Potential of lithium to reduce aluminium-induced cytotoxic effects in rat brain

Punita Bhalla · Neha Singla · D. K. Dhawan

Abstract The present study was aimed to explore the potential of an antidepressant drug lithium (Li) in reducing aluminium (Al) induced neurotoxicity. To carry out the investigations, Al was administered orally (100 mg AlCl$_3$/Kg b wt/day) whereas, Li was administered through diet (1.1 g Li$_2$CO$_3$/Kg diet, daily) for a total duration of 2 months. Al treatment resulted in a significant increase in the activity of enzyme nitric oxide synthase and the levels of L-citrulline which, however, were decreased appreciably following lithium supplementation. Al treatment also revealed an increase in DNA fragmentation as evidenced by an increase in number of comets. Interestingly, Li supplementation to Al treated rats reduced the damage inflicted on DNA by Al. Ultrastructural studies revealed an increase in chromatin condensation with discontinuity in nuclear membrane in both the cerebrum and cerebellum of Al treated rats which showed improvement following Li supplementation. Alterations in the structure of synapse and mitochondrial swelling were also seen. The present study shows the potential of Li in containing the damage inflicted by Al on rat brain.

Introduction

Aluminium (Al) is one of the most abundant elements in the biosphere and causes adverse effects on various organs (Suwalsky et al. 2001; Reinke et al. 2003). Human population is constantly exposed to Al through various sources such as Al cooking utensils, certain beverages and drinking water (Gupta et al. 2005; Ochmanski and Barabasz 2000). High levels of Al have been linked with an increased risk of a number of pathogenic disorders such as microcytic anemia, osteomalacia as well as neurodegenerative disorders (Becaria et al. 2002; Solfrizzi et al. 2006; Santibáñez et al. 2007). Al-induced neurotoxicity is caused by various factors which include induction of nitric oxide, free radical-induced damage and neurotransmission alterations (Cordeiro et al. 2003; Kaizer et al. 2005). Both apoptosis and necrosis are suggested to be the mechanisms involved in cellular death resulting from Al toxicity (Brenner 2002). Suárez-Fernández et al. (1999) have reported DNA fragmentation and changes in nuclear morphology after Al exposure. Further, mutagenic effects of Al toxicity have also been observed by various researchers (Synzynys et al. 2004; Varella et al. 2004). Al-induced free

P. Bhalla · N. Singla · D. K. Dhawan (✉)
Department of Biophysics, Panjab University,
Chandigarh 160014, India
e-mail: dhawan@pu.ac.in

radicals load may be responsible for initiating the process of apoptosis. Nitric oxide is one such free radical (Schmidt and Walter 1994) which apart from playing vital physiological functions (Garthwaite 1991; Lipton et al. 1993) also causes cytotoxic effects by reacting with superoxide anions (Estévez et al. 1995).

Various drugs/compounds have been examined for their role against aluminium-induced neurotoxicity in rat and mice models (Gong et al. 2005; Jyoti et al. 2007; Luo et al. 2007; Pan et al. 2008). In the recent studies, lithium has been shown to exert neuroprotective effects against various stimuli both in vitro and in vivo (Li et al. 2002; Yuan et al. 2004; Aghdam et al. 2007). Further, lithium has also been shown to inhibit cell death, DNA fragmentation and lipid peroxidation (Tandon et al. 1998; Shao et al. 2005). The present study was designed to explore further the potential of lithium in reducing cytotoxic effects induced by Al in rat brain.

Materials and methods

Animals

Sprague-dawley rats in the weight range of 130–150 g were obtained from the central animal house of Panjab University and were housed in polypropylene cages under hygiene conditions. The animals had free access to food and water throughout the treatment schedule. All the procedures were carried out in accordance with the ethical guidelines for care and use of laboratory animals, and protocols were approved by the institute's ethical committee.

Treatments

Forty animals were randomly and equally segregated into four main groups.

Animals in group I-served as normal controls and were provided free access to water and diet.

Animals in group II were administered Al, in the form of aluminium chloride, everyday at a dose of 100 mg/kg b wt in drinking water through oral gavage (Nehru et al. 2007).

Animals in group III received Li in the form of lithium carbonate daily at a dose of 1.1 g/Kg in diet (Tandon et al. 2006).

Animals in group IV were given combined Al and Li treatments as were given to group II and group III animals, respectively.

All the treatments were continued for a total duration of 2 months. Lithium levels in the serum were also estimated by flame photometer and were found to be in the range of 0.6–0.8 mEq/l. At the end of treatment schedules, all the animals were sacrificed by decapitation immediately after anesthetization by diethyl ether. Brains were immediately removed, placed in ice-cold isotonic saline and dissected into two regions viz cerebrum and cerebellum. Tissue homogenates (10% w/v) were prepared in ice-cold 10 mM PBS (phosphate-buffered saline, 0.15 M NaCl), pH 7.4. The homogenates were centrifuged at $2,000g$ for 10 min at 4°C to remove the cell debris and nuclear pellets. The supernatants so obtained were again homogenized at $10,000g$ for 30 min. at 4°C to obtain post-mitochondrial supernatants (PMS). In the present study, PMS' were used to carry out the estimations of nitric oxide synthase enzyme activity and L-citrulline levels.

Nitric oxide synthase

Nitric oxide synthase (NOS) activity was determined in terms of nitric oxide (NO) production by using the method described by Raddassi et al. (1994). NOS converts L-arginine to L-citrulline and NO which then reacts with oxygen to yield nitrite. Nitrite, thus formed reacts with the Griess reagent which produces color. To carry out the experiment, 0.1 ml of tissue homogenates, 0.1 ml of Griess reagent [(a) 0.1% solution of *n*-Napthyl ethylenediamine dihydrochloride prepared freshly in double distilled water (b) 1% Sulphanilamide prepared in 2.5% *o*-phosphoric acid solution, Mixed solution a and b in equal volume] were added into the wells of ELISA plate. The ELISA plate was then incubated in dark at 37°C for 30 min. The pink color so obtained was read at 540 nm on ELISA plate reader.

L-Citrulline

Estimation of L-citrulline was carried out by using the method of Boyde and Rahmatullah (1980). Determination of L-citrulline in proteins and peptides utilizes the reaction of ureido groups with doacetylmonoxime to give pink color after acid hydrolysis of proteins. To

0.1 ml of homogenates, 0.05 ml of zinc sulphate (30%) was added and the contents were mixed well to precipitate proteins. The tubes were then subjected to centrifugation at $10,000 \times g$ for 10 min. About 0.1 ml of supernatants were taken and 0.4 ml of 0.1N HCl were added followed by an addition of 1.5 ml of chromogenic solution [(a) 85% H_3PO_4 (b) 0.5% (w/v) diacetylmonoxime + 0.1% (w/v) thiosimocarbazide. Subsequently both a and b were mixed in the ratio 2:1]. After through mixing, the tubes were put in boiling water bath for 10 min. The tubes were then cooled at room temperature and the absorbance was measured against a reagent blank at 530 nm.

Protein estimation

The protein contents in the samples were measured by following the method of Lowry et al. (1951). For the assay of protein, 0.01 ml of sample, BSA standard (10–200 µg) and buffer (blank) were taken in respective tubes and the final volume was made to 0.5 ml with distilled water. About 5 ml of reagent (prepared by mixing 48 ml of 2% sodium carbonate, 1 ml of 1% copper sulphate and 1 ml of 2% potassium sodium tartarate) was then added. It was thoroughly mixed and allowed to stand for 10 min at room temperature. About 0.25 ml of 1N Folin's reagent was added followed by immediate mixing. The contents were vigorously shaken and allowed to stand for 30 min at room temperature and the absorbance was measured at 680 nm.

Alkaline comet assay

Comet assay was performed according to the method of Singh et al. (1988). The assay works upon the principle that strand breakage of the supercoiled duplex DNA leads to the reduction of the size of the large molecule and these strands can be stretched out by electrophoresis. Briefly, 130 mg of brain tissue was homogenized in PBS (phosphate buffer saline with pH 7.4) using a tip homogenizer. The homogenate (80 µl) was then mixed with 100 µl of 1% low melting temperature agarose at 37°C and then placed on a slide pre-coated with thin layer of 1% regular melting agarose. It was immediately covered with coverslips and the slides were kept at 4°C for 45 min to allow solidification of the agarose. After removing the coverslips, the cells were lysed in a lysing solution (2.5 mM NaCl, 100 mM EDTA, 10 mM Tris base, 1% Triton-X-100 and 10% DMSO) for 1 h. After washing in double distilled water, the slides were placed in a horizontal gel electrophoresis chamber. The chamber was filled with cold alkaline buffer (300 mM NaOH, 1 mM EDTA with pH >13.0) and the slides were kept at 4°C for 20 min to allow the DNA to unwind. Electrophoresis was performed for 30 min (25 V/cm, 300 mA). After electrophoresis, the slides were washed three times with neutralization buffer (0.4 M Tris buffer with pH 7.5) for 5 min. All preparative steps were conducted in dark to prevent additional DNA damage. The slides were stained with ethidium bromide for 10 min and analyzed under a fluorescence microscope at $450\times$ for comet assay using 550–560 nm excitations and 590 nm barrier filters.

DNA fragmentation studies

Tissue slices were placed in the digestion buffer (10 mM Tris–Cl with pH 8.0; 0.1 M EDTA with pH 8.0; 1% SDS and proteinase K with concentration of 1 mg/10 ml) and were incubated (14–18 h, 55°C) in a shaking water bath. The DNA contents were extracted, precipitated, and stored as described below for the analysis by agarose gel electrophoresis. After digestion, samples were extracted three times with 10% saturated phenol/chloroform/isoamyl alcohol (24:24:1) and were precipitated using ethanol. The precipitates were rinsed two times with 70% ethanol, air dried, and resuspended in Tris EDTA buffer. DNA contents were measured using a spectrophotometer (A260/A280), and only samples with 1.8 ratios were used. Agarose gel electrophoresis was then carried out to analyze the fragmentation.

Electron microscopic studies

Electron microscopic studies were also carried out to assess the effects of various treatments at the subcellular level. A very small portion of brain was incised out and immersion fixed in the fixative having formaldehyde and glutaraldehyde made in 0.2 M sodium cacodylate buffer (pH 7.2) for 4–5 h at 4°C. The specimens were then thoroughly washed thrice in 0.1 M cacodylate buffer and post fixed for 1 h in 1% osmium tetraoxide prepared in the same buffer. The specimens were then washed, dehydrated (in the

ascending series of acetone), infiltered and subsequently embedded in resin. Specimen blocks were made out by polymerization of the pure embedding resin at 60°C for 48–72 h. Ultra thin sections were prepared using ultramicrotome. Initially, semi-thin sections of thickness about 1 μm were cut using very sharp glass knives to locate the area of interest in samples belonging to different treatment groups. These semi-thin sections were then stained with 0.5% toludine blue prepared in 1% borax solution. Ultrathin sections of interference colors from golden to silver were cut and loaded on fine copper grids of 100–300 mesh size. Sections were double stained with uranyl acetate and lead citrate. These ultrathin sections were finally viewed under transmission electron microscope at AIIMS, New Delhi.

Aluminium estimation

Al levels were also determined in cerebrum and cerebellum by wet acid digestion method of Zumkley et al. (1979) using the Atomic Absorption Spectrophotometer (Perkin Elmer, India).

Statistical analysis

The statistical significance of the data has been determined using one-way analysis of variance (ANOVA) followed by multiple post-hoc test (Student–Newman–Keuls).

Chemicals

Aluminium chloride and lithium carbonate were procured from CDH (Central Drug House) Chemicals Ltd, New Delhi, India. All other chemicals were of high analytical grade.

Results

Table 1 show the body and brain weights of the normal control and treated animals. A significant decrease ($P \leq 0.001$) in the body and brain weights was observed after Al treatment as compared to normal control animals. Lithium treatment alone did not cause any significant change in the body and brain weights of the animals when compared with normal control animals. Whereas, lithium co-administration

Table 1 Effect of Lithium supplementation on body and brain weights of rats subjected to Aluminium treatment

Groups	Body wt (g)	Brain wt (g)
Normal control	240.00 ± 8.36	1.99 ± 0.04
Al treated	187.00 ± 12.04[c]	1.83 ± 0.03[c]
Li treated	236.00 ± 8.94	2.01 ± 0.07
Al + Li treated	206.66 ± 10.32[c,y]	1.96 ± 0.08[y]

All the values are expressed as means ± SD

[c] $P < 0.001$ by Newman–Keuls test when the test values are compared with normal control group

[y] $P < 0.01$, by Newman–Keuls test when the values of Al + Li treated group are compared with Al treated group

with Al was able to check the weight loss and both the body and brain weights were found to be significantly improved ($P \leq 0.01$) when compared to Al treated group.

The diet consumption in normal and rats subjected to different treatments varied between 5 and 8 g per day per rat throughout the period of study.

The nitric oxide synthase enzyme activity was measured by estimating the levels of nitric oxide and was found to be significantly increased ($P \leq 0.001$) in both the regions after Al treatment in comparison to normal control animals (Table 2). Similarly, an increase in the levels of L-citrulline levels was also seen in both the cerebrum ($P \leq 0.001$) and cerebellum ($P \leq 0.01$) when compared to normal controls (Table 3). Lithium supplementation to Al treated animals significantly decreased the enzyme activity as well as the levels of L-citrulline when compared to Al treated animals, however, the enzyme activity was significantly high in both the cerebrum ($P \leq 0.05$) and cerebellum ($P \leq 0.01$) in comparison to normal controls. No significant change in the enzyme activity as well as in L-citrulline levels were observed in animals given only lithium treatment.

The DNA damage caused in the cell as a result of Al treatment was examined by single cell gel electrophoresis (comet assay) (Table 4). The results indicated that the DNA of Al treated cell showed a comet tail indicating the DNA damage arising from the genotoxicity in the Al treated cell as compared to DNA of normal cell. The average % of DNA damage in the Al treated group was 70% as compared to normal control (28%). In the combined treatment group, damage to DNA was appreciably less (42%) as compared to Al treated rats.

Table 2 Effect of Lithium supplementation on the nitric oxide synthase activity in cerebrum and cerebellum of rats subjected to Aluminium treatment

Groups	Cerebrum (nM of nitrite accumulated/g tissue)	Cerebellum (nM of nitrite accumulated/g tissue)
Normal control	0.15 ± 0.007	0.16 ± 0.008
Al treated	0.20 ± 0.009[c]	0.21 ± 0.009[c]
Li treated	0.15 ± 0.006	0.16 ± 0.006
Al + Li treated	0.16 ± 0.009[a,z]	0.18 ± 0.007[b,z]

All the values are expressed as means ± SD

[a] $P \leq 0.05$, [b] $P \leq 0.01$, [c] $P \leq 0.001$ by Newman–Keuls test when the test values are compared with normal control group

[z] $P \leq 0.001$ by Newman–Keuls test when the values of Al + Li treated group are compared with Al treated group

Table 3 Effect of Lithium supplementation on the levels of L-citrulline in cerebrum and cerebellum of rats subjected to Aluminium treatment

Groups	Cerebrum (µmoles of L-citrulline/g tissue)	Cerebellum (µmoles of L-citrulline/g tissue)
Normal control	10.52 ± 0.92	8.42 ± 1.91
Al treated	14.01 ± 1.03[c]	11.64 ± 2.62[b]
Li treated	11.57 ± 2.10	7.88 ± 1.19
Al + Li treated	11.15 ± 1.24[y]	8.34 ± 1.35[y]

All the values are expressed as means ± SD

[b] $P \leq 0.01$, [c] $P \leq 0.001$ by Newman–Keuls test when the test values are compared with normal control group

[y] $P \leq 0.01$ by Newman–Keuls test when the values of Al + Li treated group are compared with Al treated group

Table 4 Effect of Lithium supplementation on DNA damage index observed by single cell gel electrophoresis (Comet assay) in brain of rats subjected to Aluminium treatment

Groups	No. of comets observed	Total no. of cells	DNA damage (%)
Normal control	10	35	28
Al treated	29	41	70
Li treated	8	32	25
Al + Li treated	16	38	42

As shown in Fig. 1, fragments of DNA were observed in Al treated animals (Lane 2) as compared to normal control animals (Lane 1). However, no significant fragmentation could be observed in Al treatment animals which were simultaneously subjected to lithium treatment (Lane 4).

A significant increase in the levels of Al was observed in both the regions after Al treatment in comparison to normal controls. In the combined treatment group, Al levels were significantly decreased when compared with Al treated animals. However, the Al levels were significantly high in cerebrum ($P \leq 0.01$) when compared to normal animals (Table 5).

Figure 2a shows the rat cerebrum from the normal rat with a well defined nucleus and nucleolus. Myelin sheath as well as the nuclear membrane can also be clearly seen. Figure 3a shows the effect of Al treatment at the subcellular level. An increase in the chromatin condensation was observed along with the discontinuity of nuclear membrane. Vacuoles and mitochondrial swelling can also be seen. No change at the cellular level was visible after treatment with lithium (Fig. 4a). However, in the combined treatment
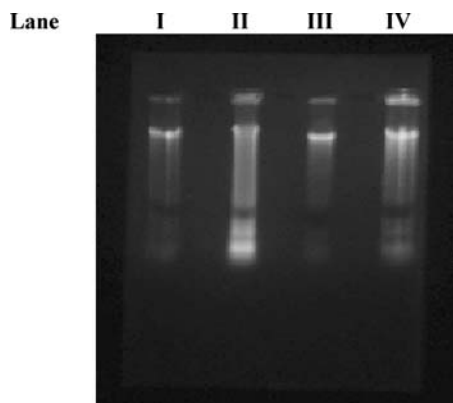
Fig. 1 Agarose gel electrophoresis of genomic DNA extracted from rat brain from various treatment groups. (6 µg DNA/lane). *Lane I* DNA from the control group. *Lane II* DNA from the Al treated group. *Lane III* DNA from lithium treated group. *Lane IV* DNA from Al and Li combined treated group

Table 5 Effect of Lithium supplementation on the levels of aluminium in cerebrum and cerebellum of rats subjected to Aluminium treatment

Groups	Cerebrum (µg/mg tissue)	Cerebellum (µg/mg tissue)
Normal control	0.33 ± 0.01	0.38 ± 0.05
Al treated	0.70 ± 0.04^c	0.64 ± 0.05^c
Li treated	0.33 ± 0.002	0.36 ± 0.05
Al + Li treated	$0.43 \pm 0.06^{b,z}$	0.42 ± 0.08^z

All the values are expressed as means $\pm$ SD

[b] $P \leq 0.01$, [c] $P \leq 0.001$ by Newman–Keuls test when the test values are compared with normal control group

[z] $P \leq 0.001$ by Newman–Keuls test when values of Al + Li treated group are compared with Al treated group

Fig. 2 a Electron micrograph from control rat showing a portion of cerebrum from frontal part with a well defined nucleus (*1*) and nucleolus (*2*) and mylinated nerve fibers (*3*). **b** Electron micrograph from control rat showing a portion of cerebellum dendrite (*1*), sine and synapse (*2*) as well as mitochondria (*3*)

Discussion

Exposure to heavy metals cast a shadow of great concern as heavy metals are associated with the etiology of various diseases. Amongst the list of metals, adverse effects caused by Al exposure are a matter of great concern and investigations are warranted so as to contain them. Thus, in the present study, the effects of Al alone or in combination with lithium at a cellular level in brain were examined. Al exposure resulted in a significant decrease in the body and brain weights. Similar results were also observed by earlier researchers (Ondreicka et al. 1996; Baydar et al. 2003; Azzaoui et al. 2008). Baydar et al. (2003) have attributed the decrease in body weights to the interference of Al with the hormonal status and/or

group, a decrease in the chromatin condensation was observed and the continuity of nuclear membrane was also seen (Fig. 5a).

Figure 2b shows the portions of cerebellum from normal control animal with a nucleus, dendrites, synapse point and a number of mitochondria. Chromatin condensation along with the disruption of synapse structure was observed in cerebellum after Al treatment (Fig. 3b). A curvature could be observed at synapse. No change at the cellular level was visible in cerebellum after lithium treatment (Fig. 4b). Lithium co-treatment with Al significantly decreased the chromatin condensation in cerebellum also, as compared to Al treated animals (Fig. 5b).
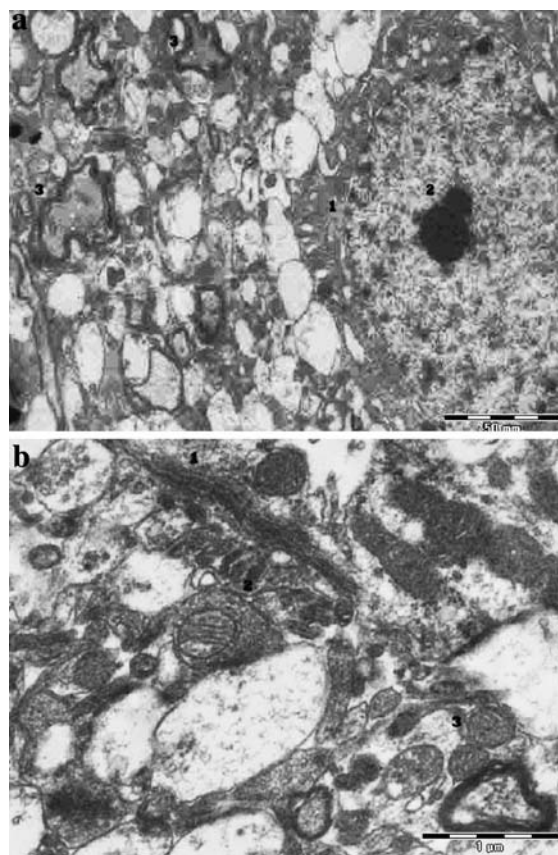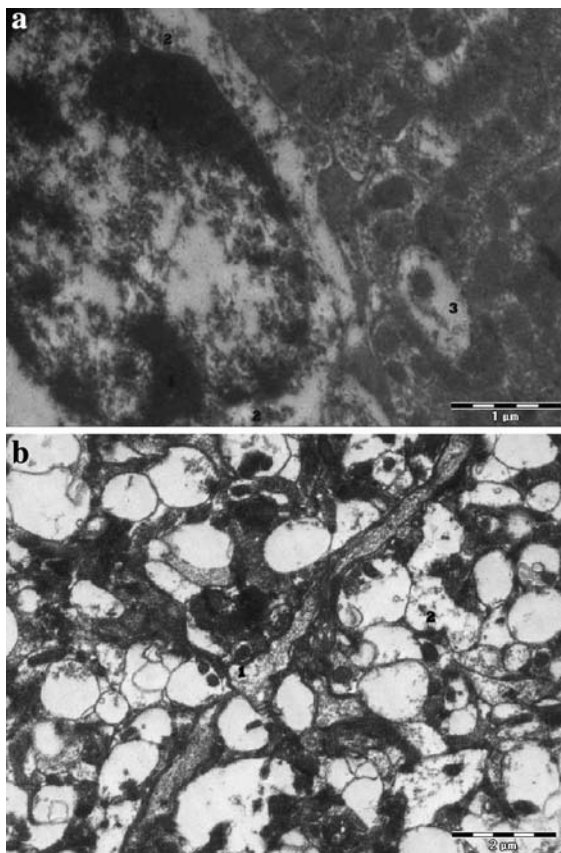
Fig. 3 a Electron micrograph from Al-treated rat cerebrum showing chromatin condensation (*1*), discontinuity of nuclear membrane (*2*) and vacuolar spaces (*3*). **b** Electron micrograph from Al-treated rat cerebellum showing distorted dendrite structure (*1*) as well as curvature synapses (*2*)

Fig. 4 a Electron micrograph from lithium treated rat cerebrum showing well defined nucleolus (*1*) and nucleus (*2*). **b** Electron micrograph of Li treated rat cerebellum showing the well defined nucleus

protein synthesis. In our study, as we did not observe any appreciable change in the diet consumption of the rats subjected to Al treatment so it can be anticipated that this effect could possibly be due to increased peroxidation of lipids as a consequence of oxidative stress in the animals following Al treatment, which has also been pointed out in our earlier studies (Bhalla and Dhawan 2009). Further, inhibition in the glycolytic pathway would also result in decreased body weights as in such toxic conditions there is an enhanced synthesis of glucose from non-exhausted carbohydrate sources thereby leading to mobilization of fat deposits resulting in decreased body weights. Lithium co-administration was, however, able to restore the body weight loss and a recovery in the weights could be seen. Lithium administration has been shown to cause increase in body weights
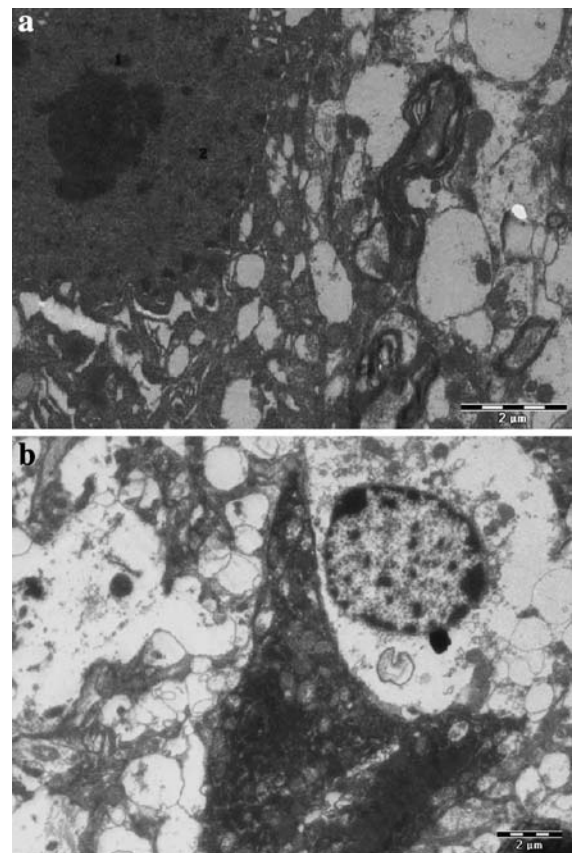
(Canolty and Johnson 1987; Geoffroy et al. 1991) and moreover lithium therapy has been shown to decrease lipid peroxidation which may result in an improvement in body weights.

Nitric oxide is a known signaling molecule and its actions are the result of its influence on a variety of protein functions (Jaffrey et al. 2001; Lane et al. 2001). Nitric oxide along with L-citrulline are the principal products of nitric oxide synthase (NOS) enzyme activity (Griffith and Stuehr 1995; Chinje and Startford 1997). In the present study, an increase in the levels of nitrite and L-citrulline were observed following Al exposure in both cerebrum and cerebellum (Tables 2, 3). Increase in nitrite levels signifies an increase in nitric oxide production. Similar increase was also observed in an earlier study by Satoh et al. (2007). Acute and chronic exposure to Al stimulates nitric-oxide synthase activity mostly
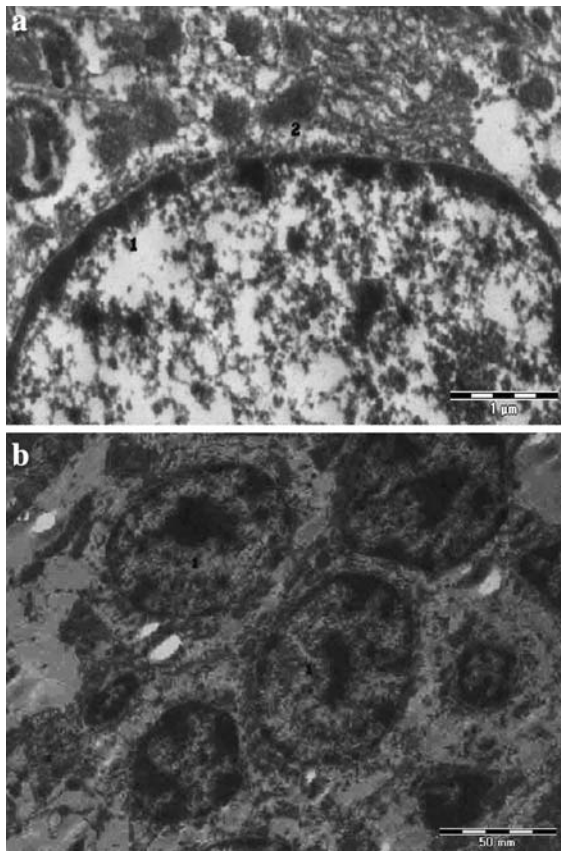
Fig. 5 a Electron micrograph from Al + lithium treated animal cerebrum showing less of chromatin condensation (*1*) and well defined nuclear membrane (*2*). **b** Electron micrograph of Al + Li co-treated rat cerebellum indicating decrease in chromatin condensation (*1*) and a continuity in nuclear membrane

present in glial cells whose contribution to the total activity is around 70% under resting conditions (Bondy et al. 1998). NOS is found to coexist with neurofibrillary tangle bearing neurons (Smith et al. 1997, 1998) and thus signifies the importance of nitric oxide in Alzheimer's disease where Al-induced neurotoxicity is also one of the causative factor. Nitric oxide is a well known free radical (Schmidt and Walter 1994) and after reacting with superoxide anion radials forms peroxynitrite (Estévez et al. 1995) which then adversely acts on various macromolecules. Further, an important regulator of NOS activity is free Ca^{2+} which via calmodulin can activate the NOS enzyme activity and thus leads to the increased amount of NO (Esplugues 2002). Al toxicity has been reported to cause an increase in calcium levels (Kaur

and Gill 2005). The increased amount of Ca^{2+} will bind to calmodulin, which further gets associated with NOS and activates the enzyme thus leading to the increased production of NO in brain regions. Co-administration of lithium was able to decrease the levels of nitric oxide and L-citrulline in both the regions. Studies have shown that antidepressant activity of lithium is mediated through the regulation of nitric oxide levels. Thus, lithium treatment is able to regulate the nitro stress induced by Al toxicity in brain.

Effects of Al toxicity on DNA were also investigated. Al treatment caused an increased DNA damage as indicated by the increased fragmentation of DNA and the number of comets observed. DNA fragmentation and increase in the appearance of comets have also been reported in other studies as a consequence of Al exposure (Lima et al. 2007). Al is known to increase the levels of reactive oxygen species (Bondy and Kirstein 1996; Bondy et al. 1998) as well as nitrogen species as seen in the present study also, which are known to cause damage to various macromolecules and also to DNA. An increase in the Al levels was also observed, which may result in more production of free radicals and thus contribute to the cytogenetic effects resulting from Al toxicity. Damage to DNA is one of the markers and typical characteristic of apoptosis (Wyllie 1993) and the present study shows that Al toxicity can lead to faster apoptosis as seen in the electron micrographs which clearly revealed disruption of cells. Lithium, on the other hand revealed neuroprotective effects as evidenced by decreased DNA damage in the combined treatment group. A similar inhibition of DNA fragmentation was observed in primary cultured rat cerebral cortical cells (Shao et al. 2005) after lithium treatment. Studies have shown that lithium significantly reduces cytochrome c release, which is a key initial event in apoptosis (Lai et al. 2006) and also inhibits DNA fragmentation (Ghribi et al. 2002) thus showing anti-apoptotic properties, which is further strengthened by the present study. Further, lithium has been shown to possess antioxidative properties (Tandon et al. 1998; Schafer et al. 2004) and hence may decrease the levels of free radicals and ultimately the damage to DNA.

In microscopic observations, disruption at the cellular level was seen after Al treatment thus

indicating its adverse effects at the histoarchitecture level. Chromatin condensation (Fig. 3a, b) in both the cerebrum and cerebellum can be seen. Jyoti et al. (2007) have also observed similar changes at the cellular level after Al treatment. Changes in the structure of synapse was also seen after Al treatment, which may affect neurotransmission and hence learning and memory processes in rats and the same is also observed in a number of studies (Rifat et al. 1990; Hänninen et al. 1994; Amador et al. 2001). Al has been shown to alter the cholesterol, phospholipids, total lipids contents of the membranes and thus may be responsible for the membrane damage as seen in an earlier study from our lab (Bhalla et al. 2009). Lithium co-administration proved beneficial and is supported by the fact that in combined treatment group an appreciable improvement was seen in structure of both the regions (Fig. 5a, b). Lithium significantly reduced the levels of ROS thereby preventing lipid peroxidation, composition and fluidity of the membranes which may account for the structural improvement as seen in the present study.

The present study thus shows the protective effects of lithium by containing the cytotoxic effects inflicted by Al.

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