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Curcumin attenuates aluminium-induced functional neurotoxicity in rats

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ABSTRACT

Curcumin is a polyphenol extracted from the rhizome of *Curcuma longa* and well known as a multi-functional drug with antioxidative, anti-cancerous and anti-inflammatory activities. Curcumin's antiageing and neuroprotective potential is widely reported. In the present study, effect of curcumin treatment dose 30 mg kg^{-1} day^{-1} was investigated against aluminium neurotoxicity in young and old animals. Direct and indirect intakes of aluminium have been reported to be involved in the etiology of several neurodegenerative disorders like Alzheimer's and Parkinson's diseases. Long term Al was administered through drinking water at a dose of 50 mg/kg/day for 6 months in both young (4 months) and old (18 months) male Wistar rats. Result obtained demonstrates that curcumin treatment attenuates the Al-induced alterations at biochemical, behavioral and ultrastructural levels which was well reflected in the electrophysiological recordings. Our results indicate that curcumin's ability to bind redox active metals and cross the blood–brain barrier could be playing crucial role in preventing against Al-induced neurotoxicity.

1. Introduction

Aluminium (Al) is the most abundant metal on the earth crust, reported to gain access to the body via the gastrointestinal tract and lung tissue. It is a commonly exposed neurotoxin and possesses multiple mechanisms of action on the central nervous system (reviewed by Miu and Benga, 2006). Al is capable of increasing the blood–brain barrier (BBB) permeability (Exley, 2001) and crossing BBB (Banks and Kastin, 1985), which leads to an increase of Al-concentration, in the hippocampus (Struys-Ponsar et al., 1997), cortex, singulated bundles and corpus callosum (Platt et al., 2001). Several epidemiological, neuropathological, and biochemical studies have suggested a possible link between the neurotoxicity of aluminium and pathogenesis of Alzheimer's disease (AD) (Kawahara et al., 2001). Aluminium promotes accumulation of insoluble amyloid-β-protein, aggregation of hyper phosphorylated tau-protein which comprises neurofibrillary tangles (NFTs) (Kawahara, 2005) and causes detrimental changes to cholinergic neurotransmission (Johnson and Jope 1986). Al also potentiates oxidation caused by several transition metals example: chromium (Cr) and copper (Cu) (Bondy et al., 1998). Al is also known to have negative effects on learning abilities in Wistar rats (Bilkei-Gorzó, 1993), mice (Kaneko et al., 2006) and rabbits (Yokel et al., 1994). In our previous studies, we have reported that long term chronic Al-intake induces oxidative stress related damages in the CA1 and CA3 field of hippocampus brain regions (Sethi et al., 2008).

Curcumin is a member of the curcuminoid family, known to possess diverse anti-inflammatory (Lim et al., 2005) and anti-cancerous properties (Aggarwal, 2008) following topical or oral administration (Sharma et al., 2005). Curcumin is a well known antioxidant and reported as a natural replacement for vitamin E therapy (Kelloff et al., 1996). Polyphenolic structure permits curcumin to cross blood–brain barrier and bind redox metal ions which makes it a promising drug for prophylaxis as well as treatment of neurodegenerative diseases in future (Garcia-Alloza et al., 2007). Among neuroprotective properties, curcumin has been shown to protect against amyloid-β-aggregation (Frautschy et al., 2001), cerebral ischemia (Shukla et al., 2008) in rats and gerbils (Ghoneim et al., 2002; Thiyagarajan and Sharma, 2004; Wang et al., 2005). Recent studies have demonstrated that curcumin has protective effects against lead (Daniel et al., 2004), iron and copper (Baum and Ng, 2004), cadmium (Dairam et al., 2007) induced neurotoxicity in the brain. None of these studies has demonstrated effect of curcumin against Al-neurotoxicity at electrophysiological, behavioral, biochemical and electron microscopic levels.

In the present study treatment of curcumin dose 30 mg kg^{-1} day^{-1} (in corn oil) for 6 months was investigated for its effect on Al-toxicity. Experimentation was performed on young (4 months) and old (18 months) aged rats to investigate the effect of curcumin on aging as well as Al-toxicity. In the electrophysiological study, multiple unit activity (MUA) recordings were performed on cerebral cortex of the brain to determine effect of curcumin on Al-induced hyperexcitability and increased neuronal firing. The effect of curcumin was assessed on Al-toxicity induced memory deficit and increased anxiety behavior by employing Morris water maze (MWM) tests and open field tests. In this study, we assessed whether curcumin treatment was effective in

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preventing memory decline or not. Assays like lipid peroxidation, and membrane linked proteins (Na–K ATPase activity, Ca^{2+} dependent protein kinase C activity) were used to detect disruption of biochemical parameters which plays putative role in Al-mediated neurotoxicity (Sethi et al., 2008). Neuroprotective effect of curcumin was also evaluated at the ultrastructural level. Transmission electron microscopy (TEM) was performed to visualize the effect of curcumin on Al-induced ultrastuctural alterations in different experimental groups.

2. Materials and methods

2.1. Materials

All electrodes and wires used in the electrophysiological surgery were tissue compatible and obtained from PlasticsOne, VA, USA. ^{32}P was obtained from The Board of Radiation and Isotope Technology (BRIT), Department of Atomic Energy, Government of India, Hyderabad, India. Curcumin and other chemicals used for biochemical assays were obtained from Sigma Aldrich chemical Co. USA.

2.2. Animals and treatment plan

Eighty male albino Wistar rats of two age groups, young (4 months) and old (18 months) were used for this study. Animals were housed in pairs in standard polypropylene cages ($8\times12\times5$-in) under controlled hygienic environmental conditions. Rats were maintained at room temperature (23 ± 4 °C) under a 12-hour light/12-hour dark cycle. Animals were obtained from the central animal facility of the Jawaharlal Nehru University, New Delhi, India after the approval of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and Institutional Animal Ethical Committee (IAEC). Randomly selected young and old rats were divided in four groups containing ten animals in each group. Scheme of group formation are as follows: (1) young control, (2) young Al-treated, (3) young Al + curcumin treated, (4) young curcumin treated, (5) old control, (6) old Al-treated, Group 7: old Al + curcumin treated and Group 8: old curcumin treated. Curcumin dissolved in corn oil was fed orally with the help of gavage at a dose of 30 mg/kg body wt for 6 months. Control and Al-treated young and old group received corn oil as a vehicle. Al-treated groups received a dose of 50 mg/kg/day $AlCl_3.6H_2O$ in double distilled drinking water for 6 months. Body weight and water intakes were measured daily to adjust the treatment doses to achieve a constant intake of aluminium. Same protocol was used to induce aluminium toxicity in our previous studies (Jyoti and Sharma, 2006; Jyoti et al., 2007; Sethi et al., 2008). Six animals from each group were used for biochemical studies and rest of them was used for histochemical studies.

2.3. Electrophysiological study

2.3.1. Surgery procedure

Experimental rats were anesthetized with standard dose of Ketamine (80 mg/kg) and Xylazine (10 mg/kg). Under deep anesthesia, surgery was performed for the placement of EEG electrodes. Four stainless steel epidural screw electrodes were placed bilaterally over the parietal cortex with the help of stereotaxic apparatus following the atlas of Paxinos and Watson (1982). One screw was placed upon the frontal sinus to serve as animal ground. Electromyogram (EMG) and electro-oculogram (EOG) were placed on the antigravity (neck) muscle and epicanthus muscle of eye. Free ends of each electrode were connected to a 15-pin connector, which was then affixed to the surface of skull with dental acrylic cement to make a robust platform. Proper post-operative care was undertaken to avoid infection and minimize pain resulted from surgery. After 5 days of habituation, when animals did not show any sign of pain, infection, or discomfort electrophysiological recordings were performed with the help of Grass Polygraph recorder.

2.3.2. EEG recordings

After recovery, rats were prepared for continuous synchronized EEG recording with the help of preamplifier, and signals were filtered at high cut off 100 Hz and low cut off at 1 Hz. For MUA recording, composite extracellular signals from the same EEG electrodes were routed through high impedance probe (Grass HIP511 with FET) signals were amplified and filtered (300 Hz to 10 KHz) by Grass P511 J preamplifiers, electronically and displayed on an oscilloscope. The standard transistor–transistor logic (TTL) spikes pulses from the window discriminator were simultaneously recorded on the polygraph. Using grass integrator preamplifier (P10) cumulative mathematical integration of EEG traces was recorded on one of the polygraph channels. The recordings were limited to the awake immobile state in which a rat sits quietly but remain awake as described in previous studies from our lab (Sharma et al., 1993, Sethi et al., 2008).

2.4. Behavioral study

2.4.1. Morris water maze test

Spatial learning and memory were assessed using the Morris water maze previously described by Sethi et al. (2008). Briefly, the testing system was composed of a black circular pool (168 cm in diameter and 50 cm deep) filled with water (temperature 20 ± 2 °C) and surrounded by extra maze distal visual cues of different shape, size and color. The pool was divided in four quadrants. A black circular hidden platform was placed in the northwest (NW) quadrant 2 cm under the water surface so that rat could escape from swimming. Experimental rats were screened for their swimming ability by recording the latency to reach the visible platform. Rats were trained to exit the water tank onto the platform by using the visual cues. Each rat was placed inside the water tank facing the tank wall, at one of the four randomly selected entry points, once in every block of four trials. The test was performed on four consecutive days (8 trials per day). The starting position was changed randomly for each trial and the animal was allowed to search for 60 s to find the hidden platform. Rat was guided to the platform, if failed to find the platform within 60 s. At the end of the trials, the rat was allowed to remain on the platform for 30 s. Morris water maze training was recorded using a web camera mounted to the ceiling. Recording was performed from 11:00AM to 2:00PM to exclude variations in performance resulting from circadian rhythmicity.

2.4.2. Open field test

Open field was performed by using the protocol described by Sethi et al. (2008). Test was performed in a white colored square arena, $70\times70\times106$ cm in diameter, divided into 49 identical squares. To observe the exploratory behavior animals were placed in the center of the field and the following variables were recorded for 3 min: the number of squares crossed, rearing and the number of feacal boli (defeacation). Before each trial, field was cleaned thoroughly with 0.1% acetic acid solution. Furthermore, a defecation index was calculated by counting the number of fecal boli. The open field is a very popular animal model of anxiety-like behavior (Prut and Belzung, 2003).

2.5. Biochemical studies

2.5.1. Preparation of tissue homogenate

After the electrophysiological recordings and behavioral trials, rats ($n=6$) sacrificed by cervical dislocation for biochemical assays. The whole brain was immediately removed and washed with cold saline. Cortical tissue was homogenized with Potter-elvehijam type homogenizer

fitted with Teflon plunger in cold 50 mM Tris buffer (pH 7.4) using the Protease Arrest™ Kit from Geno Technology Inc. (St. Louis, Mo, USA). Homogenate as then centrifuged at 6000 rpm for 10 min in Sorvall RCS or RC5C centrifugation unit. The supernatant (S1) was further ultra-centrifuged at 25,000 rpm for 25 min to form mitochondrial pellet (P2). The resulting supernatant (S2) was used as such as cytosolic fraction. Mitochondrial pellet contains mitochondrial membranes, synaptosomes and microsomes. This fraction was considered as membranous fraction (M1). Na–K-ATPase activity was measured in the crude synaptosomal fraction S1, whereas lipid peroxidation and cytosolic fraction S2.

2.5.2. Estimation of lipid peroxidation and membrane-associated protein activity

Lipid peroxidation, Na–K ATPase and bound PKC activity assays were performed in the membranous fractions (M1) of the brain homogenate. Thio-barbituric acid reactive substances (TBA-RS) content was measured to observe alterations in lipid peroxidation by following the method described by Jyoti et al. (2009). Tetra-methoxy propane (TMP) was used as a standard to estimate the level of TBA-RS content. Lipid peroxidation was expressed as TBS-RS content per mg protein. Ouabain sensitive Na–K ATPase activity was measured as iP released mg^{-1} protein $hour^{-1}$ performed by method as described previously by Kaur et al., 2003. The amount of inorganic phosphate released was quantified by the colorimetric method described by Fiske and Subbarow (1925), and Na–K ATPase activity was calculated by subtracting the ouabain-sensitive activity from the overall ATPase activity (in the absence of ouabain). PKC activity was assayed according to a modified procedure of Hetherington and Trewavas (1982) and as described in our previous report (Sethi et al., 2008). Both cytosolic and membrane-bound PKC activities were determined in an effort to analyze two distinct populations of membrane-associated PKC. In this investigation, PKC activities are reported and determined separately in the cytosolic (S1) and membranous fraction (M1) respectively. Radioactivity was measured by a Beckman-L-700 scintillation counter. Activity was expressed as β-counts per mg protein/min. Protein estimation in the cytosolic and syneptosomal fractions was performed following the protocol of Bedford, with some minor modifications, using bovine serum albumin (BSA) standard.

2.6. TEM studies

Four animals from each group were sacrificed for TEM studies to analyze the ultrastructural alterations in different experimental groups. Under anesthesia dose of Ketamine (50 mg/kg) rats were perfused with physiological saline and than fixed by 0.1M phosphate buffer (pH = 7.5) containing 2% paraformaldehyde and 2.5% glutaraldehyde. Thin sections (1 cm^2) of the somatosensori cortex were fixed in Kanovsky's fixative (2% paraformaldehyde + 2.5% glutaraldehyde in PBS) for 18 h at 4 °C. Tissues were postfixed in 1% osmium tetraoxide (OsO_4) for 2 h (4 °C) and then dehydrated in acetone. After clearing the tissue with xylene, infiltration was carried out using resins and the component araldite plus hardener (10 ml) plus accelerator (0.4 ml). Ultrathin sections were double-stained using uranyl acetate and lead citrate. These sections were observed under Fei-Philips Morgagni 268D (100 kV) TEM.

2.7. Statistical analysis

Data were expressed as mean ($N = 6$) ±SEM. For the analysis of MUA, biochemical and open field tests parameters, three-way ANOVA was conducted with the help of SigmaPlot 11.0 software (Systat Software, Inc. SigmaPlot for Windows). Three-way ANOVA was performed on age (Young vs. old), drug (corn oil vs. curcumin) and treatment (plain water vs. aluminium water) as between group factors. Post hoc mean comparisons were performed by the Holm–Sidak method for pairwise comparisons. For MWM tests, two-way RM ANOVA was performed on the young and old data with the help of GraphPad Prism 5.00 (GraphPad Prism Inc., San Diego, CA). Bonferroni posthoc test was performed to compare replicate means by row. The analysis was performed to investigate the effect of treatment, trial days and interaction between these parameters. The level of significance was accepted at $p<0.05$.

3. Results

Curcumin was tested for its ability to attenuate Al-induced alterations at electrophysiological, biochemical, behavioral and microscopic observations.

3.1. Electrophysiological study

Fig. 1 represents the sample EEG recordings showing the MUA recording and their corresponding EEG and Integrative amplitudes recorded from different channels of the Grass polygraph. The MUA recorded were manually counted to quantify the alterations in the EEG associated with aging, Al-toxicity and cur-treatment. Age [$F_{(1,40)} = 183$; $p<0.001$], drug [$F_{(1,40)} = 39.9$; $p<0.001$] and toxicity [$F_{(1,40)} = 104.7$; $p<0.001$] have a significant effect on the MUA activity in different experimental groups. Three-way AVOVA confirms that age × drug × toxicity does not show a significant interaction [$F_{(1,40)} = 0.2$; $p = 0.652$] in the recorded multiple unit activity. No significant interaction between age × drug [$F_{(1,40)} = 0.899$; $p = 0.349$], age × toxicity [$F_{(1,40)} = 3.37$; $p = 0.074$] and drug × toxicity [$F_{(1,40)} = 2.4$; $p = 0.129$] was also observed in the three-way ANOVA. To isolate which group(s) differ from the others Holm–Sidak multiple comparison procedure was used. Al-treatment significantly enhances ($p<0.001$) and curcumin treatment significantly decreases ($p<0.001$) the MUA activity in aging rats (Fig. 2). significance obtained selected paired comparisons are represented in the Fig. 2.

3.2. Biochemical study

TBA-RS content was measured as an index of lipid peroxidation (Fig. 3A). A significant effect of age [$F_{(1,40)} = 187.9$; $p<0.001$], drug [$F_{(1,40)} = 51.5$; $p<0.001$] and toxicity [$F_{(1,40)} = 116$; $p<0.001$] was observed at the level of lipid peroxidation. Three-way ANOVA indicates a significant interaction between age × drug × toxicity [$F_{(1,40)} = 5.1$; $p<0.05$]. Results of lipid peroxidation indicates a significant interaction between age × toxicity [$F_{(1,40)} = 10.4$; $p = 0.002$] and drug × toxicity [$F_{(1,40)} = 25.12$; $p<0.001$] but non-significant effect of age × drug [$F_{(1,40)} = 1.82$; $p = 0.18$] was evident. Holm–Sidak post-test confirms that age significantly affects the level of lipid peroxidation ($p<0.001$) in control and curcumin treated groups (Fig. 3A). Al-treatment significantly increases the lipid peroxidation in aging rats ($p<0.001$) and curcumin treatment significantly inhibited lipid peroxidation both in young and old curcumin treated groups($p<0.001$).

A significant effect of age, drug and toxicity was observed on the Na–K-ATPase activity. Three-way ANOVA confirms a significant effect of age × drug × toxicity [$F_{(1,40)} = 13.65$; $p<0.001$], shows that all these factors significantly affects the activity of Na–K-ATPase. Age × drug interaction depends upon toxicity (water vs. aluminium). No significant interaction between age × drug [$F_{(1,40)} = 2.18$; $p = 0.147$] while significant interaction between age × toxicity [$F_{(1,40)} = 8.7$; $p = 0.005$] and drug × toxicity [$F_{(1,40)} = 58.05$; $p<0.001$] was observed. Holm–Sidak post-test shows that curcumin treatment was observed to significantly increase Na–K-ATPase activity in both young and old Al + curcumin treated group compared to Al-treated group (Fig. 3B). Therefore, results indicate that curcumin protects Na–K-ATPase activity in both Al-induced metal toxicity and aging.

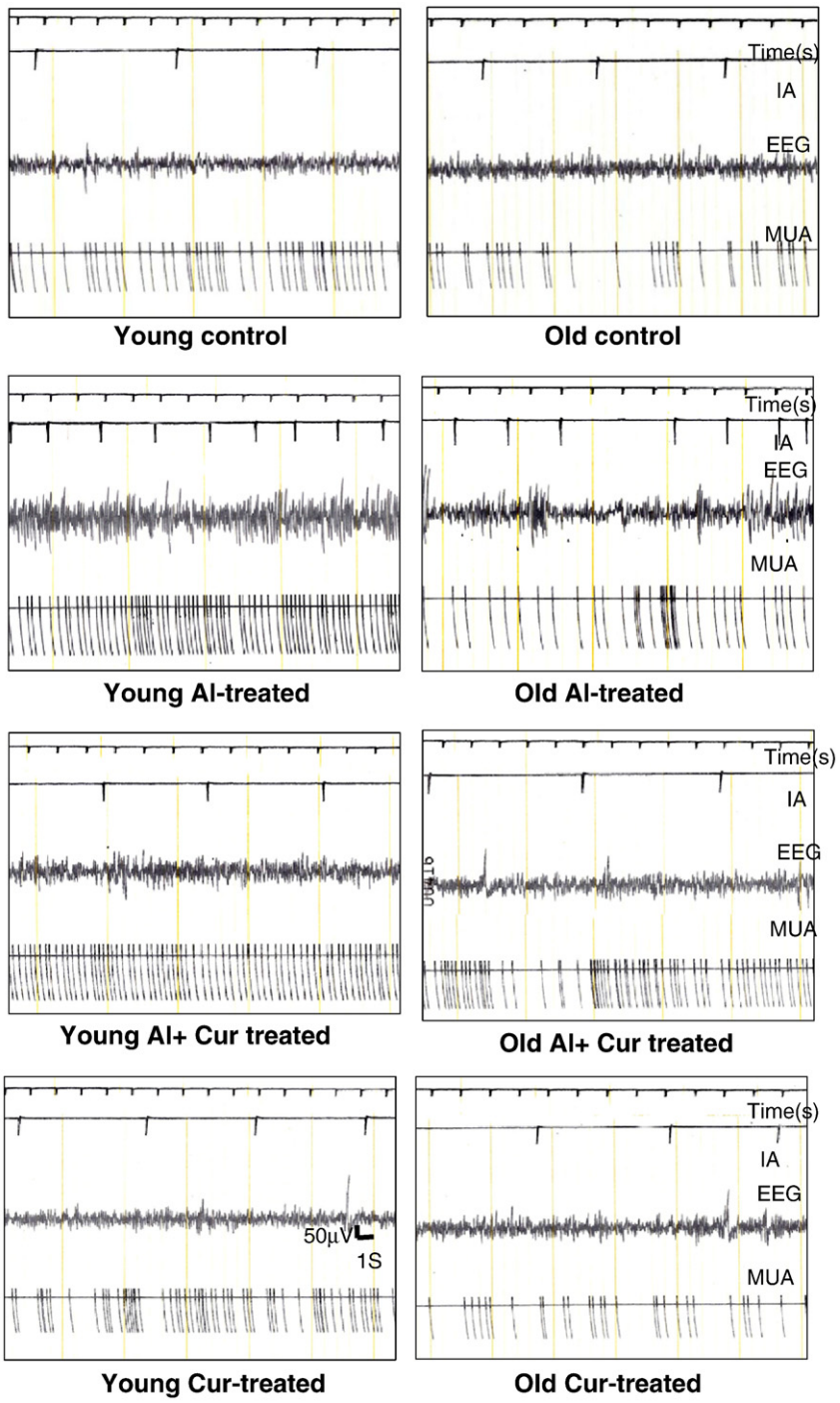

Fig. 1. Representative electrophysiological traces showing electrocorticogram (EEG), integrative amplitude (IA) and multiple unit activity (MUA) recording from parietal cortex of different experimental groups of young and old rats. Al-toxicity increased the MUA and IA while curcumin treatment in Al-toxicated group inhibited altered increase of MUA activity is evident.

Three-way ANOVA confirms a significant interaction between age × drug × toxicity ($p = 0.002$) in the observed cytosolic PKC activity. Age [$F_{(1,40)} = 2.97$; $p = 0.09$] and drug [$F_{(1,40)} = 0.724$; $p = 0.4$] have non-significant effect while significant effect of toxicity [$F_{(1,40)} = 178$; $p < 0.001$] was observed in the cytosolic PKC. Significant interaction between age × drug [$F_{(1,40)} = 47.08$; $p < 0.001$], age × toxicity [$F_{(1,40)} = 21.34$; $p < 0.001$] and drug × toxicity [$F_{(1,40)} = 16.19$; $p < 0.001$] was also observed. Holm–Sidak comparison shows that a significant decrease of cytosolic PKC activity ($p < 0.001$) occurs with age whereas curcumin significantly elevates ($p < 0.01$) the enzyme activity in old age. Curcumin treatment significantly decreases cytosolic PKC activity in young ($p < 0.001$) while elevates in old Al-treated group ($p < 0.05$) (Fig. 3C).

Age [$F_{(1,40)} = 18.3$; $p < 0.001$] and drug [$F_{(1,40)} = 28.04$; $p < 0.001$] have significant effect while non-significant effect of toxicity [$F_{(1,40)} = 0.692$; $p = 0.41$] was observed on the activity of bound PKC. Three-way interaction analysis confirms that a significant interaction between age × drug × toxicity [$F_{(1,40)} = 8.68$; $p = 0.005$] was observed in bound PKC activity. A significant interaction between drug × toxicity [$F_{(1,40)} = 5.69$; $p < 0.05$] while non-significant effect of age × drug [$F_{(1,40)} = 1.79$; $p = 0.18$] and age × toxicity [$F_{(1,40)} = 3.57$; $p = 0.06$] was evident. Age × drug treatment significantly interacts

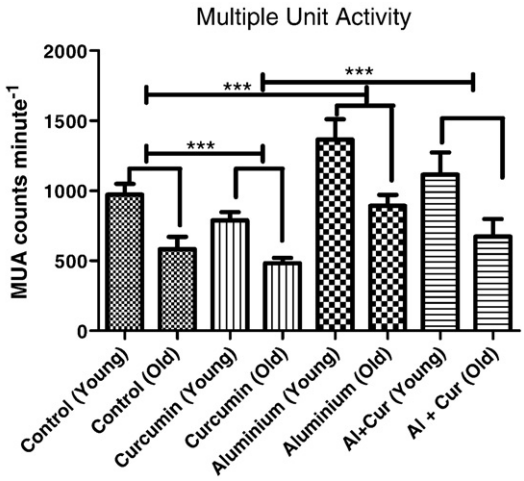

Fig. 2. Effect of curcumin treatment on aging and Al-toxicity related alterations on multiple unit activity recorded from cortex of young and old rat. Curcumin treatment suppresses the increased MUA activity linked with Al-toxicity is evident. Each data points represent mean plus SEM ($n=6$) [$p<0.001$*** $p<0.01$**, $p<0.05$* represents significant values while $p>0.05^{S}$ represents non-significant difference].

in control ($p=0.004$) while non-significant interaction was observed in the Al-treated group ($p=0.262$). All pairwise multiple comparison by Holm–Sidak method shows that curcumin treatment elevates the bound PKC activity in old age-group only. A significant age-related ($p<0.01$) and Al-toxicity ($p<0.001$) related decrease of bound PKC activity is evident (Fig. 3D).

3.3. Behavioral study

MWM (Cue-based spatial learning) tests show that average latency to find the hidden platform by different experimental rats. Our observations indicate that all experimental groups learned to find hidden platform in four experimental days. This implies that all experimental rats learn to escape swimming by searching hidden platform using visual cues. Results of MWM test in young experimental animals confirmed a significant effect of Al-toxicity [$F_{(1,30)}=85.45$; $p<0.001$], curcumin treatment [$F_{(1,30)}=7.6$; $p<0.05$] and Al + curcumin treatment [$F_{(1,30)}=23.39$; $p<0.001$] compared to control on latency to acquire hidden platform. In young, Two-way RM ANOVA confirmed a significant interaction between treatment (control vs. Al-treated) × trial days [$F_{(3,30)}=19.77$; $p<0.001$] and Al + curcumin treated × trial days [$F_{(3,30)}=12.42$; $p<0.001$]. Non-significant interaction was observed between (control vs. curcumin treated) × trial days [$F_{(3,30)}=2.6$; $p>0.05$] (Fig. 4A).

In old age-group, MWM task showed a significant effect of treatment on latency to reach the hidden platform in Al-treated [$F_{(1,30)}=83.8$; $p<0.001$], Al + curcumin treated [$F_{(1,30)}=5.56$; $p<0.05$] and curcumin treated [$F_{(1,30)}=20.88$; $p<0.01$] groups compared to their age-matched controls. Two-way RM ANOVA confirmed a significant interaction between treatment × trial days in the performance of Al-treated [$F_{(3,30)}=14.42$; $p<0.001$], Al + curcumin treated [$F_{(3,30)}=20.5$; $p<0.001$] and curcumin treated [$F_{(3,30)}=7.9$; $p<0.001$] rats compared to their age-matched controls (Fig. 4B).

3.3.1. Open field tests

Rearing, ambulatory and defaecation index was observed in open field test according to the procedure described in Materials and methods. Results of rearing activity indicate a significant effect of age [$F_{(1,40)}=21.7$; $p<0.001$] and toxicity [$F_{(1,40)}=17.42$; $p<0.001$] but non-significant effect of drug [$F_{(1,40)}=0.57$; $p=0.45$]. Non-significant interaction was observed between age × drug [$F_{(1,40)}=0.10$; $p=0.75$] and age × tocixity [$F_{(1,40)}=0.97$; $p=0.32$] while a significant interaction was observed between drug × toxicity [$F_{(1,40)}=6.97$; $p=0.012$]. Three-way AVOVA confirms that age × drug × toxicity does not show a significant interaction [$F_{(1,40)}=0.05$; $p=0.81$] in the rearing activity. To isolate which group(s) differ from the others Holm–Sidak multiple comparison procedure was used. A significant effect of curcumin treatment ($p<0.001$) was observed with in young and old age groups. Similarly a significant effect of Al-treatment was observed in young ($p<0.001$) and old ($p<0.01$) age (Fig. 5A).

Three-way ANOVA confirms a significant effect of age [$F_{(1,40)}=84$; $p<0.001$], drug [$F_{(1,40)}=20.8$; $p<0.001$] and toxicity [$F_{(1,40)}=244$; $p<0.001$] on the observed ambulatory activity of different groups. A significant interaction between age × drug × toxicity [$F_{(1,40)}=3.83$; $p<0.05$] was evident. Results of ambulatory activity indicates a significant interaction between drug × toxicity [$F_{(1,40)}=20.8$; $p<0.001$] and age × drug [$F_{(1,40)}=8.83$; $p<0.005$] but non-significant interaction of age × toxicity [$F_{(1,40)}=0.57$; $p=0.45$] was observed (Fig. 5B).

A significant effect of drug [$F_{(1,40)}=31.16$; $p<0.001$] and toxicity [$F_{(1,40)}=158$; $p<0.001$] and non-significant effect of age [$F_{(1,40)}=0.06$; $p=0.8$] was observed on the defaecation index of different groups as a result of three-way ANOVA. A non-significant interaction between age × drug × toxicity [$F_{(1,40)}=2.8$; $p=0.10$] was observed. Defaecation index indicates a significant interaction between age × toxicity [$F_{(1,40)}=16.07$; $p<0.001$] and drug × toxicity [$F_{(1,40)}=21.6$; $p<0.001$] but non-significant interaction between age × toxicity [$F_{(1,40)}=0.83$; $p=0.36$] was evident (Fig. 5C).

3.4. Histological assessment

Al-intoxicated group exhibited moderately shrunken neuronal cell bodies with extensive cytoplasmic vacuolation, with several cells exhibiting increased electron density (Fig. 6C and D). These degenerating neurons exhibited dilated endoplasmic reticulum cisternae and several necrosis like changes such as coarse and clumpy chromatin, multi-vesicular bodies, age pigment accumulation (Fig. 6C and D). In contrast, curcumin + Al-treated group exhibited homogeneous cytoplasm without vacuolization in cytoplasm and major alterations in cellular organelles (Fig. 6E and F). Curcumin treated groups appeared quite similar to the control age-matched groups (Fig. 6G and H). These micrographs show that curcumin treatment inhibits sub-cellular alterations linked with Al-toxicity (Fig. 6).

4. Discussion

Curcumin is a yellow hydrophobic polyphenol isolated from the rhizome of *Curcuma longa*. Since, time immemorial turmeric has been used in traditional Indian medicinal system "Ayurveda." In the present study, we have investigated whether oral intake of curcumin can inhibit Al-induced neurotoxicity in the cerebral cortex of rat brain. We have found that Al-toxicity enhanced MUA activity as well as hyperexcitability in the cortical region. This increased MUA in the ECoG correlates with elevated anxiety observed in the open field test parameters like high ambulation and increased defaecation index in Al-intoxicated rats. Treatment of curcumin significantly inhibited the Al-induced increased multiple unit activity (hyperexcitability) observed in the electrocorticogram (ECoG). MUA represents the cumulative neuronal firing from a group of neurons (Sethi et al., 2008). Therefore, suppression of MUA, implicates that curcumin has potential to modulate neuronal firing. Anxiolytic effect of curcumin was also reflected on the open field parameters as it significantly inhibited the defaecation index. The cortical hyperexcitability may be linked with the increased oxidative damage to membrane lipids, decrease of Na–K-ATPase activity and alterations of PKC activity.

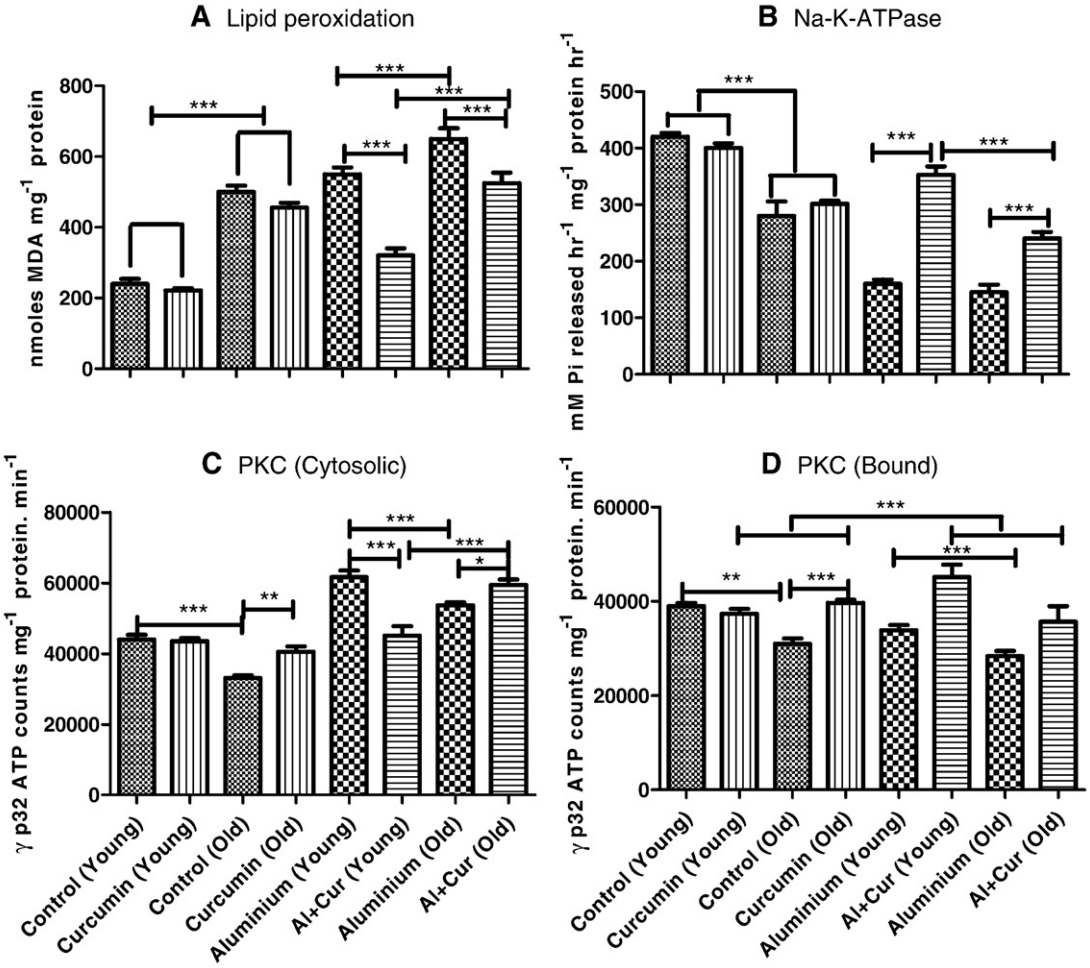

Fig. 3. Effect of curcumin treatment on Al-treatment induced biochemical alterations in lipid peroxidation (A), Na–K ATPase activity (B), PKC cytosolic (C) and PKC bound (D). Both young and old experimental groups exhibit significant inhibition of Al-induced biochemical changes. Each data point represents the mean ($n=6$) plus SEM [$p<0.001^{***}$ $p<0.01^{**}$, $p<0.05^{*}$ represents significant values while $p>0.05^{\$}$ represents non-significant difference].

Aluminium being an inert metal, mediates its neurotoxic effects by potentiating the redox active metals like ferrous ions (Oteiza et al., 1993, chromium and copper (Bondy et al., 1998). Therefore, observed electrophysiological effects of curcumin could be linked with its ability to cross BBB (Frautschy et al., 2001) and bind to redox metal like copper and iron (Baum and Ng, 2004).

In our previous study we have found that lipid peroxidation, Na–K ATPase, cytosolic and bound PKC activity significantly correlates with Al-induced alterations of neuronal firing (Sethi et al., 2008). Hence, we have performed these biochemical assays in cortex as well to investigate whether curcumin has efficacy to modulate these biochemical parameters. A generalized increase of lipid peroxidation and decrease of Na–K ATPase activity was observed in Al-toxicated young and old animals. Both LP and Na–K ATPase plays pivotal role in maintaining the potential across the membrane. Increased lipid peroxidation has been invariably correlated with hyperexcitability or seizure manifestation in different animal model of epilepsies (Willmore, 1990). Similarly decreased Na–K ATPase activity is involved in the disruption of membrane potential gradient linked with seizure menifestation in cerebral ischemia, epilepsy (Grisar, 1984), various neurodegenerative disorders (Lees, 1991), Ca^{2+} mediated excitotoxicity (Goddard and Robinson, 1976) and enhanced glutamate release (Lees, 1991). Curcumin's ability to neutralize free radicals (Bishnoi et al., 2008) must be responsible for inhibiting the Al-induced increase of lipid peroxidation. Curcumin treated groups have significantly higher Na–K ATPase activity as compared to age-matched Al-toxicated groups. The maintained level of Na–K ATPase must be responsible for the suppression of hyperexcitability or MUA activity observed in the curcumin treated groups.

The PKC family of serine/threonine kinases is reported to be involved in a variety of physiological and pathophysiological processes in the brain including development (Oster et al., 2004), synaptic plasticity (Sossin, 2007), cerebral ischemia (Bright and Mochly-Rosen, 2005), and neuronal cell death (Zhang et al., 2007). Al-induced increase in cytosolic PKC activity and decreased bound PKC activity was observed in the cortex. The observed shift of PKC homeostasis has been linked with decrease of learning abilities (Wehner et al., 1990; Sethi et al., 2008). Altered activation of cytosolic PKC has been reported to cause hyperexcitability and seizures in experimental model of epilepsies like kainate-induced seizures (Guglielmetti et al., 1997), electrical kindling model (Chen et al., 1992; Vernet et al., 1992). In this study, we have observed that treatment of curcumin significantly inhibited increase of cytosolic PKC activity and decrease of bound PKC. Hence, curcumin treatment maintains the PKC homeostasis which could be one of the reasons behind the observed therapeutic effects like suppression of hyperexcitability (MUA) and increased learning abilities in the MWM tasks.

Aluminium toxicity mediated alterations of learning abilities are highly controversial. Several studies suggest a general decline in learning abilities (Julka et al., 1995; Golub and Germann, 1998; Wu

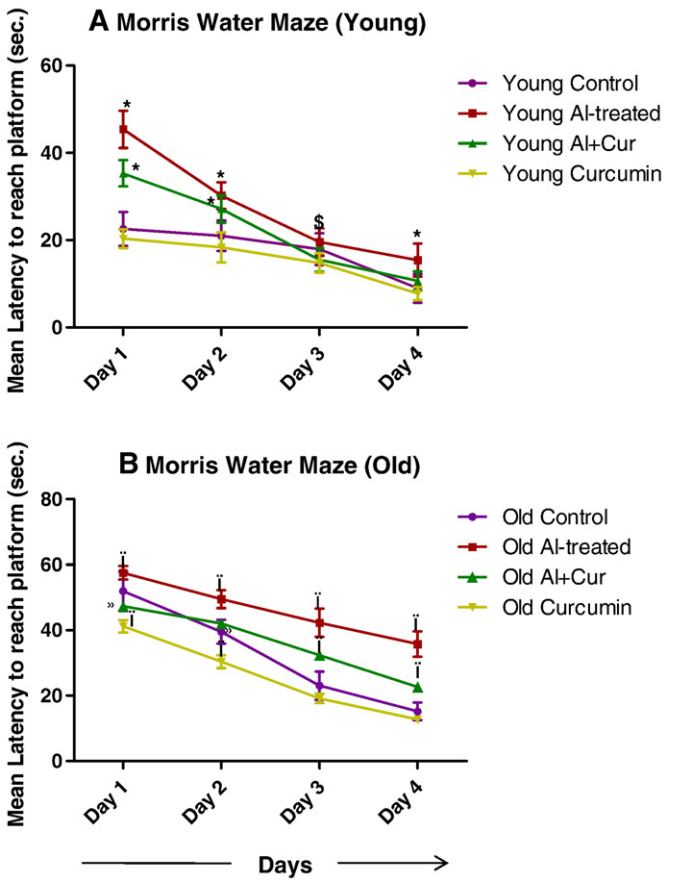

Fig. 4. Mean latency to find hidden platform in MWM trials for young and old experimental rats. Water maze tasks were performed to evaluate effect of curcumin treatment on the spatial memory abilities. Each data point represents the mean (±SEM) latency of the 5 trials for a minimum of six rats performed each day. (*) Level of significance ($p<0.05$) in comparison to control young. (•) Level of significance ($p<0.05$) in comparison to control old [$p>0.05^{\$}$ N.S. compared to control young; $p>0.05^{\psi}$ N.S compared to control old].

et al., 1998; Shi-Lei et al., 2005) while other proposes no effect on memory consolidation (Domingo et al., 1996; Colomina et al., 2002). These contradictory findings may be related to different methods, doses and routes used to induced Al-toxicity. We have previously reported that long term chronic Al-intake significantly decreases the visuo-spatial learning abilities in both young and old Al-intoxicated groups (Sethi et al., 2008). In this study, we have assessed whether curcumin treatment inhibits the Al-induced memory decline as well as increased anxiety level. Co-administration of curcumin with Al-treatment was effective in preventing memory decline observed in MWM tasks. Antidementic effect of curcumin against Al-neurotoxicity is a novel finding. Curcumin ability to modulate lipid peroxidation, acetylcholinesterase activity (Kuhad and Chopra, 2007), BDNF and its downstream effectors synapsin I and CREB (Wu et al., 2006) could be responsible for inhibiting the memory decline observed in MWM tasks. Other studies have shown antidementic effects of curcumin in experimentally induced diabetic encephalopathy (Kuhad and Chopra, 2007), amyloid β induced cognitive damage (Frautschy et al., 2001) and fluid percussion induced cognitive impairment (Wu et al., 2006).

Our TEM studies indicate that aluminium enhances neurotoxicity by inflicting damage at sub-cellular structures. In accordance to previous reports we observed increased vacuolation, swollen mitochondria, and hyper-electron dense cells in Al-toxicated young and old rats compared to age-matched controls (Jyoti et al., 2007). Curcumin treated Al-toxicated rats exhibited normal cellular structure like homogeneous cytoplasm with normal sub-cellular structures. This confirms the neuroprotective effect of curcumin at ultrastructural level. Curcumin treatment was also effective in preventing the aging associated alterations like increase of lipofuscin accumulation, cytoplasmic vacuolation, chromatin condensation etc. This further supports the anti-aging potential of curcumin reported in the past (Bala et al., 2006). Hence, the therapeutic effect of curcumin on biochemical and microscopic alterations could be responsible for the observed electrophysiological and behavioral outcome.

Our overall data indicates that curcumin could be beneficial in countering the Al-induced neurotoxicity at electrophysiological, biochemical, behavioral and ultrastructural levels. The antioxidative potential of curcumin might be playing key role in the observed therapeutic outcome against Al-induced neurotoxicity. In conclusion,

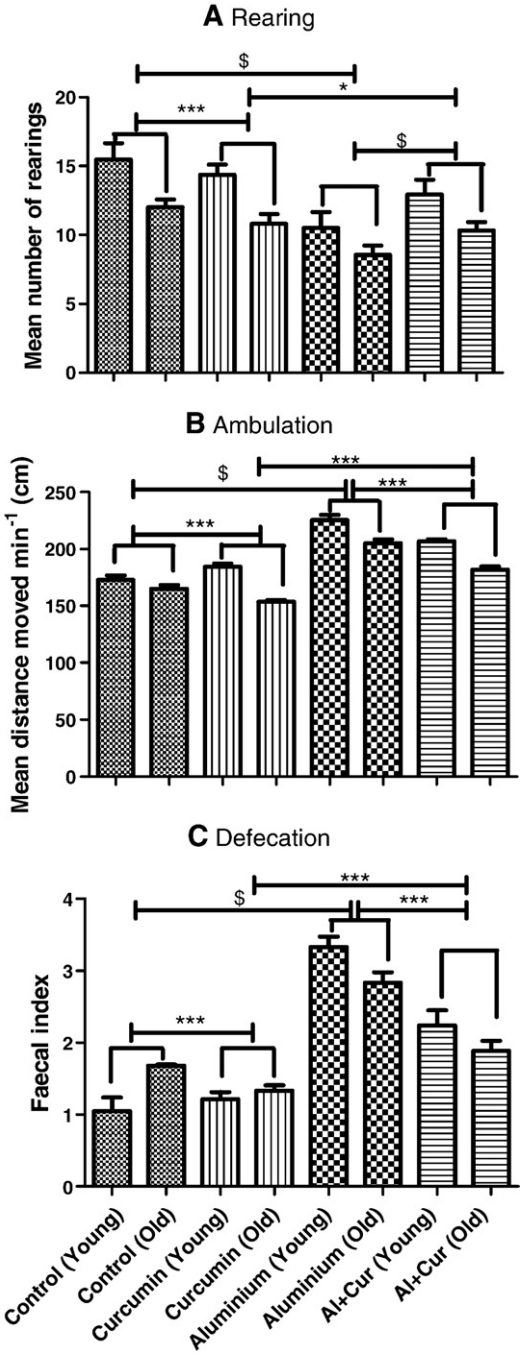

Fig. 5. Open field test results exhibit significantly decreased ambulatory activity and defaecation index in both age groups compared to Al-treated age-matched control. Each point represents the mean ($n=6$) + SEM [$p<0.001^{***}$ $p<0.01^{**}$, $p<0.05^{*}$ represents significant values while $p>0.05^{\$}$ represents non-significant difference].

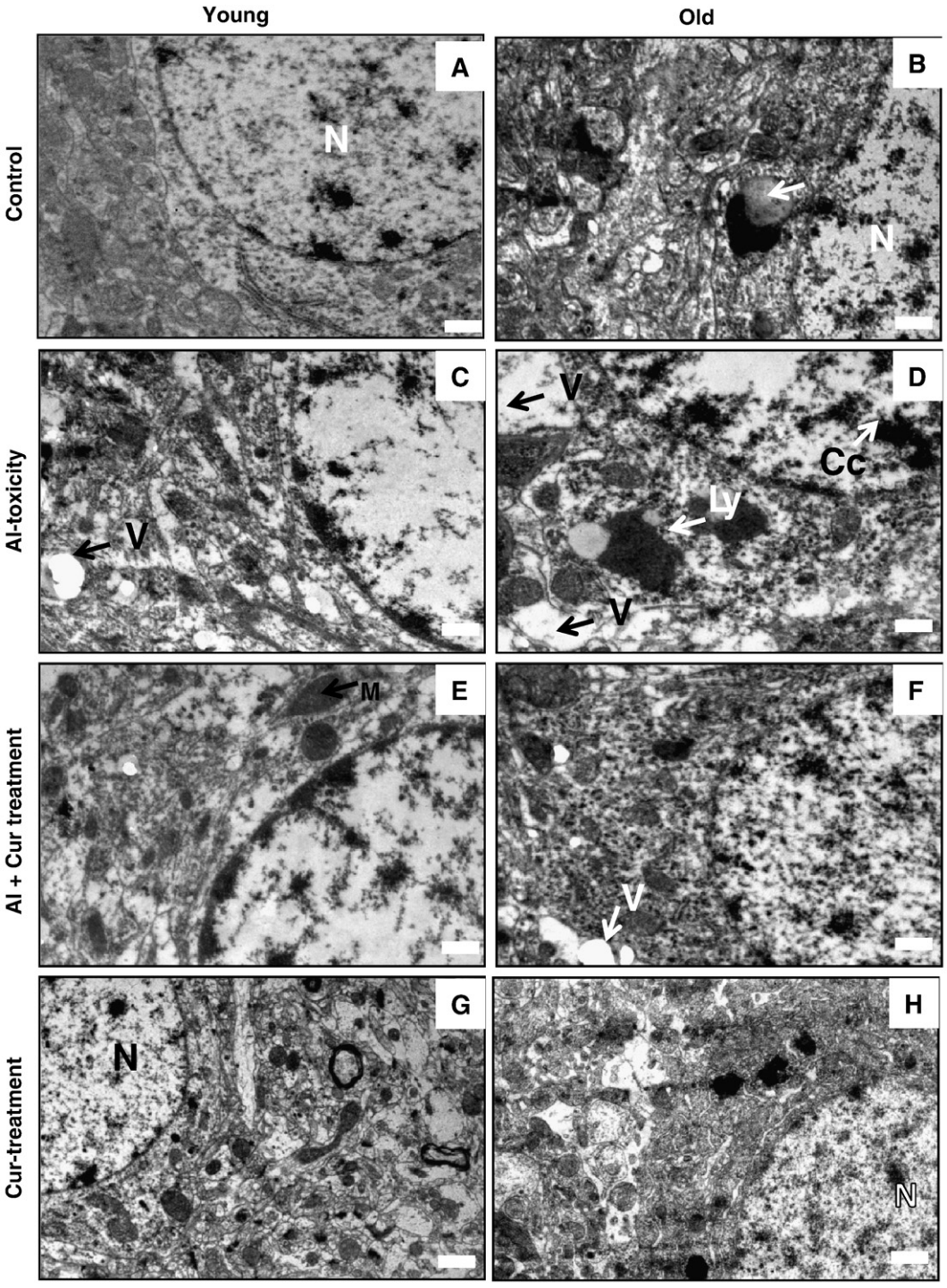

Fig. 6. Electron photomicrographs (magnification, ×2400) showing effect of curcumin treatment on Al-toxicity on sub-cellular structure of cortical cells. Cur-treatment effectively inhibited cellular alterations linked with Al-toxicity in both young and old rats. Nucleus (N), lipofuscin (Ly), condensed chromatin (Cc), hyper-electron dense cells (H), vacuolation (V), and mitochondria (M). Scale bar = 2 μM.

since curcumin is a natural polyphenol without any reported side effects, it could be used as a safe option for treatment in Al-associated neurotoxicity.

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References

Aggarwal BB. Prostate cancer and curcumin: add spice to your life. Cancer Biol Ther 2008;7:1436–40 Epub 2008 Sep 21. No abstract available.
Bala K, Tripathy BC, Sharma D. Neuroprotective and anti-ageing effects of curcumin in aged rat brain regions. Biogerontology 2006;7:81–9.
Banks WA, Kastin AJ. Aluminum alters the permeability of the blood–brain barrier to some non-peptides. Neuropharmacology 1985;24:407–12.
Baum L, Ng A. Curcumin interaction with copper and iron suggests one possible mechanism of action in Alzheimer's disease animal models. J Alzheimers Dis 2004;6:367–77.
Bilkei-Gorzó A. Neurotoxic effect of enteral aluminium. Food Chem Toxicol 1993;31:357–61.

Bishnoi M, Chopra K, Kulkarni SK. Protective effect of Curcumin, the active principle of turmeric (*Curcuma longa*) in haloperidol-induced orofacial dyskinesia and associated behavioural, biochemical and neurochemical changes in rat brain. Pharmacol Biochem Behav 2008;88:511–22.
Bondy SC, Guo-Ross SX, Pien J. Mechanisms underlying the aluminum-induced potentiation of the pro-oxidant properties of transition metals. Neurotoxicology 1998;19:65–71.
Bright R, Mochly-Rosen D. The role of protein kinase C in cerebral ischemic and reperfusion injury. Stroke 2005;36:2781–90.
Chen SJ, Desai MA, Klann E, Winder DG, Sweatt JD, Conn PJ. Amygdala kindling alters protein kinase C activity in dentate gyrus. J Neurochem 1992;59:1761–9.
Colomina MT, Roig JL, Sánchez DJ, Domingo JL. Influence of age on aluminum-induced neurobehavioral effects and morphological changes in rat brain. Neurotoxicology 2002;23:775–81.
Dairam A, Limson JL, Watkins GM, Antunes E, Daya S. Curcuminoids, curcumin, and demethoxycurcumin reduce lead-induced memory deficits in male Wistar rats. J Agric Food Chem 2007;55:1039–44.
Daniel S, Limson JL, Dairam A, Watkins GM, Daya S. Through metal binding, curcumin protects against lead- and cadmium-induced lipid peroxidation in rat brain homogenates and against lead-induced tissue damage in rat brain. J Inorg Biochem 2004;98:266–75.
Domingo JL, Llorens J, Sanchez DJ, Gomez M, Llobet JM, Corbella J. Age-related effects of aluminum ingestion on brain aluminum accumulation and behavior in rats. Life Sci 1996;58:1387–95.
Exley C. Aluminum and Alzheimer's disease. J Alzheimers Dis 2001;3:551–2.
Fiske CH, Subbarow J. The colorimetric determination of phosphorus. J Biol Chem 1925;66:375–400.
Frautschy SA, Hu W, Kim P, Miller SA, Chu T, Harris-White ME, et al. Phenolic anti-inflammatory antioxidant reversal of Abeta-induced cognitive deficits and neuropathology. Neurobiol Aging 2001;22:993-1005.
Garcia-Alloza M, Borrelli LA, Rozkalne A, Hyman BT, Bacskai BJ. Curcumin labels amyloid pathology in vivo, disrupts existing plaques, and partially restores distorted neurites in an Alzheimer mouse model. J Neurochem 2007;102:1095–104.
Ghoneim AI, Abdel-Naim AB, Khalifa AE, El-Denshary ES. Protective effects of curcumin against ischaemia/reperfusion insult in rat forebrain. Pharmacol Res 2002;46:273–9.
Goddard GA, Robinson JD. Uptake and release of calcium by rat brain synaptosomes. Brain Res 1976;110:331–50.
Golub MS, Germann SL. Aluminum effects on operant performance and food motivation of mice. Neurotoxicol Teratol 1998;20:421–7.
Grisar T. Glial and neuronal Na+–K+ pump in epilepsy. Ann Neurol 1984;16:128–34.
Guglielmetti F, Rattray M, Baldessari S, Butelli E, Samanin R, Bendotti C. Selective up-regulation of protein kinase C epsilon in granule cells after kainic acid-induced seizures in rat. Brain Res Mol Brain Res 1997;49:188–96.
Hetherington A, Trewavas A. Calcium-dependent protein kinase in pea shoot membranes. FEBS Lett 1982;145:67–71.
Johnson GV, Jope RS. Aluminum impairs glucose utilization and cholinergic activity in rat brain in vitro. Toxicology 1986;40:93-102.
Julka D, Sandhi R, Gill KD. Altered cholinergic metabolism in rat CNS following aluminum exposure: implications on learning performance. J Neurochem 1995;65:2157–64.
Jyoti A, Sethi P, Sharma D. Bacopa monniera prevents from aluminium neurotoxicity in the cerebral cortex of rat brain. J Ethnopharmacol 2007;111:56–62.
Jyoti A, Sharma D. Neuroprotective role of Bacopa monniera extract against aluminium-induced oxidative stress in the hippocampus of rat brain. Neurotoxicology 2006;27:451–7.
Jyoti A, Sethi P, Sharma D. Curcumin protects against electrobehavioral progression of seizures in the iron-induced experimental model of epileptogenesis. Epilepsy Behav 2009;14(2):300–8.
Kaneko N, Takada J, Yasui H, Sakurai H. Memory deficit in mice administered aluminum–maltolate complex. Biometals 2006;19:83–9.
Kaur J, Singh S, Sharma D, Singh R. Neurostimulatory and antioxidative effects of l-deprenyl in aged rat brain regions. Biogerontology 2003;4:105–11.
Kawahara M. Effects of aluminum on the nervous system and its possible link with neurodegenerative diseases. J Alzheimers Dis 2005;8:171–82.
Kawahara M, Kato M, Kuroda Y. Effects of aluminum on the neurotoxicity of primary cultured neurons and on the aggregation of beta-amyloid protein. Brain Res Bull 2001;55:211–7.
Kelloff GJ, Boone CW, Crowell JA, Steele VE, Lubet RA, Doody LA, et al. New agents for cancer chemoprevention. J Cell Biochem Suppl 1996;26:1-28 Review.
Kuhad A, Chopra K. Curcumin attenuates diabetic encephalopathy in rats: behavioral and biochemical evidences. Eur J Pharmacol 2007;576:34–42.
Lees GJ. Inhibition of sodium–potassium-ATPase: a potentially ubiquitous mechanism contributing to central nervous system neuropathology. Brain Res Brain Res Rev 1991;16:283–300 Review.
Lim CS, Jin DQ, Mok H, Oh SJ, Lee JU, Hwang JK, et al. Antioxidant and antiinflammatory activities of xanthorrhizol in hippocampal neurons and primary cultured microglia. J Neurosci Res 2005;82:831–8.
Miu AC, Benga O. Aluminum and Alzheimer's disease: a new look. J Alzheimers Dis 2006;10:179–201.
Oster H, Eichele G, Leitges M. Differential expression of atypical PKCs in the adult mouse brain. Brain Res Mol Brain Res 2004;127:79–88.
Oteiza PI, Fraga CG, Keen CL. Aluminum has both oxidant and antioxidant effects in mouse brain membranes. Arch Biochem Biophys 1993;300:517–21.
Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1982.
Platt B, Fiddler G, Riedel G, Henderson Z. Aluminium toxicity in the rat brain: histochemical and immunocytochemical evidence. Brain Res Bull 2001;55:257–67.
Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 2003;463:3-33.
Sethi P, Jyoti A, Singh R, Hussain E, Sharma D. Aluminium-induced electrophysiological, biochemical and cognitive modifications in the hippocampus of aging rats. Neurotoxicology 2008;29:1069–79.
Sharma D, Maurya AK, Singh R. Age-related decline in multiple unit action potentials of CA3 region of rat hippocampus: correlation with lipid peroxidation and lipofuscin concentration and the effect of centrophenoxine. Neurobiol Aging 1993;14:319–30.
Sharma RA, Gescher AJ, Steward WP. Curcumin: the story so far. Eur J Cancer 2005;41:1955–68 Review.
Shi-Lei S, Guang-Yu MA, Bachelor LH, Bachelor ZY, Dong HM, Xu XH. Effect of naloxone on aluminum-induced learning and memory impairment in rats. Neurol India 2005;53:79–82.
Shukla PK, Khanna VK, Ali MM, Khan MY, Srimal RC. Anti-ischemic effect of curcumin in rat brain. Neurochem Res 2008;33:1036–43.
Sossin WS. Isoform specificity of protein kinase Cs in synaptic plasticity. Learn Mem 2007;14:236–46.
Struys-Ponsar C, Kerkhofs A, Gauthier A, Soffie M, Van den Bosch de Aguilar P. Effects of aluminum exposure on behavioral parameters in the rat. Pharmacol Biochem Behav 1997;56:643–8.
Thiyagarajan M, Sharma SS. Neuroprotective effect of curcumin in middle cerebral artery occlusion induced focal cerebral ischemia in rats. Life Sci 2004;74:969–85.
Vernet O, Rostworowski K, Sherwin AL. Protein kinase C activity and subcellular distribution in rat brain following repeated electroconvulsive seizures. Exp Neurol 1992;115:297–301.
Wang Q, Sun AY, Simonyi A, Jensen MD, Shelat PB, Rottinghaus GE, et al. Neuroprotective mechanisms of curcumin against cerebral ischemia-induced neuronal apoptosis and behavioral deficits. J Neurosci Res 2005;82:138–48.
Wehner JM, Sleight S, Upchurch M. Hippocampal protein kinase C activity is reduced in poor spatial learners. Brain Res 1990;523:181–7.
Willmore LJ. Post-traumatic epilepsy: cellular mechanisms and implications for treatment. Epilepsia 1990;31:67–73.
Wu A, Ying Z, Gomez-Pinilla F. Dietary curcumin counteracts the outcome of traumatic brain injury on oxidative stress, synaptic plasticity, and cognition. Exp Neurol 2006;197:309–17.
Wu YH, Zhou ZM, Xiong YL, Wang YL, Sun JH, Liao HB, et al. Effects of aluminum potassium sulfate on learning, memory, and cholinergic system in mice. Zhongguo Yao Li Xue Bao 1998;19:509–12.
Yokel RA, Allen DD, Meyer JJ. Studies of aluminum neurobehavioral toxicity in the intact mammal. Cell Mol Neurobiol 1994;14:791–808.
Zhang F, Li C, Wang R, Han D, Zhang QG, Zhou C, et al. Activation of GABA receptors attenuates neuronal apoptosis through inhibiting the tyrosine phosphorylation of NR2A by Src after cerebral ischemia and reperfusion. Neuroscience 2007;150:938–49.