Neuroprotective effect of *Allium cepa* L. in aluminium chloride induced neurotoxicity

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A R T I C L E   I N F O

A B S T R A C T

The present study was envisaged to investigate the neuroprotective potential of *Allium cepa* (*A. cepa*) in aluminium chloride induced neurotoxicity. Aluminium chloride (50 mg/kg/day) was administered orally in mice supplemented with different doses of *A. cepa* hydroethanolic extract for a period of 60 days. Various behavioural, biochemical and histopathological parameters were estimated in aluminium exposed animals. Chronic aluminium administration resulted in significant motor incoordination and memory deficits, which were also endorsed biochemically as there was increased oxidative stress as well as elevated acetylcholinesterase (AChE) and aluminium levels in the brain. Supplementation with *A. cepa* in aluminium exposed animals significantly improved muscle coordination and memory deficits as well as reduced oxidative stress, AChE and decreased abnormal aluminium deposition in the brain. Histopathologically, there was marked deterioration visualized as decreased vacuolated cytoplasm as well as decreased pyramidal cells in the hippocampal area of mice brain which were found to be reversed with *A. cepa* supplementation. Administration of BADGE (PPARγ antagonist) in aluminium exposed animals reversed the neuroprotective potential of *A. cepa* as assessed with various behavioural, biochemical, neurochemical and histopathological estimations. In conclusion, finding of this study suggested significant neuroprotective potential of *A. cepa* in aluminium induced neurotoxicity. Further, the role of PPARγ receptor agonism has also been suggested as a putative neuroprotective mechanism of *A. cepa*, which needs further studies for confirmation.

1. Introduction

Experimental evidence of aluminium induced neurotoxicity subsists since 1965, whereby administration of aluminium has been reported to induce formation of neurofibrillary tangles in rabbits similar to that found in Alzheimer's disease (Klatzo et al., 1965). As per WHO reports, humans get inevitably exposed to aluminium through food, cooking utensils, deodorants, antacids (Kaur and Gill, 2006) apart from occupational exposure in gun, automobile, aerospace and defense related factories (Sińczuk-Walczak et al., 2003; Sharma and Sharma, 2012), increasing the

risk of neurodegenerative diseases such as Alzheimer's, Parkinsonism, etc. (Becaria et al., 2003).

Aluminium gains access to the brain through transferrin mediated transport, which subsequently leads to neurotoxicity (Yokel et al., 1999). Although, the mechanism of aluminium induced neurotoxicity remains elusive, but recent reports suggest elevated oxidative and inflammatory stress markers (Kumar et al., 2009) to be majorly responsible for disruption of intraneuronal metal homeostasis (Julka and Gill, 1995) as well as axonal transport and long term potentiation (Wenting et al., 2014). Thus, involvement of multiple mechanisms in aluminium induced neurotoxicity warrants multitargeted approach for effective treatment. Thus, the use of medicinal plants having multiple pharmacotherapeutic actions appears to be a more rational approach rather than using monotargeted synthetic drugs (Efferth and Koch, 2011; Singh et al., 2014).

A. cepa, a bulbous plant of genus *Allium* has potential to reduce the risk of various chronic diseases, such as cardiovascular, cancer, asthma, diabetes, etc. (Stajner and Varga, 2003; Gabler et al., 2005). Recently, Shri and Bora (2008) reported significant neuroprotective potential of *A. cepa* in ischaemia reperfusion injury induced in

mice. Most of these pharmacotherapeutic effects of A. cepa have been attributed to the presence of various phytoconstituents such as flavonoids (Patil et al., 1995; Cook and Samman, 1996; Kris-Etherton PM et al., 2002), organonosulphur compounds and anthocyanins (Dorsch and Wagner, 1991; Fitzpatrick et al., 1993; Goldman et al., 1996). As oxidative stress plays a significant role in induction of aluminium chloride induced toxicity (Kumar and Gill, 2009), therefore, A. cepa with strong antioxidant and neuroprotective potential was hypothesized to be show ameliorative effects in aluminium chloride induced neurotoxicity. Hence, the present study was envisaged.

2. Materials and methods

2.1. Animals

This study was carried out in Swiss albino male mice weighing 22–28 g, obtained from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana. The animal was housed in groups of 10 mice/cage at an ambient room temperature of 22 ± 2 °C and relative humidity of $50 \pm 5\%$ and maintained under a 12:12 h dark–light cycle (lights on at 07:00 h). Food and water were available ad libitum except during specific experimental protocols. The experimental protocol was approved by the institutional animal ethics committee. All procedures were conducted according to the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), ministry of environment and forest, Government of India.

2.2. Drugs and chemicals

Aluminium chloride was obtained from CDH, India, while Bisphenol A diglycidyl Ether (BADGE) and Quercetin was obtained from Sigma Chemicals Co., St. Louis, MO, USA. All other chemicals used for the biochemical estimations were of AR grade and were freshly prepared before the start of each experiment.

2.3. Plant collection

A. cepa bulbs var. agrifound dark red (ADR) were procured from "National Horticulture Research and Development Foundation" (NHRDF), Karnal (Haryana), India. The collected plant material was authenticated by Dr. L.R. Verma, Director NHRDF, Karnal (Haryana).

2.4. Preparation of extract

The hydroethanolic extraction of dried A. cepa bulbs was carried out using Soxhlet apparatus for 16 h. The extract was dried on rotary evaporator, lyophilized and stored at 4° C for further use. The percentage yield of the extract was found to be 17.6% w/w.

2.5. HPTLC standardization of the Allium cepa hydroethanolic extract

The hydroethanolic extract of A. cepa ADR variety, was standardized using quercetin as a biomarker. The standard marker showed an excellent coefficient of correlation (0.9915). HPTLC chromatographic analysis parameters for quercetin were shown in Table 1. TLC densitometric studies quantified 6.7 mg/g of quercetin in A. cepa hydroethanolic extract.

2.6. Experimental design

Total 56 mice, divided into 7 groups were given different treatments for 60 days as summarized in Table 2. Group 1 served as naïve and treated with saline only. In group 2, aluminium chloride

Table 1
HPTLC chromatographic analysis parameters for standardization of quercetin in A. cepa extract.

Parameter	Estimation of quercetin in Allium cepa L. hydroethanolic extract
Plate material	HPTLC precoated plates Silica Gel 60 F 254
Solvent system	Toluene:ethyl acetate:formic acid (8.5:5.5:1)
Syringe	100 μL Hamilton syringe (Bonadzu, Switzerland)
Application mode	Camag LINOMAT V automatic TLC applicator
TLC Chamber	TLC developing chamber (20 cm × 10 cm)
Development mode	Ascending
Scanning	CAMAG TLC Scanner 4 with WinCATS Software 1.4.8

(50 mg/kg/day) was administered orally. Group 3, 4 and 5 were administered different doses (50, 100 and 200 mg/kg/day) of A. cepa orally with aluminium chloride (50 mg/kg/day). In group 6, animals were administered BADGE (5 mg/kg/day i.p.), A. cepa (200 mg/kg/day) and aluminium chloride (50 mg/kg/day). In group 7, animals were administered with BADGE (5 mg/kg/day i.p.) and aluminium chloride (50 mg/kg/day). At the end of protocol period, behaviour was evaluated and brain was removed and stored at -4 °C for various biochemical and histopathological assays.

2.7. Behavioural estimations

2.7.1. Muscle coordination test using Rota rod apparatus

The Rota rod apparatus was used to determine the motor integrity and muscle coordination of animals as an index of neurotoxicity (Abdel-Aal et al., 2011). The apparatus consists of rotating rod of 1 cm in diameter; 24 cm from the base separated by flanges, rotating at a constant speed of 10 RPM for 5 min. Motor integrity and coordination were assessed by the time latency from placement of the animal on the rotating rod until it falls. Every animal from each group is placed on the rotating rod on the first day of the treatment and on the last day (60th day). Neurotoxicity was assessed as the inability of the animal to maintain equilibrium on the rotating rod for at least 5 min in each of the three trials.

2.7.2. Evaluation of memory using passive shock avoidance paradigm

The Passive shock avoidance behaviour based on negative reinforcement was employed to examine long-term memory (Jarvik and Kopp, 1965). The apparatus (Rolex, Ambala) consisted of a Plexiglas box (27 cm × 27 cm × 27 cm) with a grid floor (3 mm stainless steel rods set 8 mm apart), having a shock free zone (SFZ) (central platform 10 cm × 7 cm × 1.7 cm) on the centre of the grid floor. Electric shock (20 V AC) was delivered to the grid floor. Each mouse was trained to stay on the SFZ for at least 120 s on the 59th day of treatment period; for this the animals were gently placed on

Table 2
Schematic representation for 60 days experimental protocol.

Group	N	Treatment	Experimental design
1	8	NAÏVE	Saline p.o.
2	8	AL 50	AlCl₃ (50 mg/kg/day) p.o.
3	8	AC 50	A. cepa hydroethanolic extract (50 mg/kg/day p.o.) + AlCl₃ (50 mg/kg/day) p.o.
4	8	AC 100	A. cepa hydroethanolic extract (100 mg/kg/day p.o.) + AlCl₃ (50 mg/kg/day) p.o.
5	8	AC 200	A. cepa hydroethanolic extract (200 mg/kg/day p.o.) + AlCl₃ (50 mg/kg/day) p.o.
6	8	BADGE + AC 200	BADGE (5 mg/kg/day i.p.) + A. cepa hydroethanolic extract (200 mg/kg/day) p.o. + AlCl₃ (50 mg/kg/day) p.o.
7	8	BADGE	AlCl₃ (50 mg/kg/day) p.o. + BADGE (5 mg/kg/day i.p.)

Abbreviations: A. cepa – Allium cepa L., AlCl₃ – aluminium chloride, i.p. – Intraperitoneal, p.o. – per oral.

the central platform, and when the mouse stepped down and placing all the paws on the grid floor, shocks were delivered for 15 s. The process was repeated several times until the animal learned to stay on the SFZ for at least 90 s. The number of trials required to learn this task was noted. Retention was tested on the 60th day of the treatment period whereby each mouse was again placed on the SFZ, and then the step down latency (SDL) was recorded. SDL was defined as the time (s) taken by the mouse to step down from the central platform to the grid floor with all paws. SDL was recorded, with an upper cutoff time of 120 s, for 5 min. Increased SDL compared to the control group are the index of memory improving effect (Mishra and Goel, 2012).

2.8. Biochemical estimations

At the end of the protocol, animals were sacrificed by decapitation and the brain was removed. Brain homogenized in 10% w/v 0.1 mmol/l phosphate buffer (pH 7.4) was centrifuged at 14,500 RPM for 15 min at 4 °C. The Clear supernatant obtained was used for estimation of thiobarbituric acid reactive substances, glutathione, catalase, total nitrite/nitrate and AChE levels.

2.8.1. Estimation of thiobarbituric acid reactive substances (TBARS)
The quantitative measurement of TBARS, an index of lipid peroxidation was performed using the method described by Ohkawa et al., 1979. The absorbance was measured spectrophotometrically (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA) at 532 nm. A standard calibration curve was prepared using 1–10 nM of 1,1,3,3-tetra methoxy propane ($y = 0.03x - 0.003$; $r^2 = 0.999$). TBARS value was expressed as nanomoles per mg of protein.

2.8.2. Estimation of reduced glutathione (GSH) levels
The GSH level was estimated employing the method described by Beutler et al., 1963. Absorbance was noted spectrophotometrically (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA) at 412 nm. The GSH standard curve was plotted using 10–100 μM of GSH. The results were calculated using equation ($Y = 0.001x + 0.002$; $r^2 = 0.999$). All the values were expressed as micromoles of glutathione per mg of protein.

2.8.3. Estimation of catalase
The catalase activity was estimated using the method described by Luck et al. (1971) wherein the breakdown of H_2O_2 is measured. Briefly, 3 ml of H_2O_2 phosphate buffer and 0.05 ml of the supernatant of the tissue homogenate were mixed. The change in absorbance was recorded for 2 min at 30 s intervals at 240 nm. The results were expressed as micromoles of hydrogen peroxide decomposed/min/mg of protein.

2.8.4. Estimation of total nitrate/nitrite levels using microplate reader
The method was previously standardized in our laboratory (Choudhary et al., 2013). The method involves the use of the griess diazotization reaction to spectrophotometrically detect total nitrate/nitrite levels. In this method, 50 μL of brain homogenate (filtered) and the standard ($NaNO_2/NaNO_3$) was mixed with 50 μL of the Griess reagent in triplicate using a 96-well plate. In the supernatant well, Cu/Cd alloy was added to ensure the conversion of nitrates to nitrites. The 96-well plate was shaken at 150 RPM for 1 min to ensure proper mixing of the samples/standard with the Griess reagent. The plate was incubated for 30 min at room temperature. The absorbance of nitrite-containing samples was measured at 540 nm using a microplate reader (APR-4 Microplate Reader, Logotech, ISE

Group, Germany) against a photometric reference (blank: 50 μL HPLC grade water and 50 μL Griess Reagent). The total nitrate/nitrite levels were estimated using the straight line equation for nitrite ($y = 0.0008x + 0.0046$, $r^2 = 0.9961$). The results were expressed as ng/g of wet tissue.

2.8.5. Estimation of brain acetylcholinesterase (AChE) activity
Acetylcholinesterase (AChE) activity was assayed as reported in literature (Ellman et al., 1961; Choudhary et al., 2013). Briefly, 40 μL of filtered brain homogenate (source of acetylcholinesterase) was mixed with 80 μL of Ellman's reagent and 200 μL phosphate buffer (pH = 8) in a 96 well-plate and shaken properly. The absorbance of the reaction mixture was recorded prior to the addition of the substrate at 412 nm using a microplate reader (APR-4 Microplate Reader, Logotech, ISE Group, Germany). The reaction was initiated by adding 10 μL of the enzyme substrate (10 mM acetylthiocholine chloride) to each well and was allowed to incubate for 15 min. A yellow colour developed, and the absorption of the solution was measured at 412 nm. A molar extinction coefficient of 14,150 M^{-1} cm^{-1} was used to calculate enzyme activity. Enzyme activity was expressed as l M of acetylcholine hydrolyzed per mg of wet tissue.

2.8.6. Estimation of total brain protein
The brain total protein was determined by Lowry's method with slight modifications (Lowry et al., 1951) using total protein modified biuret, end point assay test kit (Span diagnostics Ltd., Surat, India)

2.9. Estimation of aluminium levels in cortex and hippocampal tissue of mice brain

The aluminium levels were analyzed in the cortex and hippocampal area of the brain collectively using inductively coupled plasma optical emission spectroscopy (ICP-OES) (Yuan et al., 2008). The sample was digested with 2% w/v 5.0 ml ultra-pure nitric acid in a pre-heated block at 65 °C for 4 h. Then, the tube was stirred and diluted to 25 ml with triple distilled water and centrifuged at 1500 × g for 5 min in acid cleaned polypropylene centrifuge tube. Supernatant was collected for determination of aluminium at the emission wavelength 309.27 nm.

2.10. Histopathological studies using haematoxylin and eosin (H&E) staining

After pretreatment with increasing grades of alcohol, paraffin embedding was carried out. Subsequently, coronal section of 15 μm thickness was stained using H&E staining procedure as reported by Ha et al. (2011).

2.11. Statistical analysis

Statistical analysis was carried out using sigma stat statistical software version 3.5. Results were expressed as mean ± SEM and were compared using one-way analysis of variance ANOVA followed by bonferroni multiple comparison test. All results were expressed as mean ± S.E.M. Statistical significance was considered at $p < 0.05$.

3. Results

3.1. Effect of different interventions on motor coordination and memory

Aluminium chloride administration for 60 days significantly ($p < 0.05$) decreased the fall off time in Rota rod apparatus and step down latency (SDL) in passive shock avoidance paradigm
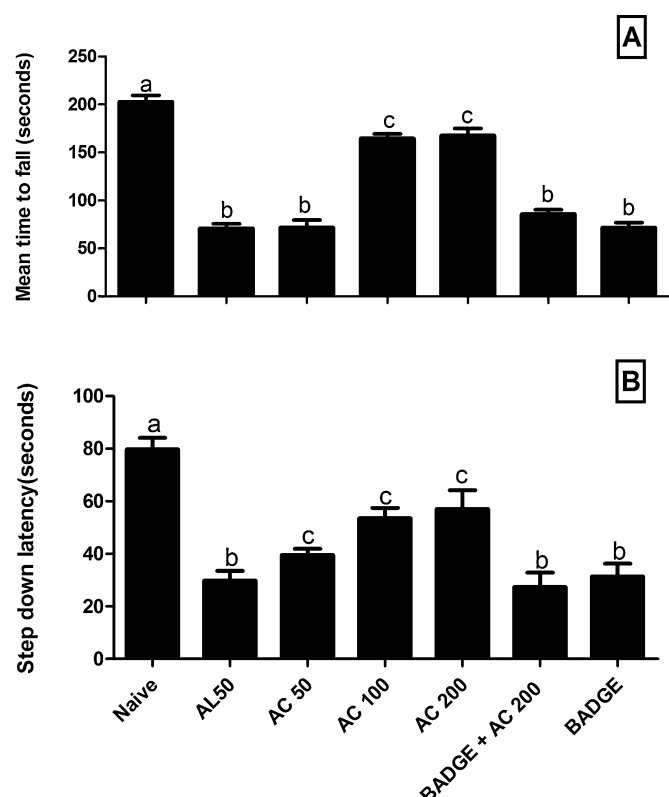
Fig. 1. Effect of *A. cepa* treatment on motor coordination (A) and memory (B) of mice subjected to 60 days of aluminium exposure. All values are represented as mean ± SEM; *n* = 8, Groups (including naïve) not significantly different from naïve are labelled as "a"; groups significantly (*p* < 0.05) different from naïve are labelled as "b" and groups significantly (*p* < 0.05) different from naïve and AL 50 are labelled as "c". Naïve – saline; AL 50 – aluminium chloride (50 mg/kg/day); AC 50 – *A. cepa* (50 mg/kg/day) + aluminium chloride (50 mg/kg/day); AC 100 – *A. cepa* (100 mg/kg/day) + aluminium chloride (50 mg/kg/day); AC 200 – *A. cepa* (200 mg/kg/day) + aluminium chloride (50 mg/kg/day); BADGE + AC200 – BADGE (5 mg/kg i.p.) + *A. cepa* (200 mg/kg/day) + aluminium chloride (50 mg/kg/day) and BADGE–BADGE (5 mg/kg i.p) + aluminium chloride (50 mg/kg/day).

apparatus (*p* < 0.05) in comparison to naïve animals. *A. cepa* co-administration with aluminium exhibited significant (*p* < 0.05) dose dependent improvement in motor coordination and SDL (*p* < 0.05) as compared to aluminium control group. Pretreatment of BADGE (5 mg/kg, i.p.) again decreased the fall off time and SDL in comparison to *A. cepa* treated aluminium exposed group as shown in Fig. 1(a) and (b).

3.2. Biochemical estimations

3.2.1. Effect of different drug interventions on lipid peroxidation, total nitrate/nitrite, reduced glutathione and catalase levels activity in mice brain

Chronic administration of aluminium chloride has significantly elevated lipid peroxide and nitrate/nitrite levels with decreased GSH and catalase activity as compared to naïve animals (*p* < 0.05). However *A. cepa* co-administration (50, 100 and 200 mg/kg) with aluminium chloride significantly attenuated oxidative damage, indicated by reduction in lipid peroxidation, nitrate/nitrite levels with elevated GSH and catalase activities. In BADGE pretreated (5 mg/kg, i.p.) group, the neuroprotective effect of *A. cepa* was reversed as shown in Table 3.

3.2.2. Effect of different drug interventions on brain acetylcholinesterase (AChE level)

Chronic aluminium chloride exposure for 60 days significantly increased AChE activity in comparison to naïve animals. However *A. cepa* dose dependent (50, 100 and 200 mg/kg) treatment with aluminium chloride administration significantly attenuated AChE activity (*p* < 0.05). Pretreatment with BADGE (5 mg/kg, i.p.) showed reversal of memory improving effects in *A. cepa* treated aluminium exposed animals, as there was significant (*p* < 0.05) decrease in AChE activity shown in Fig. 2.

3.2.3. Effect of different drug interventions on aluminium levels in hippocampal and cortical area of mice brain

Aluminium administration had significantly (*p* < 0.05) increased abnormal aluminium deposition in cortical and hippocampal area in comparison to naïve animals. However, on treatment with *A. cepa* (50, 100 and 200 mg/kg) abnormal aluminium deposition was significantly reduced. On pretreatment with BADGE (5 mg/kg, i.p.) the abnormal aluminium deposition was found to be significantly increased as shown in Fig. 3.

3.3. Effect of different drug interventions on histopathological studies

Chronic aluminium chloride treated animals showed marked deterioration in hippocampal region as visualized by increased vacuolated cytoplasm as well as decreased pyramidal cells in comparison of saline treated animals. Treatment with *A. cepa* revealed marked improvement in a dose dependent manner. Co-treatment with BADGE reversed the neuroprotective effect of *A. cepa* as visualized in Fig. 4.

Table 3
Effect of different drug interventions on oxidative stress parameters in aluminium chloride exposed animals.

Treatment group	Lipid peroxidation (nanomoles/mg protein)	Nitrate/nitrite levels (ng/g of wet tissue)	Reduced GSH levels (micromoles/g of wet tissue)	Catalase (μ moles of H_2O_2 oxidized/mg of protein)
Naïve	7.316 ± 0.33[a]	407.08 ± 20.83[a]	40.29 ± 0.56[a]	2.76 ± 0.08[a]
AL 50	30.72 ± 0.75[b]	830.01 ± 18.75[b]	20.07 ± 0.59[b]	1.24 ± 0.06[b]
AC 50	25.47 ± 0.56[c]	727.91 ± 19.27[c]	26.01 ± 0.52[c]	1.53 ± 0.02[c]
AC 100	20.24 ± 0.53[c]	611.25 ± 18.25[c]	30.72 ± 0.45[c]	1.92 ± 0.02[c]
AC 200	10.57 ± 0.46[c]	534.16 ± 24.87[c]	35.50 ± 0.61[c]	2.22 ± 0.06[c]
BADGE + AC 200	30.71 ± 0.71[b]	840.41 ± 22.51[b]	20.92 ± 0.81[b]	1.05 ± 0.22[b]
BADGE	31.67 ± 0.44[b]	829.76 ± 22.36[b]	19.98 ± 0.49[b]	1.25 ± 0.11[b]

All values are represented as mean ± SEM; *n* = 8, groups (including naïve) not significantly different from naïve are labelled as "a"; groups significantly (*p* < 0.05) different from naïve are labelled as "b" and groups significantly (*p* < 0.05) different from naïve and AL 50 are labelled as "c". Naïve – saline treatment; AL 50 – aluminium chloride (50 mg/kg/day); AC 50 – *A. cepa* (50 mg/kg) + aluminium chloride (50 mg/kg/day); AC 100 – *A. cepa* (100 mg/kg/day) + aluminium chloride (50 mg/kg/day); AC 200 – *A. cepa* (200 mg/kg/day) + aluminium chloride (50 mg/kg/day); BADGE + AC200 – BADGE (5 mg/kg i.p.) + *A. cepa* (200 mg/kg/day) + aluminium chloride (50 mg/kg/day) and BADGE–BADGE (5 mg/kg i.p) + Aluminium chloride (50 mg/kg/day).
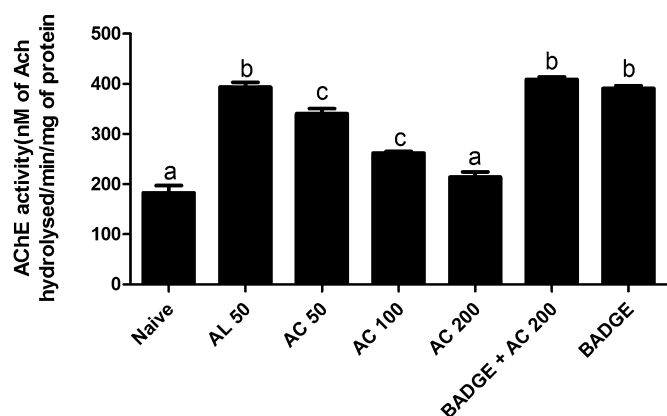
Fig. 2. Effect of *A. cepa* treatment on brain acetylcholinesterase (AChE) levels of mice subjected to 60 days of aluminium exposure. All values are represented as mean ± SEM; n = 8, Groups (including naïve) not significantly different from naïve are labelled as "a"; groups significantly ($p < 0.05$) different from naïve are labelled as "b" and groups significantly ($p < 0.05$) different from naïve and AL 50 are labelled as "c". Naïve – saline; AL 50 – aluminium chloride (50 mg/kg/day); AC 50 – *A. cepa* (50 mg/kg/day) + aluminium chloride (50 mg/kg/day); AC 100 – *A. cepa* (100 mg/kg/day) + aluminium chloride (50 mg/kg/day); AC 200 – *A. cepa* (200 mg/kg/day) + aluminium chloride (50 mg/kg/day); BADGE + AC200 – BADGE (5 mg/kg i.p.) + *A. cepa* (200 mg/kg/day) + aluminium chloride (50 mg/kg/day) and BADGE–BADGE (5 mg/kg/day i.p) + aluminium chloride (50 mg/kg/day).

4. Discussion

The present study was envisaged to investigate the neuroprotective potential of *A. cepa* in aluminium chloride induced neurotoxicity. Aluminium chloride (50 mg/kg/day) administered orally for 60 days possesses significant neurotoxicity as assessed by behavioural, biochemical and histopathological estimations. *A. cepa* treatment significantly prevented the altered behavioural, biochemical and histopathological outcomes associated with aluminium induced neurotoxicity. The detailed outcome of the
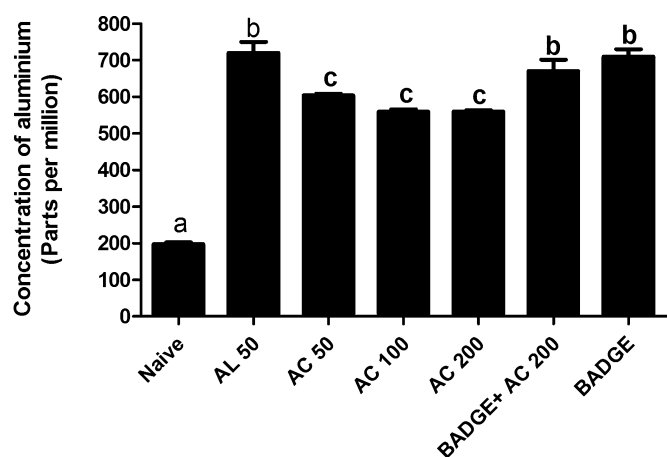

Fig. 3. Effect of *A. cepa* treatment on brain aluminium levels of mice subjected to 60 days of aluminium exposure using ICP-OES. All values are represented as mean ± SEM; n = 8, groups (including naïve) not significantly different from naïve are labelled as "a"; groups significantly ($p < 0.05$) different from naïve are labelled as "b" and groups significantly ($p < 0.05$) different from naïve and AL 50 are labelled as "c". Naïve – Saline; AL 50 – aluminium chloride (50 mg/kg/day); AC 50 – *A. cepa* (50 mg/kg/day) + Aluminium chloride (50 mg/kg/day); AC 100 – *A. cepa* (100 mg/kg/day) + aluminium chloride (50 mg/kg/day); AC 200 – *A. cepa* (200 mg/kg/day) + aluminium chloride (50 mg/kg/day); BADGE + AC200 – BADGE (5 mg/kg i.p.) + *A. cepa* (200 mg/kg/day) + aluminium chloride (50 mg/kg/day) and BADGE–BADGE (5 mg/kg/day i.p) + aluminium chloride (50 mg/kg/day).

study with justification of adopted methodology is discussed herewith.

As the available literature is incoherent regarding dose, duration and chemical form of aluminium to induce neurotoxicity, therefore, a pilot study was conducted to establish the dose and duration of aluminium administration for significant induction of neurotoxicity (Lakshmi et al., 2014; Sethi et al., 2008; Prakash et al., 2013). Aluminium chloride exposure at 50 mg/kg/day p.o. for 60 days was found to pose significant neurotoxicity as evidenced by a decrease in motor coordination and memory deficits, which are recognized parameters of aluminium induced neurotoxicity (Sethi et al., 2008). So, this protocol was followed to evaluate the neuroprotective potential of *A. cepa* in aluminium induced neurotoxicity.

In our study, chronic administration of aluminium chloride for 60 days resulted in marked behavioural deficits viz. motor incoordination and memory deficits, which were also supported biochemically as there was, marked increase in lipid peroxidation, total nitrate/nitrite, AChE levels with reduced GSH and catalase levels. Histopathological studies of CA3 area of hippocampus endorsed marked deterioration visualized as increased vacuolated cytoplasm and decreased pyramidal cells. These observations have been found in line with most of the previous aluminium induced neurotoxicity studies (Sethi et al., 2008; Bhalla et al., 2010; Kumar et al., 2009; Abdel-Aal et al., 2011).

A. cepa treatment in aluminium exposed animals significantly improved behavioural deficits viz. motor incoordination as well as memory impairment. There was significant reduction in lipid peroxidation and AChE levels while marked improvement in GSH and catalase levels, supporting observed behavioural improvements. Chronic *A. cepa* treatment markedly improved histopathological alterations associated with chronic aluminium exposure as evidenced from decreased vacuolated cytoplasm as well as increased number of pyramidal cells in comparison of aluminium control group. *A. cepa* has reported to have significant antioxidant activity, possibly due to the presence of bioactive constituents like flavonoids (quercetin, kaemferol, luteolin). Moreover, volatile sulphur-containing amino acids, S-alkenyl-cysteine sulphoxides, i.e. (+)-S-methyl-, (+)-S-propyl-, trans-(+)-S-(1-propenyl)-ʟ-cysteine sulphoxide and cycloalliin, also reported to have strong free radical scavenging activity (Xiao and Parkin, 2002; Kumari and Augusti, 2002; Rose et al., 2005). Thus, strong antioxidant potential of *A. cepa* might be responsible for improvement in behavioural, biochemical and histopathological outcomes in aluminium chloride induced neurotoxicity.

Another significant finding of the study was decreased abnormal aluminium deposition in the cortex and hippocampal area of mice brain after chronic exposure with *A. cepa* supplementation. PPARγ agonism due to various bioactive constituents viz. luteolin (Park et al., 2009), metabolites of quercetin and kaempferol (Yeh et al., 2011), phytosterols like lophenol, cycloartanol, 24-methyl lophenol and 24-ethyl cycloartanol (Tanaka et al., 2006) has been found to inhibit transcription of genes like S14, FAS, apolipoprotein CIII, transferrin (Hertz et al., 1995; Jump et al., 1997; Hertz et al., 1996; Ren et al., 1996). As transfer of aluminium is primarily transferrin mediated (Joshi et al., 1994), its inhibition might have decreased aluminium transport into the brain. Thus, decreased aluminium transfer across BBB may be another possible neuroprotective mechanism of *A. cepa* apart from its strong antioxidant potential. To further consolidate our hypotheses, we administered BADGE (PPARγ antagonist) in *A. cepa* treated animals (AC 200). BADGE cotreatment reversed the neuroprotective potential of *A. cepa*, which was supported by behavioural, biochemical and as well as histopathological outcomes. More importantly, increased aluminium deposition due to BADGE cotreatment support our hypothesis that PPARγ
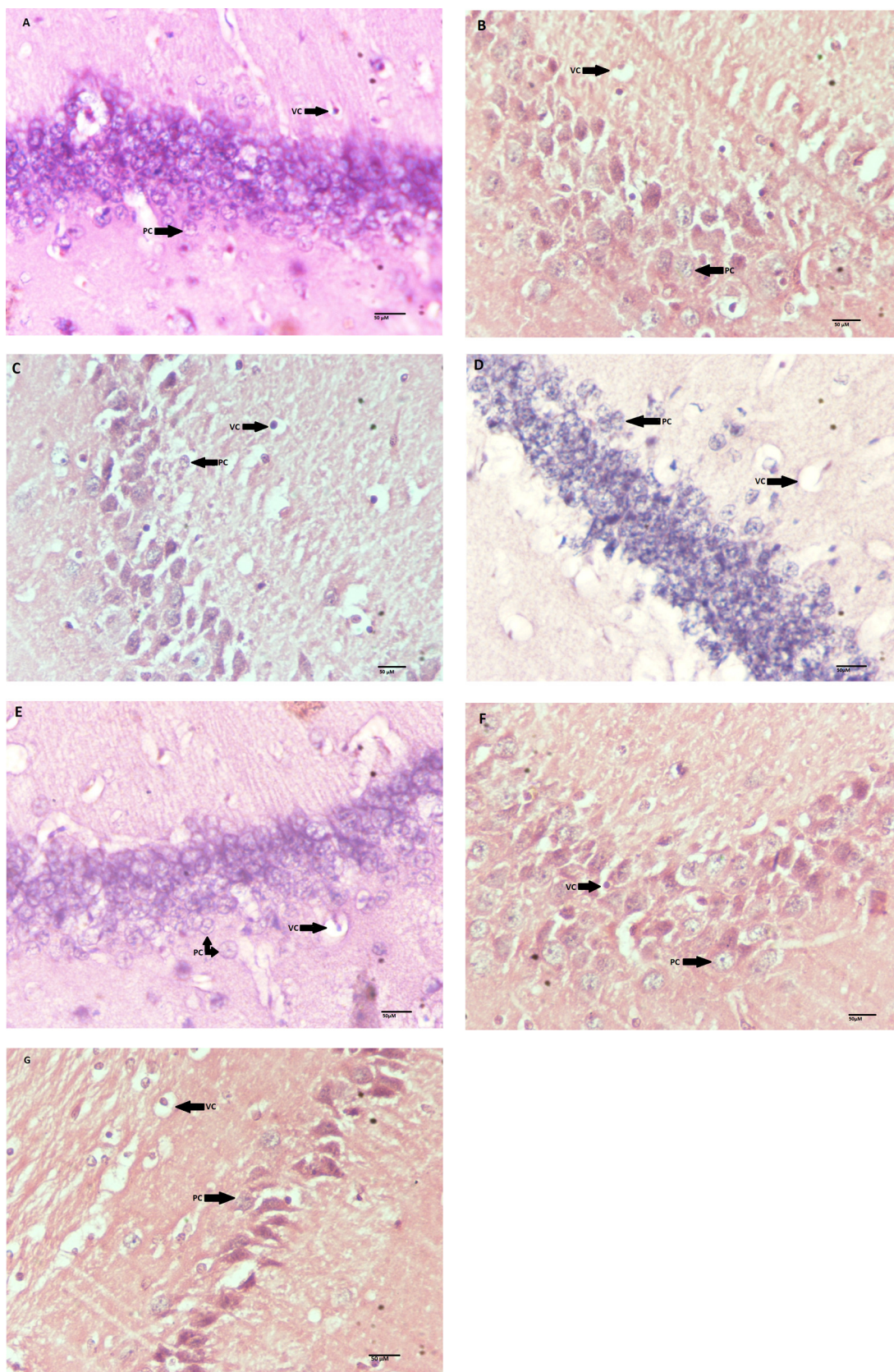
Fig. 4. H&E stained slides depicting effect of different pharmacological interventions on hippocampal CA3 area at 400× magnification. (A) Naïve – Saline; (B) AL 50 – aluminium chloride (50 mg/kg/day); (C) AC 50 – *A. cepa* (50 mg/kg/day) + aluminium chloride (50 mg/kg/day); (D) AC 100 – *A. cepa* (100 mg/kg/day) + aluminium chloride (50 mg/kg/day); (E) AC 200 – *A. cepa* (200 mg/kg/day) + aluminium chloride (50 mg/kg/day); (F) BADGE + AC 50 – BADGE (5 mg/kg i.p.) + *A. cepa* (200 mg/kg/day) + aluminium chloride (50 mg/kg/day); (4G) BADGE – BADGE (5 mg/kg i.p.) + aluminium chloride (50 mg/kg/day); BADGE – Bisphenol A diglycidyl ether, PC – pyramidal cells, VC – vacuolated cytoplasms (as markers of neurodegenration).

might have some important role to play in the neuroprotective potential of *A. cepa* in aluminium chloride induced neurotoxicity.

5. Conclusion

The present study concludes that *A. cepa* substantially reduced aluminium induced neurotoxicity. *A. cepa* treatment reduced aluminium deposition in the brain, which could be the major mechanism responsible for its neuroprotective potential besides inhibiting the ROS production. Our results also suggested the role of the PPARγ receptor in decreasing the abnormal transfer of aluminium across the BBB. But further studies are warranted to substantiate this notion.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

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