to the control mice, the levels of Serotonin was significantly elevated in BME treated mice whereas in AD induced mice, their levels were inhibited at all selected time periods. Restoration of normal levels of was observed during the subsequent period of treatment of AD induced mice with BME indicated the neuroprotective role on neurotransmitter system.

Abnormalities of serotoninergic system have been specifically implicated in some BPSD (Behavioural and Psychological Symptoms of Dementia), namely depressed mood, anxiety, agitation, restlessness and aggressiveness. Reduced 5-HT concentration in the presubiculum has been found in AD patients with psychotic symptoms. It is conceivable that, due to the complexity and diversity of BPSD, more than one neurotransmitter system may contribute to a particular behavioural syndrome. Balance between pairs of neurotransmitters may be important. For instance, reduced serotonergic and increased noradrenergic activities are linked to aggressive behavior (20). In AD, previous reports have shown extensive serotonergic denervation (21) although its clinical signification has been only partially defined. 5-HT has also been involved in several functions such as

aggression, depression, anxiety or psychosis that are relevant to BPSD (20). Serotonergic pathways interact extensively with the cholinergic, noradrenergic, GABAergic and dopaminergic systems and therefore serotonergic drugs may affect indirectly other neurotransmitters systems to alleviate BPSD.

Concentration of serotonin (5-HT) is significantly reduced in several brain areas in patients with AD. In the present study, the concentration of Serotonin 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 NaNO2. The chemistry of *Bacopa monniera* was investigated in detail and it was observed that the cognitive effects were possibly related to the modulatory effects on cholinergic (22) and Serotonergic (23) systems in the brain along with the antioxidant effect (2). According to scientists at the CDRI, a number of 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. *Bacopa monniera* also increases serotonin levels, a neurotransmitter that promotes relaxation (5). *Bacopa monniera* has also adaptogenic properties (24) which are helpful in attaining the general homeostatic response under various physiological conditions. Initial hypothesis of depression has been formulated about 40 years ago, proposing that the main symptoms of depression are due to functional deficiency of cerebral monoaminergic transmitters such as norepinephrine, 5-HT and Dopamine located at synapses (25). The observations in the present investigation on Morphometric, Behavioural aspects and on the Serotonin content 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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Fig. 1: Graphical representation of differences in the **body weights** of Control and Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME at selected time intervals.

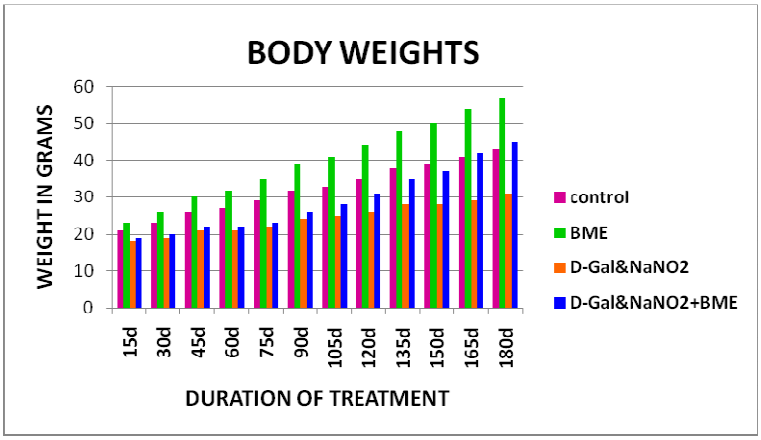


Fig. 2: Graphical representation of **Morris Water Maze** test results of Control and Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose & $NaNO_2$ + BME at selected time intervals.

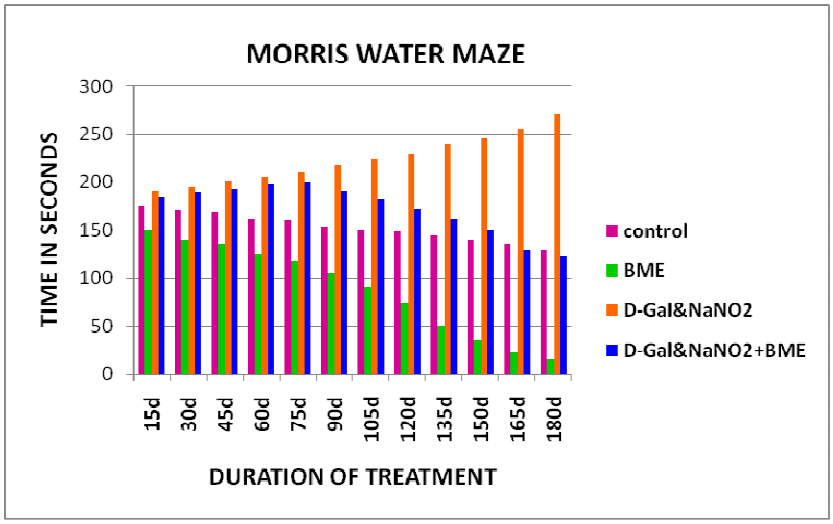
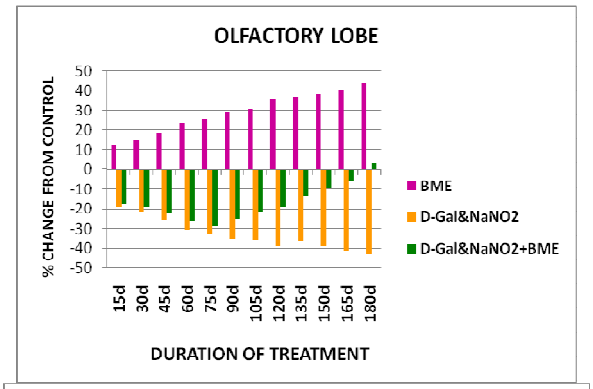
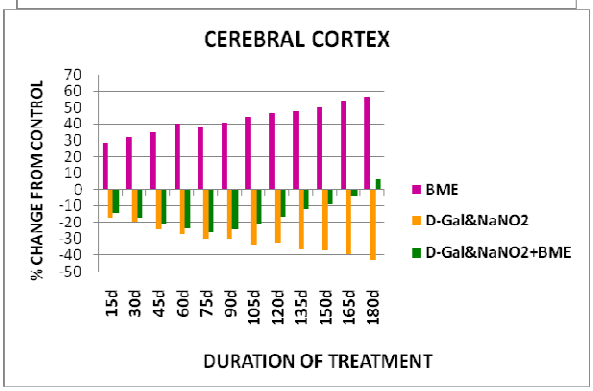
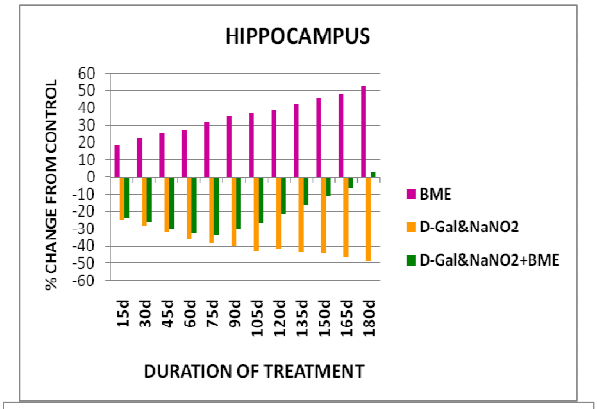


Fig. 3.1-3.6: Graphical representation of percent changes in the content of **Serotonin** (*invivo*) in Olfactory lobe(OL), Cerebral cortex(CC), Hippocampus(Hc), Cerebellum(Cb), Ponsmedulla(Pm) and Spinal cord(Spc) regions of Experimental groups of mice treated with BME, D-Galactose & $NaNO_2$ and D-Galactose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



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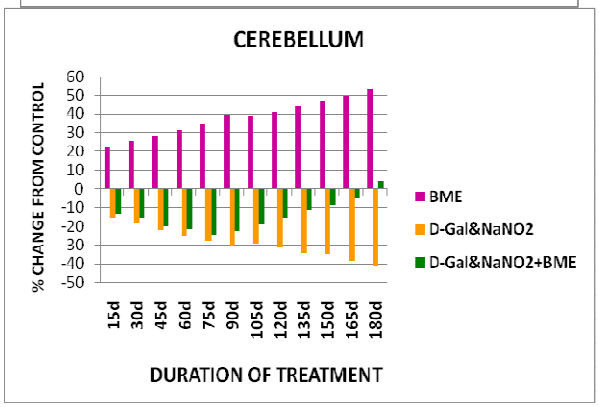
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DURATION OF TREATMENT
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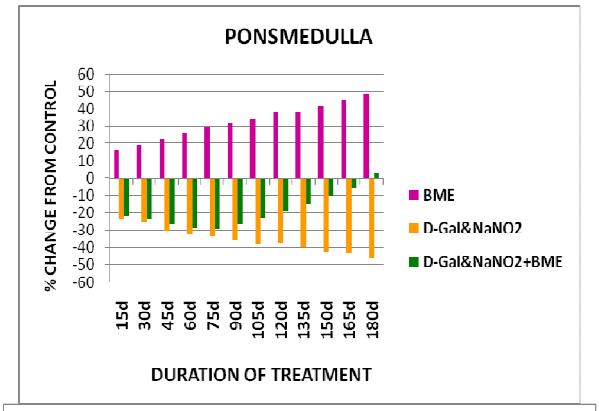
D-Gal&NaNO2+BME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DURATION OF TREATMENT
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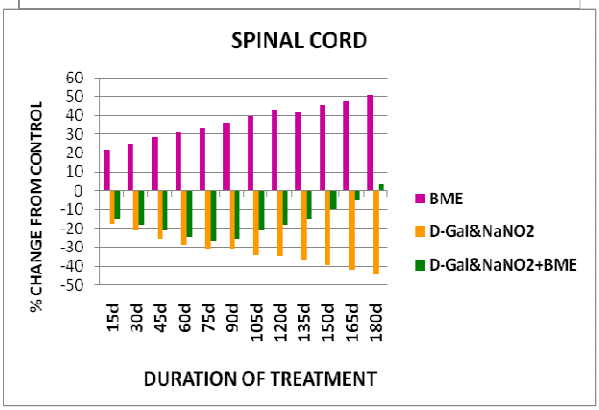
D-Gal&NaNO2+BME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DURATION OF TREATMENT
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D-Gal&NaNO2
D-Gal&NaNO2+BME