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Centella asiatica suppresses inflammation and apoptosis and regulates the Gsk-3 β signaling pathway in aluminum chloride-induced neurodegeneration in rats

Shaymaa M. Eissa^{1*}, Shymaa G. Hassan^{1*}, Azza M. Gawish¹ and Hanan R. H. Mohamed¹

Abstract

Background Over 50 million people worldwide are affected by neurodegenerative diseases, and finding effective treatments is crucial. *Centella asiatica* (CA) is a regenerating herbal medicine rich in triterpenes and phenolic compounds, traditionally used to stimulate memory. CA extract possesses antioxidant and anti-inflammatory properties. Therefore, we aimed to elucidate the underlying molecular mechanisms of CA extract as a neuroprotector against AlCl₃-induced neurodegeneration in rats.

Methods Forty-eight rats were categorized into eight groups. The control group administered distilled water, whereas the aluminum (Al) group received aluminum chloride (AlCl₃) at 100 mg/kg. Three supplementary groups received *Centella asiatica* (CA) at (200, 400, and 600 mg/kg). Three more groups were administered both CA and AlCl₃. All treatments were administered orally over 5 weeks. Oxidative stress biomarkers (MDA, GSH, and SOD) and DNA damage extent were measured in the brain tissues. In addition, the mRNA expression of apoptotic and inflammatory genes (*Bax/Bcl2*, *Caspase3*, *Il-1 β* , *Gsk3 β*) and immunohistochemical staining of B-amyloid and tau protein were evaluated.

Results The CA extract improved oxidative stress response by raising the levels of GSH and SOD while diminishing the MDA levels and DNA damage parameters. It also reduced the expression of genes responsible for inflammation and apoptosis. CA decreased the accumulation of both B-amyloid and tau proteins in the brain tissue. Further, the histopathological examination of brain tissues revealed a significant improvement in the CA-pretreated groups.

Discussion CA effectively reduced aluminum chloride neurotoxicity by decreasing apoptotic and inflammatory gene expression and lowering *Gsk-3 β* levels, which helps minimize brain amyloid-beta and tau protein accumulation.

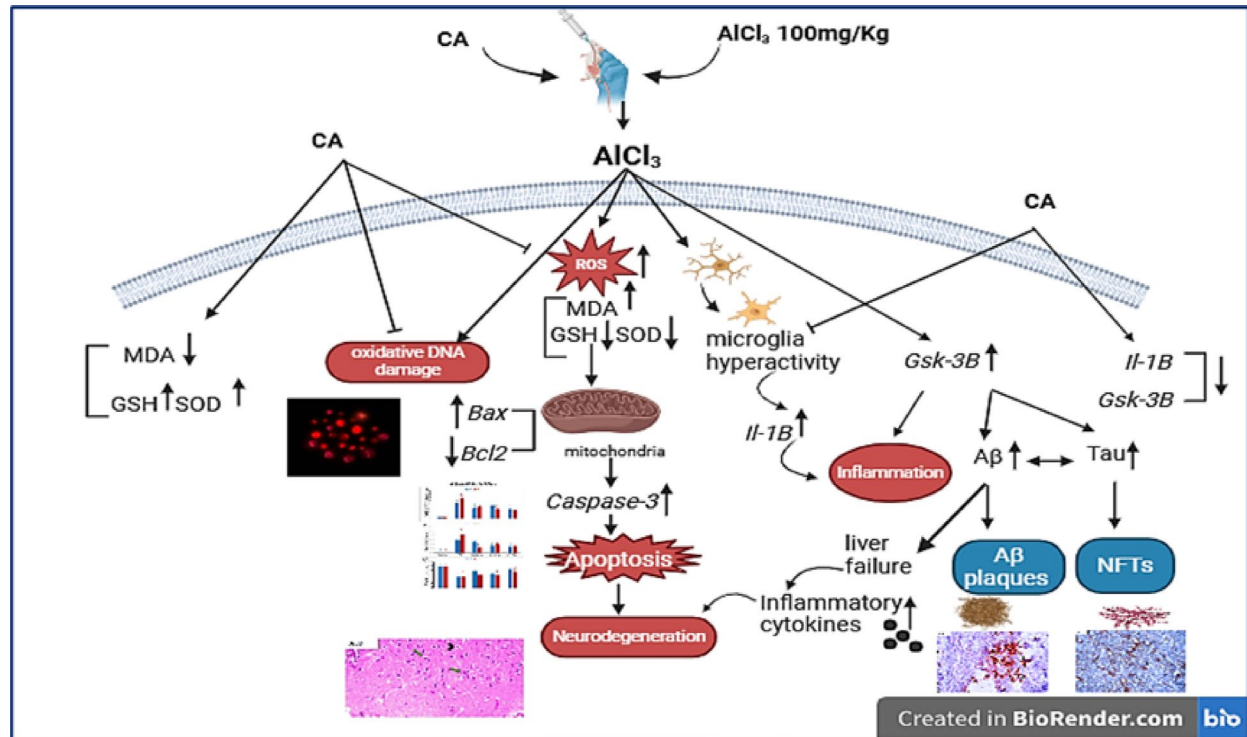
Keywords *Centella asiatica*, Rat, Inflammation, Neurodegeneration, Apoptosis, Oxidative stress

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Graphical Abstract



Background

Neurodegenerative diseases (NDs) are a significant cause of illness and death worldwide. They are characterized by the gradual loss of specific groups of neurons, leading to a breakdown in communication between brain cells (Erkkinen et al. 2018). As a result, memory, cognition, behavior, sensory, and motor functions are reduced (Wilson et al. 2023, Dugger and Dickson 2017). Over 55 million people globally are estimated to suffer from neurodegenerative diseases, with more than 60% of them living in deprived and moderate-income countries. Alzheimer's disease (AD) is the most frequent type of NDs and is responsible for sixty to seventy of all cases (Organization 2023). In 2021, it was reported that Alzheimer's was the sixth predominant cause of mortality worldwide, with about 1.6 million deaths in 2020 (Organization 2021). According to the International Alzheimer's Disease Report (2021), the cost of this disease and other dementias has gone beyond \$1 trillion. This has created a significant burden on healthcare systems and economies worldwide. These statistics highlight the global impact of neurodegenerative diseases and emphasize the pressing necessity for investigating innovative treatments and collaborative efforts to mitigate the social, economic, and healthcare challenges associated with these debilitating conditions (Gauthier et al. 2021).

Apoptosis and inflammation are interconnected biological processes that significantly contribute to the pathophysiology of neurodegenerative disorders. Apoptosis, or programmed cell death, is crucial for eliminating damaged or malfunctioning neurons, thus averting the buildup of cellular waste that could provoke an immunological response. Excessive or dysregulated apoptosis can result in substantial neuronal death, exacerbating disease development (Shoshan-Barmatz et al. 2023). Concurrently, persistent neuroinflammation has become a defining characteristic of numerous neurodegenerative conditions, such as Alzheimer's and Parkinson's diseases. Activated microglia and astrocytes secrete pro-inflammatory cytokines, including *IL-1β*, *TNF-α*, and *IL-6*, which may intensify neuronal damage and apoptosis. This establishes a detrimental loop, wherein inflammation induces apoptosis, and apoptotic cell death subsequently exacerbates inflammatory responses (Heneka et al. 2014). Comprehending the interaction between apoptosis and inflammation is crucial for formulating therapeutic strategies to impede the advancement of neurodegeneration. Simultaneously targeting both systems may provide a more effective strategy for alleviating the pathogenic cascades underlying these complicated diseases.

Centella asiatica (CA), or "Gotu kola," is a tropical plant with proven medicinal properties. It has been cultivated successfully for centuries in various countries

(Meulenbeld and Wujastyk 2001). The plant contains abundant triterpenes, phenolic substances including flavonoids and tannins, minerals, carbohydrates, alkaloids, and vitamins (Sabaragamuwa et al. 2018, Siddiqui et al. 2007). Previous studies have shown that CA has a neuroprotective function, and can decrease the production of amyloid plaque, dopamine neurotoxicity, and oxidative stress in brains (Mohandas Rao et al. 2006). Additionally, it encourages dendritic arborization in neuronal cells, which is important for normal brain function, intelligence, memory enhancement, and longevity (Mohandas Rao et al. 2006, Meyer et al. 2016, Rao et al. 2005). The aqueous extract of CA improves cognitive function and diminishes oxidative stress in the brains of rats (Kumar and Gupta 2002). Moreover, the entire plant has been found to benefit memory and improve the overall mental capacity of children with cognitive impairments (Appa Rao et al. 1973, Kakkar 1990, Mukerji 1953, Vaidyaratnam 1994).

Aluminum (Al) is a neurotoxin associated with various neurological illnesses (Hu et al. 2007, Zatta et al. 2002). It enters the brain through multiple avenues, including nutrition, cosmetics, pharmaceuticals, toothpaste, potable water, and inhalation of vapors. Upon entering the brain, the Al accumulates in different regions of the brain, causing cognitive decline and a deterioration of memory functions (Hosney et al. 2022). Studies have shown that aluminum can cause alterations in neuropathology, neurobehavioral, neurophysical, and neurochemical processes (Miu et al. 2003, Walton 2012, Walton 2013). It also functions as a cholinotoxin, which affects cholinergic activity, the primary neurochemical marker of AD (Gulya et al. 1990, Rakonczay et al. 2005).

The present research is intended to assess the efficiency of *Centella asiatica* (CA) supplementation in mitigating aluminum chloride-induced neurotoxicity in rats, administered at three distinct dosages (200, 400, and 600 mg/kg) for five weeks. The study assessed the core biological pathways by examining the expression of genes related to apoptosis (*Bax*, *Caspase-3*, and *Bcl2*) and genes associated with inflammation (*Il-1β* and *Gsk-3β*) in the brain of

rats. Furthermore, the study evaluated the brain's levels of DNA damage and oxidative stress markers, including MDA, GSH, and SOD. The study investigated the histological changes in the brain and conducted an immunohistochemical analysis of tau and β-amyloid proteins.

Methods

The experimental procedures were carried out following the applicable guidelines and regulations.

Chemicals

Gotu Kola extract was purchased from Amazon (Product code: SWV-14031). AlCl₃, formaldehyde, saline, RNA later, agarose, low-melting agarose, Triton X-100, Tris-(hydroxymethyl)-aminomethane (Tris-base), 100% ethanol, Ethidium Bromide (EtBr), and solution of Na₂EDTA were all acquired from Sigma-Aldrich (Saint Louis, the state of Missouri, USA). Kits for each biochemistry parameter have been purchased from the Bio-Diagnostic Company in Giza, Egypt.

Experimental animals

We acquired 48 male adult Wistar rats, weighing between 100 and 150 g. Following a week of acclimatization, the rats were situated in a climate-controlled setting featuring 12-hour light/dark cycles and free access to food and drink. The rats were classified into eight groups. The animal trials were conducted following ARRIVE guidelines. The Institutional Animal Care and Use Committee (CU-IACUC) (CU/I/F/78/19) approved the experimental animal protocol.

Experimental design

CA and AlCl₃ were dissolved in distilled water and taken orally in the morning for 5 weeks. CA and AlCl₃ dosages were based on previous investigations (Chiroma et al. 2019, Hosny et al. 2019). Table 1 shows that the 48 rats were randomly assigned to eight groups of six rats. Group 1 (control) got distilled water orally, while group 2 received 100 mg/kg/day of AlCl₃. The third, fourth, and fifth groups received 200, 400, and 600 mg/kg/day of CA over 5 weeks. For the sixth, seventh, and eighth groups, CA at doses of 200, 400, and 600 mg/kg/day was administered first, followed 2 h later by AlCl₃ at 100 mg/kg/day, continuing for 5 weeks.

In each group, 50% of the rats were euthanized following 3 weeks of treatment, while the remaining half received treatment for 5 weeks. Subsequently, the animals were anaesthetized through inhalation of isoflurane (1–4%) before being euthanized via cervical dislocation. Brain tissues were harvested and kept at -80 °C for molecular and biochemical studies, while segments were preserved in buffered formalin for histological and immunohistochemical analysis.

Table 1 Experimental animal groups

Groups	Description	Treatment, orally
1	Control	Distilled water
2	AlCl ₃	100 mg/kg per day of AlCl ₃
3	CA200	200 mg/kg per day of CA
4	CA400	400 mg/kg per day of CA
5	CA600	600 mg/kg per day of CA
6	CA200 + AlCl ₃	200 mg/kg per day of CA plus 100 mg/kg per day of AlCl ₃
7	CA400 + AlCl ₃	400 mg/kg per day of CA plus 100 mg/kg per day of AlCl ₃
8	CA600 + AlCl ₃	600 mg/kg per day of CA plus 100 mg/kg/day of AlCl ₃

Assessment of oxidative stress biomarkers

The tissues were homogenized in fifty mM phosphate buffer at pH 7.4 to quantify the amounts of Malondialdehyde (MDA), Glutathione (GSH), and Superoxide dismutase (SOD), depending on the previous literatures (Ohkawa et al. 1979, Beutler and Mary 1963, Nishikimi et al. 1972). The homogenate was centrifuged for ten minutes at 3000 rpm and 4 °C. The resulting supernatants were used to evaluate the previously mentioned oxidative stress indicators.

Assessment of genetic damage by the comet assay

DNA damage in brain tissues was assessed using the alkaline single-cell gel electrophoresis (SCGE) comet assay, as detailed in references (Tice et al. 2000, Nandhakumar et al. 2011). The slides were prepared and examined with a fluorescence microscope (Leica, Germany) to capture images of approximately 50 cells. The data were then assessed with Comet Score™ Version 1.5 software.

Gene expression analysis

Our research evaluated the expressions of *Caspase-3*, *Bax*, *Bcl2*, *IL-1β*, *Gsk-3β*, and *GAPDH* in brain tissue samples kept at -80 °C before analysis. Primers were designed utilizing the Primer Blast program in NCBI and manufactured in Carlsbad by Invitrogen, CA, USA, as specified in Table 2. *GAPDH* served as a housekeeping gene to normalize gene expression. RNA extraction was conducted utilizing a specific GeneJET RNA purification kit; then, cDNA synthesis was performed using the RevertAid First Strand cDNA Synthesis Kit. All these kits were obtained from Thermo-Scientific, USA. An analysis of samples from the control and treated groups was conducted.

We followed the kit instructions to perform qPCR, using a 25 μL reaction mixture that included cDNA, primers, an optimized PCR buffer Maxima Hot Start Taq DNA Polymerase, and dNTPs (Thermo Scientific Maxima SYBR Green qPCR Master Mix). The PCR method

comprised an initial heat activation phase at 95 °C for 15 min, followed by 40 denaturation cycles at 95 °C for 15 s, and subsequent annealing and elongation for 1 min at 60 °C. We conducted triple analyses of each gene and executed relative quantification of all genes employing the Comparative Ct method (ΔΔCT) to evaluate each gene’s expression in relation to the reference gene.

Immunohistochemical analysis of β-amyloid and tau protein

Paraffin-embedded brain sections measuring 5 mm were dewaxed with xylene and then re-moistened through a gradually increasing range of alcohols. Sections were treated with a 3% H2O2 solution to inhibit peroxidase activity, with anti-β-amyloid and anti-tau antibodies, and with HRP-conjugated secondary antibody and DAB chromogen. After counterstaining the sections with hematoxylin, the slides underwent examination using a Zeiss light microscope (Gad et al. 2022). Protein accumulation was evaluated using semi-quantitative visual scoring based on staining intensity compared to the control group. The scores were assigned as (+), (++), and (+++) for mild, moderate, and severe accumulation, respectively, under a standardized microscope setting to ensure consistency. Scoring was done carefully to minimize bias, and images were assessed under the same light and magnification conditions.

Histopathological examination

Fresh brain samples were restored for 24 h in 10% buffered formalin. After that, they were washed with tap water, dehydrated using an increasing series of alcohol, and cleared with xylene, then immersed in molten paraffin wax (56 degrees) and incubated in a hot air oven for a full day. After being sectioned by a rotary LEITZ microtome to a thickness of 5 μm, the tissue sections were placed on glass slides, deparaffinized, rehydrated,

Table 2 The primer sequences of the analyzed genes

Gene	Primers Sequences (5'-3')	
	Forward	Reverse
<i>Caspase3</i>	Gagcttgaacgcgaagaaa	Gagtccatcgacttgcttcca
<i>Bax</i>	Gaaccatcatgggctggaca	Gggtcccgaagtaggaaagg
<i>Bcl2</i>	Ctgggtggacaacatcgctct	Gcatgctggggccatatagt
<i>GSK-3B</i>	Ctggccaccatccttatecc	Agaagcggcggtattggtct
<i>IL-1B</i>	Agcagctttcgacagtgagg	Tcatctggacagcccaagtc
<i>GAPDH</i>	Gtatcggaagcctggttac	Cttgccgtgggtagagtc

and stained with hematoxylin and eosin. Afterward, they were examined using a light microscope (Bancroft 2013).

Analysis of statistical data

The analysis utilized IBM-SPSS version 23 software and confirmed a normal distribution. A two-way ANOVA was carried out to evaluate the effects of time and/or treatment on the variables under study. Duncan's test assessed homogeneity among the groups, and the least significant difference (LSD) method was used to identify significant differences between time intervals.

Brain

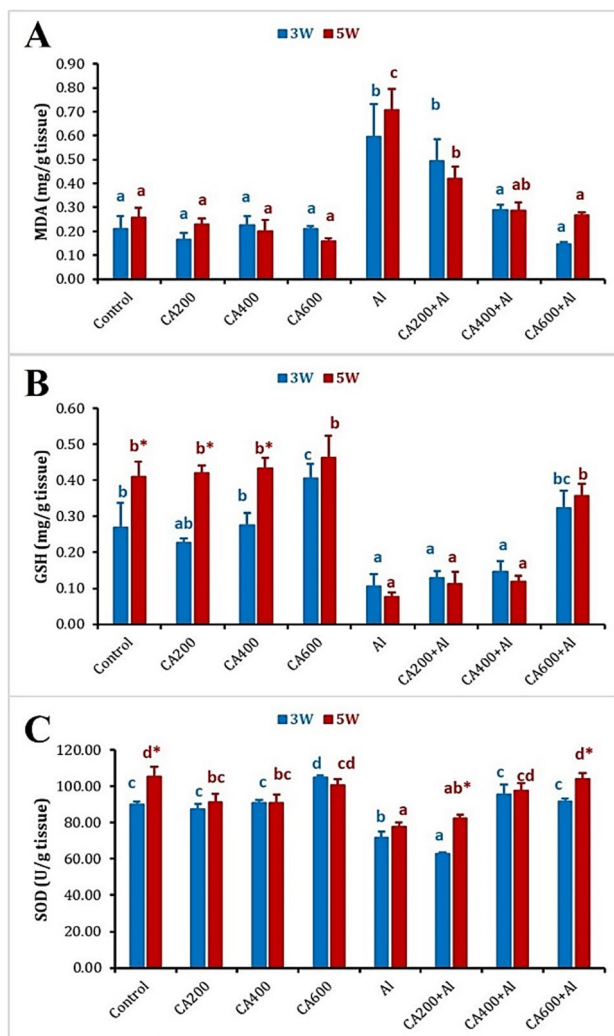


Fig. 1 The levels of malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD) in the brain tissue of rats were assessed after oral administration of 200, 400, and 600 mg/kg of CA, and 100 mg/kg of AlCl_3 , and CA combined with Al daily over periods of 3 and 5 weeks. Identical superscript letters indicate no considerable difference ($P > 0.05$), while different letters signify a significant difference ($P < 0.05$). * ($P < 0.05$) refers to comparisons made between the 3-week and 5-week assessments. CA stands for *Centella asiatica*, and Al for AlCl_3

Results

CA extract protects the brain against oxidative stress

The outcomes of oxidative stress parameters in the brain following three and five weeks of treatment are shown in Fig. 1. The MDA levels in all groups treated with CA extract alone exhibited no statistical differences ($P > 0.05$) in comparison to the control at both periods. GSH and SOD activities were mainly insignificant ($P > 0.05$) in the examined groups, except for CA200 (GSH) and CA600 (GSH&SOD), which were statistically different ($P < 0.05$). Additionally, there is a significant ($P < 0.05$) difference among all examined groups when comparing the two examined durations.

After both 3 and 5 weeks, rats treated with AlCl_3 exhibited a significant increase ($P < 0.05$) in brain MDA levels, along with a noticeable decrease in the antioxidant enzymes SOD and GSH, compared to the control group. Interestingly, co-administration of CA with AlCl_3 helped restore the oxidative balance, as reflected by a significant reduction in MDA levels and an increase in SOD and GSH activity ($P < 0.05$). However, the elevation in GSH was not statistically significant ($P > 0.05$) in the CA200+Al and CA400+Al groups. Notably, the highest dose CA600+Al showed the most prominent protective effect, particularly after 5 weeks, indicating a dose-dependent improvement in oxidative stress markers.

CA extract protects brain tissue against DNA damage

The study found that rats treated with CA200, CA400, and CA600 did not display significant differences in TL, %DNA, and TM measurements related to the control group after 3&5 weeks, except CA400 (TL&TM) revealed a statistically different ($P < 0.05$). However, OTM values in brain tissues of rats treated with CA400 and CA600 were markedly increased ($P < 0.05$) after 3 weeks, but after 5 weeks, the OTM values in all three groups were like the control group.

Nevertheless, the experimental animals administered aluminium chloride exhibited a substantial ($P < 0.05$) elevation in all parameters shown in Fig. 2. The values of TL, %DNA, TM, and OTM in the brain cells of rats administered CA and Al progressively aligned with the normal levels of the control group after 3 and 5 weeks. Following the explanation of the statistical results, the findings were further supported by fluorescent microscopy images, as shown in Fig. 3, which visually corroborated the statistical data. These images demonstrated an apparent reduction in DNA damage markers in the CA-treated group, aligning with the significant differences observed in the statistical analysis ($P < 0.05$) as shown in Fig. 2.

Brain

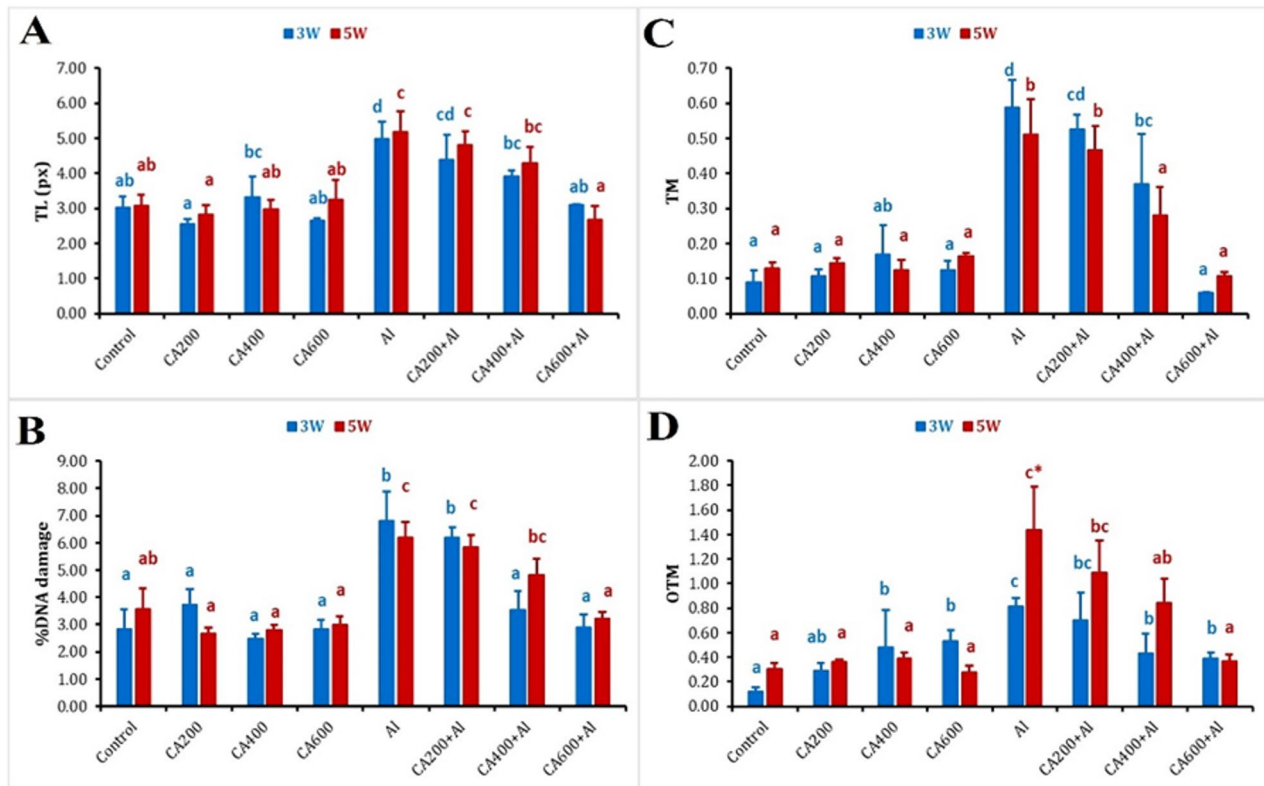


Fig. 2 The changes in tail length (TL, px), the percent DNA damage in the tail (%DNA), the changes in the tail moment (TM), and the changes in the olive tail moment (OTM) in the brain tissue of rats were assessed after oral administration of 200, 400, and 600 mg/kg of CA, and 100 mg/kg of AlCl_3 , and CA combined with Al daily over periods of 3 and 5 weeks. Identical superscript letters indicate no considerable difference ($P > 0.05$), while different letters signify a significant difference ($P < 0.05$). * ($P < 0.05$) refers to comparisons made between the 3-week and 5-week assessments. CA stands for *Centella asiatica*, and Al for AlCl_3

CA extract reduces the expression of apoptotic and inflammatory genes

Casp-3 and *Bax* mRNA expression were increased ($P < 0.05$), and the *Bcl2* mRNA amount was down-regulated in the Al-treated group contrasted to the sham at both intervals. In addition, CA extracts regulated the gene expression ($P < 0.05$) in the examined groups; CA upregulated the levels of *Bcl2* and downregulated the levels of *Bax* & *Casp-3* at both durations. However, there were no significant differences between CA doses in all examined genes, as shown in Fig. 4.

In addition, the inflammatory gene *Il-1 β* and *Gsk-3 β* mRNA levels were upregulated in the aluminum group compared to the sham group ($P < 0.05$) in the brain tissues. CA extract notably decreased the levels of *Il-1 β* and *Gsk-3 β* in a dose-dependent way compared to the Al-treated group at both durations, as shown in Fig. 5. Moreover, CA600 reported significant ($P < 0.05$) down-regulation in the *Gsk-3 β* gene expression compared to CA200 and CA400 groups. Both CA400 and CA600 groups significantly ($P < 0.05$) reduced the expression of

Il-1 β compared to the group treated with 200 mg/kg of CA.

CA extract decreased the accumulation of Beta-amyloid plaques

Figure 6 shows the Beta-amyloid protein's expression level in the brain's hippocampus region. The group treated with Al showed increased Beta-amyloid protein with ($P < 0.05$) after 3 and 5 weeks compared to the control. However, the extract of CA leads to decreased Beta-amyloid accumulation in the hippocampus sections compared to the Al-treated group

CA extract downregulates the expression of the tau protein

Figure 7 shows the amounts of tau protein in the hippocampal area of the brain. The group treated with Al significantly increased Tau protein expression after 3 and 5 weeks compared to the sham group. However, an increase in the dose of CA led to a gradual decrease in the Tau protein level in the brain tissue of the CA + Al groups

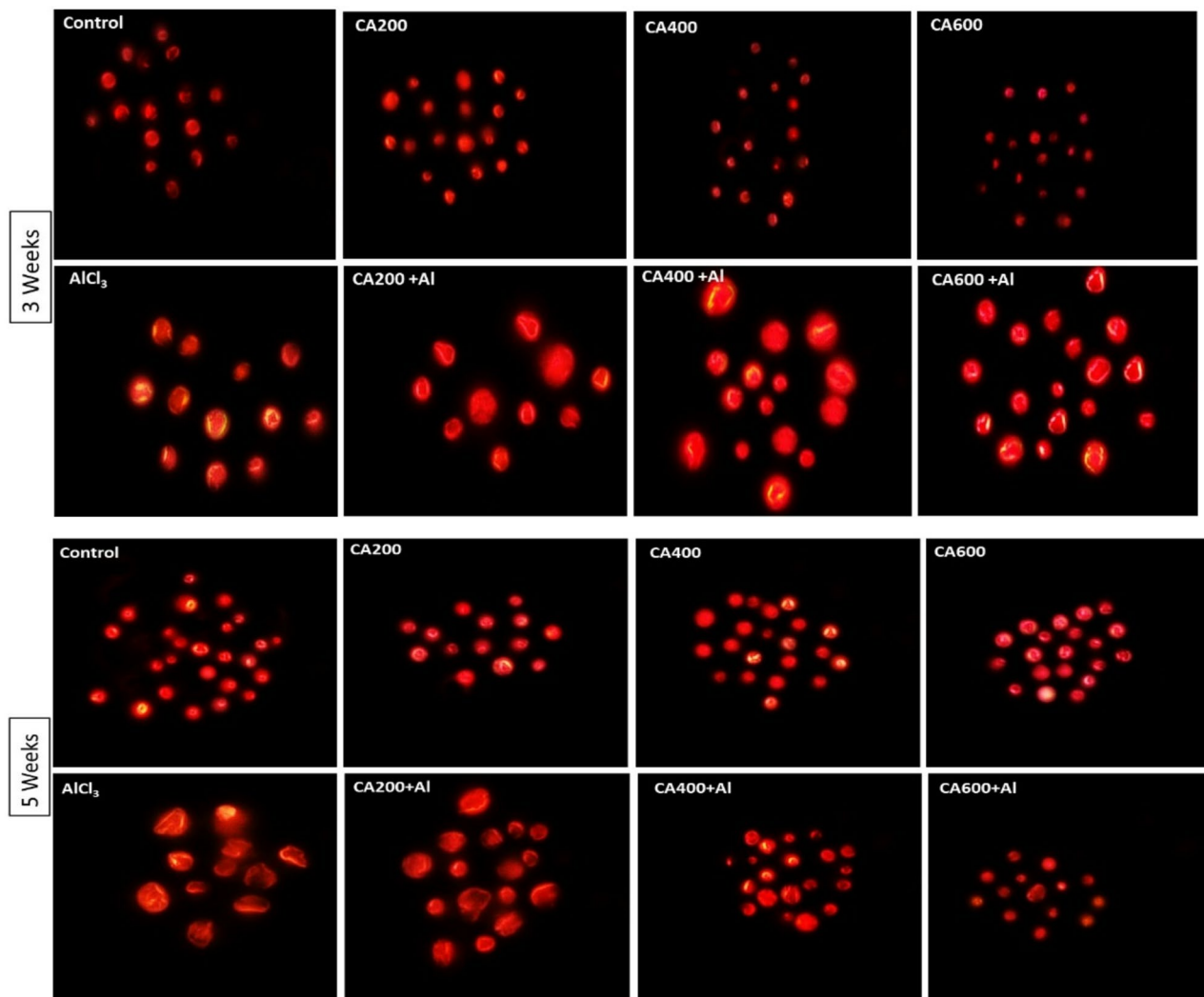


Fig. 3 Fluorescent microscope micrographs depicting the degree of DNA break in brain tissue evaluated by the alkaline comet test after oral administration of 200, 400, and 600 mg/kg of CA, and 100 mg/kg of AlCl_3 , and CA combined with Al daily over periods of 3 and 5 weeks (200x). CA: *Centella asiatica* and Al: AlCl_3

compared to the Al-treated group. The CA600 + Al group showed negative expression after 3 and 5 weeks

Ameliorative effect of CA on brain tissue

Cerebral cortex

The brain's cerebral cortex was examined under a light microscope to investigate the histological structure, as in Fig. 8. At both durations, 3&5 weeks, each of the control and treated groups, CA200, CA400, and CA600, had a normal histological structure of the cortex with regular neuron architecture. Rats treated with AlCl_3 alone showed widespread neuronal nuclear pyknosis, gliosis, and degeneration. In the CA200 + AlCl_3 group, most neurons still exhibited nuclear pyknosis and gliosis, whereas in the CA400 + AlCl_3 group, nuclear pyknosis remained, but only a few neurons were degenerated. The CA600 + Al group significantly improved the brain's

histological architecture, with many normal cells close to the control.

Hippocampus

Histopathological examination was conducted on the dentate gyrus in the hippocampus. After 3&5 weeks of treatment, the results indicated no histopathological changes, and the neurons had a typical architecture in both control and treated groups with CA200, CA400, and CA600. However, the group treated with AlCl_3 showed nuclear pyknosis, gliosis, and neurodegeneration. Furthermore, the group treated with CA200 + Al displayed nuclear pyknosis in most of the neurons and a lot of degenerated cells after 3 weeks, while it had degeneration in a few neurons after 5 weeks. The CA400 + Al and CA600 + Al groups had nucleus pyknosis seen in neurons and a few degenerated cells after 3 weeks of treatment,

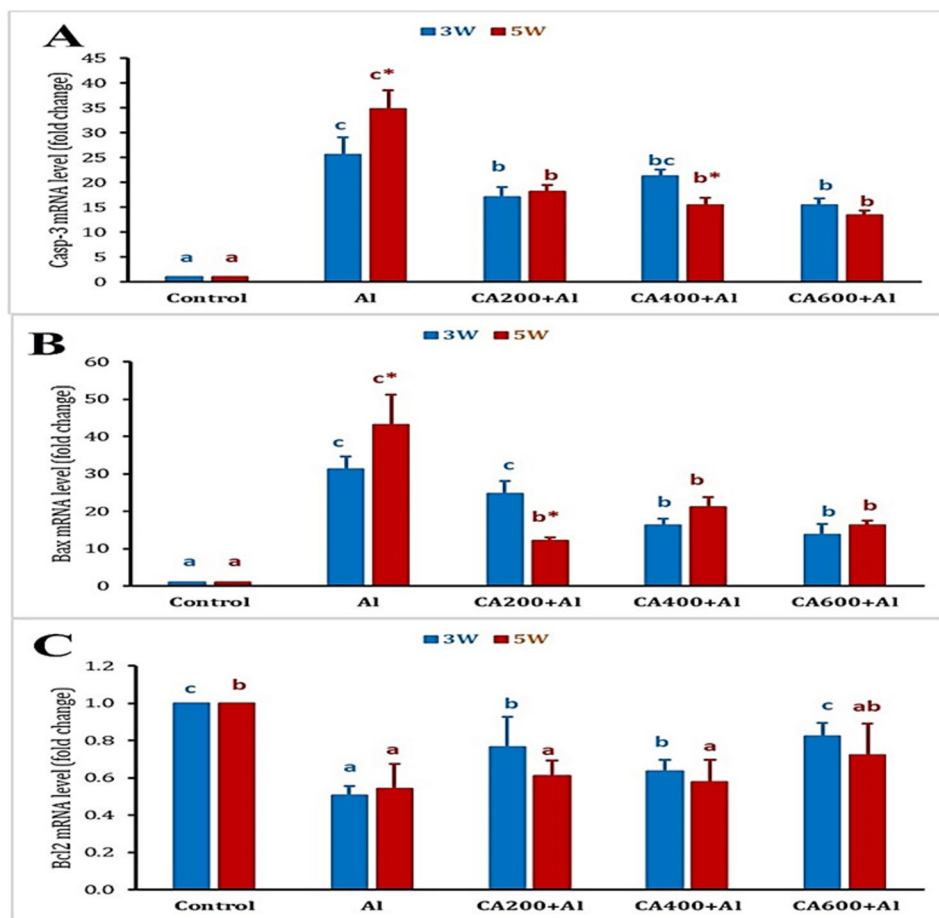


Fig. 4 The mRNA levels of *casp-3*, *Bax*, and *Bcl2* in the brain tissue of rats were treated orally with CA (200, 400, and 600 mg/kg), and (100 mg/kg) AlCl_3 daily for three and five weeks. Identical superscript letters indicate no considerable difference ($P > 0.05$), while different letters signify a significant difference ($P < 0.05$). * ($P < 0.05$) refers to comparisons made between the 3-week and 5-week assessments. CA stands for *Centella asiatica*, and AI for AlCl_3

while they exhibited no histopathological changes and preserved a normal histology architecture of neurons. as in Fig. 9.

Striatum

The striatum was examined, as shown in Fig. 10. There were no changes, and the neurons had a typical architecture in the control and treated groups CA200, CA400, and CA600 at both durations. However, the AlCl_3 group showed a nucleus with pyknosis, gliosis, and focal eosinophilic plaque formation in some neurons. In the CA200+AI group, by week 3, neurons displayed nuclear pyknosis, gliosis, and intracellular edema. By week 5, some neurons still exhibited nuclear pyknosis and gliosis, now accompanied by the appearance of multiple focal eosinophilic plaques. Meanwhile, the CA400+AI and CA600+AI groups showed nuclear pyknosis and gliosis in a few neurons after 3 weeks. However, after 5 weeks, these groups demonstrated gliosis, but with many normal cells.

Discussion

The research assessed the neuroprotective effects of *Centella asiatica* against aluminium chloride-induced neurodegeneration. Findings demonstrated that the extract mitigated aluminium-induced neurotoxicity, with a 600 mg/kg dosage exhibiting the most significant benefit in brain tissue. The *Centella asiatica* extract restored biochemical homeostasis by dose-dependently reducing oxidative stress and enhancing the antioxidant activities of GSH and SOD. It contributed to reestablishing tissue homeostasis by significantly decreasing all parameters of DNA damage. The treatment reduced the accumulation of β -amyloid plaques and Tau protein, downregulated the apoptotic genes *Casp-3* and *Bax*, as well as the inflammatory mediators $\text{IL-1}\beta$ and $\text{Gsk-3}\beta$, and, in a dose-dependent manner, upregulated the anti-apoptotic gene *Bcl2*. Histopathological examination of the brain indicated an ameliorative effect of *Centella asiatica* against aluminium chloride-induced neurodegeneration and toxicity.

The brain consumes a high amount of oxygen but has a weak antioxidant system, making it vulnerable to stress

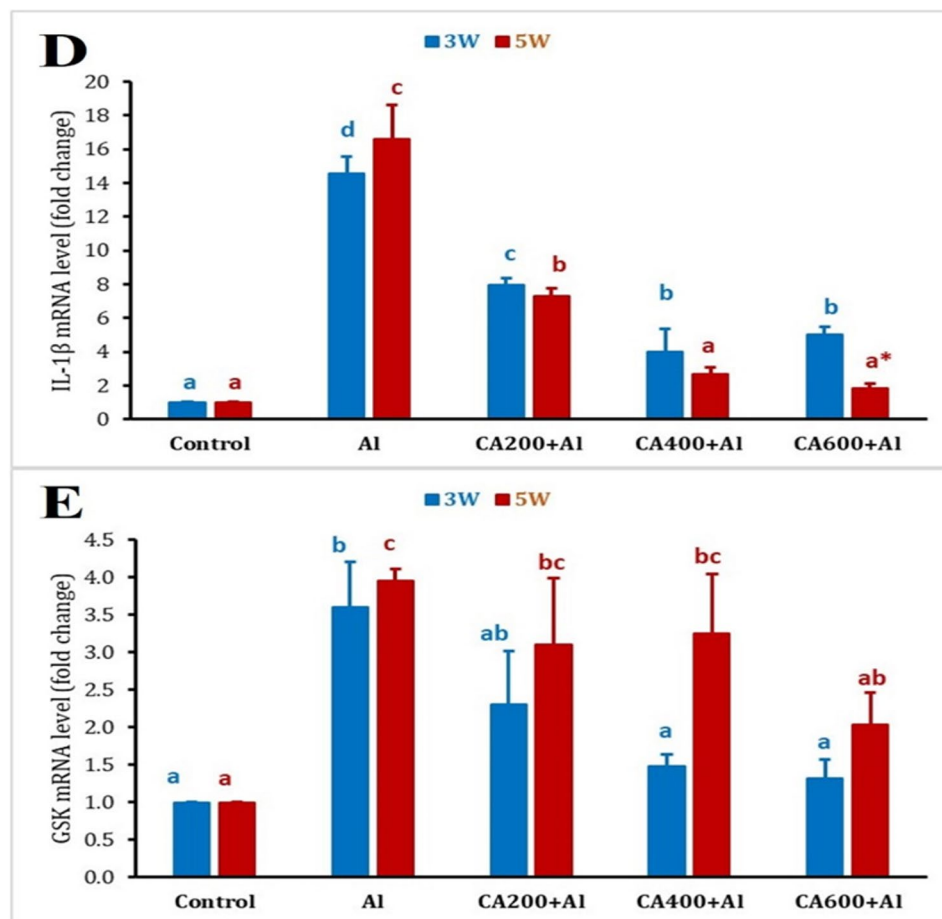


Fig. 5 The mRNA levels of *Il-1β* and *Gsk-3β* in the brain tissue of rats were treated orally with CA (200, 400, and 600 mg/kg), and (100 mg/kg) $AlCl_3$ daily for three and five weeks. Identical superscript letters indicate no considerable difference ($P > 0.05$), while different letters signify a significant difference ($P < 0.05$). * ($P < 0.05$) refers to comparisons made between the 3-week and 5-week assessments. CA stands for *Centella asiatica*, and Al for $AlCl_3$

from oxidation, particularly in the cortex and hippocampus (Gofir et al. 2021). The accumulation of aluminum can harm membrane lipids, proteins, and antioxidant defences, leading to oxidative cell injury. This occurs due to the tissue's high oxygen demands and abundance of fatty acids, particularly susceptible to peroxidation (Kaiser et al. 2005). Aluminium is a potent Lewis acid that can react with superoxide anions, forming AlO_2^{2+} ; this complex is a powerful oxidant than the superoxide anion itself. The complex accelerates the formation of hydrogen peroxide and hydroxyl radicals, which participate in creating an oxidizing environment (Exley 2004).

In the current study, rats administered with $AlCl_3$ showed an increase in MDA levels, while the GSH and SOD activities were reduced in the brain throughout the examined durations. This aligns with previous studies indicating that aluminium (Al) causes lesions in the hippocampus by disrupting the balance between the production of reactive oxygen species (ROS) and the cells' antioxidant capacity. This imbalance causes oxidative

stress, eventually resulting in cell death (Kumar and Gill 2014, Lu et al. 2013).

The results revealed normal levels of MDA, GSH, and SOD in all groups treated with CA extract alone. Co-administration of CA with aluminum markedly attenuated aluminum-induced oxidative stress in neural cells. Compared with the Al-only group, MDA levels fell while GSH and SOD activities rose in a dose-dependent way at both 3 and 5 weeks, demonstrating CA's protective effect against $AlCl_3$ -mediated cytotoxicity. This benefit likely reflects CA's high antioxidant content, including phenolic compounds such as quercetin and catechin (Tripathy and Srivastav 2023). Furthermore, this agrees with previous studies indicating that the aqueous extract of CA enhances tissue homeostasis and exhibits antioxidant properties (Kumar and Gupta 2002, Gupta and Flora 2006, Kumar et al. 2020).

Oxidative stress results from an imbalance between reactive oxygen species (ROS) and a weakened antioxidant defense system (Pryor 1986). ROS, such as hydrogen

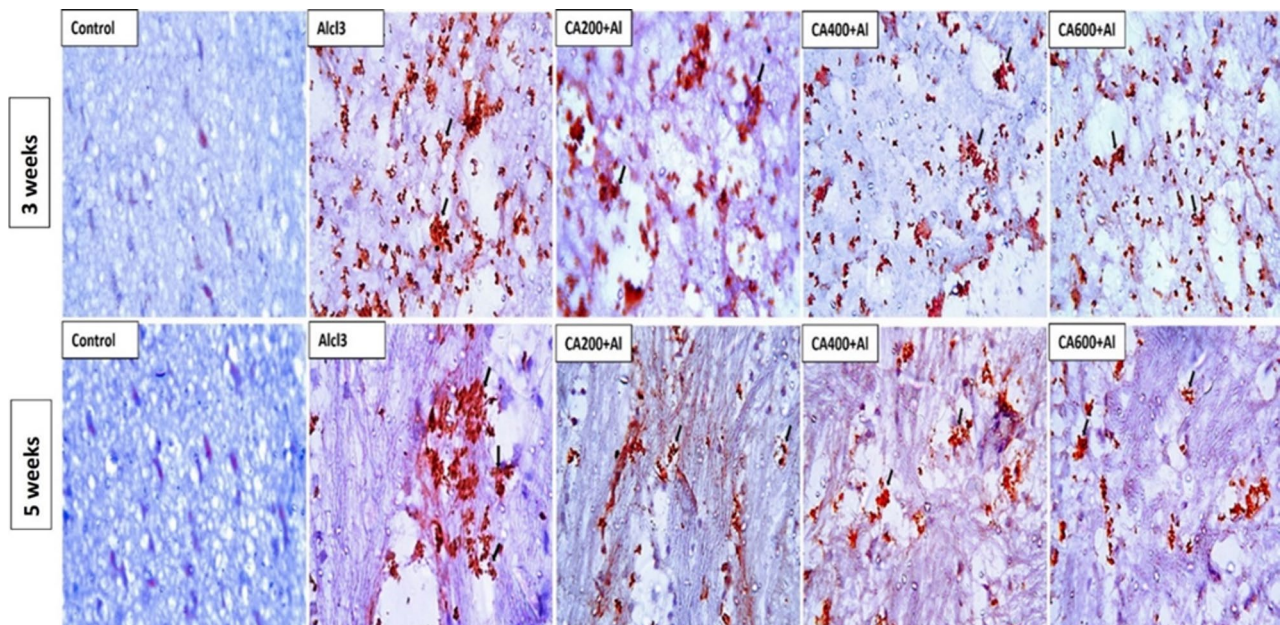


Fig. 6 Immunohistochemical photomicrographs demonstrate Beta-amyloid amounts in brain sections of rats treated orally with CA (200, 400, and 600 mg/kg), and (100 mg/kg) Aβ13 daily for three and five weeks. The control rat section showed a negative expression. The Aβ13 group showed severe expression (+++). CA200 + AI group showed moderate expression (++). CA400 + AI group showed moderate expression (++). CA600 + AI group showed mild expression (+). After 5 weeks of treatment, the control rat section showed negative expression. The Aβ13 group showed severe expression (+++). CA200 + AI group showed moderate expression (++). CA400 + AI group showed mild expression (+). CA600 + AI group showed mild expression (+). CA Centella asiatica and AI: Aβ13 (80x)

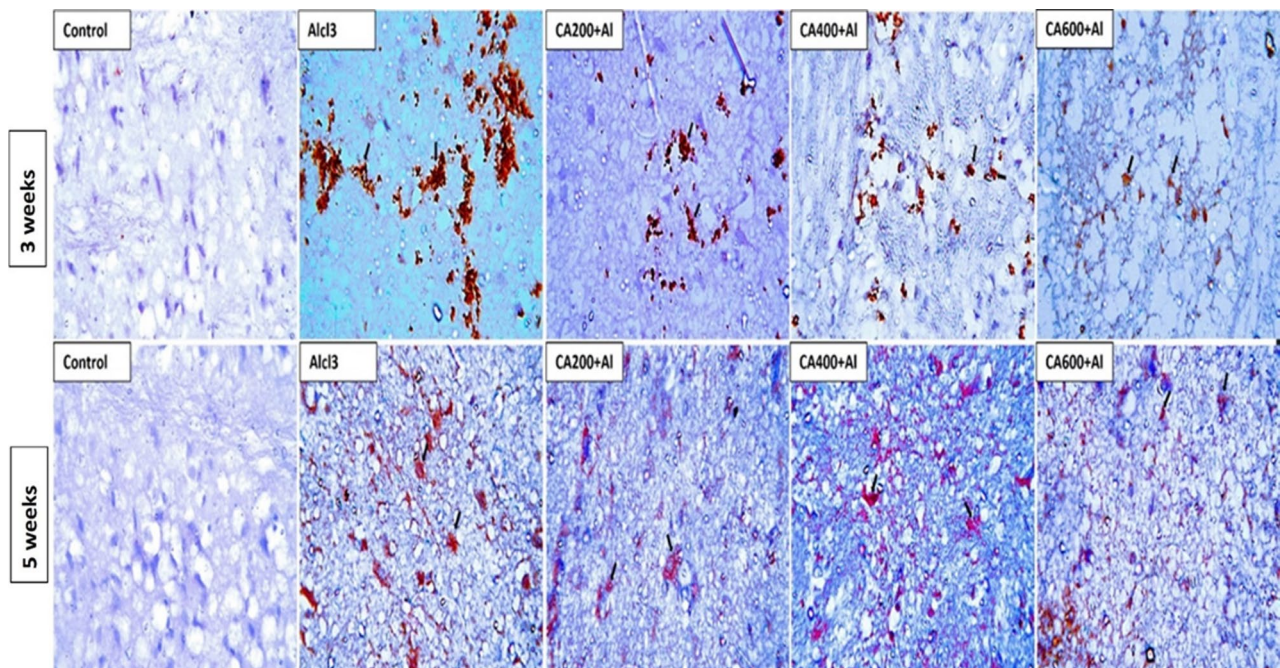


Fig. 7 Immunohistochemical photomicrographs demonstrate the Tau protein amounts in the brain sections of rats treated orally with CA (200, 400, and 600 mg/kg), and (100 mg/kg) Aβ13 daily for three and five weeks. The control rat section showed a negative expression. The Aβ13 group showed moderate expression (++). CA200 + AI group showed mild expression (+). CA400 + AI group showed mild expression (+). CA600 + AI group showed a negative expression (-). After 5 weeks of treatment, the control rat section showed negative expression. The Aβ13 group showed moderate expression (++). CA200 + AI group showed moderate expression (++). CA400 + AI group showed mild expression (+). CA600 + AI group showed a negative expression (-). CA Centella asiatica and AI: Aβ13 (80x)

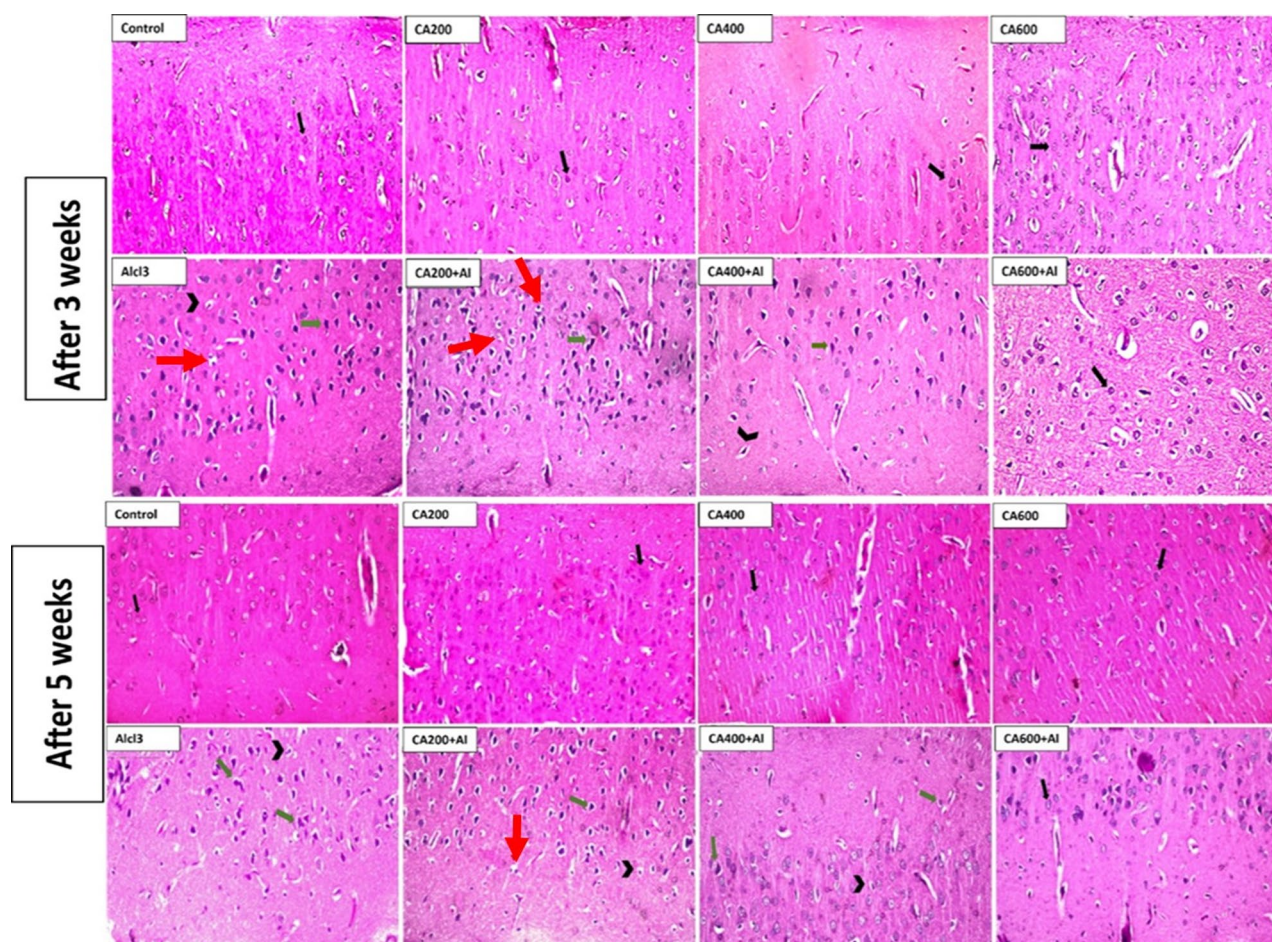


Fig. 8 Photomicrographs of cerebral cortex sections of rats treated orally with CA extract (200, 400, and 600 mg/Kg per day) with and without 100 mg/Kg per day of AlCl_3 for 3 & 5 weeks. The control, the CA200, 400, and 600 groups have the typical histological features of the brain's cerebral cortex section (black arrow). The AlCl_3 group showed nuclear pyknosis (green arrow), gliosis (red arrow), and degeneration (arrowhead) in most of the neurons. The CA200 + AI group showed nuclear pyknosis (green arrow) and gliosis (red arrow) in most neurons. The CA400 + AI group had nuclear pyknosis (green arrow) and a few degenerated cells (arrowhead). The CA600 + AI group showed more improvement in the brain with many normal cells close to the control (black arrow) (H&E, 40x)

peroxide (H_2O_2) and hydroxyl radicals ($-\text{OH}$), can interact with nucleophilic centers in cells, leading to covalent attachment to nucleic acids, RNA, and proteins. This can cause single-strand breaks, double-strand breaks, and DNA adducts, ultimately resulting in DNA damage (Cadet et al. 2017). The comet assay is a well-established technique for detecting various DNA alterations, including single-strand and double-strand breaks, alkali-labile sites, cross-links, and incomplete repair sites (Boeck et al. 2000). DNA damage is a hallmark of apoptosis, and the percentage of DNA in the tail is the best parameter for assessing induced DNA damage (Sumathi et al. 2013).

The present study indicated an elevation in all DNA damage markers: Tail length (TL), %DNA, tail moment (TM), and olive tail moment (OTM) in AI-treated groups. This agrees with the previous studies, indicating that AI treatment induces ROS production and oxidative DNA damage (Sumathi et al. 2013, Abbas et al. 2019). In

addition, co-administration of CA with AI resulted in a significant reduction in all DNA damage parameters in a dose-dependent manner in both durations. This agrees with the previous studies, which showed that CA protects against DNA damage. According to reports, CA can stop DNA damage and lipid peroxidation by acting as an antioxidant and free radical scavenger (Anand et al. 2010, Rashid et al. 2023, Yahya and Nurrosyidah 2020).

AlCl_3 treatment leads to a disruption in the balance between ROS production and the antioxidant system, potentially causing oxidative DNA damage. Additionally, the intake of AI can lead to neuroinflammation and impairment of cognitive function (Exley et al. 2006, Batool et al. 2016). Neuroinflammation can change the density of dendritic spines, which in turn can affect cognitive function (Cao et al. 2016). In this study, AI treatment resulted in the upregulation of an inflammatory-related gene (*IL-1 β*), which was consistent with

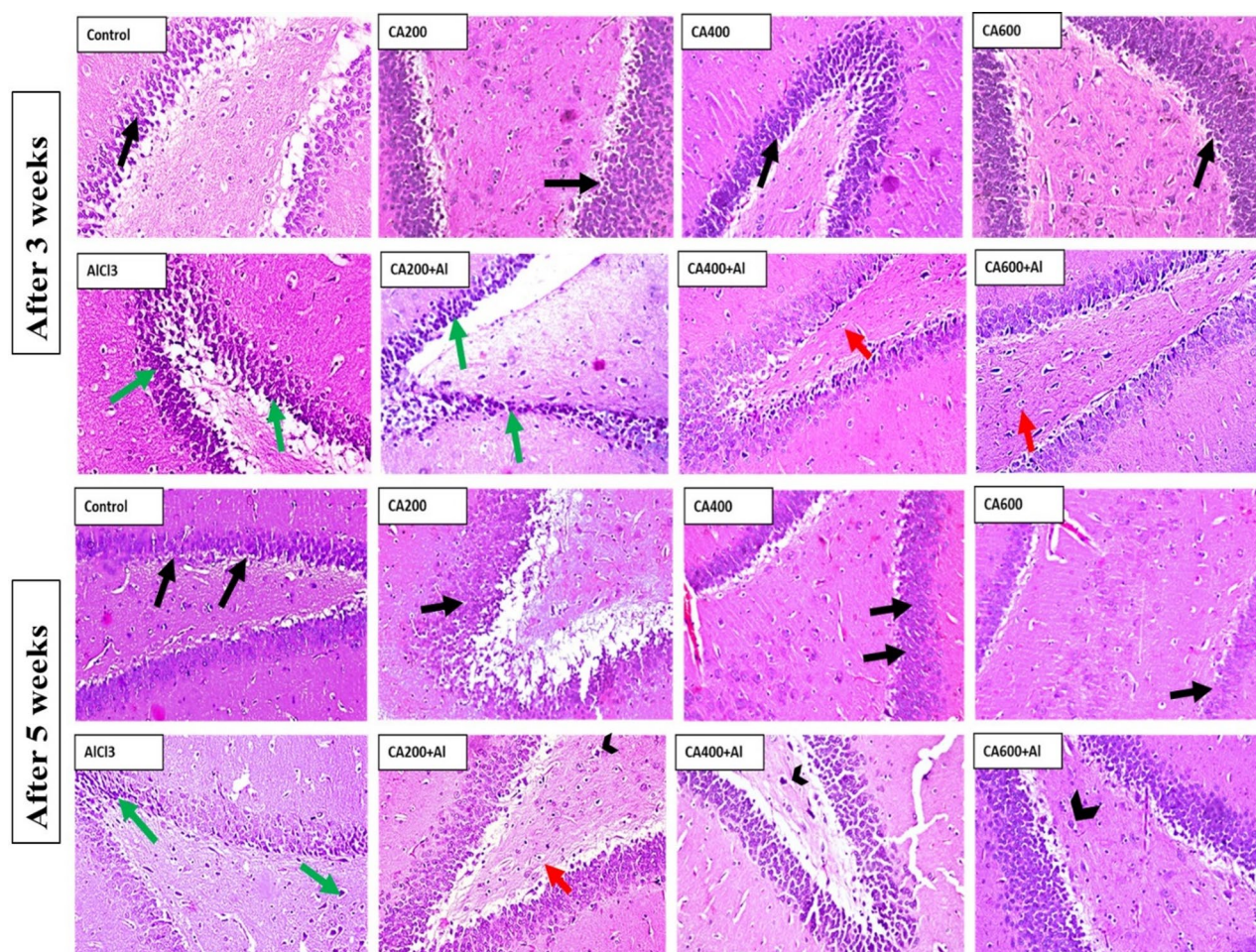


Fig. 9 Photomicrographs of the dentate gyrus in the hippocampus of rats with varying oral dosages of CA extract (200, 400, and 600 mg/kg per day) with and without 100 mg/Kg per day of AlCl_3 for 3&5 weeks. The control rat section showed the standard histological structure of the dentate gyrus (black arrow). CA200, CA400, and CA600 groups showed no histopathological change and normal architecture of neurons (black arrow). The AlCl_3 -treated rats illustrated nuclear pyknosis (green arrow) and degeneration (arrowhead) in brain tissue cells. The CA200 + Al group showed nuclear pyknosis in most neurons (green arrow) and many degenerated cells (arrowhead). CA400 + Al group showed a nucleus with pyknosis in a few neurons (green arrow) and a few degenerated cells (arrowhead). CA600 + Al group showed a nucleus with pyknosis (green arrow) and a few degenerated cells (arrowhead) after 3 weeks and standard histological structure after 5 weeks (H&E. 40x)

findings from (Cao et al. 2019, Milnerowicz et al. 2015), indicating that AlCl_3 can induce the production of inflammatory cytokines. In addition, Aluminum can activate microglia, which release inflammatory mediators, worsening neuroinflammation and potentially leading to neurodegeneration (Renke et al. 2023). Moreover, the co-administration of CA with Al leads to downregulation in the expression of $\text{IL-1}\beta$ in all treated groups in a manner dependent on the dosage in the examined durations. Recent studies indicated the anti-inflammatory effect of CA extract and its ability to downregulate the expression of inflammatory cytokines (Yan et al. 2023, Mairuae et al. 2019). This may be due to CA constituents of polyphenolic compounds, Triterpenes, and Asiatic acids.

Apoptosis is a natural mechanism of programmed cell death essential for both embryonic development and the maintenance of adult tissues. It selectively eliminates

old, unhealthy, and unnecessary cells, maintaining tissue homeostasis. Improper regulation of apoptosis is associated with various human diseases, including neurodegenerative disorders like Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). The overproduction of reactive oxygen species (ROS), DNA damage, and neuroinflammation can all lead to the induction of apoptosis (Shoshan-Barmatz et al. 2023, Elmore 2007).

Rats treated with AlCl_3 showed upregulation in the apoptotic genes (*Bax* and *Caspase-3*) and downregulation in the anti-apoptotic gene level (*Bcl2*) in the brain tissue. Animal groups treated with CA and Al indicated an inverse in the levels of these genes compared to the Al-treated group. Ahmed Rather et al. illustrated downregulation in the level of *Bax* and *casp-3* and upregulation in the level of the *Bcl2* gene in rats after administration of

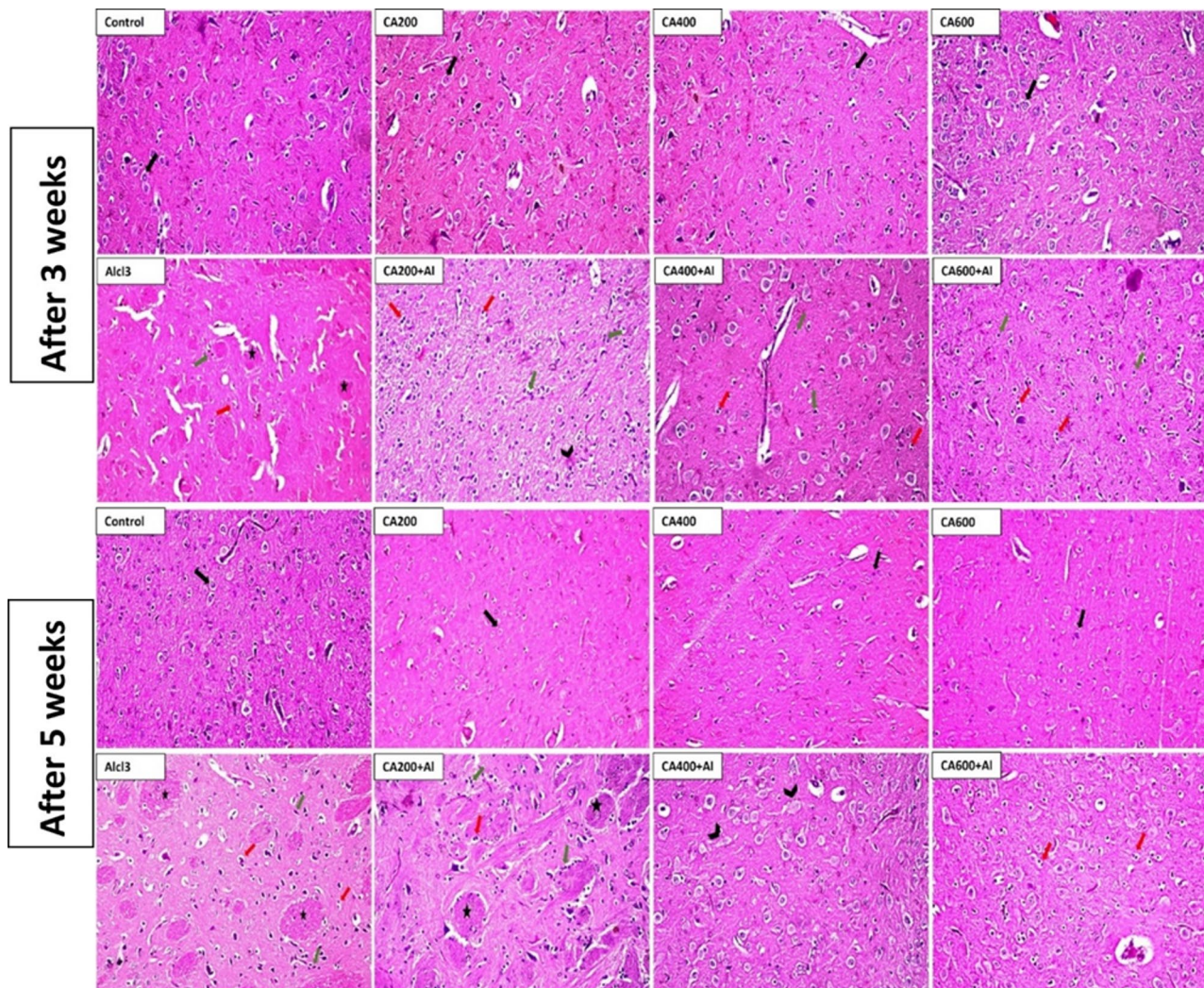


Fig. 10 Photomicrographs of the striatum of rats treated orally with CA (200, 400, and 600 mg/kg per day) with and without AlCl_3 (100 mg/kg per day) for 3&5 weeks. The control, CA200, 400, and 600 groups showed normal histological structure of brain striatum sections at both durations (black arrow). The AlCl_3 group had nuclear pyknosis (green arrow) and gliosis (red arrow) in some neurons with multiple focal eosinophilic plaques (green arrow), gliosis (red arrow), and intracellular edema between the neurons. CA400 + Al and CA600 + Al groups, respectively, showed nuclear pyknosis (green arrow) and gliosis (red arrow) in a few neurons (H&E, 40x)

CA constituent Asiatic acid (Ahmad Rather et al. 2019). In addition, the Alzheimer's rat model treated with CA extract indicated upregulation in the *Bcl2* (Chiroma et al. 2019).

It was reported that aluminum can increase the concentrations of beta-amyloid ($\text{A}\beta$), leading to the buildup of $\text{A}\beta$ plaques. Furthermore, it induces the phosphorylation of the tau protein, therefore reducing its solubility and hence enhancing the possibility of its aggregation. Rats treated with AlCl_3 exhibited the buildup of β -amyloid plaques and tau protein, along with an increase in the expression of glycogen synthase kinase-3 β (*Gsk-3 β*) in the brain cells. Previous research has suggested that exposure to Al can result in neurotoxicity and the accumulation of $\text{A}\beta$ plaques due to dysregulation of kinase enzymes such

as *Gsk-3 β* (Ahmad Rather et al. 2019, Hashimoto et al. 2000, Blennow et al. 2006, Thenmozhi et al. 2015).

In rats, the co-treatment of CA with AlCl_3 resulted in a reduction of $\text{A}\beta$ plaques, Tau proteins, and *Gsk-3 β* levels in a dose-dependent manner. This effect was observed in both small and extended periods of time, as demonstrated in the immunohistochemical and gene expression graphs in the results section. Chiroma et al. and Rather et al. illustrated downregulation in the expression of *Gsk-3 β* and a decrease in $\text{A}\beta$ plaque accumulation after administration of CA extract (Chiroma et al. 2019, Rather et al. 2018). According to reports, *Gsk-3 β* is responsible for the hyperphosphorylation of tau protein and the accumulation of $\text{A}\beta$ plaques (Snitow et al. 2021, Abu-Elfotuh et al. 2023). The current study demonstrated a downregulation in *Gsk-3 β* and $\text{A}\beta$ plaques, which led to less accumulation

of tau protein, depending on the dosage during the examined durations after CA treatment. Reduced accumulation of amyloid-beta and tau proteins in brain tissues, confirmed by immunohistochemical analysis, further supports the involvement of *Gsk-3 β* , as this pathway is well-documented to regulate A β and tau phosphorylation and aggregation in neurodegeneration (Hooper et al. 2008). The observed downregulation of *Gsk-3 β* in CA-treated groups may be attributed to the anti-inflammatory and antioxidative properties of *Centella asiatica* extract. *Gsk-3 β* is a key modulator of neuroinflammation and oxidative stress, primarily through its regulation of the *NF- κ B* pathway and apoptotic signaling (Beurel et al. 2015, Kanninen et al. 2011). Previous studies have shown that asiaticoside, a major active constituent of CA, can inhibit *Gsk-3 β* activity, thereby reducing tau phosphorylation, A β accumulation, and inflammation (Liu et al. 2023).

All the earlier illustrations demonstrated the neuroprotective properties of CA, highlighting its function as an antioxidant and anti-inflammatory agent, along with its capacity to restore tissue homeostasis following neurotoxin exposure. The histopathological examination of different brain regions of the cerebral cortex, the hippocampus, and the striatum illustrated the incidence of pyknosis, gliosis, focal eosinophilic plaque formation, and neurodegeneration due to AlCl₃ administration. This agrees with the previous studies that described the hazardous effect of AlCl₃ in brain tissue (Abbas et al. 2019, Chiroma et al. 2019, Rather et al. 2018).

Recent studies have shown that the administration of CA extract can enhance the elongation of neurites and accelerate the regeneration of nerve cells in vitro (Farhana et al. 2016). The current investigation found that the simultaneous administration of CA significantly reduced dementia and morphological alterations induced by AlCl₃ in rats, with the intensity of the effects varying with dosage. The extract works by promoting cholinergic transmission and diminishing oxidative stress, hence decelerating neurodegeneration in the brain, thereby maintaining memory and cognitive abilities, as detailed by Firdaus et al. (Firdaus et al. 2022). Furthermore, the ethanolic extract of CA has reduced the behavioral, biochemical, and neuroanatomical abnormalities, shielded the rat brain against cognitive impairment caused by AlCl₃, and prevented memory loss and cognitive impairment (Firdaus et al. 2022, Firdaus et al. 2022). Earlier studies had also shown that CA can prevent hippocampus neuronal death and maintain its ultrastructure, thereby reducing cognitive deficits in D-gal and AlCl₃-induced mice, as reported in (Chiroma et al. 2019).

Conclusion

The research investigated the neuroprotective effect of CA against neurodegeneration. CA works through various mechanisms, including modulation in the expression of genes related to apoptosis (*Bax*, *Bcl2*, and *Caspase-3*). Additionally, the observed neuroprotective effects of *Centella asiatica* may be partially mediated through modulation of the *Gsk-3 β* signaling pathway, which is known to influence tau phosphorylation, A β production, and neuroinflammation via *IL-1 β* . The observed reduction in pro-inflammatory (*IL-1 β*) and apoptotic (*Bax*, *Caspase-3*) gene expression, along with enhanced antioxidant defenses ($\uparrow$ GSH, $\uparrow$ SOD, $\downarrow$ MDA), suggests that CA modulates interconnected cellular mechanisms regulated by *Gsk-3 β* , particularly in the context of neuroinflammation and cell survival. Therefore, CA is a promising herbal medicine that protects against neurodegeneration. Further studies are required to evaluate the safety of CA. Future studies should include isolation of asiaticoside from the extract to evaluate its independent effects and explore different dosages using well-characterised animal models of specific neurodegenerative diseases. More studies are needed to assess the safety of CA.

Abbreviations

CA	<i>Centella asiatica</i>
SOD	Superoxide dismutase
A β	Beta-amyloid
ROS	Reactive oxygen species
Al	Aluminum
AlCl ₃	Aluminum chloride
NDs	Neurodegenerative diseases
AD	Alzheimer's disease
PD	Parkinson's disease
HD	Huntington's disease
MDA	Malondialdehyde
ALS	Amyotrophic lateral sclerosis
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
GSH	Glutathione
<i>Gsk-3β</i>	Glycogen synthase kinase-3
<i>Bax</i>	Bcl-2-associated X protein
<i>Bcl2</i>	B-cell leukemia 2 protein
<i>Caspase3</i>	Cysteine-aspartic acid protease
<i>IL-1β</i>	Interleukin-1 beta
<i>GADH</i>	Glyceraldehyde 3-Phosphate Dehydrogenase
EtBr	Ethidium Bromide
IACUC	Institutional Animal Care and Use Committee
SPSS	Statistical Package of the Social Sciences
ANOVA	Analysis of Variance
TL	Tail length
%DNA	The percent DNA damage in the tail
TM	Tail moment
OTM	Olive tail moment
<i>TNF-α</i>	Tumor necrosis factor α
<i>IL-6</i>	Interleukin-6

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Author contributions

S. G. H. and S. M. E. conducted the experiments, examined and created graphs of the data, and authored the first version of the paper. S. M. E., A. M. G., and H. R. H. M. supervised the work and critically revised the manuscript.

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Data availability

Corroborating data for the findings presented in the paper can be obtained from the corresponding author upon a reasonable request.

Declarations

Ethics approval and consent to participate

We carefully followed the ARRIVE guidelines while conducting the experiments on animals. Additionally, the research received approval from the Institutional Animal Care and Use Committee (CU-IACUC) at the Faculty of Science, Cairo University. (CU/IF/78/19).

Consent for publication

None.

Competing interests

The authors declare no competing interests.

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References

- Abbas, O. A., Ibrahim, I. G., & Ismail, A.-G.-E. (2019). Therapeutic effects of nano-HAP in a rat model of A β 13 induced neurotoxicity. *Iranian Journal of Pharmaceutical Research: IJPR*, 18, 1309.
- Abu-Elfotuh, K., Tolba, A. M., Hussein, F. H., Hamdan, A. M., Rabeh, M. A., Alshahri, S. A., Ali, A. A., Mosaad, S. M., Mahmoud, N. A., & Elsaed, M. Y. (2023). Anti-Alzheimer Activity of Combinations of Cocoa with Vinpocetine or Other Nutraceuticals in Rat Model: Modulation of Wnt3/ β -Catenin/GSK-3 β /Nrf2/HO-1 and PERK/CHOP/Bcl-2 Pathways. *Pharmaceutics* 15:2063.
- Ahmad Rather, M., Justin-Thenmozhi, A., Manivasagam, T., Saravanababu, C., Guillemain, G. J., & Essa, M. M. (2019). Asiatic acid attenuated aluminum chloride-induced tau pathology, oxidative stress and apoptosis via AKT/GSK-3 β signaling pathway in wistar rats. *Neurotoxicity research*, 35, 955–968.
- Anand, T., Mahadeva, N., Phani, K. G., & Farhath, K. (2010). Antioxidant and DNA Damage Preventive Properties of Centella asiatica (L) Urb. *Pharmacognosy journal*, 2, 53–58.
- Appa Rao, M., Srinivasan, K., Rao, K. T., & Rao, T. (1973). The effect of Mandoopaparni (Centella asiatica) on the general mental ability (Medhya) of mentally retarded children. *J Res Indian Med*, 8, 16.
- Bancroft, J. D. (2013). *Histochemical techniques*. Butterworth-Heinemann.
- Batool, Z., Sadir, S., Liaquat, L., Tabassum, S., Madiha, S., Rafiq, S., Tariq, S., Batool, T. S., Saleem, S., & Naqvi, F. (2016). Repeated administration of almonds increases brain acetylcholine levels and enhances memory function in healthy rats while attenuates memory deficits in animal model of amnesia. *Brain research bulletin*, 120, 63–74.
- Beurel, E., Grieco, S. F., & Jope, R. S. (2015). Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. *Pharmacology & therapeutics*, 148, 114–131.
- Beutler, E., & Mary, K. Y. (1963). Erythrocyte glutathione reductase. *Blood*, 21, 573–585.
- Blennow, K., de Leon, M. J., & Zetterberg, H. (2006). Alzheimer's disease. *The Lancet*, 368, 387–403.
- Cadet, J., Davies, K. J., Medeiros, M. H., Di Mascio, P., & Wagner, J. R. (2017). Formation and repair of oxidatively generated damage in cellular DNA. *Free Radical Biology and Medicine*, 107, 13–34.
- Cao, Z., Wang, P., Gao, X., Shao, B., Zhao, S., & Li, Y. (2019). Lycopene attenuates aluminum-induced hippocampal lesions by inhibiting oxidative stress-mediated inflammation and apoptosis in the rat. *Journal of inorganic biochemistry*, 193, 143–151.
- Cao, Z., Yang, X., Zhang, H., Wang, H., Huang, W., Xu, F., Zhuang, C., Wang, X., & Li, Y. (2016). Aluminum chloride induces neuroinflammation, loss of neuronal dendritic spine and cognition impairment in developing rat. *Chemosphere*, 151, 289–295.
- Chiroma, S. M., Baharuldin, M. T. H., Mat Taib, C. N., Amom, Z., Jagadeesan, S., Ilham Adenan, M., Mahdi, O., & Moklas, M. A. M. (2019). Centella asiatica protects d-galactose/A β 13 mediated Alzheimer's disease-like rats via PP2A/GSK-3 β signaling pathway in their Hippocampus. *International journal of molecular sciences*, 20, 1871.
- Chiroma, S. M., Baharuldin, M. T. H., Taib, C. N. M., Amom, Z., Jagadeesan, S., Adenan, M. I., & Moklas, M. A. M. (2019). Protective effect of Centella asiatica against D-galactose and aluminium chloride induced rats: Behavioral and ultrastructural approaches. *Biomedicine & Pharmacotherapy*, 109, 853–864.
- De Boeck, M., Touil, N., De Visscher, G., Vande, P. A., & Kirsch-Volders, M. (2000). Validation and implementation of an internal standard in comet assay analysis. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 469, 181–197.
- Dugger, B. N., & Dickson, D. W. (2017). Pathology of neurodegenerative diseases. *Cold Spring Harbor perspectives in biology*, 9, a028035.
- Elmore, S. (2007). Apoptosis: a review of programmed cell death. *Toxicologic pathology*, 35, 495–516.
- Erkkinen, M. G., Kim, M.-O., & Geschwind, M. D. (2018). Clinical neurology and epidemiology of the major neurodegenerative diseases. *Cold Spring Harbor perspectives in biology*, 10, a033118.
- Exley, C. (2004). The pro-oxidant activity of aluminum. *Free Radical Biology and Medicine*, 36, 380–387.
- Exley, C., Begum, A., Woolley, M. P., & Bloor, R. N. (2006). Aluminum in tobacco and cannabis and smoking-related disease. *The American journal of medicine*, 119, 276.e9–276.e11.
- Farhana, K. M., Malueka, R. G., Wibowo, S., & Gofir, A. (2016). Effectiveness of gotu kola extract 750 mg and 1000 mg compared with folic acid 3 mg in improving vascular cognitive impairment after stroke. Evidence-Based Complementary and Alternative Medicine 2016.
- Firdaus, Z., Kumar, D., Singh, S. K., & Singh, T. D. (2022). Centella asiatica alleviates A β 13-induced cognitive impairment, oxidative stress, and neurodegeneration by modulating cholinergic activity and oxidative burden in rat brain. *Biological Trace Element Research*, 200, 5115–5126.
- Firdaus, Z., Singh, N., Prajapati, S. K., Krishnamurthy, S., & Singh, T. D. (2022). Centella asiatica prevents D-galactose-Induced cognitive deficits, oxidative stress and neurodegeneration in the adult rat brain. *Drug and Chemical Toxicology*, 45, 1417–1426.
- Gad, H. A., Mansour, M., Abbas, H., Malatani, R. T., Khattab, M. A., & Elmowafy, E. (2022). Plurol will not miss the boat: A new manifesto of galantamine conveyance. *Journal of Drug Delivery Science and Technology*, 74, 103516.
- Gauthier, S., Rosa-Neto, P., Morais, J., & Webster, C. (2021). Journey through the diagnosis of dementia. World Alzheimer Report 2021.
- Gofir, A., Wibowo, S., & Hakimi, M. (2021). Potential neurological applications of Centella asiatica: a brief review. *Indonesian Journal of Pharmacology and Therapy* 2.
- Gulya, K., Rakonczay, Z., & Kasa, P. (1990). Cholinotoxic effects of aluminum in rat brain. *Journal of neurochemistry*, 54, 1020–1026.
- Gupta, R., & Flora, S. (2006). Effect of Centella asiatica on arsenic induced oxidative stress and metal distribution in rats. *Journal of Applied Toxicology: An International Journal*, 26, 213–222.
- Hashimoto, R., Nakamura, Y., Komai, S., Kashiwagi, Y., Tamura, K., Goto, T., Aimoto, S., Kaibuchi, K., Shiosaka, S., & Takeda, M. (2000). Site-specific phosphorylation of neurofilament-L is mediated by calcium/calmodulin-dependent protein kinase II in the apical dendrites during long-term potentiation. *Journal of neurochemistry*, 75, 373–382.
- Heneka, M. T., Kummer, M. P., & Latz, E. (2014). Innate immune activation in neurodegenerative disease. *Nature Reviews Immunology*, 14, 463–477.
- Hooper, C., Killick, R., & Lovestone, S. (2008). The GSK3 hypothesis of Alzheimer's disease. *Journal of neurochemistry*, 104, 1433–1439.
- Hosney, M., Sakraan, A., & Elzayat, E. M. (2022). Therapeutic Potential of Adipose-Derived Mesenchymal Stem Cells (ADMSCs) with/without Taurine in Aluminum Chloride-Induced Alzheimer's Disease Rat Model. *Egyptian Academic Journal of Biological Sciences B Zoology*, 14, 211–229.
- Hosny, E. N., Sawie, H. G., Elhadidy, M. E., & Khadrawy, Y. A. (2019). Evaluation of antioxidant and anti-inflammatory efficacy of caffeine in rat model of neurotoxicity. *Nutritional neuroscience*, 22, 789–796.

- Hu, W.-P., Li, X.-M., Chen, J.-G., & Li, Z.-W. (2007). Potentiation of the nicotinic acetylcholine receptor by aluminum in mammalian neurons. *Neuroscience*, 149, 1–6.
- Kaizer, R. R., Corrêa, M. C., Spanevello, R. M., Morsch, V. M., Mazzanti, C. M., Gonçalves, J. F., & Schetinger, M. R. (2005). Acetylcholinesterase activation and enhanced lipid peroxidation after long-term exposure to low levels of aluminum on different mouse brain regions. *Journal of inorganic biochemistry*, 99, 1865–1870.
- Kakkar, P. (1990). *Mandukaparni-medicinal uses and therapeutic efficacy*. Probe:196–182.
- Kanninen, K., White, A. R., Koistinaho, J., & Malm, T. (2011). Targeting Glycogen Synthase Kinase-3 β for Therapeutic Benefit against Oxidative Stress in Alzheimer's Disease: Involvement of the Nrf2-ARE Pathway. *International Journal of Alzheimer's Disease*, 2011, 985085.
- Kumar, A., Choudhary, A., & Kaur, H. (2020). Centella asiatica: The paragon of medicinal values under threat.
- Kumar, M. V., & Gupta, Y. (2002). Effect of different extracts of Centella asiatica on cognition and markers of oxidative stress in rats. *Journal of ethnopharmacology*, 79, 253–260.
- Kumar, V., & Gill, K. D. (2014). Oxidative stress and mitochondrial dysfunction in aluminium neurotoxicity and its amelioration: a review. *Neurotoxicology*, 41, 154–166.
- Liu, S., Chen, L., Li, J., Sun, Y., Xu, Y., Li, Z., Zhu, Z., & Li, X. (2023). Asiaticoside Mitigates Alzheimer's Disease Pathology by Attenuating Inflammation and Enhancing Synaptic Function. *International Journal Of Molecular Sciences*, 24. <https://doi.org/10.3390/ijms241511976>
- Lu, H., Hu, J., Li, J., Pang, W., Hu, Y., Yang, H., Li, W., Huang, C., Zhang, M., & Jiang, Y. (2013). Optimal dose of zinc supplementation for preventing aluminum-induced neurotoxicity in rats. *Neural regeneration research*, 8, 2754.
- Mairuae, N., Cheepsunthorn, P., & Buranrat, B. (2019). Anti-inflammatory and anti-oxidative effects of Centella asiatica extract in lipopolysaccharide-stimulated BV2 microglial cells. *Pharmacognosy Magazine*, 15, 140–146.
- Meulenbeld, G. J., & Wujastyk, D. (2001). *Studies on Indian medical history*. Motilal Banarsidass Publ.
- Meyer, J. C., Hariri, P., & Schellack, N. (2016). Overview of Alzheimer's disease and its management. *SA Pharmaceutical Journal*, 83, 48–56.
- Milnerowicz, H., Sciskalska, M., & Dul, M. (2015). Pro-inflammatory effects of metals in persons and animals exposed to tobacco smoke. *Journal of Trace Elements in Medicine and Biology*, 29, 1–10.
- Miu, A. C., Andreescu, C. E., Vasiu, R., & Olteanu, A. I. (2003). A behavioral and histological study of the effects of long-term exposure of adult rats to aluminum. *International Journal of Neuroscience*, 113, 1197–1211.
- Mohandas Rao, K., Muddanna Rao, S., & Gurumadhva Rao, S. (2006). Centella asiatica (L.) leaf extract treatment during the growth spurt period enhances hippocampal CA3 neuronal dendritic arborization in rats. *Evidence-Based Complementary and Alternative Medicine*, 3, 349–357.
- Mukerji, B. (1953). The Indian pharmaceutical codex-council of scientific and industrial research. New Delhi:223,228.
- Nandhakumar, S., Parasuraman, S., Shanmugam, M., Rao, K. R., Chand, P., & Bhat, B. V. (2011). Evaluation of DNA damage using single-cell gel electrophoresis (Comet Assay). *Journal of pharmacology & pharmacotherapeutics*, 2, 107.
- Nishikimi, M., Rao, N. A., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and biophysical research communications*, 46, 849–854.
- Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, 95, 351–358.
- Organization, W. H. (2021). Global status report on the public health response to dementia.
- Organization, W. H. (2023). Dementia.
- Pryor, W. A. (1986). Cancer and Free Radicals. In D. M. Shankel, P. E. Hartman, T. Kada, A. Hollaender, C. M. Wilson, & G. Kuny (Eds.), *Antimutagenesis and Anticarcinogenesis Mechanisms* (pp. 45–59). Springer US.
- Rakonczay, Z., Horváth, A., Juhász, A., & Kálmán, J. (2005). Peripheral cholinergic disturbances in Alzheimer's disease. *Chemico-biological interactions*, 157, 233–238.
- Rao, K. M., Rao, S. M., & Rao, S. G. (2005). Centella asiatica (linn) induced behavioural changes during growth spurt period in neonatal rats. *Neuroanatomy*, 4, 18–23.
- Rashid, M. H. O., Akter, M. M., Uddin, J., Islam, S., Rahman, M., Jahan, K., Sarker, M. M. R., & Sadiq, G. (2023). Antioxidant, cytotoxic, antibacterial and thrombolytic activities of Centella asiatica L.: possible role of phenolics and flavonoids. *Clinical Phytoscience*, 9, 1.
- Rather, M. A., Thenmozhi, A. J., Manivasagam, T., Nataraj, J., Essa, M. M., & Chidambaram, S. (2018). Asiatic acid nullified aluminium toxicity in in vitro model of Alzheimer's disease. *Frontiers in Bioscience-Elite*, 10, 287–299.
- Renke, G., Almeida, V. B. P., Souza, E. A., Lessa, S., Teixeira, R. L., Rocha, L., Sousa, P. L., & Starling-Soares, B. (2023). Clinical Outcomes of the Deleterious Effects of Aluminum on Neuro-Cognition, Inflammation, and Health: A Review. *Nutrients* 15:2221.
- Sabaragamuwa, R., Perera, C. O., & Fedrizzi, B. (2018). Centella asiatica (Gotu kola) as a neuroprotectant and its potential role in healthy ageing. *Trends in Food Science & Technology*, 79, 88–97.
- Shoshan-Barmatz, V., Arif, T., & Shteiinfer-Kuzmine, A. (2023). Apoptotic proteins with non-apoptotic activity: expression and function in cancer. *Apoptosis*, 28, 730–753.
- Siddiqui, B., Aslam, H., Ali, S., Khan, S., & Begum, S. (2007). Chemical constituents of Centella asiatica. *Journal of Asian natural products research*, 9, 407–414.
- Snitow, M. E., Bhansali, R. S., & Klein, P. S. (2021). Lithium and therapeutic targeting of GSK-3. *Cells*, 10, 255.
- Sumathi, T., Shobana, C., Mahalakshmi, V., Sureka, R., Subathra, M., Vishali, A., & Rekha, K. (2013). Oxidative stress in brains of male rats intoxicated with aluminium and neuromodulating effect of Celastus paniculatus alcoholic seed extract. *Asian J Pharm Clin Res*, 6, 80–90.
- Thenmozhi, A. J., Raja, T. R. W., Janakiraman, U., & Manivasagam, T. (2015). Neuro-protective effect of hesperidin on aluminium chloride induced Alzheimer's disease in Wistar rats. *Neurochemical research*, 40, 767–776.
- Tice, R. R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J. C., & Sasaki, Y. (2000). Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environmental and molecular mutagenesis*, 35, 206–221.
- Tripathy, S., & Srivastav, P. P. (2023). Encapsulation of Centella asiatica leaf extract in liposome: Study on structural stability, degradation kinetics and fate of bioactive compounds during storage. *Food Chemistry Advances*, 2, 100202.
- Vaidyaratnam, P. (1994). *Varier's Indian medicinal plants, a compendium of 500 species*. Arya Vaidya Sala. Orient Longman Ltd.
- Walton, J. (2012). Aluminum disruption of calcium homeostasis and signal transduction resembles change that occurs in aging and Alzheimer's disease. *Journal of Alzheimer's Disease*, 29, 255–273.
- Walton, J. (2013). Aluminum involvement in the progression of Alzheimer's disease. *Journal of Alzheimer's Disease*, 35, 7–43.
- Wilson, D. M., Cookson, M. R., Van Den Bosch, L., Zetterberg, H., Holtzman, D. M., & Dewachter, I. (2023). Hallmarks of neurodegenerative diseases. *Cell*, 186, 693–714.
- Yahya, M. A., & Nurrosyidah, I. H. (2020). Antioxidant activity ethanol extract of gotu kola (Centella asiatica (L.) Urban) with DPPH method (2, 2-Diphenyl-1-Pikrilhidrazil).
- Yan, Z.-H., Shi, X.-Y., & Jiang, H. (2023). *Phytochemistry, pharmacology, and clinical applications of Centella asiatica*. L.) Urban.
- Zatta, P., Ibn-Lkhyat-Idrissi, M., Zambenedetti, P., Kilyen, M., & Kiss, T. (2002). In vivo and in vitro effects of aluminum on the activity of mouse brain acetylcholinesterase. *Brain research bulletin*, 59, 41–45.

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