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Therapeutic Effect of *Bacopa monniera* Against Aluminum Induced toxicity in Medulla Oblongata of Albino rat

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In the present study the pro-oxidant activity of aluminum (Al) and the protective role of *Bacopa monniera* extract (BME) were determined in the medulla oblongata of albino rats. Albino rats were divided into four groups. First group of rats was used as control, second group of rats received oral dose of Aluminum maltolate only, third group of animals received *Bacopa monniera* extract (BME) and fourth group of animals received concurrently Aluminum maltolate (Al-M) plus *Bacopa monniera* (BME) extract respectively, for 4 weeks. At the end of the treatment, the medulla oblongata was removed and processed to examine the thiobarbituric acid reactive substances (TBA-RS) and antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx). Oxidative stress was promoted in medulla oblongata following Aluminum administration. In contrast, BME extract exerted an antioxidant action which was related with an increase in the levels of antioxidant enzymes. Moreover, evidences from light microscopic images clearly demonstrating that Al-M-induced neuronal changes, which were minimized by BME treatment, architecture of medulla oblongata in Al-M+BME treated group was almost similar to the control.

Key words: Neurodegenerative disease, herbal medicine, *Bacopa monniera*, lipid peroxidation, antioxidant status

INTRODUCTION

Some of the medicinal herbs have been traditionally used as brain/nerve tonic (Soundararajan and Karrunakaran, 2011). *Bacopa monniera* is a perennial creeping herb and extensively used for centuries as an ayurvedic medicinal plant, it is used for the treatment of different disorder like epilepsy, insomnia, anxiety, memory enhancer (Tripathi *et al.*, 1996; Ernst, 2006; Jyoti *et al.*, 2007). It exhibits antioxidant property (Jyoti *et al.*, 2007) antinociceptive property (Biswas *et al.*, 2012) and antimicrobial activity (Sampathkumar *et al.*, 2008; Sengupta *et al.*, 2008). In India, certain ayurvedic preparations like “Brahmighritam” and “Brahmimirasayanam” are prepared by using Bramhi (Govindarajan *et al.*, 2005). The ethanolic extract also inhibit the electroshock and immobilization of stress and significantly improve the speed of visual processing, learning rate and memory consolidation (Chowdhuri *et al.*, 2002). Chelation of metal ions (Tripathi *et al.*, 1996) and scavenging of free radical (Russo *et al.*, 2003) properties of BM extract shows the neuroprotective and cognitive enhancing effect of BME extract.

Aluminum is the most abundant metal in the earth's crust does not have any biological function (Farina *et al.*, 2002), moreover it affects the human health (Osinska *et al.*, 2004). Aluminum widely spread through food additives, toothpastes and packed foods etc., aluminum having antacids provides approximately 50-100 mg per day (Reinke *et al.*, 2003). Pressure cookers are also one of the sources that contain Aluminum (Sengupta *et al.*, 2006). Being the cheap, aluminum wares are commonly used in India (Nayak *et al.*, 2010). Aluminum neurotoxicity in animals has been clearly established and involved in the etiology of several neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease. In Alzheimer's disease patient's brain, high amount of aluminum has been reported (Kawahara, 2005). Due to high level of tissue oxygen consumption, brain is considered to be most sensitive to oxidative damage (Kaneko *et al.*, 2004). Medulla oblongata controls autonomic functions such as breathing, digestion, heat and blood vessel functions swallowing and sneezing. Hence, the present investigation has been carried-out to examine the protective role of BME extract as a therapeutic agent against Al-M-induced oxidative stress and cellular damage of medulla oblongata in albino rats.

MATERIALS AND METHODS

Preparation of *Bacopa monniera* whole plant extract: The whole plant of *Bacopa monniera* was dried in shade

and then powdered. The powder was extracted with distilled water. The aqueous extract was discarded and the residual plant material was extracted thrice with 90% ethanol. The residue obtained after removing the solvent, dried in vacuum and macerated with acetone to give free flowing powder.

Chemicals: Aluminum maltolate was purchased from the Sigma Chemical Co; H₂O₂, BSA and other chemicals were obtained from Merck, Qualigen and SD Fine Chemical Company.

Animals: In this study male albino rats of 3 months old with body weight 200-250 g were used. The rats were procured from an authorized vendor (Sri Venkateswara enterprises, Bangalore, India), randomized six per group in polypropylene cages (47×34×20 cm) containing sterile paddy husk as bedding and maintained at 22-25°C under a bell regulated light and dark (12h:12h). The rats were fed on standard rat chow (Sai durga feeds and foods, India) and water *ad libitum*.

Experimental design: Animals were equally randomized to four groups of 6 animals each:

Group I: Control: administered with (0.9%) Saline solution

Group II: Aluminum maltolate treated rats: Aluminum maltolate was dissolved in (0.9%) saline solution and administered orally at a dose of 100 mg kg⁻¹ b.wt. for one month

Group III: *Bacopa monniera* treated rats: *Bacopa monniera* was administered orally at a dose of 40 mg kg⁻¹ b.wt. for one month

Group IV: Aluminum maltolate and *Bacopa monniera* treated rats: aluminum maltolate was administered simultaneously with *Bacopa monniera* orally for one month

Tissue collection and preparation of tissue homogenates:

Six rats of each group were sacrificed and dissected after the treatment period. Tissue was removed, collected in ice cold medium and homogenate was prepared. Tissue homogenate was made in 10 Mm sodium phosphate buffer using electric motor with Teflon glass and pestle. All procedures were carried out in ice cold conditions.

Determination of lipid peroxidation index: Thiobarbituric acid reactive substances (TBA-RS), an index of lipid peroxidation was estimated in brain, liver and kidney tissues as described by Ohkawa *et al.* (1979). The amount of TBA-RS was determined spectrophotometrically by

UV-Vis spectrophotometer UV-2450 (Shimadzu) at 532 nm and the values were expressed as nano moles of TBA-RS per mg protein.

Measurement of antioxidant enzyme activities: Superoxide dismutase (SOD) activity was estimated in tissue homogenates as described by Misra and Fridovich, 1972. SOD activity was measured as the inhibition of photoreduction of Nitro Blue Tetrazolium (NBT) by the enzyme. Enzyme activity was expressed as unit of SOD/min/mg protein.

Catalase (CAT) activity was assayed spectrophotometrically using the method Aebi (1984). The decrease in absorbance was observed for 60 sec with 15 sec interval at 240 nm. CAT activity was expressed as $\mu\text{mol H}_2\text{O}_2$ decomposed/min/mg protein.

Glutathione peroxidase activity (GPx) was measured by the method of Flohe and Gunzler (1984). GPx activity is expressed as $\mu\text{mol/mg/min}$.

Protein estimation: Protein concentration in all tissue homogenates was done by the standard protocol of Lowry *et al.* (1951). Bovine serum albumin (BSA) was used as standard and the color developed was read at 660 nm.

Histopathological studies: The brain, liver and kidney tissues from all the groups were fixed at 10% formaldehyde for 24 h and dehydrated in gradual ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections (4-5 μm thick) were prepared and then stained with hemotoxylin and eosin (H-E) dye for photomicroscope observation.

Statistical analysis: Results are expressed as Mean \pm SD (standard deviation). All the data were analyzed using the one way Analysis of Variance (ANOVA) followed by Scheffe contrast. The level of significance was set at $p < 0.05$.

RESULTS

In the present study, Fig. 1 shows TBA-RS levels were significantly elevated in Al-M administered rats compared to control rats, where as TBA-RS levels were significantly inhibited with simultaneous administration of Al-M and BME. TBA-RS levels in BME only treated rats were almost similar to the control rats. There was a significant decrease in SOD, CAT and GPx in Al-M administered group compared with the control group, where as BME treatment noticeably restored the decreased antioxidant enzymes in medulla oblongata (Fig. 2-4).

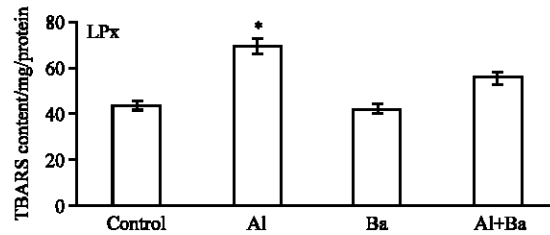


Fig. 1: Effect of *Bacopa monniera* on thiobarbituric acid reactive substance (TBARS) levels in medulla oblongata of rats exposed to Aluminum. *Significant compared to control. Results are expressed as Mean \pm SD (standard deviation of the mean). The level of probability less than 0.05 used as the criterion of significance in all cases

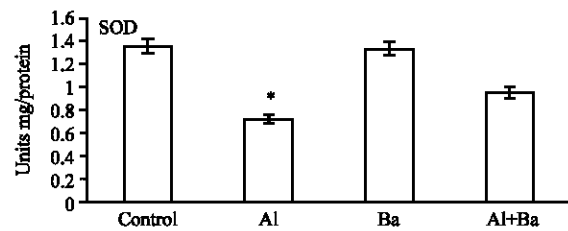


Fig. 2: Effect of *Bacopa monniera* on superoxide dismutase (SOD) levels in medulla oblongata of rats exposed to Aluminum. *Significant compared to control. Results are expressed as Mean \pm SD (standard deviation of the mean). The level of probability less than 0.05 used as the criterion of significance in all cases

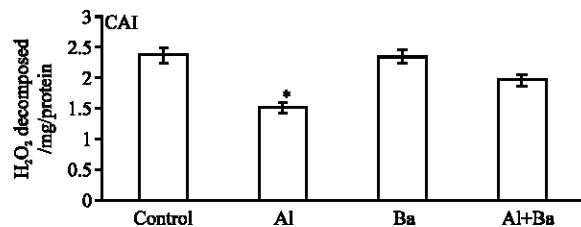


Fig. 3: Effect of *Bacopa monniera* on catalase (CAT) levels in medulla oblongata of rats exposed to Aluminum. *Significant compared to control. Results are expressed as Mean \pm SD (standard deviation of the mean). The level of probability less than 0.05 used as the criterion of significance in all cases

We performed the histopathological studies by light microscope to evaluate the protective effects of BME treatment against Al-M-induced damage. Photomicrograph of medulla oblongata with Al-M intoxication showed degenerated nucleus, degenerated

neurons and vacuolation (Fig. 5b). These structural changes elucidate the impaired medulla oblongata function by Al-M exposure. The key findings of this study reveals that Al-M alone induced medulla oblongata damage was decreased by BME treatment. The degenerative changes occurred due to Al-M exposure was reversed by the co-administration of *Bacopa monnieri* extract (Fig. 5d). BME treated rat's shows normal texture as control rats (Fig. 5c).

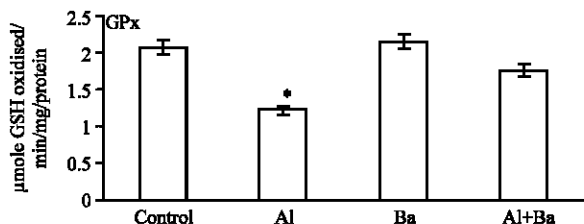


Fig. 4: Effect of *Bacopa monnieri* on glutathione peroxidase (GPx) levels in medulla oblongata of rats exposed to Aluminum. * significant compared to control. Results are expressed as Mean±SD (standard deviation of the mean). The level of probability less than 0.05 used as the criterion of significance in all cases

DISCUSSION

Aluminum increases the production of reactive oxygen species in brain, so that it induces oxidative stress in biological systems (Shahriari *et al.*, 2007) resulting in age related neurodegenerative disorders such as Alzheimers disease and Parkinson's disease etc. In the present study, there is a significant increase in lipid peroxidation and decrease in SOD, CAT and GPx activities in Al-M administered rats. Our results are in consonance with the earlier reports (Al-Kahtani, 2010; Mohammadirad and Alodollahi, 2011; Mathur *et al.*, 2010; Julka and Gill, 1996). In recent years a number of authors have demonstrated that aluminum administration increases lipid peroxidation in rat and mouse brain (Tanino *et al.*, 2000; Pratico *et al.*, 2002; Sushma *et al.*, 2006). We found that the co-administration of ethanolic extract of BM in rats during aluminum treatment at a dose of 40 mg/kg/day inhibited the lipid peroxidation product and increased the levels of antioxidant enzymes such as SOD, CAT and GPx. This may be due to antioxidant property of BM extract which can protect tissues against free radicals (Yazdanparast *et al.*, 2007). The results are in agreement with the previous reports (Jyoti *et al.*, 2007; Bhattacharya *et al.*, 2000; Chowdhuri *et al.*, 2002).

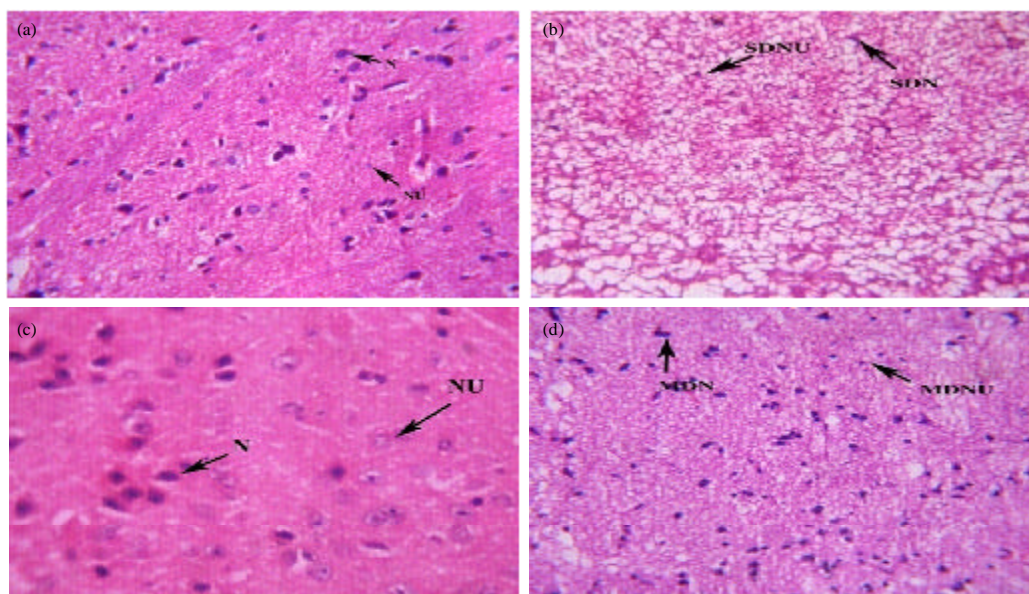


Fig. 5(a-d): (a) Control rat medulla oblongata showing nucleus (N) and neurons (NU). H and E. 400X, (b) Aluminum treated rat medulla oblongata showing severe degenerative changes in nucleus (SDN) and severe degenerative changes in neurons (SDNU). H and E. 400X, (c) *Bacopa monnieri* treated rat medulla oblongata showing nucleus (N) and neurons (NU). H and E. 400X, (d) Aluminum and *Bacopa monnieri* treated rat medulla oblongata showing mild degenerative changes and (MDN) in nucleus and mild degenerative changes in neurons (MDNU). H and E. 400X

In living system, production of free radicals and antioxidant defense mechanism is a normal phenomenon, but sometimes the free radicals may not be removed by antioxidant system (Sharma *et al.*, 2012). In such conditions membrane damage and neuronal death occurs due to substantial increase in the rate of phospholipids peroxidation (Mohammadirad and Alodollahi, 2011). Antioxidants protects from several diseases by scavenging free radicals (Ranjbar *et al.*, 2007). Accumulation of free radicals is controlled by different antioxidant enzymes like CAT, SOD and GPx (Gill *et al.*, 2011; Hussein *et al.*, 2012). It was also proven that the expression of certain enzymes involved in generation and scavenging of relative oxygen species in the brain have been modified by BM extract Chowdhuri *et al.* (2002). The pharmacological effect of BM may be due to the presence of saponins (Chakravarty *et al.*, 2003).

In histopathological observations by light microscope medulla oblongata with Al-M intoxication showed degenerated nucleus, degenerated neurons and vacuolation (Fig. 5b). The results are in consonance with the (Sushma *et al.*, 2006). Co-administration of BME along with Al-M reduces the tissue damage to some extent compared to Al-M alone treatment.

Hence the present study shows that aluminum toxicity is mediated through oxidative damage and BM extract has potential to counter this oxidative stress.

CONCLUSION

From the present results it is concluded that *Bacopa monniera* has good anti oxidant activity. The ethanol extract of *Bacopa monniera* shows protective effect against aluminum induced toxicity.

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