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Research report

Protective effect of curcumin (*Curcuma longa*), against aluminium toxicity: Possible behavioral and biochemical alterations in rats

Anil Kumar*, Samrita Dogra, Atish Prakash

Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India

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ABSTRACT

Aluminium is a potent neurotoxin and has been associated with Alzheimer's disease (AD) causality for decades. Prolonged aluminium exposure induces oxidative stress and increases amyloid beta levels in vivo. Current treatment modalities for AD provide only symptomatic relief thus necessitating the development of new drugs with fewer side effects. The aim of the study was to demonstrate the protective effect of chronic curcumin administration against aluminium-induced cognitive dysfunction and oxidative damage in rats. Aluminium chloride (100 mg/kg, p.o.) was administered to rats daily for 6 weeks. Rats were concomitantly treated with curcumin (*per se*; 30 and 60 mg/kg, p.o.) daily for a period of 6 weeks. On the 21st and 42nd day of the study behavioral studies to evaluate memory (Morris water maze and elevated plus maze task paradigms) and locomotion (photoactometer) were done. The rats were sacrificed on 43rd day following the last behavioral test and various biochemical tests were performed to assess the extent of oxidative damage. Chronic aluminium chloride administration resulted in poor retention of memory in Morris water maze, elevated plus maze task paradigms and caused marked oxidative damage. It also caused a significant increase in the acetylcholinesterase activity and aluminium concentration in aluminium treated rats. Chronic administration of curcumin significantly improved memory retention in both tasks, attenuated oxidative damage, acetylcholinesterase activity and aluminium concentration in aluminium treated rats ($P < 0.05$). Curcumin has neuroprotective effects against aluminium-induced cognitive dysfunction and oxidative damage.

1. Introduction

Alzheimer's disease (AD) is an age related neurodegenerative disorder comprising complex neurobiochemical and neuropathological events. It is characterized by extracellular Aβ deposits [27] intraneuronal neurofibrillary tangles [14] and selective neuronal loss. The etiology of this disorder is multifactorial including genetics, head trauma, oxidative stress, inflammation and environmental factors including aluminium toxicity. The potential association of aluminium and AD began with the observations of Klatzo et al. [33] who demonstrated neurofibrillary degeneration in rabbits following aluminium exposure. Experimentally it has been demonstrated that chronic exposure to aluminium not only causes neurologic signs which mimic progressive neurodegeneration but also results in neurofilamentous changes in the hippocampus, cerebral cortex, brain stem, spinal cord, and biochemical changes which are seen in AD [5]. However, substantial evidence also exists which contradicts the link between AD and aluminium [6,40] thereby catalyzing controversies on whether aluminium is trivial or a significant factor involved in AD [42]. Besides, increased concentration of aluminium in brain has also been observed in neuritic deposits, plaques and neurofibrillay tangles in Alzheimer's brain [41]. It has been reported that aluminium accumulates significantly in hippocampus following chronic exposure of aluminium [28]. Aluminium gains access to the brain via the specific high affinity receptors for transferrin (TfR) expressed in the blood brain barrier [50]. Upon entering the brain it affects the slow and fast axonal transports, induces inflammatory responses [9], inhibits long-term potentiation, causes synaptic structural abnormalities thereby resulting in profound memory loss [31]. At the molecular level, it influences DNA topology, gene transcription [39] and cellular energy metabolism. It induces misfolding and self-aggregation of highly phosphorylated cytoskeletal proteins such as neurofilaments or microtubule-associated proteins and Aβ which are implicated in AD [29,30]. It is a potent cholinotoxin [25] and causes apoptotic neuronal loss which is a characteristic symptom of neurodegeneration associated with AD [21]. Aluminium is a non-redox active metal which is capable of increasing the cellular oxidative milieu by potentiating the pro-oxidant properties of transition metals such as iron and copper [6]. It leads to progres-

Abbreviations: AD, Alzheimer's disease; DNA, deoxyribonucleic acid; ROS, reactive oxygen species; DTNB, dithiobisnitrobenzoic acid; H_2O_2, hydrogen peroxide; GSH, reduced glutathione; LOX, lipooxygeanse; NO, nitric oxide.

* Corresponding author. Tel.: +91 172 2534106; fax: +91 172 2541142.
E-mail address: kumaruips@yahoo.com (A. Kumar).

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sive deterioration of mitochondrial function [3] which culminates into excessive free radical generation eventually resulting in DNA damage, nitration of protein residues and lipid peroxidation.

Antioxidants and plant phenolics are being tried as chemoprotective agents in epidemiological and experimental studies to regulate the progression of oxidative stress related diseases. Curcumin is a hydrophobic polyphenol derived from the rhizome of herb *Curcuma longa* belonging to family zingiberaceae. It has been shown to exhibit wide variety of biological and pharmacological activities namely antioxidant, anti-inflammatory [51,55], antimicrobial and anticarcinogenic [32,37] activities. Curcumin has been reported to be a dual inhibitor of COX-2 and LOX thus acting as a potent anti-inflammatory agent. It can intercept and neutralize potent pro-oxidants and carcinogens. In fact curcumin can suppress oxidative damage, inflammation, cognitive deficits and amyloid accumulation [59] which is the characteristic features of AD. Further, curcumin has been reported to inhibit the formation of amyloid oligomers, fibrils, bind plaques and reduce amyloid in vivo [1]. Apart from AD, therapeutic benefits of curcumin have also been demonstrated in ethanol induced oxidative injury in brain, CCl_4-induced hepatic injury [19], cadmium-induced oxidative damage [17] and cyclosporine-induced renal dysfunction [56]. Based on this background, present study was designed to investigate the neuroprotective effect of curcumin against aluminium-induced cognitive impairment and associated oxidative damage in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (180–200 g) procured from Central Animal House, Panjab University, Chandigarh were used. Animals were acclimatized to the laboratory conditions at room temperature prior to the experimentation. Animals were kept under standard conditions of a 12 h light/dark cycle with food and water ad libitum in plastic cages with soft bedding. All the experiments were carried out between 9.00 and 15.00 h. The protocol was approved by the Institutional Animal Ethics Committee and was carried out in accordance with the Indian National Science Academy Guidelines for the use and care of animals.

2.2. Drugs and treatment schedule

Aluminium chloride (CDH, India) and curcumin (Sigma chemicals Co., St. Louis, MO, USA) solutions were made freshly at the beginning of each experiment. For oral administration, aluminium chloride was dissolved in drinking water and curcumin was dissolved in 0.5% carboxymethyl cellulose and administered in a dose of 0.5 ml/100 g body weight. Animals were randomized into six groups based on their body weight. Each group having minimum seven number of animals. The groups were as follows:

Group 1: naïve (received vehicle for aluminium chloride and curcumin) ($n = 7$)
Group 2: aluminium chloride ($AlCl_3$) treated (100 mg/kg; p.o.) + vehicle for curcumin ($n = 7$)
Group 3: curcumin (30 mg/kg; p.o.) + vehicle for aluminium chloride ($n = 7$)
Group 4: curcumin (60 mg/kg; p.o.) + vehicle for aluminium chloride ($n = 7$)
Group 5: curcumin (30 mg/kg; p.o.) + aluminium chloride (100 mg/kg; p.o.) ($n = 7$)
Group 6: curcumin (60 mg/kg; p.o.) + aluminium chloride (100 mg/kg; p.o.) ($n = 7$)

The doses of curcumin and aluminium chloride were selected based on those reported in literature. The study was carried out for a period of 42 days (6 weeks). The drug was administered orally 1 h after aluminium chloride administration.

2.3. Behavioral assessment

2.3.1. Assessment of cognitive performance

2.3.1.1. Spatial navigation task. The acquisition and retention of a spatial navigation task was evaluated by using Morris water maze [18]. Animals were trained to swim to a visible platform in a circular pool (180 cm in diameter and 60 cm in height) located in a test room. In principle rats can escape from swimming by climbing onto the platform and over time the rats apparently learn the spatial location of the platform from any starting position at the circumference of the pool. The pool was filled with water (28 ± 2 °C) to a height of 40 cm a movable circular platform (9 cm diameter), mounted on a column was placed in a pool 2 cm above the water level during the acquisition phase. A similar platform was placed in the pool 2 cm below the water level for the maze retention phase. During both the phases the platform was placed in the centre of one of the quadrants. The water was made opaque by adding a non-toxic dye. Four equally spaced locations around the edge of the pool (N, S, E, and W) were used as starting points and this divided the pool into four equal quadrants.

2.3.1.1.1. Maze acquisition phase (training). Animals received a training session consisting of four trials on day 20. In all four trials, the starting position was different. A trial began by releasing the animal into the maze facing towards the wall of the pool. The latency to find the escape platform was recorded to a maximum of 90 s. If the rat did not escape onto the platform within this time it was guided to the platform and was allowed to remain there for 20 s. The time taken by rat to reach the platform was taken as the initial acquisition latency (IAL). At the end of the trial the rats were returned to their home cages and a 5 min gap was given between the subsequent trials.

2.3.1.1.2. Maze retention phase (testing for retention of the learned task). Following 24 h (day 21) and 21 days (day 42) after IAL, rat was released randomly at one of the edges facing the wall of the pool and tested for retention of response. The time taken to find the hidden platform on day 21 and day 42 following start of aluminium chloride administration was recorded and termed as first retention latency (1st RL) and second retention latency (2nd RL), respectively.

2.3.1.2. Elevated plus maze paradigm. The elevated plus maze consisted of two opposite black open arms (50 cm × 10 cm), crossed with two closed walls of the same dimensions with 40 cm high walls. The arms were connected with a central square of dimensions 10 cm × 10 cm the entire maze was placed 50 cm high above the ground. Acquisition of memory was tested on day 20 from the start of aluminium chloride administration. Rats were placed individually at one end of the open arm facing away from the central square. The time taken by the animal to move from the open arm to the closed arm was recorded as the initial transfer latency (ITL). Animals were allowed to explore the maze for 20 s after recording the ITL and were then returned to the home cages. If the animal did not enter the enclosed arm within 90 s, it was pushed on the back into one of the enclosed arm and the ITL was recorded as 90 s. Retention of memory was assessed by placing the rat in an open arm and the retention latency was noted on day 21 and day 42 of the ITL and was termed as the first retention transfer latency (1st RTL) and second retention transfer latency (2nd RTL), respectively [52].

2.3.2. Assessment of gross behavioral activity

Gross behavioral activity was observed at the end of each week for total of 6 weeks since the initiation of aluminium chloride treatment. Each animal was placed in a square (30 cm) closed arena equipped with infra-red light sensitive photocells using digital photoactometer. The animals were observed for a period of 5 min and the values were expressed as counts/5 min. The apparatus was placed in a darkened, light and sound attenuated and ventilated test room [49].

2.3.3. Biochemical assessment

Biochemical tests were conducted 24 h after the last behavioral test. The animals were sacrificed by decapitation. Brains were removed and rinsed with ice-cold isotonic saline. Brains were then homogenized with ice-cold 0.1 mmol/l phosphate buffer (pH 7.4). The homogenate (10%, w/v) was then centrifuged at 10,000 × *g* for 15 min and the supernatant so formed was used for the biochemical estimations.

2.3.3.1. Measurement of lipid peroxidation. The extent of lipid peroxidation in the brain was determined quantitatively by performing the method as described by Wills [58]. The amount of malondialdehyde (MDA) was measured by reaction with thiobarbituric acid at 532 nm using Perkin Elmer Lambda 20 UV VIS Spectrophotometer (Norwalk, CT, USA). The values were calculated using the molar extinction co-efficient of chromophore (1.56×10^5 $(mol/l)^{-1}$ cm^{-1}).

2.3.3.2. Estimation of nitrite. The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide was determined by a colorimetric assay with Greiss reagent (0.1% *N*-(1-napthhthyl) ethylene diamine dihydrochloride, 1% sulphanilamide and 5% phosphoric acid) [24]. Equal volumes of the supernatant and the Greiss reagent were mixed and the mixture was incubated for 10 min at room temperature in the dark. The absorbance was measured at 540 nm using Perkin Elmer Lambda 20 UV VIS Spectrophotometer (Norwalk, CT, USA). The concentration of nitrite in the supernatant was determined from sodium nitrite standard curve.

2.3.3.3. Estimation of reduced glutathione. Reduced glutathione was estimated according to the method described by Ellman et al. [15]. A 1 ml supernatant was precipitated with 1 ml of 4% sulphosalicylic acid and cold digested for 1 h at 4 °C. The samples were then centrifuged at 1200 × *g* for 15 min at 4 °C. To 1 ml of the supernatant obtained, 2.7 ml of phosphate buffer (0.1 mmol/L, pH 8) and 0.2 ml of 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) was added. The yellow color developed was measured at 412 nm using Perkin Elmer Lambda 20 UV VIS Spectrophotometer (Norwalk, CT, USA). Results were calculated using the molar extinction co-efficient of the chromophore (1.36×10^4 $(mol/l)^{-1}$ cm^{-1}).

2.3.3.4. Estimation of antioxidant enzyme activities.

2.3.3.4.1. Superoxide dismutase activity. SOD activity was assayed by the method of Kono [35]. The assay system consisted of EDTA 0.1 mM, sodium carbonate 50 mM and 96 mM of nitro blue tetrazolium (NBT). In the cuvette, 2 ml of the above mixture, 0.05 ml of hydroxylamine and 0.05 ml of the supernatant were

added and the auto-oxidation of hydroxylamine was measured for 2 min at 30 s interval by measuring the absorbance at 560 nm using Perkin Elmer Lambda 20 UV VIS Spectrophotometer (Norwalk, CT, USA).

2.3.3.4.2. Catalase activity. Catalase activity was assessed by the method of Luck [38], wherein the breakdown of hydrogen peroxide is measured. Briefly, the assay mixture consisted of 3 ml of H_2O_2 phosphate buffer and 0.05 ml of the supernatant of the tissue homogenate. The change in absorbance was recorded for 2 min at 30 s interval at 240 nm using Perkin Elmer Lambda 20 UV VIS Spectrophotometer (Norwalk, CT, USA). The results were expressed as micromoles of hydrogen peroxide decomposed per min per mg protein.

2.3.3.4.3. Glutathione-S-transferase activity. The activity of glutathione-*S*-transferase was assayed by the method of Habig and Jakoby [26]. Briefly the assay mixture consisted of 2.7 ml of phosphate buffer, 0.1 ml of reduced glutathione, 0.1 ml of 1-chloro-2,4-dinitrobenzene (CDNB) as substrate and 0.1 ml of supernatant. The increase in the absorbance was recorded at 340 nm for 5 min at 1 min interval using Perkin Elmer Lambda 20 UV VIS Spectrophotometer (Norwalk, CT, USA). The results were expressed as nmol of CDNB conjugated/min/mg protein.

2.3.3.5. Estimation of acetyl cholinesterase (AchE) activity. AchE is a marker of extensive loss of cholinergic neurons in the forebrain. The AchE activity was assessed by Ellman method [16]. The assay mixture contained 0.05 ml of supernatant, 3 ml of sodium phosphate buffer (pH 8), 0.1 ml of acetylthiocholine iodide and 0.1 ml of DTNB (Ellman reagent). The change in absorbance was measured for 2 min at 30 s interval at 412 nm using Perkin Elmer Lambda 20 UV VIS Spectrophotometer (Norwalk, CT, USA). Results were expressed as micromoles of acetylthiocholine iodide hydrolyzed per min per mg protein.

2.3.3.6. Protein estimation. The protein content was estimated by Biuret method [23] using bovine serum albumin as a standard.

2.3.3.7. Aluminium estimation. The aluminium was analyzed by wet acid digestion method of Zumkley et al. [62] in hippocampus and cortex of brain. A mixture of 2.5 ml of per choleric acid/nitric acid (1:4 in volume) was added to brain parts. Then, mixture with brain sample was placed in sand bath for 44 h until the point of a white ash or residue was obtained. Then residues were dissolved in 2.5 ml of 10 mM nitric acid. Then this sample (in liquid form) was placed in the sample holder of atomic absorption spectrophotometer (Perkin Elmer, India). The total concentration of aluminium was calculated in μg/gm of tissue or PPM.

2.3.4. Statistical analysis

Values are expressed as mean ± SEM. The behavioral assessment data were analyzed by a repeated measures two-way analysis of variance (ANOVA) with drug-treated groups as between and sessions as the within-subjects factors. The interaction drug treatment × session was considered to test for drug effect on retention. The biochemical estimations were separately analyzed by one-way ANOVA. Post hoc comparisons between groups were made using Tukey's test. $P < 0.05$ was considered significant.

3. Results

3.1. Effect of curcumin on memory performance in spatial navigation task paradigm in aluminium chloride treated rats

In the spatial navigation task, naïve and curcumin *per se* (30 and 60 mg/kg, p.o.) group of animals quickly learned to swim directly to the platform in the Morris water maze on day 20. Aluminium chloride treated rats showed an initial increase in escape latency, which declined with continued training during the acquisition of a spatial navigation task on day 20. There was a significant difference in the mean IAL of aluminium chloride treated group when compared to naive group on day 20 indicating that chronic administration of aluminium chloride impaired acquisition of spatial navigation task ($P < 0.05$). In contrast, concomitant administration of curcumin (30 and 60 mg/kg, p.o.) with aluminium chloride significantly decreased the IAL to reach the platform in the pre-trained rats as compared to aluminium chloride treated rats on day 20 (Table 1). Following training, the mean retention latencies (1st and 2nd RL) to escape onto the hidden platform was significantly decreased in naïve group on days 21 and 42, respectively as compared to IAL on day 20 since the initiation of aluminium chloride treatment. On the contrary, the performance in the aluminium chloride treated rats had changed after initial training in the water maze on days 21 and 42, with significant increase in mean retention latencies compared to IAL on day 20. The results suggest that aluminium chloride caused significant cognitive impairment. However, chronic curcumin treatment (30 and 60 mg/kg, p.o.) in aluminium chloride treated rats showed a significant decline in the 1st and 2nd RL as compared to aluminium chloride treated rats on days 21 and 42, respectively (Table 1) and improved the retention performance of the spatial navigation task.

Table 1
Effect of curcumin (CMN; 30 and 60 mg/kg, p.o.) on spatial navigation task in aluminium chloride treated rats.

Treatment (mg/kg)	Mean latency (s)		
	IAL	1st RL	2nd RL
Naïve	40.5 ± 1.29	11.33 ± 1.4	8.16 ± 2.16
$AlCl_3$ (100)	85.0 ± 1.93[a]	77.0 ± 1.43[a]	69.33 ± 1.80[a]
CMN (30)	61.33 ± 1.51	13.83 ± 1.945	11.16 ± 2.16
CMN (60)	60.8 ± 1.077	11.83 ± 1.09	9.66 ± 1.421
CMN (30) + $AlCl_3$	69.6 ± 2.470[b]	39.65 ± 1.577[b]	32.15 ± 0.475[b]
CMN (60) + $AlCl_3$	63.4 ± 1.438[b,c]	24.0 ± 0.569[b,c]	20.5 ± 1.576[b,c]

The initial acquisition latencies (IAL) on day 20 and retention latencies on days 21 (1st RL) and 42 (2nd RL) following aluminium chloride treatment were observed in Morris water maze. Values are mean ± SEM. *Note*: $AlCl_3$: aluminium chloride; CMN: curcumin.

[a] $P < 0.05$ as compared to naive group.

[b] $P < 0.05$ as compared to aluminium chloride treated group.

[c] $P < 0.05$ as compared to CMN (30) + $AlCl_3$ group; repeated measures two-way ANOVA followed by Tukey's test for multiple comparisons.

3.2. Effect of curcumin on memory performance in elevated plus maze task paradigm in aluminium chloride treated rats

In the elevated plus maze task, mean ITL on day 20 for each rat was relatively stable and showed no significant variation. All the rats entered the closed arm within 90 s. Following training, naïve and curcumin-treated (30 and 60 mg/kg) rats entered closed arm quickly and mean retention transfer latencies (1st RTL and 2nd RTL) to enter closed arm on days 21 and 42 were shorter as compared to ITL on day 20 of each group, respectively. In contrast, aluminium chloride treated rats performed poorly throughout the experiment and did not show any change in the mean retention transfer latencies on days 21 and 42 as compared to pre-training latency on day 20, demonstrating that chronic aluminium chloride administration induced marked memory impairment. Chronic administration of curcumin (30 and 60 mg/kg) following aluminium chloride administration significantly decreased the mean retention latencies on days 21 and 42 ($P < 0.05$ vs aluminium chloride treated group) (Table 2). The mean transfer latencies of curcumin (30 and 60 mg/kg, p.o.) + aluminium chloride treated groups were signifi-

Table 2
Effect of curcumin (CMN; 30 and 60 mg/kg, p.o.) on memory performance in elevated plus maze paradigm in aluminium chloride treated rats.

Treatment (mg/kg)	Mean transfer latency (s)		
	ITL	1st RTL	2nd RTL
Naïve	61.16 ± 1.79	17.33 ± 4.28	15.34 ± 5.16
$AlCl_3$ (100)	66.76 ± 1.53[a]	79.33 ± 1.33[a]	74.33 ± 1.20[a]
CMN (30)	64.16 ± 1.51	19.16 ± 0.945	13.16 ± 1.16
CMN (60)	63.8 ± 1.077	17.16 ± 1.79	10.16 ± 0.844
CMN (30) + $AlCl_3$	66.3 ± 1.470	44.33 ± 0.83[b]	40.8 ± 0.478[b]
CMN (60) + $AlCl_3$	65.8 ± 1.238	30.6 ± 0.577[b,c]	27.44 ± 0.958[b,c]

The initial transfer latencies (ITL) on day 20 and retention transfer latencies on days 21 (1st RTL) and 42 (2nd RTL) following aluminium chloride treatment were observed. Values are mean ± SEM. *Note*: $AlCl_3$: aluminium chloride; CMN: curcumin.

[a] $P < 0.05$ as compared to naïve group.

[b] $P < 0.05$ as compared to aluminium chloride treated group.

[c] $P < 0.05$ as compared to CMN (30) + $AlCl_3$ group; repeated measures two-way ANOVA followed by Tukey's test for multiple comparisons.

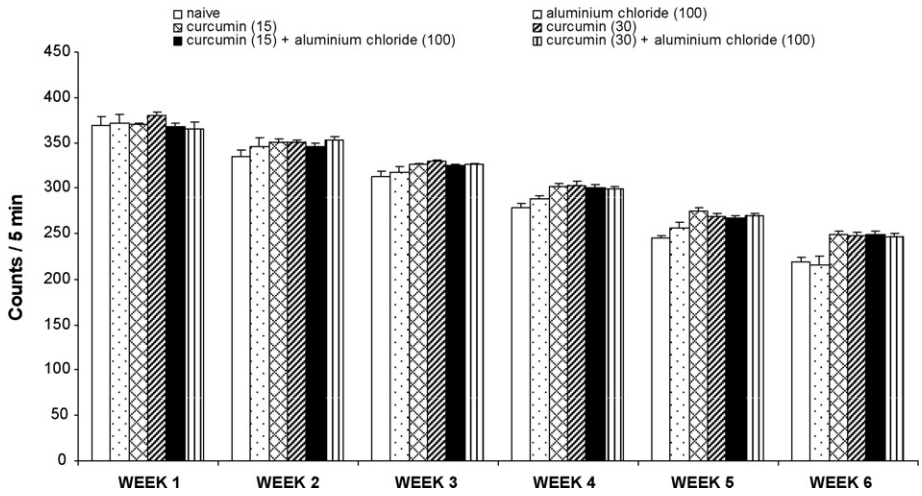

Fig. 1. Effect of curcumin (CMN; 30 and 60 mg/kg, p.o.) on locomotor activity in aluminium chloride treated rats. Values are mean ± SEM. Data were analyzed by two-way ANOVA.

cantly different from that of curcumin *per se* groups on days 21 and 42 ($P < 0.05$) (Table 2).

3.3. Effect of curcumin on locomotor activity in aluminium chloride treated rats

In the present series of experiments, the mean scores of locomotor activity for each rat were relatively stable and showed no significant variation. The mean scores in naïve and aluminium chloride treated rats remained unchanged. Chronic administration of curcumin (30 and 60 mg/kg, p.o.) had no effect on the locomotor activity as compared to naïve rats throughout the study period (Fig. 1). Further, both the dose of curcumin (30 and 60 mg/kg, p.o.) in aluminium chloride treated rats did not cause any alteration in the locomotor activity as compared to aluminium chloride treated rats alone during the study (Fig. 1).

3.4. Effect of curcumin on brain lipid peroxidation, nitrite and reduced glutathione levels in aluminium chloride treated rats

Chronic administration of aluminium chloride caused marked increase in free radical generation and significant rise in brain MDA, nitrite levels and decrease of reduced GSH levels as compared to naïve rats ($P < 0.05$). Further, there were no alteration in the brain MDA levels, nitrite level and reduced GSH levels due to curcumin (30 and 60 mg/kg, p.o.) *per se* treatment as compared to naïve rats. However, simultaneous chronic curcumin (30 and 60 mg/kg, p.o.) administration to aluminium chloride treated rats significantly prevented the increase in MDA, nitrite levels and depletion of reduced GSH (Table 3).

3.5. Effect of curcumin on brain antioxidant enzyme activities in aluminium chloride treated rats

Chronic administration of aluminium chloride caused marked oxidative and nitrosative stress which led to decrease in the antioxidant enzyme activities namely glutathione-*S*-transferase, superoxide dismutase and catalase as compared to naïve rats ($P < 0.05$). Further, curcumin (30 and 60 mg/kg, p.o.) *per se* treatment did not cause any significant alteration in the glutathione-*S*-transferase, superoxide dismutase and catalase activities when compared to naïve rats. However, concomitant chronic curcumin (30 and 60 mg/kg, p.o.) administration to aluminium chloride treated rats caused a significant increase in the levels of glutathione-*S*-transferase, superoxide dismutase and catalase activities (Table 3).

3.6. Effect of curcumin on brain acetylcholinesterase activity in aluminium chloride treated rats

Chronic aluminium chloride administration in rats showed significant decline in the brain AChE activity as compared to naive rats. However, chronic curcumin (30 mg/kg and 60 mg/kg, p.o.) treatment significantly ameliorated the reduction in AChE activity compared to aluminium chloride treated group ($P < 0.05$) (Fig. 2).

Table 3
Effect of curcumin (CMN; 30 and 60 mg/kg, p.o.) on aluminium chloride-induced oxidative stress parameters in rat brain.

Treatment (mg/kg)	MDA levels, nmol MDA/mg protein (% of control)	Nitrite levels, μmol/mg protein (% of control)	Reduced glutathione, nmol/mg protein (% of control)	Catalase, μmol of hydrogen peroxide decomposed/min/mg protein (% of control)	Superoxide dismutase, units/mg protein (% of control)	Glutathione-*S*-transferase, nmol of CDNB conjugated/min/mg protein (% of control)
Naïve	100 ± 10	100 ± 12	100 ± 10	100 ± 12	100 ± 14	100 ± 12
$AlCl_3$ (100)	339 ± 21[a]	229.67 ± 12[a]	23.37 ± 6.8[a]	16.43 ± 12.4[a]	13.45 ± 5[a]	17.68 ± 7[a]
CMN (30)	110.7 ± 15	100.29 ± 16	95.35 ± 17	92.9 ± 13.7	96.17 ± 17	95.64 ± 14
CMN (60)	113.4 ± 28	99.44 ± 26	96.77 ± 15	98.76 ± 10.8	99.16 ± 16	98.65 ± 13
CMN (30) + $AlCl_3$	230.81 ± 27[b]	223.91 ± 8[b]	66.45 ± 5[b]	35.96 ± 6[b]	29.05 ± 8[b]	30.65 ± 8[b]
CMN (60) + $AlCl_3$	166.26 ± 16[b,c]	158.98 ± 7[b,c]	78.61 ± 6.3[b,c]	69.71 ± 5[b,c]	62.52 ± 5[b,c]	63.05 ± 6[b,c]

Values are mean ± SEM. *Note*: $AlCl_3$: aluminium chloride; CMN: curcumin.

[a] $P < 0.05$ as compared to naïve group.

[b] $P < 0.05$ as compared to $AlCl_3$ treated group.

[c] $P < 0.05$ as compared to CMN (30) + $AlCl_3$ group; repeated measures two-way ANOVA followed by Tukey's test for multiple comparisons.

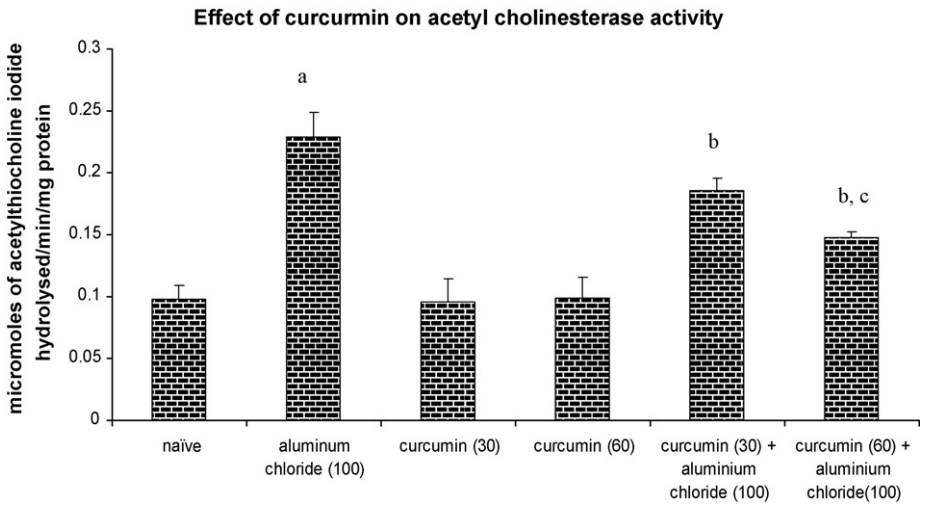

Fig. 2. Effect of curcumin (CMN; 30 and 60 mg/kg, p.o.) on acetyl cholinesterase activity in aluminium chloride treated rats. Values are mean ± SEM. [a] $P < 0.05$ as compared to naive group; [b] $P < 0.05$ as compared to aluminium chloride treated group; [c] $P < 0.05$ as compared to CMN (30) + $AlCl_3$ group; repeated measures two-way ANOVA followed by Turkey's test for multiple comparisons. *Note*: $AlCl_3$: aluminium chloride; CMN: curcumin.

Table 4
Effect of curcumin (CMN; 30 and 60 mg/kg, p.o.) on concentration of aluminium in hippocampus and cortex aluminium chloride treated rat brain.

Treatment	Hippocampus	Cortex
Naive	0.41 ± 0.02	0.46 ± 0.04
$AlCl_3$ (100)	0.97 ± 0.06[a]	0.84 ± 0.06[a]
CMN (30)	0.38 ± 0.01	0.42 ± 0.02
CMN (60)	0.37 ± 0.02	0.4 ± 0.02
CMN (30) + $AlCl_3$	0.52 ± 0.04[b]	0.72 ± 0.07[#]
CMN (60) + $AlCl_3$	0.46 ± 0.06[b]	0.66 ± 0.05[#]

Values are mean ± SEM. Concentration of Al: μg/mg tissue; repeated measures one-way ANOVA followed by Tukey's test for multiple comparisons. *Note*: $AlCl_3$: aluminium chloride; CMN: curcumin.

[a] $P < 0.05$ as compared to naïve group.

[b] $P < 0.05$ as compared to $AlCl_3$ treated group.

[#] Not significant.

3.7. Curcumin's effect on aluminium concentration in aluminium chloride treated rats

Aluminium chloride treatment significantly increased ($P < 0.05$) the levels of aluminium in both hippocampus and cortex areas of rat brain as compared to control rats. However, chronic curcumin (30 mg/kg and 60 mg/kg, p.o.) treatment significantly attenuated the rise in aluminium concentration in hippocampus as compared to control. However, curcumin treatment did not produce significant effect on brain aluminium concentration in cortex as compared to control (Table 4).

4. Discussion

Aluminium is a ubiquitous metal and has been implicated in the etiology of Alzheimer's disease where it exacerbates brain oxidative damage [2,43], causes inflammation and induces Aβ deposition. Alzheimer's disease is characterized by impairment in working memory [20], visuoperception, attention and semantic memory. In present study, chronic exposure of aluminium increased aluminium concentration in hippocampus and cerebral cortex as compared to the control animals. It has been observed that high aluminium level in brain is associated with decline in visual memory and attention concentration in hemodialysis patients [7]. The results of our study indicate that chronic administration of aluminium chloride results in progressive deterioration of spatial memory in both Morris water maze and elevated plus maze task paradigms. Experimentally, it has been shown that intracerebral administration of aluminium chloride causes learning deficits in Morris water maze task in rabbits [46] which is in concordance with our findings. Chronic aluminium treatment leads to impairment of glutamate-NO-cGMP pathway in the cerebellum of rats [10] which can explain the memory impairment and neurobehavioral deficits observed. Chronic administration of curcumin was able to reverse the cognitive deficit, suggesting its potential role as a neuroprotectant against aluminium-induced neurotoxicity.

Aluminium causes marked oxidative damage by increasing the redox active iron concentration in the brain mainly via the Fenton reaction [45]. In our study, chronic administration of aluminium chloride resulted in marked oxidative stress as indicated by increase in lipid peroxidation, nitrite levels, decrease in reduced glutathione levels, catalase, superoxide dismutase and glutathione-*S*-transferase activity. This could be due to the reduced axonal mitochondria turnover, disruption of Golgi and reduction of synaptic vesicles induced by aluminium treatment which results in release of oxidative products like malondialdehyde, carbonyls, peroxynitrites and enzymes like superoxide dismutase within the neurons [5].

Curcumin is a polyphenol found in dietary spice turmeric. It is a lipophilic molecule and structurally resembles ubiquinols and is known to possess strong antioxidant activity [4]. In the present study, curcumin alone did not have any effect on the markers of oxidative stress in brain of normal animals however it significantly attenuated the aluminium chloride-induced oxidative damage. It has been shown to inhibit iron-induced lipid peroxidation [48]. This antioxidant property of curcumin has been attributed to the presence of chain breaking or hydrogen donating phenolic groups in its molecular structure. It has also been shown to inhibit *i*NOS expression [8] and specifically scavenge NO-based radicals [54], which may explain the fact that it caused a decrease in nitrite levels in brain of aluminium chloride treated rats. Rajkrishnan et al. [47] showed that curcumin enhanced the reduced glutathione levels in ethanol intoxicated rats. This lends support to our findings that curcumin caused an increase in the reduced glutathione levels in the aluminium intoxicated rats. Curcumin treatment was able to restore the activity of the various antioxidant enzymes in aluminium chloride treated rats. It has been reported in literature that curcumin increases the levels of SOD and catalase in irradiated mice [34]. In fact curcumin has been reported to be several times more potent scavenger than vitamin E [61]. Not only the parent

compound but its major metabolite tetrahydrocurcumin is a strong antioxidant and has been demonstrated to scavenge free radicals, inhibit lipid peroxidation and formation of hydroperoxides [53]. At molecular level, curcumin is an atoxic natural inhibitor of NF-κB and as a result modulates the expression of various genes such as cyclooxygenase-2, matrix metalloproteinase-9, inducible nitric oxide synthase, interleukin-8 and anti-apoptotic proteins which are regulated by NF-κB [44]. It is also a potent inducer of protective heat shock proteins [11] and inhibitor of lipoxygenases [57]. Curcumin also inhibits amyloid formation by directly inhibiting Aβ aggregation, metal chelation, antioxidant property, hypocholesterolemic effect, modulating β-secretase activity, anti-inflammatory property and modulating the JNK signaling pathways [12]. All these effects may also contribute to its neuroprotective effect.

AD affects mainly the cholinergic system resulting in decreased activity of acetylcholinesterase [13] and choline acetyl transferase [22]. Experimentally aluminium has been shown to decrease acetylcholinesterase in mouse brain [60]. In fact it causes a biphasic effect on the acetylcholinesterase activity, with an initial increase in the activity of this enzyme during 4–14 days of exposure followed by a marked decrease. This has been attributed to the slow accumulation of aluminium in the brain [36]. This also explains the fact that aluminium chloride treatment caused a marked reduction in the acetylcholinesterase activity which was restored by chronic curcumin treatment. Our results also supports that curcumin decreases the concentration of aluminium in hippocampus particularly. The hippocampus and dentate gyrus (DG) of brain are mainly responsible for memory formation. This could be only the mechanistic pathways for the neuroprotective effect of curcumin in cognitive dysfunction of aluminium treated rats.

From the epidemiologic and experimental studies reported, there is ample evidence which supports the fact that aluminium plays a pivotal role in the neuropathology of AD. This study validates the fact that chronic exposure to aluminium causes cognitive dysfunction and related oxidative damage. It clearly demonstrates that curcumin has a neuroprotective effect against aluminium induced behavioral and biochemical changes and further warrants the need for molecular studies to elucidate the mechanisms underlying the protective effects of curcumin.

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