Centella Asiatica Extracts Regulates Aluminium Chloride-Induced Neurotoxicity in Rats: Impact on Inflammation, Apoptosis and Biogenic Amine Levels

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Abstract

Objectives and design: Aluminium (Al) is an environmental neurotoxin that affects cerebral functions and causes health complications. The antioxidant and memory enhancing effects of Centella Asiatica (CA) are well known in the past few decades. Therefore, the present study has been designed to investigate the therapeutic potential of CA against chronic Aluminium Chloride (AlCl₃) exposure induced rats. Wistar albino rats were segregated into four groups: group 1-control rats, group 2-rats received AlCl₃ (300 mg/kg body weight, every day orally) for 60 days, rats in group 3-received CA (500 mg/kg body weight, orally) and group 4 rats were initiated with both AlCl₃ and CA treatment.

Material and methods: Neurotoxicity was assessed by measuring brain biogenic amines, Al concentration in blood and brain, the levels of pro-inflammatory cytokines and later the apoptotic related components. Significant depletion of neither brain Dopamine (DA), Epinephrine (E), Nor Epinephrine (NE) nor 5-Hydroxytryptamine (5-HT) levels was observed following AlCl₃ exposure. Administration of CA raised the pro-inflammatory cytokines levels (tumor necrosis factor alpha, TNF-α and inducible nitric oxide synthase, iNOS) and modulated the levels of apoptotic proteins cytochrome c, caspases 3, with altered Bax/Bcl-2 ratio in favour of apoptosis in cortex that are determined by western blotting and immunohistochemistry.

Results: Administration of CA significantly increased biogenic amine levels, decreased Al concentration compared to Al-induced animals. CA ameliorates AlCl₃-induced inflammatory niche. Further, treatment with CA ameliorated neuronal apoptosis by reducing cytochrome c, caspase-3 expressions significantly and by modulating Bax/Bcl-2 ratio.

Conclusion: The findings of this study suggest that CA protects the cerebral cortex against AlCl₃-induced neurotoxicity. This neuroprotective effect is partially mediated by its anti-oxidant, anti-inflammatory and anti-apoptotic activities as well as elevating biogenic amine levels.

Keywords: Aluminium chloride; Apoptosis; Centella asiatica; iNOS; TNF-α

Introduction

Aluminum (Al) is a potent environmental neurotoxin and has been associated with several disorders. Al and its salts are widely exposed to human body due to their presence in several products including cosmetics, drugs, food additives (salt, spices, yellow cheese and herbs) and a powerful flocculants in water purification. Al has the potential to be toxic for humans and its compounds have neurotoxic manifestations which further shown to contribute several age-related neurodegenerative diseases [1]. Chronic exposure to Al causes pathogenesis of age-related illnesses such as the Alzheimer’s Disease (AD), the Parkinson’s disease, the Huntington’s disease, inflammation, Amyloid Beta (Aβ) deposition and plaque formation in the brain and suggested that this excess might be a possible contributing factor for the aging process by inflicting oxidative damage [2]. Evidence suggests that inhaled Al not only causes neurologic signs, which mimic progressive neurodegeneration, but also results in neurofilamentous changes in the cerebral cortex focal region where more Al accumulation takes place [3,4]. Previous studies have shown that, the excessive concentration of Al in neurotic deposits, plaques and neurofibrillary tangles in the brain of Alzheimer’s patients [5].

Al is a well-documented and experimental neurotoxicant that enhances neuroinflammatory events in the brain by multiple mechanisms. Accumulating evidence suggests that Aluminium Chloride (AlCl₃) promote free radical generation by potentiating the pro-oxidant properties of transition metals (iron and copper) [6]. In addition, AlCl₃ exerts its own pro-oxidant effect by altering calcium flux and homeostasis and to facilitate peroxidation of membrane lipids and antioxidant enzymes [7]. Beside this, various Al salt complexes can alter neuronal signal transduction pathways associated with glial cell activation, which further promote an uncontrolled inflammatory cascade in the brain. These activated glial cells secrete cytotoxic agents including Reactive Oxygen Species (ROS), Nitric Oxide (NO), inflammatory cytokines Like Tumor Necrosis Factor alpha (TNF-α), inducible Nitric Oxide Synthase (iNOS) which are toxic and inturn
cause synaptic abnormalities ultimately related to memory dysfunc-
tion. Earlier study has evidenced that AlCl₃ activates astrocytes and
glial cells which releases different inflammatory mediators due to
induction of apoptosis [8,9]. Therefore, overall effects are to reduce
pro-inflammatory mediator’s production by activated microglia and
astrocytes should be useful for prevention of neuroinflammation and
eventually neuroprotection.

Aptosis under mitochondrial control has been implicated in a
progressive and selective loss of neurons, which involve the release
of cytochrome c into the cytoplasm and initiation of the apoptosis
cascade. AlCl₃ is a potent cholinotoxin and causes aberrant apoptotic
neuronal loss by stimulating ROS production, which is a charac-
teristic symptom of neurodegeneration [10-12]. It induces apoptosis
of neurons and astrocytes via involvement of caspases activation
in rodent and in vitro models [13,14]. Reactivation of caspases has
been implicated in mediating neural death during pathological pro-
cesses causing neuronal loss in acute and chronic neurodegenerative
diseases. Caspase-induced apoptosis has been documented in Amy-
loid-β-induced Alzheimer’s and 6-hydroxydopamine-induced Par-
kinson’s neuronal apoptosis [15,16]. Restriction of apoptosis and its
associated factors during neurodegeneration is presumed to be bene-
ficial for the survival of neurons. Thus clinical trials are in progress
that anti caspase approach in neurodegeneration will be an exciting
avenue for therapeutic neuroprotective interventions [17].

The antioxidant capabilities of Centella Asiatica (CA) have been
documented previously in different experimental models. Centella
Asiatica (CA), also known as gotu kola and Indian pennywort, is a
perennial herbaceous creeper of the Apiaceae family. Recently, CA
has been reported as a neuroprotectant against different degenerative
diseases. CA modulate the immune system [18], act as antioxidant
[19], prevent alleviation of oxidative stress [20], act as anti-inflamma-
tory agent [21], and inhibit proliferation of cancer cells [22]. Yet, the
most widely reported health benefit of this herb is in improving the
brain function, particularly related to learning and memory. In fact,
CA has been reported to stimulate nerve regeneration in vitro [23],
which could explain its brain protective effect. In humans, it has been
reported to improve mental ability of the mentally retarded children
and decrease the amyloid-β levels in the brain [24,25]. Previously,
we have demonstrated that CA inhibited cognitive impairment and
improves learning and memory in AI-induced rats by reducing oxi-
dative stress and Acetyl Cholinesterase (ACHE) activity [26]. Based
on this background, the present study was carried out to investigate
the protective effect of CA against AlCl₃ induced inflammation and
oxidative stress associated mitochondria apoptosis. Therefore in this
study, we have investigated the efficacy of CA in terms of biogenic
amines, expression of pro-inflammatory cytokines TNF-α, iNOS and
apoptotic inducing factors in cortex of rat brain.

Materials and Methods

Animals

Wistar albino rats of either sex (120-150g) were procured from
King Institute and Central Animal House Facility (CAHF) of Univer-
sity of Madras, Chennai, India. Animals were fed with commercial
pellet diet and water ad libitum and maintained at an ambient tem-
perature of 25 ± 2°C with a 12-hr light: dark cycle. The experiments
were conducted in accordance with guidelines of Institutional Animal
Ethical Committee (IAEC) governed by Committee for the Purpose
of Control and Supervision of Experiments on Animals (CPCSEA)
guidelines, Government of India.

Drugs and experimental design

Aluminum Chloride Hexahydrate (AlCl₃, 6H₂O) was purchased from
Sd fine-chem, Mumbai, India. All other chemicals and reagents
used were of analytical grade.

Preparation of the plant extract: Fresh leaves of CA were collected
from regular vendor and species identification was done from Center
for Advanced Studies in Botany, University of Madras, Chennai, In-
dia. The leaves were cleaned, shade dried and coarsely ground with
grinder. The coarse powder of plant was extracted with 8 parts of
distilled water under boiling for 5 hrs and was filtered through a 400
mesh cloth to collect the extract. The extract was concentrated and
freeze dried to get a powder of greenish brown in color. The percent-
age w/w yield of the extract was 41%. Standardized aqueous extract
of CA and AlCl₃ solutions (made freshly at the beginning of each
experiment daily). For oral administration, AlCl₃ and CA were admin-
istered in a dose of 0.5 ml/100 g body weight. The animals were
divided into four groups (n=6). Group 1 control rats. Group 2 rats ad-
ministered with AlCl₃ (300 mg/kg) for 60 days. Group 3 rats received
CA alone (dissolved in water, 500 mg/kg). Group 4 rats co-treated
with CA and induced with AlCl₃ simultaneously for 60 days. The doses
of AlCl₃ [26,27] and CA [26] were selected based on published report
of our own laboratory.

Biochemical and cellular assessments

The animals were anesthetized by diethyl ether and killed by cer-
vical decapitation after an overnight fast. Brains were dissected out
by making midline incision to view the skull. Then, a small incision
from the caudal part of parietal bone and a firm cut in the anterior
part of the frontal bone were made to remove the brain more easily.
Isolated brains were rinsed with ice-cold isotonic saline then kept
on ice (using large and small curved serrated forceps) to obtain cerebral
cortex [28]. The isolated brain and its parts were homogenized using
0.1% Triton X-100 buffer (pH - 7.4), in a Potter-Elvehjem homoge-
izer and then centrifuged at 10,000xg (4°C) for 30 min. Aliquots
of the supernatants were separated, stored at -80°C and used for further
biochemical assays.

Aluminium estimation

The aluminium was analyzed by wet acid digestion method of
Zumkley et al., in serum and cortex of brain. A mixture of 2.5 ml of
perchloric acid/nitric acid (1:4 in volume) was added to brain parts
[29]. Then, mixture with brain sample was placed in sand bath for 4hr
until the point of a white ash or residue was obtained. Then residues
were dissolved in 2.5 ml of 10mm 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.

Assessment of brain monoamine neurotransmitters levels

The level of biogenic Amines Dopamine (DA), Epinephrine (E),
Norepinephrine (NE) and 5-Hydroxytryptamine (5-HT, serotonin)
were assessed in brain sample of the different treated groups by
the method described by Byers et al., [30]. Briefly, for the estimation
of monoamine neurotransmitter level, brain tissue was homogenized in
homogenizing solution containing the internal standard Dihydroxy
Dopamine (D-3,4-DAP). The levels were determined spectrophotom-
ically by using the method described by mobile phase of 50 mm
NaOH containing 1 µm potassium ferricyanide and 2 µm
potassium ferrocyanide. The absorbance was determined at 600 nm
using 1 cm quartz cells. The levels of these amines were expressed
every six hours for 60 days, and the results were represented as mean 
± SD. The data were analyzed using one-way ANOVA with tukey's
post hoc test. The level of significance was set at P<0.05.
Benzylamine (DHBA). Following tissue disruption, the samples were centrifuged, then filtered using 0.22 µm filter (Millipore, USA). The concentrations of the monoamine contents DA, E, NE and 5-HT were measured by reverse-phase High-Performance Liquid Chromatography with Electrochemical Detector (HPLC-ECD). The mobile phase consisted of 50mm phosphate buffer (pH - 3.5) containing 80mg/L Octane Sulfonic Acid (OSA), 25 mg/L Ethylenediamine Tetraacetic Acid (EDTA) and 10% v/v methanol. The mobile phase flow rate was 1ml/min. The applied oxidation potential was set at 750mV. The concentration of the neurotoxicants was determined by external standard method using peak areas. A linear standard curve was constructed by plotting peak areas versus the corresponding concentrations.

Protein extraction and Western Blotting

The proteins were isolated from cerebral cortex region from brain tissue and were homogenized in ice-cold HEPES (N-2- Hydroxyethylpiperazine-N-2-Ethane Sulfonic acid) lysis buffer (HEPES 25 mm; MgCl2 5 mm; EDTA 5 mm in dd. H2O). The homogenates were centrifuged (10,000 rpm/15 min/4°C), and the protein content of the supernatant was assayed and diluted to give equal protein concentrations of 30 µg. The protein content was estimated by Lowry et al., using BSA as standard [31]. Western blot analysis was performed according to the methods of Towbin et al., [32]. Equal concentration of protein from each sample was prepared with 6X Laemmli Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS PAGE). Sample loading buffer 0.5 mm Tris-HCl, pH 6.8; 10 % glycerol v/v; 10 % SDS w/v, 0.2 % β-mercaptoethanol v/v, 0.05 % bromophenol blue (w/v) boiled for 5 min prior to loading onto 12 % gels, resolved at a constant supply of voltage and transferred onto PVDF (Poly Vinylidene Fluoride) membranes (Millipore Corp., Bedford, MA, USA). To assess the expression of specific proteins, membranes were incubated overnight at 4°C in 10 ml PBS/Tween (0.1 % v/v) containing 1 % BSA with one of the following antibodies: goat polyclonal TNF-α, caspase-3, rabbit polyclonal iNOS, Bax, Bcl-2 and cyt c, (1:500) (Santacruz Biotech, USA). Following incubation, immunoreactive bands were detected by incubating with respective Horse Radish Peroxidase (HRP)-conjugates. Protein– antibody complexes were visualized by the addition of Diaminobenzidine (DAB) as a substrate. Relative band intensities were quantified using Image-J analysis software.

Immunohistochemical analyses

Brain sections were fixed in 4% cold paraformaldehyde in 100-mm Phosphate Buffered Saline (PBS) and were made into coronal (4µm) coated on clean slides. The sections were de-paraffinized in fresh xylene and rehydrated using graded ethanol solutions. The slides were dipped in freshly prepared solution of 1 % H2O2 in methanol for 20 min to quench endogenous peroxidase. Slides were rinsed in PBS and incubated for 1 hr in blocking solution (3 % BSA, 0.1 % Tween-20 in PBS) at Room Temperature (RT). Sections were incubated with goat polyclonal TNF-α, (1:3,000), rabbit polyclonal iNOS and cyt c (1:500) in blocking solution for 12 h at 4°C re-equilibrated to room temperature and washed with PBS, incubated with Horse Radish Peroxidase (HRP) antibody conjugates (1:2,500) in blocking solution without Tween-20 for 2 hrs at RT. Sections were washed with PBS and incubated with 0.2 % solution of 3,3'-Diaminobenzidine (DAB) until desired stain intensity develops at RT followed by washing in distilled water. Sections were counter stained with hematoxylin and mounted with Di-N-Butylphthalate-Polyestrene-Xylene (DPX). Sections were viewed by two independent observers blinded to experimental conditions and the total number of positively stained cells was counted and presented graphically.

Statistical analysis

Statistical analysis of the data was performed using SPSS/10.0 software. Data are expressed as mean ± Standard Deviation (SD). Significance of differences between groups’ mean was analyzed using two-way ANOVA followed by Least Significant Difference (LSD) test. Statistical significance was set below 5 % level (p<0.05 was considered significant).

Results

Effect of CA on Al concentration in serum and brain tissue

The concentration levels of Al detected in serum and brain tissue samples from control and experimental rats are illustrated in figure 1. AlCl3 treatment significantly increased concentration level of Al in brain cortex areas and serum as compared to control rats. However, chronic CA treatment significantly attenuated the rise in Al concentration in both as compared to control rats (p<0.05) and CA exposed groups which showed the lowest concentrations of Al.

Effect of CA on AlCl3-induced rat brain neurotransmitters

The effects of AlCl3 on brain neurotransmitters like DA, E, NE, 5-HT and their response to treatment with CA are shown in figure 2. Significant depression (p<0.05) of DA, E, NE and 5-HT neurotransmitters levels in brain homogenates of Al-exposed rats compared to control group. A remarkable induction of neurotransmitters was achieved in the AlCl3 +CA co-treated group whilst not restored to the control level. A significant enhancement of brain neurotransmitters was observed in AlCl3 +CA co-treated group compared with Al exposed group.

CA treatment decreases the AlCl3-induced expressions of TNF-α and iNOS

Activated microglia and astrocytes release proinflammatory cytokines that may eventually cause neuroinflammatory events and neuronal death [33]. We have studied the expressions of pro-inflammatory

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cytokines TNF-α and iNOS, as these are implicated in excitotoxicity [34]. Figure 3 shows the immunohistochemical analysis of TNF-α protein expression in the cortex of control group of rats. AlCl₃-induced rats (Figure 3B) showed significantly (p<0.05), increased TNF-α expression in rat brain. CA-treated groups of rats showed significantly (p<0.05), reduced expressions for TNF-α in cortex as compared to AlCl₃-induced groups (Figure 3D). No adverse changes were noticed in cortex of control and CA-alone-treated groups of rats (Figure 3C).

An abundant activation of iNOS (Figure 3F) positive cells was evident in cerebral cortex tissue from rats exposed to AlCl₃ (p<0.05), in contrast to an almost negligible expressions was found in control and CA-alone treated animals (Figure 3E). CA-treated group of rats exhibited an evident reduction of expression of iNOS (p<0.05) (Figure 3H) when compared with AlCl₃-induced and control group. No adverse changes were noticed in cortex of control and CA-alone-treated groups of rats (Figure 3G). The total number of immunoreactive cells was quantified and is represented in graph (Figure 3I).

Western blotting analyses of TNF-α and iNOS, is shown in figure 4A, which was found to be significantly (p<0.05) increased in rat brain cortical region of AlCl₃-induced group when compared to TNF-α and iNOS protein levels in control and CA alone groups. CA treatment significantly (p<0.05) reduced protein expressions of TNF-α and iNOS. Control and drug control groups showed similar levels of TNF-α and iNOS expressions. Further, quantitative data expressing the corresponding protein levels were assessed using ImageJ (NIH software), relative to β-actin, and are graphically represented (Figure 4B). Thus, CA exerts beneficial effects against neuroinflammation by limiting inflammatory cytokine production [21].

CA treatment suppressed cytochrome c expression in AlCl₃-induced rat brain

The mitochondria-mediated apoptotic pathway is characterized by mitochondrial dysfunction with the release of caspase activators including cytochrome c. Figure 5 shows the immunohistochemical analysis of cytochrome c in the control and experimental groups of rat brain. Level of cytochrome c was significantly (p<0.05) increased in cortex of AlCl₃-induced rat as compared to controls. CA treatment significantly (p<0.05) reduced the cytochrome c levels in cortex as compared to AlCl₃-administered groups of rats.
CA-alone administered groups of rats showed no appreciable changes in cytochrome c expression levels. Quantitative data expressing the corresponding protein levels were assessed using densitometer and the results were expressed as relative intensity arbitrary unit (Figure E).

**Figure 5:** (A) Representative micrographs of immune stained sections of cytochrome c expression in cortex from control and experimental groups of rats. Brain specimens were probed with antibodies specific for cytochrome c (Magnification ×40). Immunoreactivity was assessed and measured objectively by two independent observers. B) For quantification, data expressing total number of positively stained cells quantification were performed by counting the positively stained cells in 10 fields/section by two independent observers in blinded fashion, and the average was used to denote the total number of positively stained cells. Hypothesis testing method included two-way Analysis of Variance (ANOVA) followed by Least Significant Difference (LSD). Results are given as statistically significant at p<0.05; compared with *AlCl₃ vs. control; *AlCl₃ + CA vs. AlCl₃.

**CA treatment suppressed apoptotic-inducing proteins in AlCl₃-induced rat brain**

The mitochondria-mediated pathway is controlled by anti-apoptotic Bcl2 and pro-apoptotic Bax. The balance between Bcl2/Bax determines cell survival or death. Figure 6a shows the levels of Bax, Bcl2 and caspase-3 in the cortex of control and experimental groups of rats. Levels of Bax/Bcl2 were significantly (p<0.05) tuned in favor of apoptosis in AlCl₃-induced rats as compared to control rats. Treatment with CA caused significant (P<0.05) decrease in the protein level of Bax, caspase-3 and increased level of Bcl-2 protein as compared to AlCl₃-induced rats. Quantification of Bax/Bcl2 and caspase-3 expressions were represented graphically (Figure 6B) and the levels show that CA significantly protected cortex from AlCl₃-induced neuronal apoptosis.

**Discussion**

Al is a ubiquitous metal and implicated in the pathophysiology of neurodegenerative diseases. Reports suggest that AlCl₃ alters cytoskeletal dynamics of neurons and astrocytes leading to pathogenesis of neurodegeneration. In mammals, Al accumulation in the brain has been linked to neurodegenerative diseases such as PD and AD [4, 35]. Understanding the etiology of neurodegeneration and identifying ways to achieve early prevention are being considered as important approaches in the management of neurological diseases. Counteracting attempts to prevent/inhibit neurodegeneration and enhance neuronal survival has been greatly summoned. Recently, the traditional medicinal plant CA and its extracts have gained much interest for their ability to modulate neuronal function and influence learning and memory processes [36,37]. Hence, the present study was an attempt to understand the reduction of neuroinflammatory signals and apoptotic mechanisms underlying neuroprotective efficacy of CA in a rat model of neurodegeneration.

**Figure 6:** Representative immunoblots of Bax, Bcl2 and caspase-3 protein expressions in the cortex of control and experimental groups of rats. Western blot analyses were performed using probes specific for Bax, Bcl2, caspase-3 and β-actin as indicated. Lane 1 Control; Lane 2 AlCl₃, -induced; Lane 3 CA alone; Lane 4 AlCl₃-induced and CA treated. Data expressing the corresponding protein levels were assessed using densitometry and is expressed in relative intensity (arbitrary units). Hypothesis testing method included two-way Analysis Of Variance (ANOVA) followed by Least Significant Difference Difference (LSD). Results are given as statistically significant at p<0.05; compared with *AlCl₃ vs. control; *AlCl₃ + CA vs. AlCl₃.

Brain is considered a preferential site of Al accumulation mainly at some cortical regions and hippocampus via crossing the Blood-Brain Barrier (BBB) [38]. The obtained data demonstrated that the serum Al level was elevated in Al exposed rats, indicated an occurrence of serum hyper-aluminemia which resulted in an elevation of brain tissue Al content. This elevation appears to be a consequence of an alteration in the permeability of the BBB, it has been shown that Al somehow interacts with the BBB via interfering with its function at the endothelial cell level, whereas positively charged Al ions neutralize the anionic sites of the endothelial cell surface and also may consequently altering the function of the BBB [39]. Concomitant treatment with CA was associated with a significant reduction in serum and brain Al content this depletion may be due to CA's free radical scavenging potential [26]. This may preserve cell membrane function including ion transport and membrane fluidity.

In the current study, the effect of chronic Al exposure on brain monoaminergic systems as stress response indicators was assessed. Primarily, the data presented in this work elicited a marked depletion
in the brain monoaminergic system (5-HT, DA, E, NE and 5-HT) levels, which negatively correlated with the brain Al level. 5-HT is an excitatory neurotransmitter synthesized from tryptophan via the intermediate 5-hydroxytryptophane. The decrease in the brain 5-HT levels are associated with a decrease in the synthesis of 5-HT and loss of serotoninergic neurons [40]. The decline in the levels of DA, E and NE neurotransmitters may be linked to their closely related synthetic route. These neurotransmitters are derived from tyrosine, which is converted to DA by the enzymes tyrosine hydroxylase and DOPA decarboxylase. DA is converted through the action of dopamine-β-hydroxylase to NE [41]. Similar decreases were previously reported by Fernandez-Davila et al. [42]. In the current study CA supplementation might be useful in maintaining brain neurotransmitters levels to some extent.

Al intoxication leads to marked astrocytes and microglial activation that contribute to the pathogenesis of neuroinflammation and neurodegeneration [43]. Chronic administration of AlCl3 causes a greater inflammatory response over immunoreactivity of astrocytes and microglia associated with cognitive dysfunction and disease [44]. Reactive gliosis and the associated proinflammatory cytokines/chemokines could be result of inflammation and neuronal injury in the rat brain [45]. Inhibition of pro-inflammatory mediators would be a better approach in regulating the progression of neurodegenerative disorders. The pro-inflammatory cytokines, TNF-α and iNOS, contribute to neuronal damage and death in vivo and in vitro [46]. Activation of iNOS in glial cells is a key step in neuroinflammation and is often related with TNF-α release. A potent proinflammatory cytokine TNF-α is synthesized by microglia, astrocytes and neurons [47]. TNF-α together with other transcription factors (NF-kB, nuclear factor kappa B) involved in cell responses including inflammation, proliferation, cell migration and apoptosis [48]. NO, the enzymatic product of iNOS, is associated with neuronal death by inhibiting mitochondrial respiration and excitotoxicity [49]. iNOS is normally not expressed in the brain, but is induced by pro-inflammatory cytokines and pathogenic components like endotoxins. To explore the mechanism of CA on the attenuation of AlCl3-induced neuroinflammation, the expression of TNF-α and iNOS was detected in the rat brain. Reduced expressions of TNF-α and iNOS were observed in the cortex of rats treated with CA, which demonstrates that CA exerts anti-inflammatory effect. Hence, we assume that CA regulates focal microglia activation and proinflammatory cytokine production in cortex and this antioxidant is an effective experimental tool to reduce the brain lesions associated with inflammatory responses [21]. It also influences memory performance and, hence, CA can be utilized as a protective agent against neurodegenerative disorders [26].

Apoptosis is one of the mechanisms contributing to neuronal loss in AlCl3-induced neurodegeneration. Aβ oligomers are majorly contributed to neuronal cell death by inducing apoptotic proteins during AlCl3 administration. Bcl-2 is a member of apoptotic regulator protein that plays a major role in the regulation of cell death under both the physiological as well as pathological conditions. Bcl-2 over expression is perceived as a survival factor to protect against neurons during brain injury [12,13]. In the present study, induction of AlCl3 resulted in a decrease in the activity of Bcl2 where as increased the activity of Bax in cerebral cortex region of rat brain. The ratio of Bax/Bcl2 levels in cortex was turned in favour of apoptosis in AlCl3-administered rats that denotes that AlCl3 induces neuronal apoptosis in cortex region. Results of the present study are supported by documented report denoting the involvement of Bax, Bcl2 in AlCl3-induced apoptosis in rabbit [13,50]. Further, we demonstrate that treatment with CA reduces Bax/Bcl2 ratio thereby antagonizing apoptotic niche in cortex of AlCl3-administered rat brain. Mobilization of proapoptotic Bcl2 family signaling protein, Bax from cytosol to mitochondria causes mitochondrial outer membrane permeabilisation leading to cytochrome c expulsion from mitochondrial inter-membrane space leading to Caspase activation [51]. In this study, protein levels of cytochrome c and expressions of caspase-3 were significantly increased upon AlCl3-exposure in the cortex denotes that AlCl3 induces neuronal apoptosis. Results of present study are in accordance with documented reports denoting the involvement of caspase activation in many neurodegenerative disorders [52]. Treatment with CA attenuates AlCl3-induced cytochrome c expression and also its downstream effectors caspases-3. This indicates that CA exerts neuroprotective effect by inhibiting neuronal apoptosis and is further supported that dietary flavonoids are capable of inhibiting intrinsic apoptosis of neurons [53].

In summary, the present investigation sheds light on the potent neuroprotective potential of CA in AlCl3-induced neurotoxicity. Our observations suggest that CA helps to maintain brain neurotransmitters levels and further regulates apoptotic neuronal cell death, focal microglia activation and inflammation in the cortex. Therefore, it is sensible to suggest from the previous and the present studies that CA may be used as therapeutic agents in the treatment of cognitive dysfunction-associated disorders such as Alzheimer’s disease. Future research may be directed towards the use of CA in clinical trials to evaluate its neuroprotective effect on humans.

Conflict of interest:

The authors declare no conflict of interest.

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