Neurochemical Research (2019) 44:2020–2029
https://doi.org/10.1007/s11064-019-02839-9

ORIGINAL PAPER

Curcumin Acts as Post-protective Effects on Rat Hippocampal Synaptosomes in a Neuronal Model of Aluminum-Induced Toxicity

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Received: 13 May 2019 / Revised: 15 June 2019 / Accepted: 26 June 2019 / Published online: 29 June 2019

Abstract

The neurotoxic effects of aluminum are generally associated with reduced antioxidant capacity, increased oxidative stress and apoptosis, which lead to the induction of neurodegenerative processes. Curcumin has a lipophilic polyphenol character and effects of antioxidant and anti-apoptotic. The present study was undertaken to examine possible aluminum exposure in rats brain synaptosomes and to investigate whether protective and therapeutic effects of curcumin on biochemical and morphological changes in both pre- and post-treated groups. Aluminum chloride ($AlCl_3$) at 50 µM concentration and curcumin at 5 and 10 µg/mL doses were applied to hippocampal synaptosomes of rats according to experimental design. Biochemical effects were evaluated by MTT cytotoxicity, malondialdehyde (MDA) levels, nitric oxide (NO) levels, glutathione (GSH) levels, caspase 3 activities, cytochrome c levels, DNA fragmentation values and protein levels. Morphological examinations were done by TEM analysis. $AlCI_3$ exposure in the synaptosomes enhanced oxidative stress, triggered apoptosis and caused ultrastructural alterations which were well reflected in the TEM images. Curcumin pre-treatment slightly ameliorated the MDA levels, NO levels, cytochrome c levels and caspase 3 activities in $AlCI_3$-exposed synaptosomes, but these results were not statistically significant. Furthermore, curcumin post-treatment significantly improved oxidative damage and morphological alterations, and suppressed cytochrome c and caspase 3 activities. Taken together, our data showed that curcumin had more therapeutic effects than protective effects in $AlCI_3$-induced neurotoxicity. Nevertheless, the therapeutic (post-protective) effects of curcumin should be further investigated in in vivo neurodegenerative models involving behavioral tests.

Keywords Aluminum chloride · Curcumin · Oxidative stress · Apoptosis · Morphological alteration · Hippocampal synaptosomes

Introduction

Aluminum is one of the most common non-essential elements found in nature [1]. Ionic form of aluminum (Al^{+3}) is chemically highly reactive and tends to be a compound. Al^{+3} has recently been used as a component in the production of many cosmetic products, pharmaceuticals and daily products, such as food and beverages [2]. Al^{+3} is especially used as an effective stimulant of the immune system in the ingredient of many vaccines and medicines [3].

Al^{+3} is absorbed mainly from the gastrointestinal tract and spreads throughout the body, it is subsequently accumulated in different tissues [4, 5]. Al^{+3} passes through the blood brain barrier by binding to the transferrin [6]. Al^{+3} predominantly accumulates in the brain areas, such as corpus striatum, brain stem, hippocampus and frontal cortex [7]. It triggers oxidative damage and impairs cellular signaling cascades by increasing the production of reactive oxygen and reactive nitrogen species (ROS and RNS, respectively) [8, 9]. Studies have also shown that Al^{+3} treatment may play a role in neuronal degeneration processes that are associated with glucose metabolism, cellular respiration, axonal signal transduction, inhibition

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of antioxidant enzymes, lipid peroxidation and protein denaturation [10]. Al^{+3} treatments also affect cytochrome oxidase activity, which enhances neuronal death by increasing mitochondrial membrane permeability, DNA damage and apoptosis [11, 12].

In recent years, antioxidant hallmarked plants have been gained an importance in oxidative stress-related neurodegenerative diseases. From these antioxidant plant specimens Curcuma longa (turmeric), a traditional Indian spice, is a member of the ginger family. The active component of turmeric is curcumin, which is chemically called diferuloylmethane [13]. Curcumin as an alternative protective agent has effects of antimicrobial [14], anti-carcinogenic [15], anti-inflammatory [16] and antioxidant [17]. Moreover, curcumin seems to have a lot of promise in neurodegenerative diseases due to its ability to cross the blood brain barrier [18]. Previous study reported that curcumin decreased the ROS production by supporting antioxidant capacity and inhibited the neuro-apoptosis [19].

In the present study, we aimed to compare whether the pre- and post-protective effects of curcumin were through apoptotic and oxidative biomarkers in aluminum chloride ($AlCI_3$)-induced synaptosomes. Therefore, we applied pre- and post-treatment of curcumin to $AlCI_3$-induced synaptosome, then measured some biochemical parameters in rat hippocampal synaptosomes, such as MTT cytotoxicity, malondialdehyde (MDA) levels, nitric oxide (NO) levels, glutathione (GSH) levels, cytochrome c levels, caspase 3 activities, DNA fragmentation values and morphological analysis.

Material and Methods

Animals

This study was conducted with 56 healthy Wistar Albino adult male rats (weighing 250 ± 30 g) obtained from the Medical and Surgical Experimental Research Center, Eskisehir. Experimental procedures were carried out according to the decision of Animal Experiments Local Ethics Committee of Eskisehir Osmangazi University (Protocol no: 655) and with the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences. Rats were housed in polycarbonate cages with controlled temperature (25 ± 2 °C), humidity (45 ± 5%) and 12-h cycle of light/dark. Rats were anaesthetized with an intramuscular injection ketamine + xylazine (at doses of 40 mg/kg and 10 mg/kg, respectively) and sacrificed by cervical dislocation. During the experimental procedure, all steps were taken to alleviate animal pain and the least number of animals were used.

Preparation and Treatments of Synaptosomes

Rat brain synaptosomes were obtained according to the modified Whittaker et al. [20]. Briefly, after anesthesia, hippocampus of rats immediately dissected on ice and homogenized in a solution containing (1:10 w/v) 10 mM HEPES and 0.3 M sucrose. Following, homogenates were centrifuged at 3000×*g* for 10 min at 4 °C, and then pellets were removed. The supernatants obtained from the first centrifugation were re-centrifuged at 12,000×*g* for 20 min at 4 °C. After, the pellets contained the synaptosomal parts were stored in the presence of 5% dimethyl sulfoxide (DMSO, a cryoprotectant) at − 80 °C (Dunkley et al. 2008) for future biochemical analysis.

Synaptosomal fractions each containing 100 mg of tissue in 3 mL medium were divided into eight subgroups; each group containing hippocampus regions from seven animals:

- Group I (control), synaptosomes were exposed to saline for 6 h at 37 °C;
- Group II, synaptosomes were exposed to 50 µM $AlCl_3$ for 3 h, and then added with saline for another 3 h at 37 °C;
- Group III, synaptosomes were treated with 5 µg/mL curcumin for 3 h, and then added with saline for another 3 h at 37 °C;
- Group IV, synaptosomes were treated with 10 µg/mL curcumin for 3 h, and then added with saline for another 3 h at 37 °C;
- Group V (pre-protective effects), synaptosomes were pretreated with 5 µg/mL curcumin for 3 h, and then added with 50 µM $AlCl_3$ for another 3 h at 37 °C;
- Group VI (pre-protective effects), synaptosomes were pretreated with 10 µg/mL curcumin for 3 h, and then added with 100 µM $AlCl_3$ for another 3 h at 37 °C;
- Group VII (post-protective effects), synaptosomes were pre-exposed to 50 µM $AlCl_3$ for 3 h, and then added with 5 µg/mL curcumin for another 3 h at 37 °C;
- Group VIII (post-protective effects), synaptosomes were pre-exposed to 50 µM $AlCl_3$ for 3 h, and then added with 10 µg/mL curcumin for another 3 h at 37 °C.

Treated synaptosomes were used for the determination of MTT levels, MDA levels, NO levels, GSH levels, cytochrome c levels, caspase 3 activities, DNA fragmentation values and morphological analysis. Protein contents of synaptosomes were measured by the method of Lowry et al. [21].

Curcumin was freshly prepared with 5% DMSO before the experiment and administered at 5 and 10 µg/mL doses.

The curcumin doses were determined according to previous in vivo and in vitro studies [22, 23]. Similarly, $AlCl_3$ was also freshly prepared dissolving in bi-distilled water prior to the experiment and applied to synaptosomes at 50 µM concentration. This concentration was the amount of Al^{+3} detected in the hippocampus region of rats after aluminum exposure [24, 25].

MTT Assay

We utilized the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromid (MTT) to formazan crystals by mitochondrial dehydrogenase to mitochondrial activity of synaptosomes [26]. Briefly, 50 µl synaptosomal fraction was added to 500 µl MTT solution (2.5 mg/ml in PBS), and then mixture was incubated at 37 °C for 3 h. Following incubation, the mixture was centrifuged at 2000×*g* for 10 min at 4 °C and the resulting pellet re-suspended in DMSO to dissolve the formazan crystals. Finally, all samples were measured spectrophotometrically at 592 nm. The results were showed by comparison with the control.

Determination of MDA Levels in Synaptosomes

MDA levels were measured to determine lipid peroxidation in $AlCl_3$-induced rat synaptosomes. MDA levels measured according to the method reported by Ohkawa et al. [27]. MDA is a secondary product of lipid peroxidation that is accepted as an important parameter most commonly used in the determination of lipid peroxidation. According to this method, MDA forms a pink-colored complex with incubation at 95 °C for 1 h with TBA at pH 3.4. This complex was measured at 532 nm wavelength. The results were indicated as nmol/mg protein.

Determination of NO Levels in Synaptosomes

The determination of NO levels was based on the measurement of nitrite and nitrate formed by oxidation of nitric oxide. The amounts of nitrite and nitrate in the samples were determined by two consecutive reactions. Initially, nitrate was reduced to nitrite by means of enzymatic or non-enzymatic conversion. In the acidic reaction medium, the nitrite was diazotized with sulfanilamide and subsequently forms a purple azo compound with N-(1-naphthyl) ethylenediamine. The amount of nitrite was measured precisely according to the method also known as the Griess reaction [28]. The results were expressed as µmol/mg protein.

Determination of GSH Levels in Synaptosomes

GSH levels in rat brain synaptosomes were determined according to Srivastava and Beutler [29]. Briefly, the sulfhydryl group of GSH reacts with DTNB (Ellman's reagent) to form yellow colored 5-thio-2-nitrobenzoic acid (TNB). The GSH concentration was determined by measuring the absorbance of this colored product at 412 nm. Reaction medium contains 0.1 mL sample, 2 mL 100 mM Tris–HCl buffer pH 8.4 and 0.1 mL Ellman's reagent (60 mg/100 mL, 0.1 M Tris–HCl buffer pH 7.0). Synaptosomal GSH levels were reported as µmol/mg protein.

Assays for Caspase 3 Activities and Cytochrome c Levels in Synaptosomes

Caspase 3 activity and cytochrome c levels were measured to show apoptotic status in synaptosomes. Caspase-3 activities in synaptosomes were measured spectrophotometrically at 405 nm using a colorimetric caspase-3 measurement kit (Sigma-Aldrich, St. Louis, Missouri, USA). Briefly, the determination of caspase 3 activity was based on the detection of releasing p-nitroaniline (pNA), which was induced by hydrolysis of acetyl-Asp-Glu-Val-Asp p-nitroanilide with caspase 3. The concentration of the pNA released from the substrate was calculated the absorbance values at 405 nm. The results were expressed as µmol pNA/min/mL.

Measurement of cytochrome c levels was based on the quantitative sandwich enzyme immunoassay technique. The rat cytochrome c assay kit (R&D Systems, Minneapolis, USA), which contains the microplates coated with monoclonal antibodies specific for rat cytochrome c, was used according to the manufacturer's protocol. Data were indicated as ng/mL.

Measurement of DNA Fragmentation

Synaptosomal DNA fragmentation values were determined using agarose gel electrophoresis. Mitochondrial DNA in synaptosomal fractions was obtained according to the modified method of Drakulic et al. [30]. Briefly, the synaptosomal DNA following centrifugation processes was incubated at 37 °C for 3 h after mixing with 40 µl sodium dodecyl sulfate (5%), 10 µl RNase A (10 mg/mL) and 10 µl proteinase K (15 mg/mL). After incubation, DNA was precipitated by ethanol, separated by centrifugation and solved for electrophoresis in Tris–acetate-ethylenediaminetetraacetic acid (EDTA) buffer (pH 7). DNA purity was determined according to absorbance ratios of 260 nm and 280 nm by taking 1.6 and 1.8 as reference values. 5 µl DNA samples from different groups along with 100 bp marker were loaded on 1% agarose gel and the samples were allowed to migrate by electrical field at 75 V for 3 h. DNA bands were visualized using ethidium bromide under UV light (Syngene G:BOX Chemi XRQ, Cambridge, United Kingdom).

Transmission Electron Microscopic Analysis

Transmission electron microscopic (TEM) analysis was taken to show morphology in treated and untreated synaptosomes. Synaptosomes were fixed with 2.5% glutaraldehyde for 2 h at room temperature. After, synaptosomes were centrifuged at 18,000×*g* for 10 min. Pellets were washed twice times with phosphate buffer 0.1 M pH 7.4 and post-fixed in 1% osmium tetroxide for 2 h at room temperature. Following, the synaptosomes were stained with uranyl acetate and lead citrate for 4 h, and dehydrated with increasing ethanol concentrations. Finally, they were embedded into Epon resin. Sections were photographed in a JEOL JEM 1220 transmission electron microscope.

Statistical Analysis

SPSS Version 21 was applied for the statistical analysis of biochemical measurements. Our results were shown as mean ± standard deviation. All data were assessed for the normality by using the Kolmogorov-Simirnov and Shapiro–Wilk tests. Data conforming to normal distribution, one-way ANOVA was performed, but to data that is not compatible with normal distribution, Kruskal–Wallis test was performed. $P < 0.05$ was considered significant.

Results

The Effects of Pre- and Post-treatment of Curcumin on Oxidative and Apoptotic Markers in the AlCl3-Exposed Synaptosomes

$AlCl_3$ exposure showed in a dose-dependent reduction in MTT levels in the synaptosomes (Fig. 1a). The MTT levels in $AlCl_3$-induced synaptosomes was reduced by 73% compared with the control group ($p < 0.001$). However, treatment with 5 and 10 µg/mL curcumin in Group III and Group IV produced respectively a modest increase of 5% and 12% in MTT levels compared to the control ($p > 0.05$). Moreover, curcumin pre-treatments in the Group V and Group VI did not show an increase in MTT levels compared to the Group II. Noteworthy, we detected a statistically significant decrease in MTT levels in synaptosomes after curcumin post-treatment. 5 and 10 µg/mL curcumin post-treatments led to a significant increase of 59% and 75% in MTT levels compared to the Group II, respectively.

As shown in Fig. 1b, 50 µM $AlCl_3$ exposure increased lipid peroxidation in the synaptosomes. MDA levels in the Group II were significantly higher than the control group (67% of control, $p < 0.01$). Curcumin treatments in the Group III and Group IV did not show any changes in MDA levels compared to the control. Curcumin pre-treatments in the Group V and Group VI caused a slightly decrease in MDA levels compared to the Group II, while MDA levels in the post-treated curcumin groups were found similar to the control. Curcumin post-treatments in the Group VII and Group VIII caused respectively 28% and 41% decreases in MDA levels compared to the Group II.

Synaptosomes treated with 50 µM $AlCl_3$ were found about three-fold increase in NO levels compared to the control (Fig. 1c). Curcumin treatments in the Group III and Group IV did not show any changes in NO levels compared to the control. Synaptosomes pre-treated with curcumin in the Group V and Group VI were shown a decrease in NO levels comparison with the Group II (24% and 31%, respectively). On the other hand, curcumin post-treatments in the Group VII and Group VIII caused further decline in NO levels compared to Group II. In addition, synaptosomes post-treated with 5 and 10 µg/mL curcumin doses were led to 45% and %52 decreases in NO levels compared to the Group II, respectively ($p < 0.05$).

$AlCl_3$-treated synaptosomes in the Group II showed a significant decrease of 42% in GSH levels compared to the control (Fig. 1d). Curcumin treatments in the Group III and Group IV were produced a slight increase in GSH levels compared to the control (8% and 13%, respectively). GSH levels in the pre-treated curcumin groups showed no changes compared to the Group II, while curcumin post-treatments in the Group VII and Group VIII were found a significant increase in GSH levels (35% and 48%, respectively).

We measured the cytochrome c and caspase 3 levels to confirm the apoptosis in synaptosomes following treatment with $AlCl_3$ and curcumin (Fig. 1e, f). Curcumin treatments in the Group III and Group IV did not show any changes in cytochrome c and caspase 3 levels compared to the control, whereas, in the $AlCl_3$-treated group was respectively observed a remarkable increase in cytochrome c and caspase 3 levels (54% and 61% of control, $p < 0.01$). Curcumin pre-treatments in the Group V and Group VI caused a moderate reduction in cytochrome c and caspase 3 levels compared to the Group II ($p > 0.05$). However, synaptosomes in the Group VII showed a significant decrease in cytochrome c and caspase 3 levels compared to the Group II (19% and 43%, respectively). Additionally, cytochrome c and caspase 3 levels in Group VIII were found a decrease of 32% and 45%, respectively.

DNA Fragmentation Levels in the Synaptosomes

Agarose gel electrophoresis was used to demonstrate DNA fragmentation in the synaptosomal fractions. It can be seen from Fig. 2 that $AlCl_3$-exposed synaptosomes in the Group II showed an increase in DNA fragmentation levels. Consistently with cytochrome c and caspase 3 results, we found

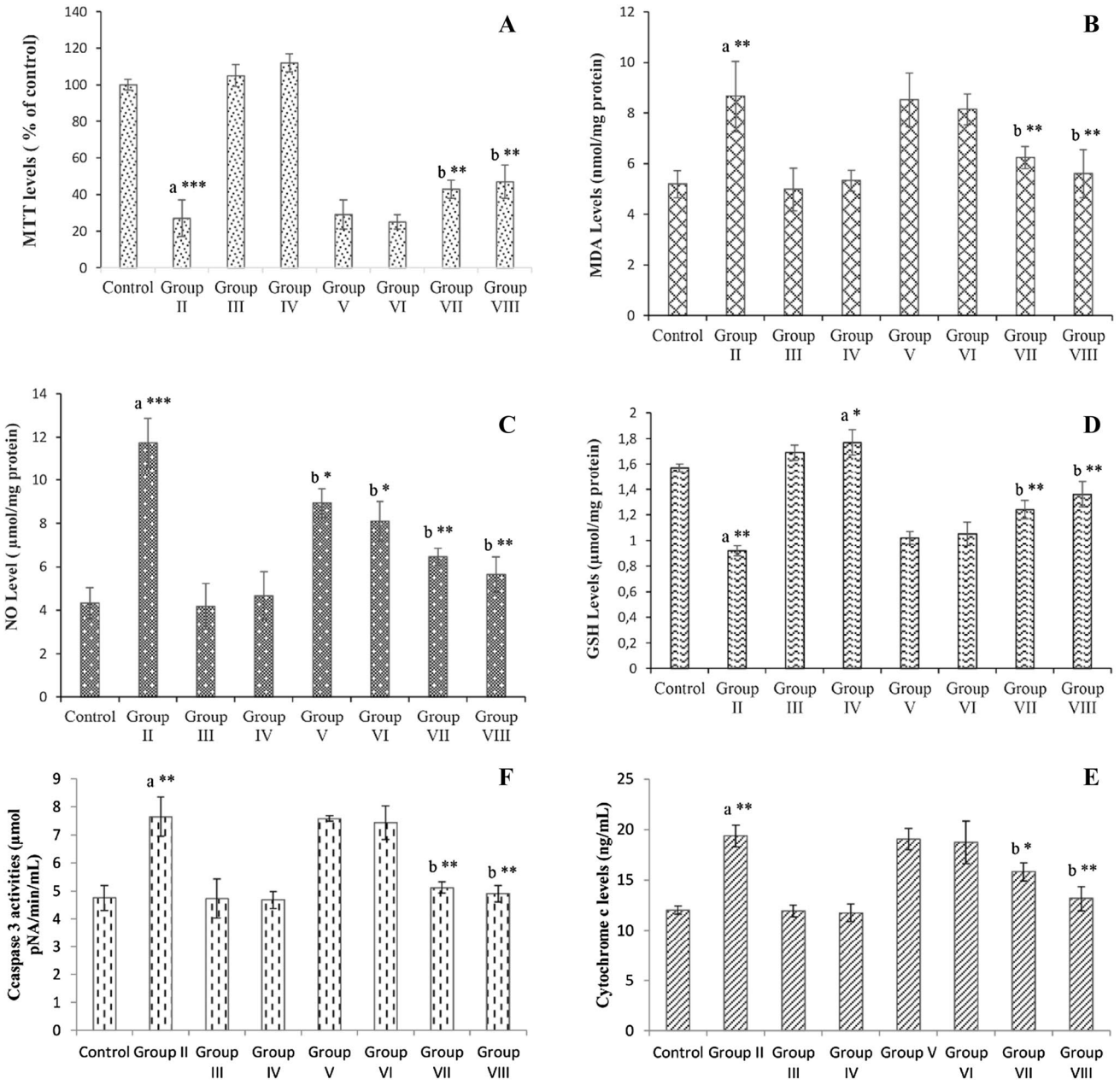

Fig. 1 Effects of pre- and post-treatment curcumin on MTT, MDA, NO, GSH, cytochrome c levels and caspase 3 activities in the $AlCl_3$-induced synaptosomes. **a** MTT levels. **b** MDA levels. **c** NO levels. **d** GSH levels. **e** Cytochrome c levels. **f** Caspase 3 activities. *a* Compared to the control group. *b* Compared to Group II. $*P<0.05$; $**P<0.01$; $***P<0.001$

that curcumin treatments led to a higher decrease in DNA fragmentation levels in the post-treatment groups.

Morphology and Integrity of Synaptosomes

Synaptosomes treated with $AlCl_3$ and curcumin showed morphological variations when compared with the control group. Synaptosomal fractions in the control group displayed organized morphological structures, such as synaptic vesicles and a clear density on synaptic junctions, which incorporates the plasma membranes of the pre- and post-synaptic regions (Fig. 3a). $AlCl_3$-treated synaptosomes in the Group II demonstrated distributed synaptosomal structures including myelin fragments (Fig. 3b). Curcumin treatments in the Group III and Group IV did not show any changes in synaptosomal structures (Fig. 3c). TEM images of synaptosomes in pre-treated groups showed to have a less clear density on the synaptic junctions and to contain vesicular disintegrations (Fig. 3d). The pos-treated synaptosomes, on the other hand, involved

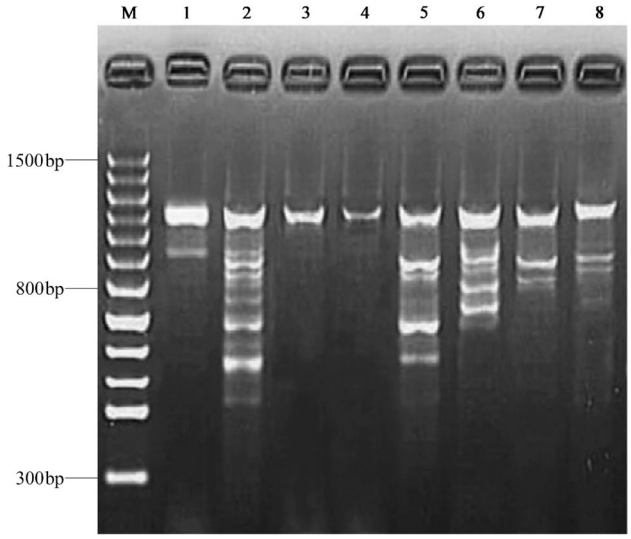

Fig. 2 The effects of curcumin and $AlCl_3$ on synaptosomal DNA fragmentation levels. (M) DNA marker (*1*) Group I (*2*) Group II (*3*) Group III (*4*) Group IV (*5*) Group V (*6*) Group VI (*7*) Group VII (*8*) Group VIII

a clearer spherical synaptic vesicles and junctions in the axon terminals (Fig. 3e).

Discussion

Al^{+3} accumulates highly in hippocampus, a brain region that plays a crucial role in memory, than the other brain regions and influences the biochemical processes associated with neurodevelopmental [24, 31, 32]. This accumulation affects a large number of cellular signaling pathways related to neurodegenerative processes like redox balance, oxidative stress and apoptosis [2, 9]. Previous studies also showed that Al^{+3} led to a marked oxidative damage in the brain by increasing ROS generation mainly via the Fenton reaction, which induces lipid peroxidation [33, 34]. Lipid peroxidation is an important biomarker associated with neuronal damage and neurochemical changes [35]. According to our results, $AlCl_3$ treatment increased the lipid peroxidation by triggering oxidative stress in the hippocampal synaptosomes of rats. Curcumin, a lipophilic polyphenol isolated from the rhizome of Curcuma longa, maintains the cell membrane integrity and acts as a strong protector against oxidative stress [36, 37]. Khan et al. [38] indicated that aluminum-treatment significantly increased the lipid peroxidation in rat brain and

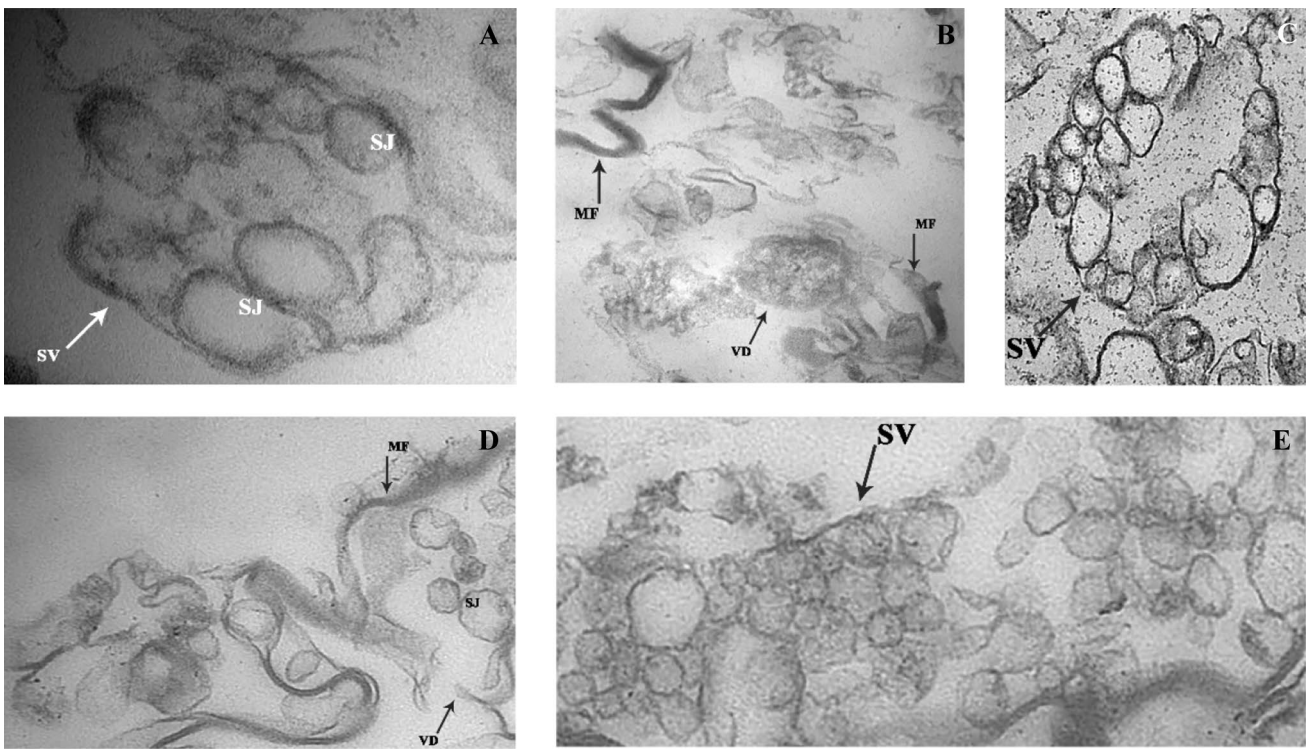

Fig. 3 Electron microscopy images of synaptosomal fractions. **a** Control group. **b** Group II. **c** Curcumin treatment. **d** Pre-treated groups. **e** Post-treated groups. *SV* synaptic vesicle, *SJ* synaptic junction, *VD* vesicular disintegration, *MF* myelin fragment. Scale bar is 200 nm. Magnification: × 150,000

co-administration of curcumin with aluminum treatment inhibited lipid peroxidation in curcumin-treated rats. It was also reported that curcumin has an ability to modulate lipid peroxidation in long term chronic aluminum-intoxicated rats [39]. Moreover, in this study, we assessed whether curcumin pre- and post-treatment inhibited the $AlCl_3$-induced lipid peroxidation. Our results showed that curcumin alleviated significantly $AlCl_3$-induced lipid peroxidation in the post-treated synaptosomes due to its antioxidant properties.

NO is produced from L-arginine and oxygen by the nitric oxide synthase (NOS) enzyme [40]. Abnormal NOS activation during aluminum treatment causes to enhance in NO levels that also contribute to deterioration of intracellular signaling mechanisms [41]. Kaizer et al. [42] reported that aluminum exposure led to oxidative damage by increasing NO levels in different mouse brain regions. Similarly, our data displayed that NO levels increased in $AlCl_3$-induced synaptosomes. On the other hand, it was shown that curcumin can play a crucial role in down-regulation of NOS expression [43] and specifically scavenge RNS in rats after aluminum exposure [34]. Consistently, we demonstrated that NO levels in $AlCl_3$-induced synaptosomes were normalized by curcumin treatment. In addition, curcumin post-treatment groups exhibited a higher decrease in NO levels.

GSH forms a large part of the intracellular thiol pool and protects the cell from oxidative stress [44]. It has been well known that the reduction of GSH levels in the brain following aluminum exposure appeared the result of the attack caused by increased ROS [45]. In our study, we observed a decrease in GSH levels in $AlCl_3$-treated synaptosomes. Besides, previous study showed that co-administration of curcumin with Al was demonstrated to support the antioxidant mechanism in the brain [46]. The presence of hydroxyl groups in curcumin scavenges the ROS by producing the electron transfer through the phenolic sites [47]. This further can be in charge of suppressing the Al-induced increase of redox instability [48]. Guangwei et al. [49] also showed that curcumin treatment elevated GSH levels in rat brain. In addition, previous study reported that curcumin treatment inhibited oxidative damage by enhancing GSH levels in rats [50]. Furthermore, in this present study, the curcumin post-treatment produced a significant increase in GSH levels in $AlCI_3$-exposed synaptosomes. According to our results, curcumin post-treatment in $AlCI_3$-exposed synaptosomes supported the antioxidant mechanisms more than curcumin pre-treatment.

Al^{+3} accumulation in the brain mediates apoptotic and genotoxic processes [51]. The subcellular concentration and translocation of apoptosis-regulating proteins can provide valuable information about the fate of cells. Cytochrome c situated between mitochondrial membranes plays a key role in the apoptotic process [52]. The release of mitochondrial cytochrome c into the cytoplasm where it activates the caspase 3 which in turn activates the apoptosis. Sood et al. [45] reported that aluminum treatment (100 mg/kg oral gavage for 8 weeks) led to mitochondrial dysfunctions in rat cerebral cortex, mid brain, and cerebellum regions. There was also reported that intracisternal administration of aluminum into rabbit brain increased cytochrome c release, activates caspase 3 and DNA fragmentation [53]. Similarly, we showed that $AlCl_3$-treated synaptosomes showed an increase in cytochrome c release, caspase 3 activation and DNA fragmentation. In the literature, curcumin supplementation was expressed to show simultaneously a significant amelioration in apoptotic, genotoxic and oxidative stress-related indices [54, 55]. Additionally, it was shown to have a potential to protect DNA damage by inhibiting oxidative stress and apoptosis [56]. Our data demonstrated that curcumin treatment inhibited the cytochrome c release and decreased caspase 3 activity and DNA fragmentation in $AlCI_3$-induced synaptosomes. A reduction in the elevated cytochrome c release and caspase 3 activity is a protective event against induction of apoptotic pathways. Amelioration in DNA fragmentation by curcumin treatment was consistent with the cytochrome c levels and caspase 3 activities. In this study, we also investigated whether pre-protective or post-protective effects of curcumin related to $AlCl_3$-induced neurotoxicity. Our results displayed that the decrease of apoptosis and DNA fragmentation in $AlCl_3$-treated synaptosomes were higher in the post-protective groups.

In recent years, synaptosomes have often been used as a model to understand the synaptic molecular mechanisms underlying neurodegenerative diseases [57]. In this study, we specially selected the brain hippocampus region of rats, which were more efficient for the isolation of synaptosomal enriched fractions. In addition, it was reported that synaptosomes isolated from hippocampus were more susceptible to neurodegenerative damage and oxidative stress [58]. Synaptosomes are membranous sacs containing synaptic components and are produced by subcellular fraction of homogenized nerve tissue. Besides, they include the post- and pre-synaptic membrane and the synaptic junctions, as well as the complete presynaptic terminal containing mitochondria and synaptic vesicles. Previous study reported that aluminum-toxicated rats exhibited vesicular disintegrations like heterogeneous cytoplasm with abnormal sub-cellular structures in TEM brain images [39]. Our TEM images indicated that $AlCl_3$ treatment caused neurotoxicity by inflicting damage in the synaptosome membrane structures (Fig. 3b). However, curcumin treatment was effective in preventing the aluminum-induced alterations like increase of lipofuscin accumulation, cytoplasmic vacuolation and mitochondrial abnormalities in rat brain cells. In this present study, curcumin treatment showed organized membrane structures including a clear density on the synaptic junctions in $AlCl_3$-induced synaptosomes (Fig. 3c, e).

Conclusion

Our data verifies that curcumin has post-protective effects rather than pre-protective effects following $AlCl_3$-induced neurotoxicity. To the best of our knowledge, the current study, which is the preliminary research, is the first data indicating that curcumin acts as a post-protective (therapeutic) agent against oxidative stress and apoptosis induced by $AlCl_3$ in hippocampal synaptosomes of rats. Therefore, we conclude that curcumin may be a suitable therapeutic agent, especially in the prognosis of neurodegenerative diseases. However, there should be further investigations for the therapeutic (post-protective) effects of curcumin with in vivo studies, especially associated with neurodegenerative disease. Therefore, in our future studies, we will firstly examine the pre- and post-protective effects of curcumin on cell lines (neurons and/or astrocytes), and then investigate behavioral tests in neurodegenerative rat models in vivo.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interests.

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