

Hyptis suaveolens-Mediated Silver Nanoparticles reversed Aluminum Chloride-Induced Cerebellar toxicity via modulation of oxidative stress, Neuroinflammation, and Neuronal Integrity

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Authors contribution

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Adekemi designed, conceptualized and did the experiment

Hassan Luqman Adepoju, Ojo Foluso Olamide, Omoola Olasheu Oluwatosin and Kolade Pelumi

Folurunso analyzed and drafted the manuscript text

Hasiya Sule Buba, Ahmad Abdul-Raheem Folorunsho, Ekom Monday Etukudo, Abdulakeem Miftahudeen

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All authors contributed to the funding of the research

Abstract

Background: Aluminum chloride (AlCl_3) exposure has been implicated in neurotoxicity through mechanisms involving oxidative stress, neuroinflammation, and neuronal degeneration, particularly within the cerebellum. Green synthesis of silver nanoparticles using medicinal plants has gained attention for their potential neuroprotective properties. This study investigated the protective effects of *Hyptis suaveolens*-mediated silver nanoparticles (HS-AgNPs) against AlCl_3 -induced cerebellar toxicity in rats.

Methods: Silver nanoparticles were synthesized using *Hyptis suaveolens* leaf extract and characterized using standard physicochemical techniques. Adult Wistar rats were randomly divided into experimental groups, including control, AlCl_3 -treated, and AlCl_3 + HS-AgNP-treated groups. Aluminum chloride was administered to induce cerebellar toxicity, while HS-AgNPs were given as therapeutic intervention. Biochemical assays were performed to assess oxidative stress markers, including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). Pro-inflammatory markers were evaluated to determine neuroinflammatory responses. Histopathological examination of cerebellar tissue was conducted to assess neuronal integrity.

Results: AlCl_3 exposure significantly increased lipid peroxidation and pro-inflammatory markers while reducing antioxidant enzyme activities compared to controls. Histological analysis revealed neuronal degeneration, disrupted Purkinje cell layers, and structural alterations in the cerebellum. Treatment with HS-AgNPs significantly reduced oxidative stress, restored antioxidant enzyme levels, attenuated neuroinflammation, and preserved cerebellar architecture. The reversal of biochemical and histological alterations suggests a protective role of HS-AgNPs.

Conclusion: *Hyptis suaveolens*-mediated silver nanoparticles exhibit significant neuroprotective effects against aluminum-induced cerebellar toxicity by modulating oxidative stress and inflammatory pathways while preserving neuronal integrity. These findings support the therapeutic potential of plant-mediated nanoparticles in managing environmentally induced neurotoxicity and warrant further translational investigation.

Keywords: Aluminum chloride, Cerebellar toxicity, *Hyptis suaveolens*, Silver nanoparticles, Oxidative stress, Neuroinflammation, Neuroprotection.

Background of the Study

Aluminum chloride (AlCl_3) is a widely distributed environmental and occupational neurotoxin associated with a broad spectrum of physiological, biochemical, and behavioral disturbances in both humans and experimental animals [1]. Exposure to aluminum chloride constitutes a persistent public health concern across developing and industrialized nations alike, owing to its ubiquitous presence in the environment and the difficulty of avoiding occupational contact [2]. Accumulating evidence suggests that aluminum chloride may serve as a contributory factor in the pathogenesis of Alzheimer's disease (AD), although the precise mechanistic relationship between aluminum exposure and AD development remains a subject of ongoing scientific debate [3]. Notably, epidemiological investigations have reported a positive association between aluminum chloride concentrations in drinking water sources and the incidence of AD, while several experimental and clinical studies have documented impairments in learning and memory function following sustained aluminum chloride exposure [4].

Alzheimer's disease is an age-related, chronic neurodegenerative condition whose neuropathological hallmarks include the intraneuronal deposition of hyperphosphorylated tau protein forming neurofibrillary tangles and the extracellular aggregation of amyloid-beta peptides into senile plaques [5,6]. It is currently understood that the aberrant accumulation of these proteinopathic species disrupts synaptic integrity, impairs neuronal connectivity, and ultimately precipitates a progressive and irreversible decline in brain

function [7]. In its earliest clinical stage, the disease manifests principally as short-term memory impairment; however, as the disease progresses, affected individuals develop a wider range of symptoms encompassing language disturbances, disorientation, mood and behavioral dysregulation, social disengagement, and eventual loss of long-term memory [8]. The clinical presentation and rate of progression vary considerably among patients [9], a heterogeneity that has been attributed to interactions among biological, genetic, and age-related factors [10]. Among the neurochemical theories proposed to explain AD pathophysiology, the cholinergic hypothesis remains the most foundational, postulating that a deficit in the biosynthesis and release of the neurotransmitter acetylcholine underlies the cognitive and memory impairments characteristic of the disease, and this hypothesis continues to inform the development of most approved pharmacological interventions [11].

Alzheimer's disease represents the leading cause of dementia worldwide, imposing a substantial and escalating burden on patients, caregivers, and healthcare systems globally. Current pharmacological options, including donepezil — a selective, reversible inhibitor of acetylcholinesterase — afford only modest symptomatic benefit without modifying the underlying neurodegenerative process or halting disease progression [12,13]. Donepezil exerts its therapeutic effect by competitively inhibiting the enzymatic degradation of acetylcholine at cholinergic synapses, thereby augmenting cholinergic neurotransmission in brain regions critically involved in memory and cognition. Its clinical utility spans mild, moderate, and severe stages of AD, as well as vascular dementia and dementia associated with Parkinson's disease; it has received regulatory approval from the United States Food and Drug Administration (FDA) in multiple formulations, including oral tablets and a transdermal patch [13]. Nevertheless, the inability of existing therapies to arrest or reverse neurodegeneration underscores the urgent need for neuroprotective agents capable of targeting multiple pathological mechanisms simultaneously.

Hyptis suaveolens (L.) Poit., vernacularly referred to as Vilayati tulsi, is a herbaceous member of the family Lamiaceae (Mint family) [14]. This plant has historically occupied an important position in traditional

medicine systems across several regions, where it has been employed in the management of diverse ailments, despite remaining relatively undercharacterized in formal pharmacological research [15]. The broad therapeutic reputation of *H. suaveolens* is supported by documented pharmacological activities that include sedative, diuretic, antispasmodic, anti-inflammatory, antipyretic, anti-rheumatic, antifungal, and antineoplastic properties, attributable to its diverse array of bioactive secondary metabolites [16,17]. Of particular relevance to the present investigation, *H. suaveolens* has been recognized in ethnopharmacological records for its cognitive-enhancing properties, and preliminary scientific evidence has begun to suggest therapeutic potential in the context of Alzheimer's disease. However, no experimental study has yet systematically evaluated its neuroprotective capacity specifically against AlCl_3 -induced cerebellar neurotoxicity.

Experimental animal models of aluminum chloride-induced neurotoxicity have consistently demonstrated significant neurobehavioral deficits, including impairments in spatial memory, associative learning, and locomotor coordination, which are attributable to aluminum-mediated oxidative injury and neuroinflammatory cascades within the central nervous system [18,19]. Nano-formulation strategies applied to botanical extracts have gained considerable scientific attention as a means of enhancing phytochemical bioavailability, improving tissue permeability, and amplifying pharmacological efficacy. The neuroprotective potential of nano-formulated *H. suaveolens* extract is hypothesized to derive primarily from its constituent flavonoids and phenolic compounds, which possess well-characterized free radical-scavenging and anti-inflammatory capacities [18,19]. Against this background, the present study was designed to evaluate the extent to which *Hyptis suaveolens*-mediated silver nanoparticles confer neuroprotection against AlCl_3 -induced cerebellar toxicity, with particular emphasis on oxidative stress biomarkers, neuroinflammatory mediators, and indices of neuronal structural integrity.

Materials and methods

Experimental Animals and Care

A total number of thirty (30) adult male Wistar rats with an average weight of 150g were procured from the animal house, Department of Anatomy, Faculty of Basic Medical Sciences, University of Ilesa, Ilesa, Osun State. The animals were randomly divided into 5 groups with each group comprising six (6) rats. The animals were caged in the animal house of the Department of Anatomy, University of Ilesa, Ilesa, Osun State and acclimatized for two weeks under standard laboratory conditions of temperature 27-30°C. They were fed with rat pellets (Top Feed Ltd) and had free access to drinking water.

Table 1: Shows the groupings of the animals with their respective treatments, dosages, route and duration of administration.

Groups	Treatment	Dosage	Route of Administration
Group 1 (Control)	Distilled water	2mls/kg.bw	Orally
Group 2 (AlCl ₃)	Aluminum chloride only	150mg/kg.bw	Orally
Group 3 (AlCl ₃ /Donepezil)	Aluminum chloride + Donepezil	150mg/kg.bw/5mg /kg.bw	Orally
Group 4 (H. <i>Suaveolens</i> AgNPs)	Suaveolens	100mg/kg. b.w	Orally
Group 5 (AlCl ₃ /H. <i>Suaveolens</i> AgNPs)	Aluminum chloride + Suaveolens	150mg/kg.bw/100 mg/kg. b.w	Orally

Hyptis suaveolens

Collection and Authentication of Leaves

Fresh and healthy leaves of *Hyptis suaveolens* was obtained from a farmland in Osogbo, Osun State, Nigeria. The plant was identified and authenticated in the Department of Plant Biology, Obafemi Awolowo University, Ile-Ife, Osun State with Authentication number IFE-18332. The leaves were washed thoroughly with distilled water three times to remove any dust particles, then air dried and the dried leaves were pulverized using an electric blender.

Preparation of Leaf Extract

Hyptis suaveolens (200 g) extract was dissolved in 1000 ml of distilled and the solution was placed in a shaker for twenty-four (24) hours for proper dissolution. The solution was then filtered using Whatmann filter paper No 42 (125 mm) to remove the residue from the filtrate. The filtrate was used as a reducing and stabilizing agent for 1 mM of AgNO₃.

Synthesis of Nanoparticles using *Hyptis suaveolens* leaf extract

For the synthesis of nanoparticles, 1mM of AgNO₃ was prepared. The *Hyptis suaveolens* filtrate was then added to AgNO₃ solution in a ratio of (1:9). The reaction mixture was prepared by adding 90 mls of 1mM of AgNO₃ to 10ml of aqueous leaf extracts separately to make up a final solution of 100 ml. The mixture was then stirred continuously at room temperature. The color change (typically from light yellow to dark brown) was observed, which indicated the formation of nanoparticles and was left for some hours to fully synthesize.

Purification of Nanoparticles

The mixture of the leaf extract and AgNO₃ was centrifuged at 10,000 rpm for 15-20 minutes, after then the supernatant was discarded and the pellet (Nanoparticles) was washed with distilled water 2-3 times. The nanoparticles were then lyophilized to get solid *Hyptis Suaveolens* silver nanoparticles.

Chemicals and drugs

Drugs and Reagents

Aluminum chloride (AlCl_3) was procured from Sigma Aldrich, United States of America (USA). All other reagents utilized for this study were of analytical grade and purity. The aluminum chloride solution was prepared by dissolving 15 g of AlCl_3 in 500 ml of distilled water at a final concentration of 100 mg/kg. Dissolved AlCl_3 was administered orally for 21 days (**Table 1**). Donepezil (10 mg) manufactured by Eisai Inc. USA.

Animal sacrifice

The animals were sacrificed 24 hours after the last administration by cervical dislocation, and the brains were rapidly removed from the skull of the rats using brain forceps. The cerebella tissues were isolated and homogenized with phosphate buffer solution (PBS) and stored at -80°C for biochemical analysis and also processed for histological and immunohistological staining procedures.

Biochemical test for Oxidative Stress and Lipid Peroxidation

Estimation of oxidative stress markers was carried out using the thiobarbituric acid reactive substance for MDA, and analysis of SOD was based on the inhibition of adrenaline. CAT activity was assayed following the decomposition of hydrogen peroxide [20–23].

Determination of Tumor Necrotic Factor α , interleukin (IL) 10 and interleukin 1 β and interleukin (IL)-10

Cerebellar concentrations of tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interleukin-10 (IL-10) were quantified using sandwich enzyme-linked immunosorbent assay (ELISA). Commercially available ELISA kits (Enzo Life Sciences Inc., NY, USA) were employed in strict accordance with the manufacturer's instructions. These kits were validated to detect and measure the total immunoreactive fraction of each cytokine, encompassing both the free (unbound) and carrier-bound forms

present within the tissue homogenate. Absorbance readings were obtained using a microplate spectrophotometer, and cytokine concentrations were subsequently interpolated from standard curves generated with recombinant protein standards supplied with each kit. Results were expressed as picograms per milligram of protein (pg/mg protein).

Determination of Acetylcholine, Dopamine, Serotonin, and Brain-Derived Neurotrophic Factor (BDNF) Levels

Neurochemical assessments of acetylcholine, dopamine, serotonin, and brain-derived neurotrophic factor (BDNF) were conducted on post-centrifugation supernatants derived from cerebellar tissue homogenates. Quantification of each analyte was performed using species-specific, commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits (Abcam, Cambridge, UK). All assay procedures were carried out in strict conformity with the protocols specified by the manufacturer, including sample dilution, incubation conditions, washing steps, and colorimetric detection. Optical densities were recorded at the manufacturer-recommended wavelength, and analyte concentrations were calculated against four-parameter logistic (4PL) standard curves. Final values were normalized to total protein content and expressed as nanograms per milligram of protein (ng/mg protein).

Photomicrography

Histologically prepared cerebellar sections were examined under a Sellon-Olympus trinocular light microscope (Model XSZ-107E; China) equipped with a coupled digital imaging camera (Canon PowerShot 2500). Representative photomicrographs were acquired from each experimental group at standardized magnifications. To ensure objective and unbiased histopathological evaluation, all slides were assessed by a board-certified pathologist who remained blinded to group allocation throughout the entire review process. Digital images were subsequently imported into ImageJ software (version 1.54k for Windows; SOFTONIC International, S.A.; <https://imagej.net/ij/>) for calibration and dimensional annotation. A spatial

scale factor, expressed in micrometers per pixel ($\mu\text{m}/\text{pixel}$), was applied to all micrographs to enable accurate morphometric referencing.

Statistical analysis

All quantitative data were subjected to statistical analysis using Chris Rorden's ANOVA software for Windows (version 0.98). Intergroup differences were evaluated by one-way analysis of variance (ANOVA), with Tukey's Honestly Significant Difference (HSD) post-hoc test applied to identify the source of significant variance both within and between experimental groups. Continuous variables are presented as mean values with standard error of the mean ($\text{mean} \pm \text{SEM}$). A probability threshold of $p < 0.05$ was adopted as the criterion for statistical significance across all comparisons.

RESULTS

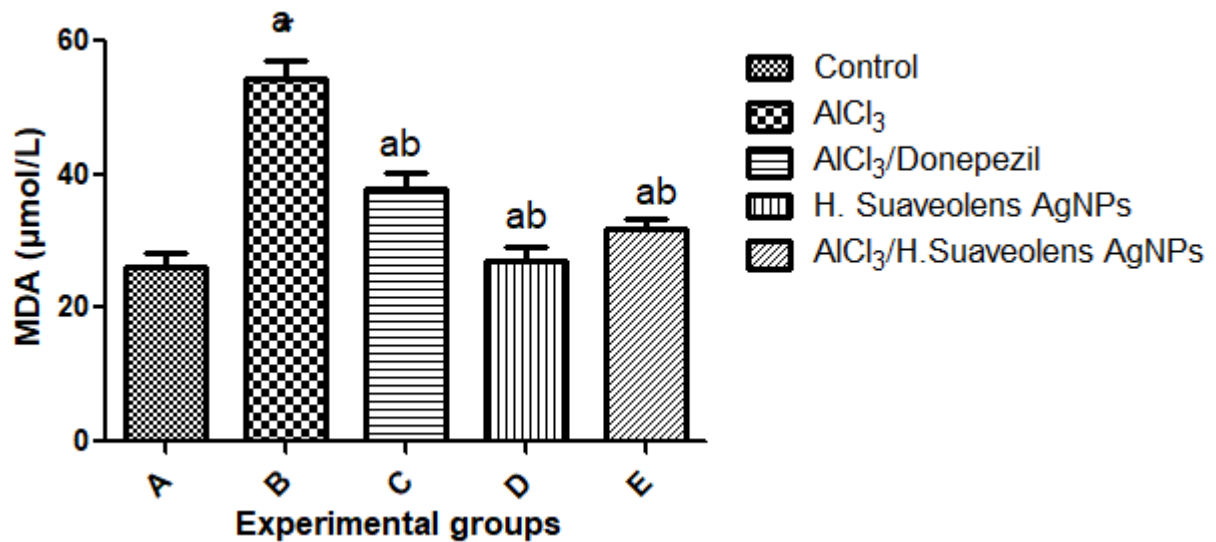


Fig. 1: Showing The effect of *Hyptis suaveolens*-Mediated Silver Nanoparticles on Levels of oxidative stress (MDA) markers in rats treated with AlCl_3

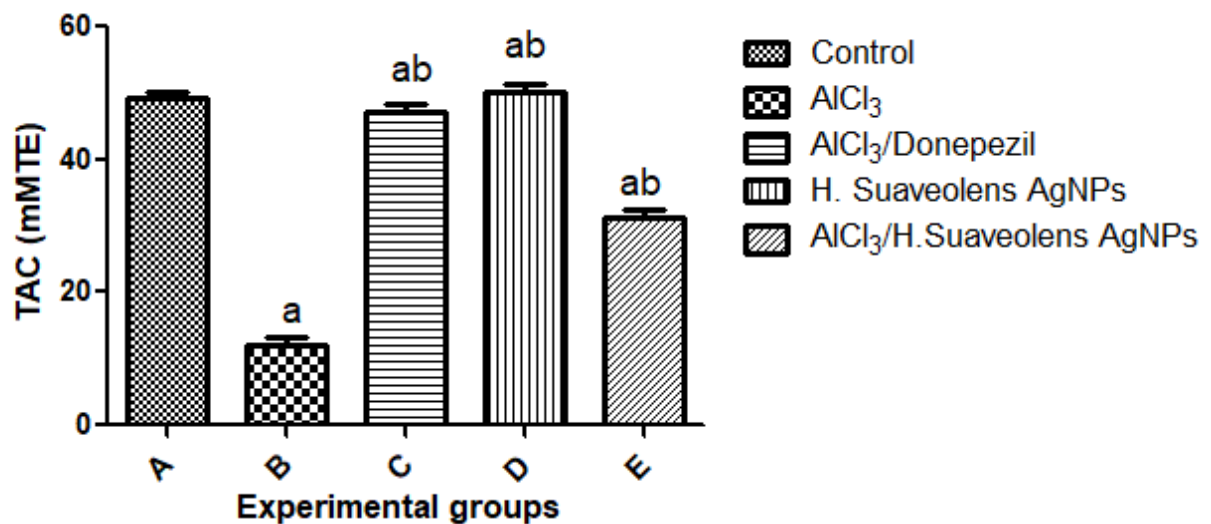


Fig. 2: Showing The effect of *Hyptis suaveolens*-Mediated Silver Nanoparticles on Levels of oxidative stress (TAC) markers in rats treated with AlCl_3

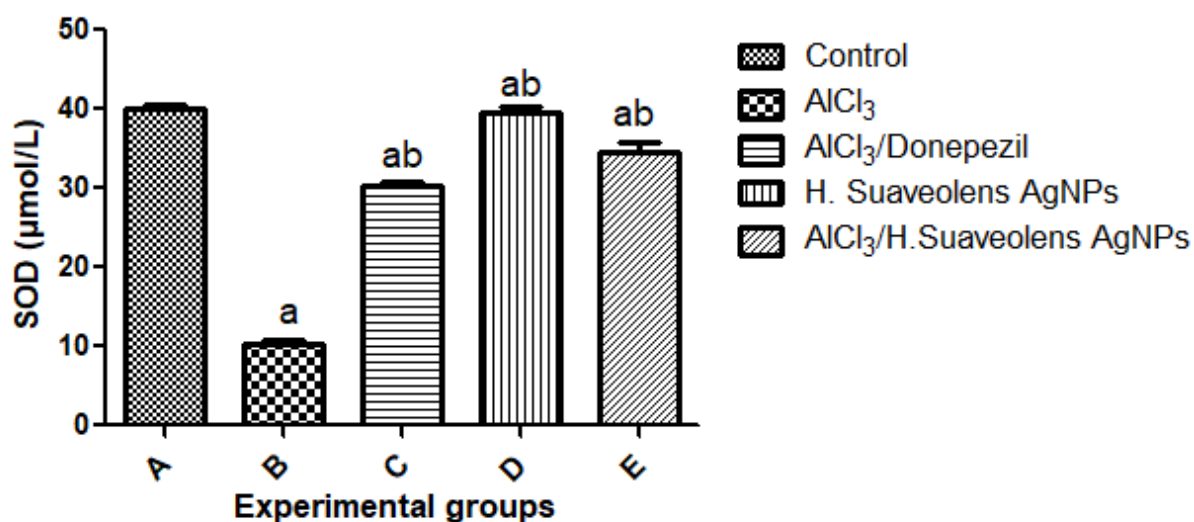


Fig. 3: Showing The effect of *Hyptis suaveolens*-Mediated Silver Nanoparticles on Levels of oxidative stress (SOD) markers in rats treated with AlCl₃

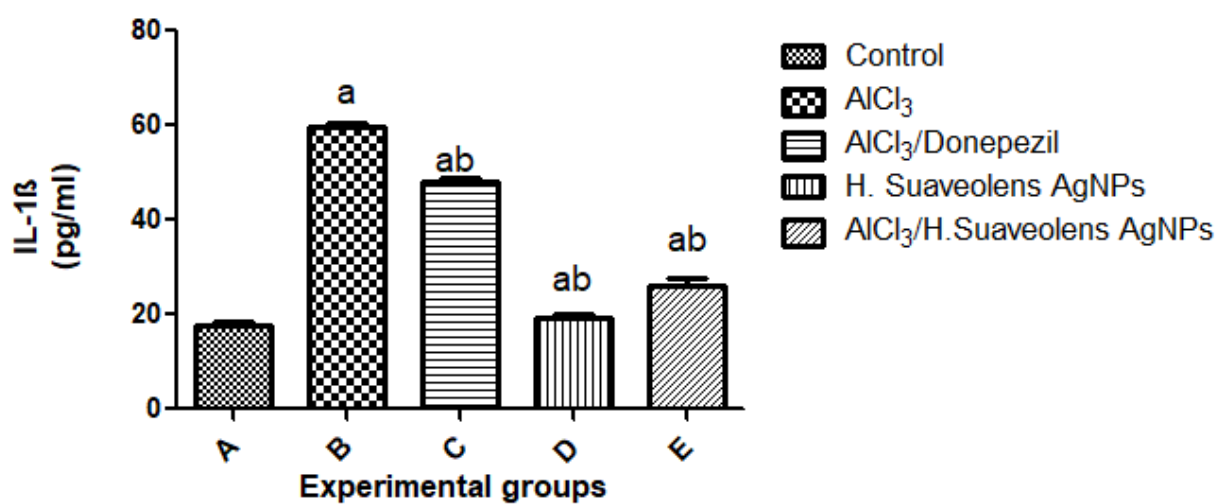


Fig. 4: The effects of *Hyptis suaveolens*-Mediated Silver Nanoparticles on levels of inflammatory cytokines (IL-1β) in AlCl₃-treated rats

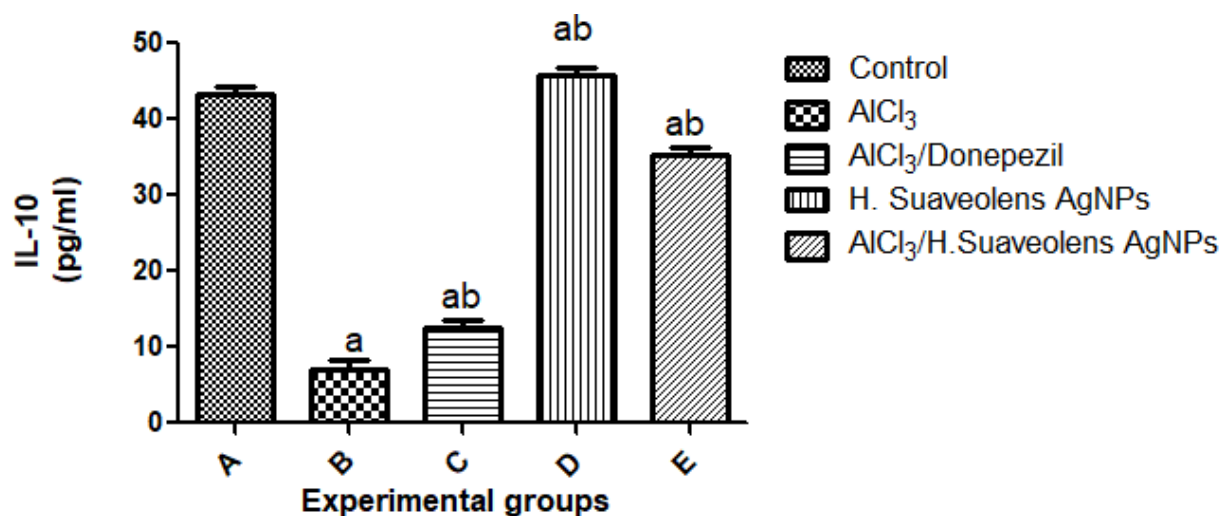


Fig. 5: The effects of *Hyptis suaveolens*-Mediated Silver Nanoparticles on levels of inflammatory cytokines (IL-10) in AlCl_3 -treated rats

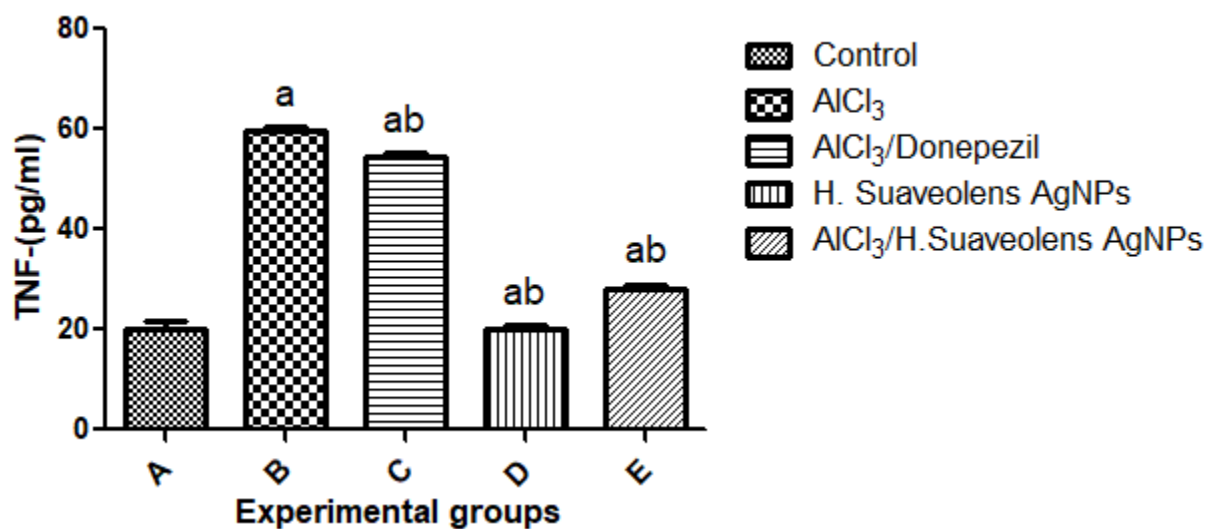


Fig. 6: The effects of *Hyptis suaveolens*-Mediated Silver Nanoparticles on levels of inflammatory cytokines (TNF) in AlCl_3 -treated rats

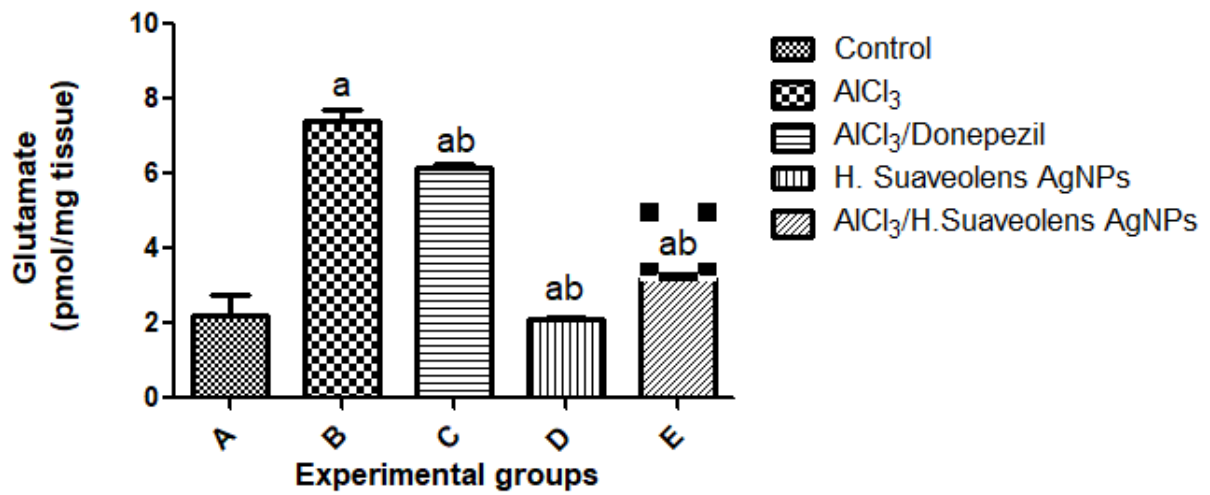


Fig. 7: The effects of cortex neurotransmitter levels *Hyptis suaveolens*-Mediated Silver Nanoparticles on cerebellar neurotransmitter (Glutamate) levels.

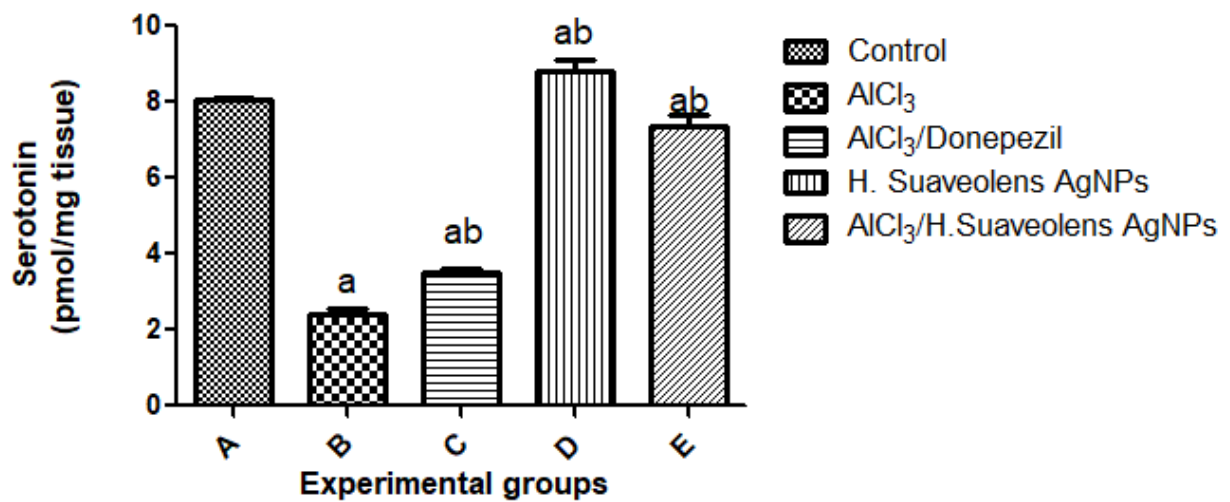


Fig. 8: The effects of cortex neurotransmitter levels *Hyptis suaveolens*-Mediated Silver Nanoparticles on cerebellar neurotransmitter (Serotonin) levels.

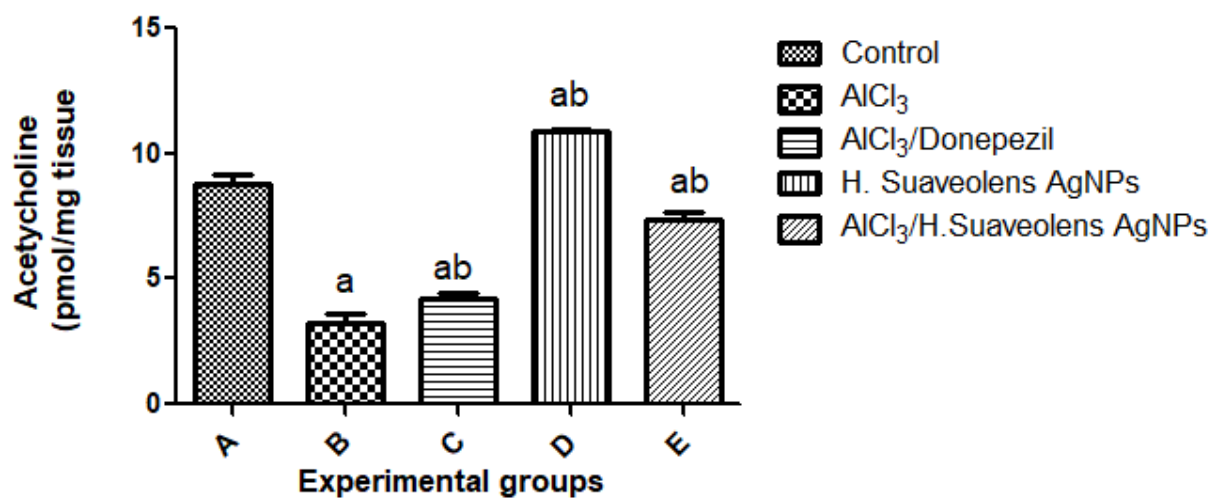


Fig. 9: The effects of cortex neurotransmitter levels *Hyptis suaveolens*-Mediated Silver Nanoparticles on cerebellar neurotransmitter (Acetylcholine) levels.

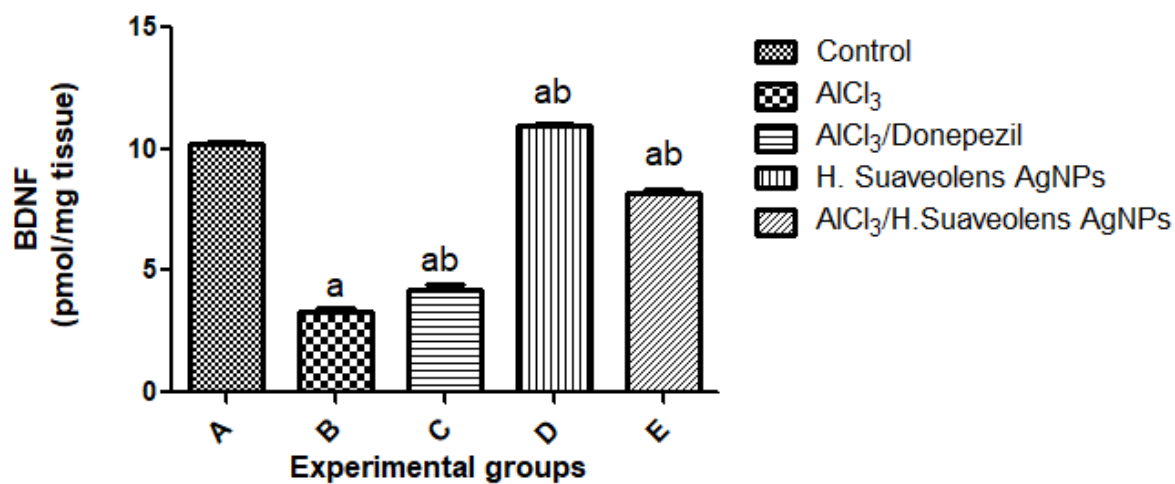


Fig. 10: The effects of cortex neurotransmitter levels *Hyptis suaveolens*-Mediated Silver Nanoparticles on cerebellar neurotransmitter (BDNF) levels.

Histological Observations

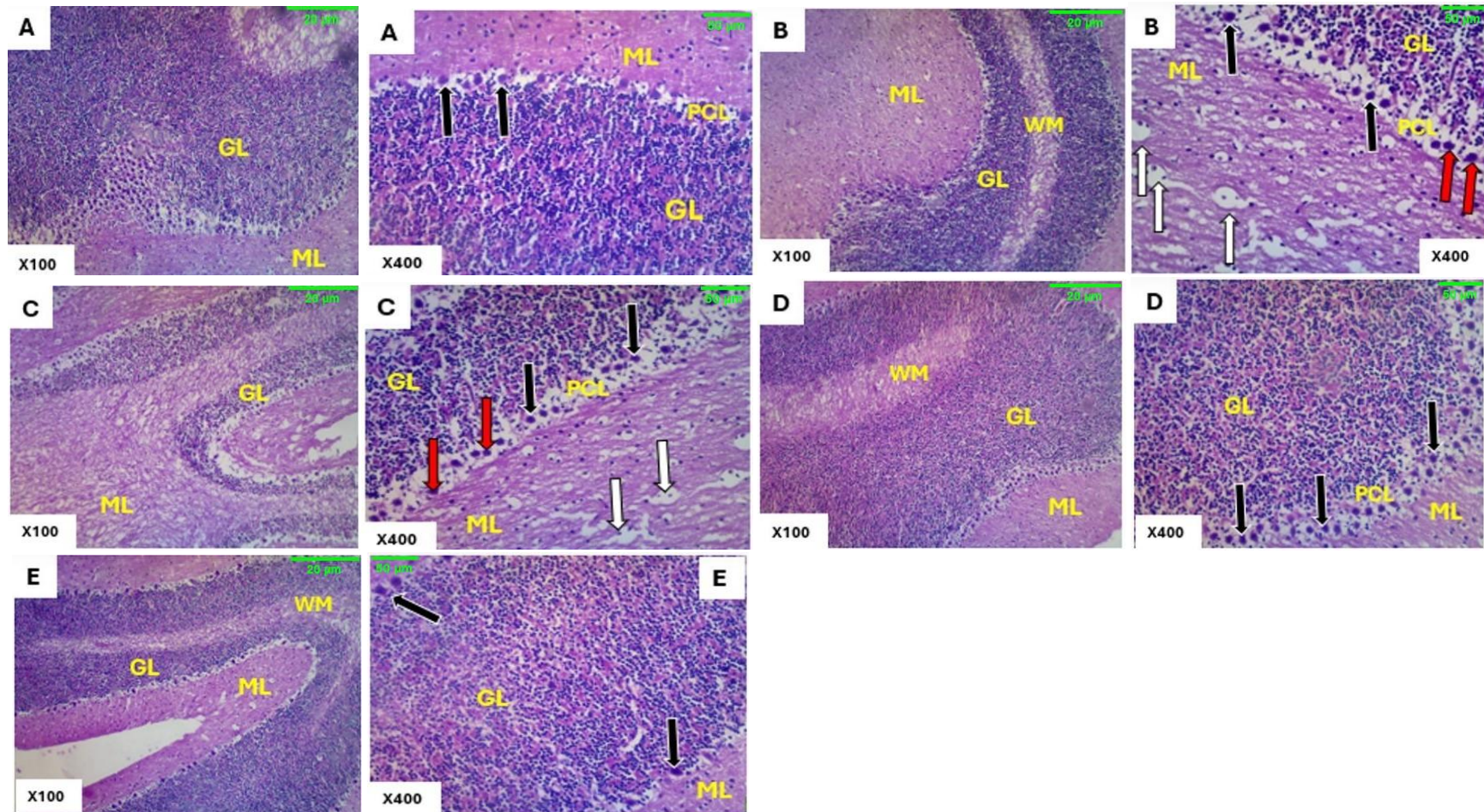


Fig. 11: Photomicrographs of the cerebellar cortex stained with Hematoxylin and Eosin (H&E) showing general histoarchitecture. (A) Control: Shows normal cortical layers with distinct Molecular Layer (ML), Granular Layer (GL), and a monolayer of flask-shaped Purkinje cells (Black arrows). (B) AlCl_3 Only: Shows severe degenerative changes characterized by spongiosis/vacuolation (White arrows) and shrunken, pyknotic Purkinje cells (Red arrows) with deeply stained nuclei. (C) AlCl_3 + Donepezil: Shows moderate restoration of the Purkinje cell layer (Black arrows), though mild vacuolation (White arrows) and some pyknotic cells (Red arrows) persist. (D) *H. suaveolens* AgNPs Only: Displays normal cytoarchitecture indistinguishable from the control group (Black arrows). (E) AlCl_3 + *H. suaveolens* AgNPs: Demonstrates significant restoration of the cortical layers with healthy, viable Purkinje cells (Black arrows) and reduced vacuolation. All micrographs x100 were scaled at $20\mu\text{m}$, and all micrographs x400 were scaled at $50\mu\text{m}$. (ML=Molecular Layer, GL=Granular Layer, PCL=Purkinje Cell Layer, WM=White Matter).

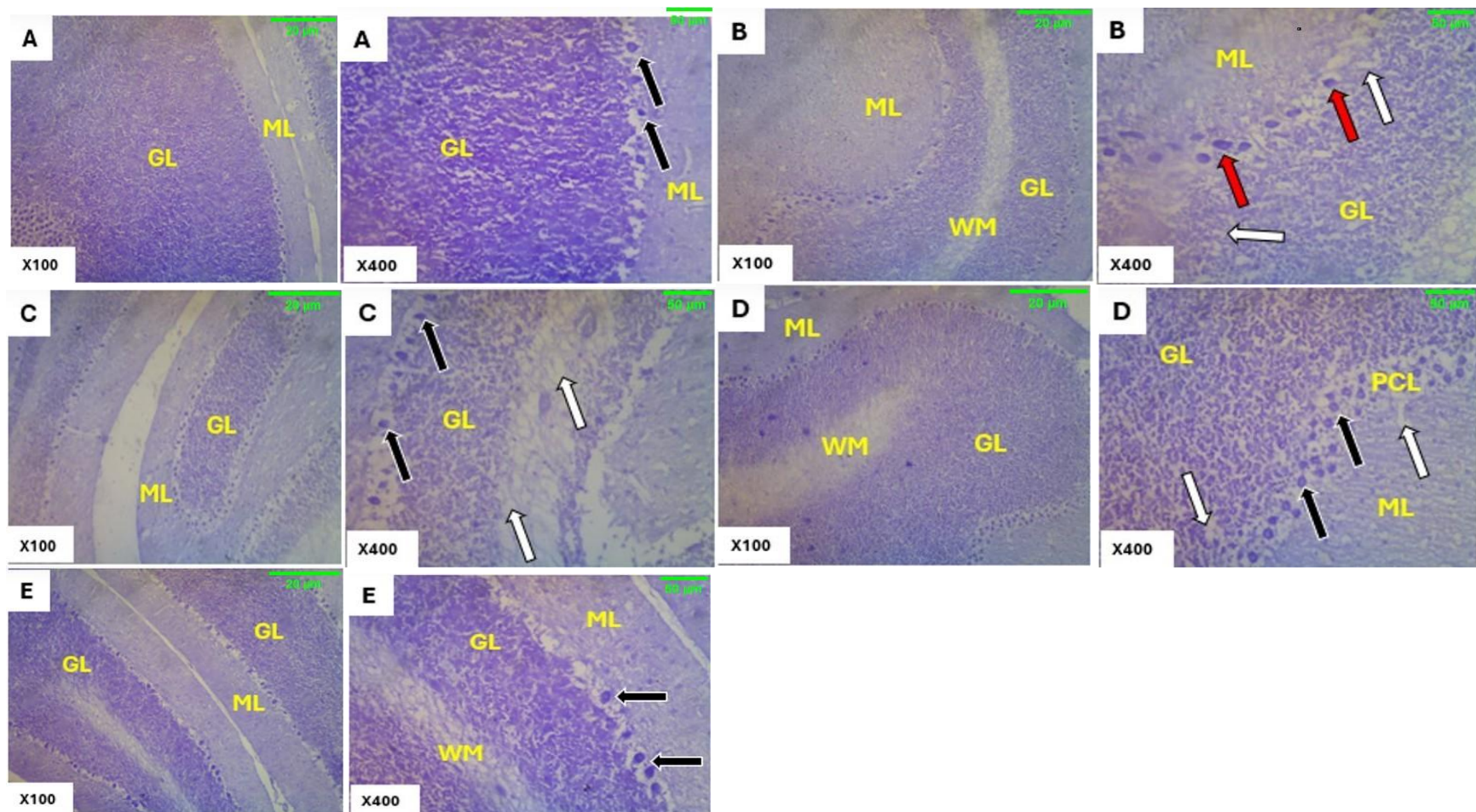


Fig. 12: Photomicrographs of the cerebellar cortex stained with Cresyl Violet (Nissl Stain) demonstrating neuronal integrity and protein synthesis (A) Control: Shows normal cytoarchitecture with deeply stained Purkinje cells (Black arrows) indicating abundant Nissl substance. (B) AlCl_3 Only: Shows severe chromatolysis (loss of Nissl staining), shrunken/pyknotic neurons (Red arrows), and perineuronal vacuolation (White arrows). (C) AlCl_3 + Donepezil: Shows amelioration of toxicity with restored staining intensity in Purkinje cells (Black arrows) and reduced vacuolation. (D) *H. suaveolens* AgNPs Only: Displays normal cerebellar morphology with distinct Nissl staining (Black arrows) comparable to the control. (E) AlCl_3 + *H. suaveolens* AgNPs: Demonstrates significant neuroprotection with preservation of Nissl substance in Purkinje cells (Black arrows) and maintenance of the granular layer density. All micrographs x100 were scaled at 20 μm , and all micrographs x400 were scaled at 50 μm . (ML=Molecular Layer, GL=Granular Layer, WM=White Matter, PCL = Purkinje cells Layer).

[Insert Fig. 13 here]

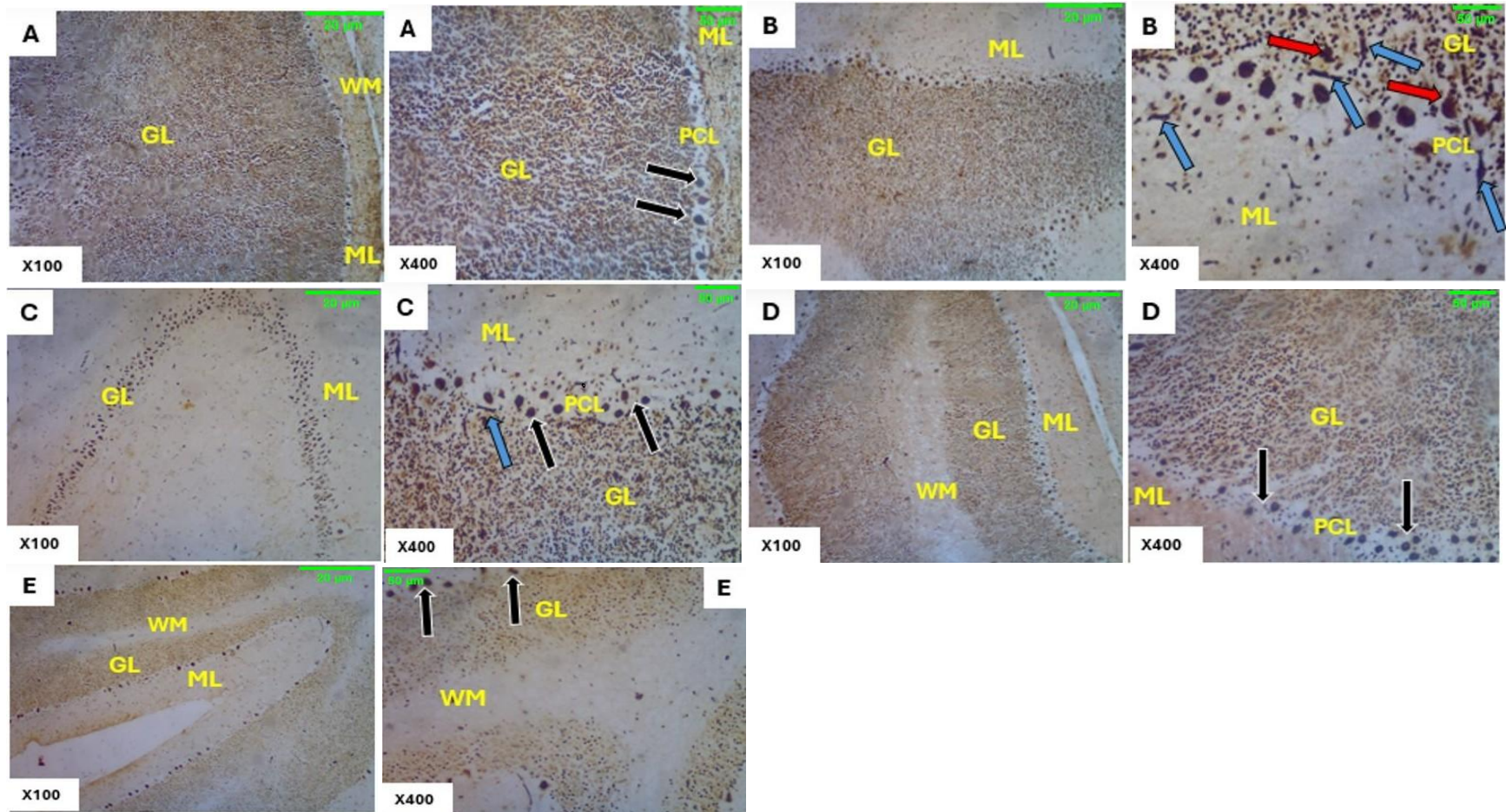


Fig. 13: Photomicrographs of the cerebellar cortex stained with Bielschowsky Silver stain to assess neurofibrillary pathology and axonal integrity. (A) Control: Shows normal neurofibrillary network with fine, intact axons and Purkinje cells free of argentophilic deposits (Black arrows). (B) AlCl_3 Only: Demonstrates severe neurotoxicity characterized by the presence of intraneuronal neurofibrillary tangles (Red arrows) and thickened, fragmented dystrophic neurites (Blue arrows). (C) AlCl_3 + Donepezil: Shows amelioration of pathology with a marked reduction in tangles and preservation of axonal structure (Black arrows), though slight axonal thickening persists (Blue arrow). (D) *H. suaveolens* AgNPs

Only: Displays normal fibrillary architecture similar to the control. (E) AlCl_3 + *H. suaveolens* AgNPs: Shows significant neuroprotection with cleared axoplasm in Purkinje cells (Black arrows) and intact axonal projections, comparable to the control group. *All micrographs x100 were scaled at 20 μm , and all micrographs x400 were scaled at 50 μm . (ML=Molecular Layer, GL=Granular Layer, PCL=Purkinje Cell Layer, WM=White Matter).*

DISCUSSION

Alzheimer's disease (AD) is a chronic, progressive neurodegenerative disorder characterized by an insidious onset and a relentlessly deteriorating clinical course [24,25]. It constitutes the predominant aetiology of dementia globally, accounting for approximately 60–70% of all dementia cases [26]. The hallmark presenting feature during the early phase of the disease is an impairment of episodic memory, most notably the inability to retain newly acquired information [27]. With disease advancement, affected individuals exhibit a broadening spectrum of cognitive and neuropsychiatric manifestations, including expressive and receptive language deficits, spatiotemporal disorientation, affective instability, diminished executive function, social withdrawal, progressive self-neglect, and a range of behavioral disturbances, all of which culminate in profound functional dependency. Global epidemiological estimates recorded in 2020 placed the number of individuals living with AD at approximately 50 million [11]. Although the condition predominantly affects individuals aged 65 years and above, early-onset variants account for up to 10% of diagnosed cases, with symptom emergence occurring as early as the fourth decade of life through the mid-sixth decade [28]. The disease disproportionately affects women relative to men, with an estimated prevalence of approximately 6% among persons aged 65 years and older [13].

In this study, treatment with aluminum chloride (AlCl_3) significantly increased MDA levels, indicating enhanced lipid peroxidation and oxidative damage, consistent with previous findings by [29] and others who reported similar increases in MDA following aluminum exposure [30]. Co-treatment with nano-formulated *Hyptis suaveolens* extract resulted in significantly lower MDA levels compared to AlCl_3 alone, indicating the extract's ameliorative effect against AlCl_3 -induced lipid peroxidation. Concurrently, activities of antioxidant enzymes SOD and CAT were reduced following aluminum chloride treatment, reflecting impaired antioxidative defense mechanisms. This decrease aligns with reports that aluminum exposure diminishes antioxidant enzyme function, contributing to elevated oxidative stress and neuronal damage [29]. The observed increase in oxidative stress markers may be due to aluminum's ability to promote ROS generation through multiple pathways, leading to the accumulation of ROS, lipid

peroxidation, and subsequent cellular damage. Importantly, co-treatment with nano-formulated *Hyptis suaveolens* extract significantly restored SOD and CAT activities compared to AlCl₃ treatment, suggesting the extract's potent antioxidant and protective effects against aluminum-induced oxidative stress.

Attenuation of Oxidative Stress and Restoration of Antioxidant Defense

The present findings demonstrate that aluminum chloride (AlCl₃) exposure in rats induced marked oxidative stress, evidenced by a significant elevation in malondialdehyde (MDA) levels alongside a pronounced reduction in key antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT). These alterations indicate enhanced lipid peroxidation and impaired antioxidant defense, consistent with earlier reports showing that aluminum promotes excessive reactive oxygen species (ROS) generation and oxidative neuronal damage [29,30]. The observed oxidative imbalance likely arises from aluminum's ability to disrupt mitochondrial function, catalyze ROS formation, and suppress endogenous antioxidant systems, thereby accelerating neuronal injury and neurodegenerative processes characteristic of AD [31–33].

Notably, co-treatment with nano-formulated *Hyptis suaveolens* extract markedly attenuated these deleterious effects. Animals treated with *H. suaveolens*-mediated silver nanoparticles (AgNPs) exhibited significantly reduced MDA levels alongside restoration of SOD and CAT activities toward normal values, indicating effective suppression of lipid peroxidation and reinforcement of endogenous antioxidant defenses. This antioxidant recovery was comparable to, and in some parameters superior to, that observed with donepezil treatment, highlighting the strong redox-modulating potential of the nanoparticles. These findings are consistent with previous studies demonstrating that phytochemical-rich plant extracts and green-synthesized nanoparticles possess potent free radical-scavenging properties and enhance cellular antioxidant capacity [34–36], thereby mitigating AlCl₃-induced neurotoxicity.

Importantly, oxidative stress is a central driver of Alzheimer's disease pathology, contributing to amyloid- β aggregation, tau hyperphosphorylation, synaptic dysfunction, and neuronal apoptosis [37,38]. The ability of *H. suaveolens* AgNPs to significantly restore total antioxidant capacity and suppress oxidative damage suggests a disease-modifying effect rather than mere symptomatic relief. This suggests modulation of

multiple oxidative stress biomarkers, providing robust biochemical evidence that *H. suaveolens*-derived AgNPs confer strong neuroprotection. This reveals the therapeutic potential of *H. suaveolens* AgNPs as a promising, plant-based nanotherapeutic strategy for mitigating oxidative stress-driven neurodegeneration, likened to that seen in Alzheimer's disease.

Mitigation of Neuroinflammation

The present study demonstrated that aluminum chloride (AlCl_3) exposure induced pronounced neuroinflammation, as evidenced by significant activation of the NF- κ B signaling pathway, elevated levels of pro-inflammatory cytokines (TNF- α and IL-1 β), and a concomitant reduction in the anti-inflammatory cytokine IL-10. These findings are consistent with earlier reports showing that aluminum triggers NF- κ B-mediated inflammatory cascades, leading to sustained neuroinflammation and neuronal injury [39,40]. The observed suppression of IL-10 further exacerbates this inflammatory state by weakening endogenous anti-inflammatory and neuroprotective mechanisms, as previously documented by [41]. Together, these alterations confirm the successful induction of an Alzheimer's disease (AD)-like inflammatory phenotype, characterized by chronic cytokine dysregulation and heightened vulnerability to neurodegeneration.

Notably, treatment with nano-formulated *Hyptis suaveolens* extract significantly attenuated these inflammatory responses. Administration of *H. suaveolens*-mediated silver nanoparticles (AgNPs) effectively downregulated NF- κ B activation, reduced TNF- α and IL-1 β levels, and restored IL-10 concentrations toward physiological values. This pattern indicates a robust anti-inflammatory effect mediated through suppression of the NF- κ B signaling axis, a central regulator of neuroinflammation in AD. The ability of the AgNPs to rebalance pro- and anti-inflammatory cytokines suggests not only symptomatic relief but also restoration of immune homeostasis within the central nervous system. These effects were comparable to, and in some cases exceeded, those observed with donepezil, highlighting the strong therapeutic potential of the nano-formulated extract.

These findings agree with previous studies demonstrating that various plant extracts and plant-derived nanoparticles exert potent anti-inflammatory and neuroprotective effects by modulating cytokine signaling and suppressing oxidative stress-driven inflammation [42,43]. Given that chronic elevation of TNF- α and

IL-1 β accelerates amyloid- β deposition, synaptic dysfunction, and neuronal loss in AD [44–46], the ability of *H. suaveolens* AgNPs to normalize these mediators underscores their disease-modifying potential. This study highlights inflammation modulation activity of *H. suaveolens*, supported by molecular and biochemical evidence, thereby positioning *H. suaveolens*–mediated AgNPs as promising multi-target neurotherapeutic agent capable of attenuating neuroinflammation and slowing the progression of Alzheimer’s disease.

Modulation of Neurotransmitters and Neurotrophic Factors

In this study, exposure to aluminum chloride (AlCl₃) caused a significant reduction in BDNF levels compared to the control, supporting previous reports that AlCl₃ impairs BDNF expression [47]. This decrease in BDNF likely contributes to the cognitive deficits and neuronal dysfunction observed following aluminum toxicity [48,49]. Conversely, treatment with nano-formulated *Hyptis suaveolens* extract, either alone or in combination with AlCl₃, significantly increased BDNF levels. This upregulation of BDNF is linked to enhanced synaptic plasticity and improved memory formation [50,51]. The protective effect of *Hyptis suaveolens* could be attributed to its rich content of antioxidant phytochemicals, such as flavonoids and phenols, which may counteract oxidative stress and neuroinflammation, both known to suppress BDNF expression [52]

Additionally, AlCl₃ exposure caused a significant increase in AChE levels compared to the control group, consistent with findings by [53] This elevation in AChE activity may contribute to the neurotoxic effects of aluminum by accelerating acetylcholine breakdown [39,54], thereby disrupting cholinergic signaling. Treatment with nano-formulated *Hyptis suaveolens* extract significantly lowered AChE levels, demonstrating a neuroprotective effect likely through its ability to inhibit AChE activity. This inhibitory effect may be attributed to bioactive compounds such as flavonoids and phenols present in *Hyptis suaveolens* [52]

These findings align with previous reports showing that plant-derived nanoparticles and polyphenol-rich extracts enhance cholinergic transmission [55–57], suppress glutamate-induced excitotoxicity [58,59], and upregulate neurotrophic factors through redox and inflammatory pathway modulation [59–61]. Importantly,

the simultaneous restoration of neurotransmitter levels and BDNF underscores the multi-target therapeutic action of *H. suaveolens* AgNPs, a highly desirable feature given the multifactorial nature of AD. This reveals an integrated demonstration of neurotransmitter regulation and neurotrophic enhancement, providing strong mechanistic evidence that *H. suaveolens* AgNPs can effectively counteract aluminum-induced neurotoxicity and hold promise as a safe, disease-modifying therapeutic strategy for Alzheimer's disease.

Preservation of Cerebellar Histoarchitecture and Neuronal Integrity

The histological findings from H&E, Cresyl Violet, and Bielschowsky silver staining collectively provide strong evidence of the neuroprotective potential of *Hyptis suaveolens*-mediated silver nanoparticles (AgNPs) against AlCl₃-induced cerebellar neurodegeneration. In the AlCl₃-treated group, severe structural abnormalities were evident, including marked spongiosis, disrupted cortical layering, and shrunken, pyknotic Purkinje cells in the H&E-stained cerebellar tissue sections. These pathological features reflect oxidative injury, excitotoxic damage, and neuronal loss, which are hallmarks of Alzheimer's disease (AD)-like neurodegeneration [62,63]. Consistent with previous reports, AlCl₃ exposure produced pronounced cerebellar degeneration characterized by neuronal loss and architectural distortion, which are closely linked to impaired synaptic transmission and motor-cognitive dysfunction [64].

Cresyl Violet staining further corroborated these findings by revealing extensive chromatolysis, loss of Nissl substance, and neuronal shrinkage in AlCl₃-exposed rats, indicating collapse of protein synthesis machinery and compromised neuronal metabolism, key early events in AD pathology [38,62]. These alterations reflect impaired ribosomal activity and reduced synaptic plasticity, both of which contribute to progressive cognitive decline. In contrast, animals treated with *H. suaveolens* AgNPs exhibited marked preservation of Nissl substance, intact Purkinje cell morphology, and maintained granular layer density, suggesting effective protection of neuronal biosynthetic and functional integrity. While donepezil produced partial recovery, the more pronounced restoration observed in the AgNP-treated group highlights the superior neurorestorative potential of the nano-formulated extract. These observations align with the

previous reports that phytochemical-rich and green-synthesized nanoparticles counteract oxidative and excitotoxic damage, stabilize neuronal metabolism, and enhance synaptic resilience [65,66].

Bielschowsky silver staining further confirmed the disease-modifying effects of *H. suaveolens* AgNPs by revealing marked attenuation of neurofibrillary pathology. AlCl_3 exposure resulted in extensive argentophilic neurofibrillary tangles, axonal fragmentation, and cytoskeletal disorganization. These are features associated with tau pathology and impaired axonal transport in AD [67,68]. In contrast, *H. suaveolens* AgNP-treated animals showed well-preserved axonal architecture, absence of neurofibrillary tangles, and intact Purkinje cell networks comparable to control animals. This preservation of axonal integrity suggests effective protection against tau-mediated neurotoxicity and microtubule destabilization. The relatively modest improvement seen with donepezil further emphasizes the enhanced structural protection conferred by *H. suaveolens* AgNPs. These findings are consistent with reports indicating that oxidative stress and neuroinflammation promote tau hyperphosphorylation, while antioxidant-based nanotherapeutics suppress these processes and preserve cytoskeletal stability [69]

Collectively, the convergence of H&E, Nissl, and Bielschowsky staining provides compelling multi-level histological evidence that *H. suaveolens*-mediated AgNPs exert robust neuroprotective and disease-modifying effects. By preserving cerebellar architecture, maintaining neuronal integrity, and preventing neurofibrillary degeneration, these nanoparticles demonstrate strong potential as a multi-target therapeutic strategy for mitigating Alzheimer's disease-related neurodegeneration.

CONCLUSION

This study revealed that nano-formulated *Hyptis suaveolens*, having the ability to cross the blood-brain barrier, shows great promise as a multi-targeted therapeutic agent against AlCl_3 -induced neurotoxicity. It provides a natural and effective approach for addressing Alzheimer's disease-related pathology by safeguarding cognitive function, restoring biochemical balance, and maintaining brain integrity. These findings indicate there is a need for further investigation of this extract as a candidate for neuroprotective interventions.

ETHICS AND CONSENT TO PARTICIPATE

All experimental procedures involving animals were conducted in strict adherence to the ethical guidelines established by the Faculty of Basic Medical Sciences, University of Ilesa. Ethical approval was obtained from the Ethics Committee of the Faculty of Basic Medical Sciences, University of Ilesa, under approval reference number BMSUNILESA001/08/2024. The research protocol further complied with the European Council Directive 2010/63/EU governing the protection of animals used for scientific purposes.

The plant material, *Hyptis suaveolens* (L.) Poit., was collected in compliance with applicable local and national botanical guidelines. Taxonomic identification and authentication were carried out at the Department of Botany, Obafemi Awolowo University, Ile Ife, Osun State, and a voucher specimen was deposited under accession number 18332. The species is not listed under any restricted, endemic, or endangered category under national legislation, and all collection activities were conducted with the full authorization of the relevant institutional authority.

DATA AVAILABILITY

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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