ACTIVITY OF CATECHIN IN THE CEREBELLUM OF MERCURY CHLORIDE-TREATED WISTAR RATS

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UNIVERSITY OF BENIN
BENIN-CITY

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CERTIFICATION

This is to certify that this M.Sc. thesis was written by Eboka, Olisaemeka Ifechukwude in the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria

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DEDICATION

To my parents, Mr and Dr. (Mrs.) Eboka.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

ABSTRACT

Reports indicate that cerebellar disorders are induced by exposure to heavy metals, such as Mercury, via oxidative stress, neuroinflammation, and Purkinje cell apoptosis, thus disrupting motor coordination and cognitive processing. Catechin (CA), a flavonoid in green tea, exhibits antioxidant, anti-tumor, and anti-inflammatory effects, making it a potential therapeutic agent against heavy metal toxicity. This study investigated the activity of catechin in the cerebellum of Wistar rats exposed to Mercury chloride. Sixty-four Wistar rats were randomly assigned into eight groups (n=8), and treated for twenty-eight days, as follows; A (control), B (5 mg/kg body weight [bw] of mercury chloride [$HgCl_2$]), C (10 mg/kg bw of CA and $HgCl_2$), D (20 mg/kg bw of CA and $HgCl_2$), E (200 mg/kg bw of Vitamin E and $HgCl_2$), F (10 mg/kg bw of Catechin only), G (20 mg/kg bw of Catechin only) and H (200 mg/kg bw of Vitamin E only). Thereafter, weights, neurobehavioral activities, mercury concentration, antioxidant enzymes activity, lipid peroxidation, and histology of the cerebellum were assessed. In-silico studies were utilized to investigate multi-targeted protective potential of catechin, by assessing its interactions with key mediators of inflammation, oxidative stress, and apoptosis. Findings showed that $HgCl_2$ treatment significantly decreased ($p<0.05$) body weight and antioxidant enzymes, while significantly increasing ($p<0.05$) lipid peroxidation, mercury concentration, and neurobehavioral deficits, with cerebellar histology revealing Purkinje cell alterations, vacuolations, and pyknotic nuclei. Conversely, groups pre-treated with CA showed an attenuation of the $HgCl_2$-induced adverse effects. In-silico results showed catechin bound more strongly to Nrf2, TNF-α, IL-6, NF-κB, and Caspase-3 than amantadine and riluzole, highlighting its multi-targeted antioxidant, anti-inflammatory, and anti-apoptotic potential. This study highlights catechin as a safe, natural therapeutic for $HgCl_2$-induced cerebellar disorder, offering a promising alternative to conventional drugs with fewer side effects Further studies exploring the translational application of catechin in clinical trials are recommended.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Cerebellar dysfunction, characterized by impaired motor coordination, tremors, and balance disorders, represents a critical neurological challenge with profound effects on daily life (Yacoubi & Christou, 2024; Xia *et al*., 2023; He *et al*., 2021; Barbuto *et al*., 2020). The cerebellum, responsible for fine-tuning motor activity and learning, is especially vulnerable to toxic insults due to its high metabolic demand and reliance on tightly regulated excitatory neurotransmission (Korczowska-Łącka *et al*., 2023). Disruption of these delicate processes often leads to excitotoxicity, oxidative stress, and progressive neurodegeneration, which are hallmarks of cerebellar damage observed in both clinical and experimental contexts (Binvignat & Olloquequi, 2020; Di Penta *et al*., 2013).

The threat of cerebellar dysfunction is amplified by the broader context of heavy metal pollution. Metals such as mercury, lead, cadmium, and arsenic, while naturally occurring, have become pervasive environmental contaminants due to industrialization, mining, agricultural runoff, and improper waste disposal (Angon *et al*., 2024; Hembrom *et al*., 2019). Once released, they infiltrate water, soil, and air, entering the food chain and bioaccumulating in organisms, including humans. Mercury (Hg) compounds, in particular, pose an acute threat because of their capacity to disrupt enzyme function, impair antioxidant defences, and accumulate in vital organs such as the brain, liver, and kidneys (Teixeira *et al*., 2018; Abdel Moneim, 2015). One of the most threatening contributors to cerebellar dysfunction is exposure to mercury chloride ($HgCl_2$), a highly toxic inorganic compound of mercury. Once inside the body, $HgCl_2$ readily crosses the blood–brain barrier via amino acid transport systems, preferentially accumulating in cerebellar neurons such as Purkinje and granule cells (Aschner & Aschner, 1990). Here, it interferes with glutamate transport, induces excitotoxicity, and

triggers neuronal apoptosis, ultimately impairing cerebellar structure and function (Fonfría *et al*., 2005; Carocci *et al*., 2013). Experimental studies further demonstrate that mercury chloride exposure results in oxidative stress, mitochondrial dysfunction, and behavioural deficits linked to motor incoordination and locomotor impairment (Enogieru & Abhelemhen, 2025; Chamoli *et al*., 2024; Cariccio *et al*., 2019).

Due to increasing environmental and workplace exposure, $HgCl_2$ neurotoxicity is not just a biological risk factor but a significant public health problem. Preventive and curative approaches are therefore urgently required. Standard treatments such as chelation therapy remain the gold standard in managing acute poisonings; however, growing interest in natural products, particularly plant-derived polyphenols like catechin, highlights their potential as adjuvant interventions. Catechin is a naturally occurring polyphenolic compound belonging to the flavonoid group and is widely distributed in green tea, cocoa, and various fruits (Li *et al*., 2024; Jalouli *et al*., 2025). It has attracted considerable scientific interest due to its broad range of biological and pharmacological properties, including antioxidant, anti-inflammatory, and neuroprotective activities (Fakhri *et al*., 2022; Winiarska-Mieczan & Kwiecień, 2023). Structurally, catechin possesses multiple hydroxyl groups capable of donating hydrogen atoms to neutralize reactive oxygen species, thereby contributing to its potent antioxidant activity (Fakhri *et al*., 2022). Through its free radical–scavenging and metal–chelating abilities, catechin can prevent lipid peroxidation and stabilize cellular membranes, mitigating oxidative damage in biological systems (Talebi *et al*., 2021). In addition to its antioxidant potential, catechin has been reported to influence several biochemical pathways, including activation of the nuclear factor erythroid 2–related factor 2 (Nrf2)/antioxidant response element (ARE) signalling pathway, which promotes the expression of key antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Khalatbary & Khademi, 2020; Winiarska-Mieczan & Kwiecień, 2023).

1.2 STATEMENT OF RESEARCH PROBLEM

Mercury pollution remains a critical global concern, with mercury chloride ($HgCl_2$) representing one of its most toxic inorganic forms. Owing to its ability to cross the blood–brain barrier, $HgCl_2$ preferentially accumulates in the cerebellum, a brain region essential for motor coordination and balance. Experimental studies have shown that $HgCl_2$ exposure induces oxidative stress, lipid peroxidation, mitochondrial dysfunction, and inflammatory responses, leading to structural alterations such as Purkinje cell loss and granule layer disorganization, which manifest as motor and cognitive deficits (Carocci *et al*., 2013; Cariccio *et al*., 2019; Kumari & Chand, 2023). These neurotoxic effects are exacerbated by mercury's bioaccumulation in neural tissue, where it disrupts antioxidant defences and impairs enzymatic activities including superoxide dismutase and catalase (Teixeira *et al*., 2018; Abdel Moneim, 2015).

Although chelation therapy remains the standard intervention for mercury poisoning, its clinical utility is limited by adverse effects and incomplete neuroprotection (Zwolak, 2021). This limitation underscores the urgent need for safer, natural alternatives. Catechin, a flavonoid abundant in green tea, possesses potent antioxidant and metal-chelating properties that have demonstrated protective effects against cadmium- and lead-induced organ toxicity (Borowska *et al*., 2018; Wongmekiat & Peerapanyasut, 2018). However, its potential role in mitigating $HgCl_2$-induced cerebellar neurotoxicity, particularly in experimental models such as Wistar rats, remains poorly explored. Addressing this research gap is essential for determining whether catechin can attenuate oxidative damage, preserve cerebellar histoarchitecture, and improve motor function, thereby offering novel insights into neuroprotective strategies against mercury-induced neurodegeneration.

1.3 AIM

The aim of the study was to investigate the activity of Catechin on mercury chloride-treated cerebellar toxicity in Wistar rats.

1.4 SPECIFIC OBJECTIVES

The objectives of the study were to:

1. investigate the activity of Catechin on the brain and body weight changes in rats treated with or without mercury chloride;
2. determine the activity of Catechin on the neurobehavioral activities (Open field, movement initiation, step, and string tests) in rats treated with or without mercury chloride;
3. determine the activity of Catechin on the antioxidant enzymes (Catalase, Superoxide dismutase and Glutathione peroxidase activity), Glutathione concentration, Malondialdehyde concentration and concentration of mercury in the cerebellum of rats treated with or without mercury chloride;
4. investigate the activity of Catechin on the histology of the cerebellum in rats treated with or without mercury chloride; and
5. determine the activity of Catechin on the key molecular targets of oxidative stress, apoptosis and inflammation using *in-silico* molecular docking techniques.

1.5 JUSTIFICATION OF STUDY

Investigating the activity of catechin in the cerebellum of mercury chloride–treated Wistar rats matters because it addresses a pressing neurotoxicological problem with broad biomedical implications. The search for natural compounds such as catechin, a bioactive flavonoid found in green tea, represents a promising avenue for mitigating mercury-induced neuronal damage. Studies have shown that catechin possesses strong antioxidant and anti-inflammatory activities that counteract heavy metal toxicity by scavenging reactive oxygen species and preserving

neuronal integrity (Ramli *et al*., 2020; Suzen *et al*., 2022). The potential benefits of this research are multifold. First, demonstrating that catechin confers neuroprotection against mercury chloride toxicity could provide a natural, dietary-based intervention for populations at risk of heavy metal exposure. For instance, polyphenolic compounds have been shown to protect against heavy-metal-induced cerebellar damage by reducing oxidative stress and maintaining Purkinje cell function, which are vital for neuroprotection (Wnuk, 2025). Second, establishing catechin's efficacy may expand the use of nutraceuticals as adjunct therapies in neurodegenerative diseases where oxidative imbalance plays a central role. This aligns with earlier findings where plant-derived compounds significantly ameliorated heavy-metal-induced cerebellar alterations in rodents (El-Tarras *et al*., 2016; Agrawal *et al*., 2025).

Furthermore, solving this problem contributes to a deeper mechanistic understanding of how natural antioxidants interact with heavy metal toxicity pathways. For example, catechin has been found to enhance glutamate uptake and metabolism in astrocytes, thereby reducing excitotoxic injury in mercury exposure models (Olawade *et al*., 2025; Ramli *et al*., 2020). This mechanistic insight not only strengthens the evidence for catechin's protective role but also provides a foundation for developing pharmacological agents inspired by dietary polyphenols. By connecting toxicology, nutrition, and neuroscience, research on catechin activity in mercury chloride–treated cerebellum offers a valuable contribution to preventive medicine and therapeutic strategies against neurotoxic and degenerative disorders.

CHAPTER TWO

LITERATURE REVIEW

2.1 THE CEREBELLUM

The cerebellum is a critical brain region essential for the regulation of motor control, coordination, balance, and the fine-tuning of movements. Unlike the cerebral cortex, which is primarily associated with the initiation of voluntary movements, the cerebellum ensures precision, fluidity, and timing of motor activity. Its contributions extend beyond motor control, influencing cognition, language, and emotional regulation, but its primary role remains deeply tied to locomotor and postural stability (Stoodley & Limperopoulos, 2016). Damage to the cerebellum frequently manifests in motor deficits such as ataxia, tremors, and impaired coordination, underscoring its indispensable role in maintaining functional motor behaviour.

Anatomically, the cerebellum is located in the posterior cranial fossa, beneath the occipital lobes of the cerebral cortex, and posterior to the brainstem. It is divided into two hemispheres connected by the vermis and comprises distinct lobes and deep nuclei. This structure is intricately connected to other brain regions through three cerebellar peduncles, which serve as conduits for afferent and efferent pathways. These pathways link the cerebellum to motor areas of the cerebral cortex, basal ganglia, and spinal cord, allowing it to integrate sensory information and modulate motor commands. Functional imaging and tracing studies have further revealed that the cerebellum participates in distributed networks that also involve associative and limbic systems, emphasizing its role in integrating motor and cognitive processes (Habas, 2021; Shah *et al*., 2019).

Connectivity is central to cerebellar function. Afferent inputs from the spinal cord provide somatosensory information, while efferent projections influence motor execution through cortical and subcortical circuits. The cerebellum's architecture, characterized by its highly

regular cytoarchitecture of Purkinje cells, granule cells, and interneurons, is optimized for processing complex patterns of input and generating fine-tuned motor outputs. Such connections not only ensure accurate timing of movements but also permit adaptive plasticity, allowing the cerebellum to adjust to changing motor demands or recover from injury (Kalia, 2008). Comparative neuroanatomical studies have further emphasized that cerebellar connectivity patterns are highly conserved across species, highlighting their evolutionary importance in motor coordination and balance (Bolon, 2000).

The importance of studying cerebellar function becomes particularly evident in the context of neurotoxic exposure. Environmental neurotoxicants, including heavy metals and organic toxins, exert significant effects on cerebellar structure and function. Experimental models demonstrate that toxins targeting the cerebellum led to histopathological changes, including Purkinje cell degeneration and altered synaptic organization, which correlate with motor dysfunction (Edalatmanesh *et al*., 2014). Heavy metals such as mercury and manganese are known to accumulate in cerebellar tissue, disrupting redox balance and impairing motor coordination. Functional imaging and histological studies confirm that exposure to neurotoxicants impairs connectivity within cerebellar motor networks, compromising both motor execution and adaptive learning (Corbo *et al*., 2025; Lucchini *et al*., 2012).

Animal studies further underscore the cerebellum's vulnerability to toxins. For instance, neurotoxic lesions in cerebellar or associated motor nuclei produce oxidative stress, synaptic disruption, and deficits in balance and locomotion (Jimenez-Martin & Blanco-Lezcano, 2015). Plant-derived toxins, such as those from *Datura metel*, also induce cerebellar damage and motor alterations in rodents, reinforcing the cerebellum's sensitivity to both natural and anthropogenic toxicants (Igben *et al*., 2025). These findings position the cerebellum as a central site for studying the impact of environmental exposures on neurological health, particularly

because behavioural impairments are often directly traceable to histological and biochemical changes in cerebellar circuits.

The broader implications of cerebellar vulnerability extend to developmental neurotoxicology. Evidence from paediatric and developmental studies shows that early-life injury to the cerebellum can profoundly disrupt motor, cognitive, and emotional development (Stoodley & Limperopoulos, 2016). Given its connectivity with higher-order brain systems, cerebellar damage during critical periods of development has long-term consequences that extend beyond motor dysfunction. Understanding how neurotoxicants affect cerebellar development and connectivity is therefore essential for addressing both neurological diseases and environmental health risks.

2.1.1 Anatomical and Histological Structure of the Cerebellum

The cerebellum represents one of the most intricately organized regions of the central nervous system, essential for fine motor control, balance, and coordination. Its architecture is distinguished by a highly ordered laminar structure, composed of the molecular layer (ML), the Purkinje cell layer (PCL), and the granular cell layer (GCL). This precise organization not only ensures efficient processing of afferent sensory and motor information but also makes the cerebellum particularly vulnerable to pathological insults. Understanding the histological features of the cerebellum, both in normal and diseased conditions, is central to unravelling its role in neurological health and disease.

The molecular layer (ML) is the outermost layer of the cerebellar cortex and consists primarily of the dendritic arbors of Purkinje cells, parallel fibres of granule cells, and various inhibitory interneurons such as stellate and basket cells. The ML provides the substrate for synaptic integration, where excitatory and inhibitory signals converge upon Purkinje cell dendrites. Histological studies have shown that during development, the molecular layer expands as

granule cells migrate inward, giving rise to dense synaptic networks critical for motor coordination (Rakic & Sidman, 1970). In normal conditions, the ML demonstrates a well-preserved balance between excitatory and inhibitory elements, but under toxic or hypoxic stress, alterations such as dendritic beading and synaptic loss are frequently observed (Asker *et al*., 2016).

The Purkinje cell layer (PCL) forms a thin, middle layer characterized by a single row of Purkinje cells, which are the sole output neurons of the cerebellar cortex. Purkinje cells send their inhibitory GABAergic projections to the deep cerebellar nuclei, which in turn project excitatory signals to motor control centres. Their strategic position and extensive dendritic trees make Purkinje cells central integrators of cerebellar function. However, this same complexity renders them highly vulnerable to neurotoxic insults. Studies have documented Purkinje cell loss or displacement in response to toxic exposures such as aflatoxin B1 and heavy metals, leading to motor deficits (Laag & Abd Elaziz, 2013; Roegge *et al*., 2006). Koeppen (2018) emphasized their selective vulnerability to hypoxia, excitotoxicity, and methylmercury, which often manifests as shrinkage, vacuolation, and eventual cell death. Pathological crowding or ectopic positioning of Purkinje cells within the ML further reflects disruptions in cerebellar architecture under toxic conditions (Abdel-aziz *et al*., 2019).

The granular cell layer (GCL), located beneath the PCL, is the innermost and most densely populated layer of the cerebellar cortex. It contains millions of small granule cells that send axons into the ML, where they bifurcate into parallel fibres that synapse with Purkinje dendrites. The GCL is critical for amplifying sensory inputs and ensuring the fidelity of motor outputs. In healthy conditions, the GCL demonstrates tightly packed granule cells and uniform layering. However, it is not immune to toxicological or pathological alterations. Methylmercury exposure, for example, has been shown to selectively damage the GCL in rats,

causing neuronal loss and impaired cerebellar signalling (Du *et al*., 2023). In contrast, some stereological studies have reported structural preservation of granule cells under certain neurotoxic exposures, suggesting differential vulnerabilities between Purkinje and granule cells (Larsen & Brændgaard, 1995).

Purkinje cells occupy a unique position within this layered architecture, serving as the final output neurons of the cerebellar cortex. Their dendritic arborizations receive tens of thousands of synaptic contacts, making them highly metabolically active and susceptible to oxidative stress. Toxic exposures, including phenytoin and polychlorinated biphenyls (PCBs), disrupt Purkinje cell development and function, resulting in altered cerebellar connectivity and motor dysfunction (Ohmori *et al*., 1999; Roegge *et al*., 2006). The vulnerability of Purkinje cells thus makes them reliable markers of cerebellar pathology in experimental and clinical studies.

Histological studies under normal conditions depict the cerebellum as highly ordered, with neatly aligned Purkinje cells, intact dendritic arbors, and tightly packed granule cells. Pathological insults, however, disrupt this harmony. Reports describe Purkinje cell shrinkage, vacuolation, and displacement into the ML, accompanied by degeneration of granule cells and widening of the ML due to dendritic loss (Mohamed & Mohamed, 2018). Exposure to nanoparticles, toxins, and environmental pollutants often results in immunohistochemical changes in Purkinje cells, including altered expression of calbindin and glial markers, reflecting neuronal stress and glial reactivity (Abdel-aziz *et al.*, 2019). Collectively, these findings underscore the cerebellum's sensitivity to toxic insults and the utility of its histological features as biomarkers of neurotoxicity.

In summary, the cerebellum's histological organization into the ML, PCL, and GCL underpins its ability to integrate complex sensory and motor information. Purkinje cells, as the primary output neurons, are central to cerebellar function but also represent the most vulnerable element

of this architecture. Normal histology demonstrates a precisely aligned and interconnected system, while pathology introduces marked disruptions, including Purkinje cell loss, granule cell degeneration, and synaptic alterations. Understanding these features not only illuminates the basic biology of the cerebellum but also provides a critical lens for evaluating the impacts of neurotoxic exposures and neurological disorders.

2.1.2 Cerebellar Physiological Functions and Cognitive Roles

The cerebellum has historically been viewed as a brain structure dedicated primarily to the regulation of motor activity. Classical neurological observations linked cerebellar injury to motor deficits such as ataxia, tremors, and impaired balance, establishing its role in movement coordination and postural control. However, contemporary research has significantly broadened this understanding, demonstrating that the cerebellum contributes to a range of cognitive and affective functions, including learning, memory, language, and social behaviour (Cory-Slechta, 2023). Its extensive connectivity with cortical and subcortical regions provides the anatomical basis for these non-motor contributions, positioning the cerebellum as a key integrator of motor and cognitive processes.

One of the most important advances in cerebellar research has been the recognition of its involvement in cognitive processing and learning. Functional neuroimaging studies have consistently shown cerebellar activation during tasks involving working memory, attention, and language. Moreover, the cerebellum plays a role in associative learning, where it contributes to error prediction and the fine-tuning of cognitive operations in much the same way as it regulates motor activity (Chamoli & Karn, 2024). Experimental lesion studies in animals reinforce this perspective, demonstrating deficits in learning paradigms such as classical conditioning and spatial navigation when cerebellar circuits are disrupted. These findings confirm that cerebellar contributions to cognition extend beyond support for motor function to include higher-order information processing.

Neurobehavioral investigations have provided additional evidence of cerebellar influence on behaviour. In animal models, cerebellar dysfunction has been linked to impairments in coordination, exploratory behaviour, and social interactions. For example, exposure to heavy metals such as mercury chloride ($HgCl_2$) in Wistar rats produces significant behavioural deficits, including reduced locomotor activity, impaired motor coordination, and diminished exploratory tendencies (Enogieru & Abhelemhen, 2025). These outcomes correlate with histological changes in cerebellar tissue, particularly Purkinje cell loss and dendritic degeneration, highlighting the link between cerebellar pathology and measurable neurobehavioral outcomes. Similarly, comparative studies of toxicant exposures confirm that cerebellar circuits mediate not only coordination but also motivation and adaptive learning, thereby reinforcing the cerebellum's broader role in behavioural regulation (Dike *et al*., 2023).

The relevance of cerebellar function becomes even clearer when considering the effects of environmental neurotoxicants. Mercury, particularly in the form of $HgCl_2$, has been shown to disrupt cerebellar structure and impair associated behavioural functions. Mechanistically, $HgCl_2$ induces oxidative stress, mitochondrial dysfunction, and apoptosis in cerebellar neurons, leading to long-lasting deficits in motor coordination and cognition (Enogieru & Olisah, 2025). Neurochemical studies further demonstrate alterations in neurotransmitter systems, including reductions in dopamine and acetylcholine, which compromise both motor and cognitive functions (Pohl *et al*., 2006). These toxicological effects align with evidence from human studies, where mercury exposure is associated with memory impairment, attention deficits, and reduced executive function (Azevedo *et al*., 2023).

Emerging research has sought to mitigate mercury-induced cerebellar dysfunction through therapeutic interventions. Natural compounds such as folic acid and Allium sativum (garlic) extracts have demonstrated neuroprotective effects in experimental models. Folic acid

supplementation attenuates oxidative damage and preserves cerebellar histology in $HgCl_2$-exposed Wistar rats, leading to improved behavioural outcomes (Enogieru & Abhelemhen, 2025). Similarly, Allium sativum has been shown to reduce caspase-3 activation, restore antioxidant defences, and protect against neurobehavioral deficits caused by $HgCl_2$ exposure (Enogieru & Olisah, 2025). These findings suggest that antioxidant therapy may represent a promising avenue for reducing cerebellar damage induced by inorganic mercury exposure. However, while such interventions demonstrate efficacy in animal models, translation into clinical settings remains limited, and further research is required to assess their long-term therapeutic potential.

In summary, the cerebellum plays a crucial role not only in motor coordination but also in cognition, learning, and behaviour. Its involvement in neurobehavioral regulation underscores its importance in understanding the full spectrum of brain function. Neurotoxic exposures such as mercury chloride highlight the cerebellum's vulnerability, producing both structural damage and functional deficits. Experimental interventions with natural compounds demonstrate potential for mitigating these effects, but significant research gaps remain in translating these findings to humans. Recognizing the cerebellum as a central mediator of both motor and cognitive outcomes emphasizes its relevance in toxicological studies and therapeutic development.

2.1.3 Cerebellar Dysfunction

Cerebellar dysfunction is a group of disorders that affect the cerebellum's motor coordination, balance, and cognitive function-coordination capability. Fine-tuning motor activity is the key function that the cerebellum occupies, and the interruption that ensues is manifested as ataxia, dysmetria, dysarthria, and nystagmus. Ataxia, as the presentation, can occur as a complication of degenerative diseases, structural lesions, as well as autoimmune and metabolic illnesses. More recent studies, as well, have brought into focus the cerebellum's cognitive and emotional

regulation functions, including the importance that extends beyond motor control (Reumers *et al*., 2025).

Pathophysiology of cerebellar impairment is heterogeneous, as is the circuitry and connectivity of the cerebellum. Focal cerebellar lesions will produce discrete impairments that vary with the anatomical site: vermian lesions commonly produce truncal ataxia and postural instability, whereas hemispheric lesions have little impact on limb coordination. Functional imaging and neurophysiological experiments demonstrate that impaired cerebellar-cortical connectivity is the substrate both for motor and non-motor symptoms and that cerebellar pathology is correlated with larger neural network impairments (Mitoma *et al.,* 2019).

Recent reports highlight the notion of "cerebellar reserve" as the cerebral capability to compensate for cerebellar lesions through plasticity and reorganisation of functions (Mitoma *et al*., 2020). This notion is critical in the creation of rehab tactics that utilise residual cerebellar functions. Occupational and physical therapy can improve motor recovery, with data proving that task-specific training and modulation of sensory feedback enhance adaptive cerebellar reactions (Bogaert *et al*., 2024).

In addition to motor symptoms, cerebellar impairment presents as cognitive and affective disturbances under the rubric of the "cerebellar cognitive affective syndrome". They consist of disturbances in executive function, spatial thinking, and emotional control, implying that cerebellar circuits converge with the prefrontal and limbic circuits. This more extensive function of the cerebellum justifies the need for multiple discipline management approaches that include both motor and cognitive rehabilitation (Ciricugno *et al*., 2024).

2.1.3.1 Types of Cerebellar Dysfunction

Degenerative cerebellar disorders, like spinocerebellar ataxias (SCAs), multiple system atrophy of the cerebellar type (MSA-C), and idiopathic late-onset cerebellar ataxia, are one of

the main types of cerebellar dysfunction. They are typically hereditary or sporadic and are defined by progressive Purkinje downturn and cerebellar shrinking. For instance, people with Type I autosomal dominant cerebellar ataxias have a mix of neurological symptoms, such as involvement of both pyramidal and extrapyramidal areas, which is a sign of damage to more than one system (ten Donkelaar *et al*., 2023). Genetic testing developments have revealed multiple mutations corresponding to specific subtypes of SAC, enhancing diagnosis as well as the possibility of directed drug design.

Another significant group is developmental and structural cerebellar disorders, including cerebellar hypoplasia, Dandy–Walker malformation, and pontocerebellar hypoplasia. They generally result from shattered embryonic development or mutations in the gene causing neuronal migration and differentiation disorders. Patients typically present with early ataxia, motor delay, and oculomotor disturbances. As Accogli *et al.* (2008) describe, developmental cerebellar disorders offer useful teachings on cerebellar patterning and plasticity. Ten Donkelaar *et al*. (2023) also underscored how structural disturbances can have varied functional outcomes based on residual cerebellar function, again underscoring the need for prompt diagnosis and supportive therapy.

2.1.3.2 Degenerative Cerebellar Disorders

Degenerative cerebellar disorders are heterogeneous conditions of the nervous system that involve progressive cerebellar neurone loss, especially Purkinje neurones, and cause ataxia, dysarthric speech, and impairment in motor coordination. Most well-known among these are the spinocerebellar ataxias (SCAs), multiple system atrophy–cerebellar type (MSA-C), and idiopathic late-onset cerebellar ataxia (ILOCA). SCAs are the collection of over 40 genetically different subtypes, each linked with distinct molecular pathogenesis, including CAG trinucleotide repeat expansion or mutations in mitochondrial or ion channel function (Iskusnykh *et al*., 2024). Atrophy of the cerebellum is the pathologic signature, along with

accompanying afferent and efferent fibre system degeneration, that causes motor as well as cognitive impairment (Lew *et al*., 2022).

At the molecular and cellular level, degenerative disorders of the cerebellum encompassed intricate pathophysiological mechanisms such as oxidative stress, mitochondrial impairment, and activation of glial cells. Anonymously, these play a part in bringing about excitotoxicity, breakage in synapses, and sequential neurodegeneration (Iskusnykh *et al*., 2024). Ageing serves as a driving force, decreasing neuroprotective mechanisms as well as cerebellar reserve, the capability of the cerebellum, like the rest of the brain, to compensate for the damage incurred. The rate and pattern of degeneration differ between disorders: as an example, MSA-C consists of disseminated glial cytoplasmic inclusions along with olivopontocerebellar atrophy, whereas SCAs have more specific vulnerability according to the affected gene. Neuroimaging evidence supports uniform grey matter loss in the cerebellar hemispheres as well as the vermis across degenerative diseases, relating symptomatically (Guo *et al*., 2021; Yang *et al*., 2024).

Treatment of degenerative cerebellar disorders is still predominantly symptomatic, with an emphasis on rehabilitation and neuroprotective approaches that maintain cerebellar function. Pharmacologic work has looked at agents that can regulate excitatory neurotransmission and oxidative homeostasis, but no definitive cures have resulted as of this date. However, with degenerative cerebellar disorders progressing through the work on cerebellar plasticity and reserve, hope is renewed that innovation in therapy can occur. Neurorehabilitation interventions, including task-specific motor training and balance therapy, can take advantage of spared cerebellar circuits and postpone the degradation of function (Mitoma *et al*., 2019). New work is also examining gene-targeted therapy in hereditary ataxias, a critical step toward

the creation of disease-modifying treatments in this stout group of diseases (Mitoma *et al*., 2020; Salem *et al.,* 2023).

2.1.3.3 Development and Structural Cerebellar Disorders

Developmental and structural disorders of the cerebellum include a broad group of diseases caused by the defect in cerebellar formation, differentiation, and embryogenic maturation. They frequently occur due to genetic mutations, environmental damage, or perinatal wounds that interfere with the precise temporal schedule of cerebellar neurogenesis and migration. The cerebellum, the earliest part of the brain that develops, undergoes a prolonged maturation process that extends into the perinatal period, making the cerebellum especially prone to developmental disruptions (ten Donkelaar *et al.*, 2023). Common structural malformations are Dandy–Walker malformation, cerebellar hypoplasia, and Joubert syndrome, all characterized by variable motor incoordination, developmental delay, and intellectual disability. Those disorders provide evidence on how premature interruption in the cerebellar primordium can have significant effects on motor as well as intellectual outcomes.

Genetic studies have elucidated the molecular mechanisms underlying the majority of developmental cerebellar afflictions. Mutations in genes that regulate either the proliferation, migration, or axonal guidepost determination of neurons like ZIC1, Atoh1, and LHX1 can produce discrete cerebellar phenotypes (Marzban *et al*., 2023; Aldinger *et al*., 2019). Transcriptome evaluations on human foetal cerebellar samples have discovered gene expression patterns that are specific to regions and essential for the development of Purkinje cells and granule neurons (Haldipur *et al*., 2022). Distortions in these gene programs can have structural impairments as outcomes, as well as impair the functional connectability between the cerebellum and the upper cortical regions, which underlies the cerebellum's increasing involvement in neurodevelopmental diseases like autism spectrum disorders (ASD) and attention-deficit/hyperactivity disorders (ADHD) (van der Heijden *et al*., 2021).

Structural cerebellar malformations are commonly characterized by the embryologic origin and site affected. Underdevelopment of the cerebellar hemispheres or vermis refers to cerebellar hypoplasia, whereas the group of autosomal recessive disorders with premature neuron loss in the cerebellum and brainstem is the pontocerebellar hypoplasia. An increase in the size of the fourth ventricle along with hypoplasia of the cerebellar vermis is characteristic in Dandy–Walker malformation, often combined with hydrocephalus and cortical malformations. Advances in neuroimaging have been critical in establishing these conditions and relating structural lesions with the functional outcomes (Ibrahim & Hachem, 2020; D'Arrigo *et al*., 2021). They provide further evidence that severity and localisation of cerebellar malformation establish the extent and severity of neurological and intellectual impairments.

Functional considerations in developmental cerebellar disorders go beyond motor control into cognitive, affective, and behavioural areas. Cerebellar cognitive affective syndrome such as executive impairment, language impairment, and emotional dysregulation has been noted in children with congenitally induced cerebellar anomalies, highlighting the integrative function of the cerebellum in the development of the brain and behaviour (Stoodley & Limperopoulos, 2016). In addition, longitudinal reviews indicate that initial cerebellar damage abolishes critical periods in the maturation process of the cortex, with lasting neurodevelopmental effects (Sathyanesan *et al*., 2019). Taken together, these observations underscore the key contribution of the cerebellum in the process of arranging the development of the brain and the significance of premature diagnosis, genetic counselling, and specific useful treatments in the prevention of disability in later life.

2.1.4 Cerebellar Vulnerability to Toxicants and Neurodegeneration

The cerebellum, while traditionally recognized for its essential role in motor coordination and balance, has emerged as one of the most vulnerable regions of the central nervous system to

environmental toxicants. Its high neuronal density, metabolic demands, and reliance on finely tuned neurotransmission render it susceptible to oxidative stress, excitotoxicity, and apoptotic pathways triggered by heavy metals and other neurotoxic agents. Among these, mercury chloride ($HgCl_2$) represents a critical toxicant due to its capacity to cross the blood-brain barrier, accumulate in neural tissue, and induce persistent structural and functional alterations.

The susceptibility of the cerebellum to mercury chloride toxicity has been demonstrated across multiple experimental models. Long-term exposure leads to deposits of mercury within cerebellar tissue, particularly targeting Purkinje cells, which are the sole output neurons of the cerebellar cortex and central to motor regulation. These cells exhibit a unique vulnerability due to their large dendritic arbors and intense metabolic requirements. Experimental evidence has shown marked reductions in Purkinje cell density, accompanied by dendritic degeneration and functional impairments in motor coordination (Bittencourt *et al*., 2021). Similar findings have been reported in both in-vivo and in-vitro studies, where mercury exposure triggers oxidative stress, mitochondrial dysfunction, and apoptotic cascades (Teixeira *et al*., 2018).

Mechanistically, the primary pathways of mercury-induced cerebellar damage involve oxidative stress, inflammation, and apoptosis. Mercury readily generates reactive oxygen species (ROS), leading to lipid peroxidation, protein oxidation, and DNA damage. This oxidative burden overwhelms endogenous antioxidant systems such as glutathione, disrupting neuronal homeostasis (Fonnum & Lock, 2004). Concurrently, neuroinflammatory responses are activated, with microglial and astrocytic reactivity exacerbating neuronal injury. Apoptotic signalling pathways, including caspase-3 activation, have been documented in mercury chloride-exposed cerebella, confirming programmed cell death as a major endpoint of toxicity (Enogieru & Olisah, 2025). Together, these mechanisms converge to compromise Purkinje cell survival and undermine cerebellar circuitry integrity.

Histological evidence provides strong support for these mechanistic insights. In rat models exposed to $HgCl_2$, degeneration and necrosis of Purkinje cells have been observed alongside vacuolization and disruption of cortical layers (Ibegbu *et al*., 2014). Methylmercury, a related organic compound, has also been extensively studied and shown to induce apoptotic degeneration of Purkinje cells and granule neurons, with in-vitro models of cerebellar granule cells further confirming excitotoxic and oxidative stress-mediated mechanisms (Suñol & Rodríguez-Farré, 2012; Ceccatelli *et al*., 2010). These alterations translate into measurable motor deficits, including ataxia and impaired locomotion, reinforcing the functional consequences of structural damage.

In addition to animal models, in-vitro studies have provided valuable insights into cellular-level changes. Cultured neurons exposed to mercury display glutamate transporter dysfunction, glutathione depletion, and increased vulnerability to DNA damage, which collectively contribute to excitotoxic and apoptotic cascades (Fonnum & Lock, 2004). Such findings have been crucial for delineating molecular events preceding cell death and for identifying potential targets for intervention.

Human studies, although less controlled, have corroborated these findings by linking mercury exposure to cerebellar dysfunction and broader neurodegenerative outcomes. Epidemiological data associate occupational and environmental mercury exposure with tremors, impaired coordination, and cognitive decline (Cariccio *et al*., 2019). Autopsy reports from cases of chronic exposure reveal Purkinje cell loss and gliosis in the cerebellar cortex, consistent with animal and in-vitro observations. Importantly, these findings extend to developmental exposures, where prenatal and early childhood mercury exposure has been linked to long-term deficits in motor and cognitive outcomes, reflecting the cerebellum's heightened vulnerability during critical developmental periods.

The implications for human health are significant. Mercury-induced cerebellar degeneration contributes not only to overt motor impairments but also to subtle cognitive and behavioural deficits, highlighting the cerebellum's role beyond motor control. The chronicity of mercury accumulation and its ability to cross the placental barrier underscore the intergenerational risks associated with exposure (Cariccio *et al*., 2019). While therapeutic strategies such as antioxidant supplementation and stem cell therapies have been investigated, their translational potential remains limited. Experimental studies suggest that compounds such as ascorbic acid and Allium sativum can attenuate oxidative stress and apoptosis in $HgCl_2$-exposed cerebella (Ibegbu *et al*., 2014; Enogieru & Olisah, 2025), but further clinical validation is necessary.

In conclusion, the cerebellum's susceptibility to toxicants, particularly mercury chloride, is well-established across human, animal, and in-vitro studies. Mechanisms of injury converge on oxidative stress, inflammation, and apoptosis, with Purkinje cells emerging as the principal neuronal targets. The consistency of findings across models underscores the translational significance for human health, where mercury exposure continues to pose environmental and occupational risks. The integration of mechanistic insights with histological and behavioural outcomes provides a robust framework for understanding cerebellar neurodegeneration and identifying avenues for therapeutic intervention.

2.1.5 Current Research and Gaps in Cerebellar Protection

The cerebellum's vulnerability to environmental toxicants has prompted extensive research into neuroprotective strategies, particularly the use of natural compounds with antioxidant and anti-inflammatory properties. Recent studies underscore the ability of plant-derived molecules such as catechin, flavonoids, and polyphenols to counteract oxidative damage and preserve cerebellar histology under toxicant exposure. However, while evidence supports its protective role in various models of neurotoxicity, significant gaps remain in the specific evaluation of

catechin against mercury chloride ($HgCl_2$)-induced cerebellar damage. Addressing these gaps provides both the rationale and direction for current investigations into histological and behavioural outcomes.

Several studies demonstrate the broad protective effects of natural compounds on cerebellar tissue exposed to heavy metals and environmental pollutants. For example, Owoeye and Gabriel (2016) reported that extracts of *Telfairia occidentalis* preserved cerebellar and hippocampal histology and reduced oxidative stress in mercury-exposed rats. Similarly, Enogieru and Idemudia (2024) compared ginger and cocoa extracts in lead-exposed rats, finding both capable of restoring antioxidant balance and ameliorating cerebellar histopathology. Gallic acid has also been shown to attenuate tebuconazole-induced cerebellar injury, with histopathological and immunohistochemical evidence of Purkinje cell protection (Ismail & Hassanin, 2024). These findings support the hypothesis that dietary polyphenols and antioxidants can effectively mitigate cerebellar neurotoxicity.

Catechin, abundant in green tea, is of particular interest because of its neuroprotective properties. Mousa and Ibrahim (2023) observed that it reduced oxidative markers such as malondialdehyde (MDA) in methylmercury-exposed rats, protecting Purkinje cells and preserving cerebellar architecture. Likewise, Singh *et al*. (2021) found that it reduced oxidative stress in arsenic-intoxicated mice, highlighting its potential across different toxicants. These studies suggest that catechin exerts its effects through antioxidant activity, mitochondrial stabilization, and modulation of apoptotic pathways. Despite these encouraging findings, most existing work has focused on methylmercury or other heavy metals, rather than inorganic mercury such as $HgCl_2$, which remains a key toxicant in environmental and laboratory settings.

The gap in knowledge surrounding catechin's specific protective effects under $HgCl_2$ exposure is critical. $HgCl_2$ differs from methylmercury in its solubility, transport, and tissue distribution,

and thus may interact differently with cerebellar neurons. While Tams *et al*. (2018) reported neurobehavioral improvements following treatment with *Salacia reticulata* extract in $HgCl_2$-exposed rats, there remains a lack of mechanistic studies examining how catechin interacts with oxidative, inflammatory, and apoptotic pathways in this context. Furthermore, evidence of catechin penetration across the blood–brain barrier and its bioavailability within cerebellar tissue under mercury chloride exposure is sparse (Granda & de Pascual-Teresa, 2018).

Another important research gap lies in the integration of histological and behavioural outcomes. Most studies have focused on biochemical markers of oxidative stress or histological assessments, but relatively few have combined these with functional behavioural assays. Given the cerebellum's central role in motor coordination and exploratory behaviour, assessing behavioural outcomes alongside histological integrity provides a more complete understanding of neuroprotection. Gamoudi (2019) emphasized this in studies of rooibos tea, where neuroprotection against bisphenol-A included both histological preservation and improved exploratory behaviour in Wistar rats. Bridging these methodological approaches will clarify whether histological protection translates into functional recovery.

In summary, current research highlights the promise of natural compounds, particularly catechin, in protecting cerebellar integrity against toxicant exposure. However, there is a striking lack of studies directly evaluating catechin's protective role against $HgCl_2$-induced cerebellar damage. Understanding whether catechin can mitigate both histological and behavioural alterations is essential for translating findings into effective interventions. These gaps justify the focus of the present study, which seeks to integrate histological evidence with behavioural and biochemical outcomes and *in-silico* studies to provide a comprehensive assessment of catechin's protective potential in mercury chloride neurotoxicity.

2.2 MERCURY

Mercury, a naturally occurring heavy metal, persists in the environment through natural processes like volcanic eruptions and anthropogenic activities such as mining, coal combustion, and industrial discharges, contaminating air, water, and soil (WHO, 2024; EPA, 2023). Its toxicity varies by form: elemental mercury (Hg^0), inhaled as vapor from dental amalgams or industrial spills, causes respiratory and neurological damage; organic mercury, primarily methylmercury from contaminated fish, bioaccumulates in the food chain, leading to severe central nervous system impairments; and inorganic mercury, such as mercuric chloride ($HgCl_2$), is ingested or absorbed dermally, targeting kidneys and the gastrointestinal tract (Cleveland Clinic, 2024; Cappelletti *et al*., 2019). The World Health Organization estimates that over 19 million people, particularly in developing regions, face health risks from mercury exposure, with symptoms ranging from fatigue and irritability to tremors and cognitive deficits (WHO, 2024). This pervasive exposure underscores mercury's public health significance, necessitating a focused examination of its neurotoxic effects.

This review centres on mercury's neurotoxicity, with a particular emphasis on cerebellar dysfunction induced by $HgCl_2$, an inorganic form known for its high solubility and reactivity (Branco *et al*., 2021). $HgCl_2$'s ability to cross the blood-brain barrier and accumulate in the cerebellum triggers oxidative stress, inflammation, and apoptosis, leading to histological damage like Purkinje cell loss and weight changes, as observed in rat models (Egba *et al*., 2022; Asuku *et al*., 2024). These mechanisms align with this study's findings, which include elevated biochemical markers and cerebellar damage mitigated by antioxidants like catechin. By exploring human exposure, cerebellar effects, and current treatments, this review highlights $HgCl_2$'s role in neurotoxicity, building on general mercury toxicity to address its specific cerebellar impact.

2.2.1 Human Exposure and Metabolism

Mercury exposure in humans varies by its chemical form, elemental, organic, and inorganic, each with distinct pathways and metabolic fates that influence toxicity. Elemental mercury (Hg^0), primarily encountered through inhalation of vapours from dental amalgams or industrial spills, is rapidly absorbed in the lungs (80–100%) and oxidizes to Hg^{2+} in red blood cells, distributing to kidneys and brain with a half-life of 40–60 days and excretion via urine and faeces (Clarkson and Magos, 2006; ATSDR, 2024). Occupational exposure, such as in mining, often exceeds safe levels (0.025 mg/m³), causing neurological symptoms like tremors (WHO, 2024). Organic mercury, mainly methylmercury from contaminated fish, exhibits near-complete gastrointestinal absorption (95%), demethylates slowly in the liver, and bioaccumulates in the brain, with a longer half-life of 50–70 days, posing risks for chronic neurotoxicity (Farina *et al*., 2011). Inorganic mercury, including mercuric chloride ($HgCl_2$), is absorbed through ingestion (7–15% bioavailability) from contaminated water or occupational dermal contact (2–10%), binding to sulfhydryl groups in proteins and accumulating predominantly in kidneys (50% body burden) (ATSDR, 2024; Bjørklund *et al*., 2017).

Metabolism of these forms differs significantly. Elemental mercury's oxidation to Hg^{2+} facilitates urinary excretion, but its volatility increases inhalation risks. Methylmercury forms complexes with cysteine, mimicking methionine to cross the blood-brain barrier (BBB), leading to prolonged neural retention (Branco *et al*., 2021). Inorganic forms like $HgCl_2$ undergo minimal metabolism, primarily forming glutathione conjugates in the liver for biliary and renal excretion, with a biphasic half-life (1–2 days rapid, 1–2 months terminal) (Clarkson and Magos, 2006). Recent studies highlight occupational $HgCl_2$ exposure in artisanal mining, with blood levels reaching 50–100 µg/L, exceeding safe thresholds (<10 µg/L) and causing systemic toxicity (Budnik and Casteleyn, 2019). Despite low oral bioavailability, $HgCl_2$'s high solubility enhances its distribution to the cerebellum, inducing oxidative stress and neuronal damage, as

evidenced in rat models and human case studies (Asuku *et al*., 2024). This cerebellar accumulation underpins $HgCl_2$'s neurotoxic potential, distinguishing it from other forms and linking exposure to the cerebellar effects explored subsequently.

2.2.2 Mechanisms and Effect of Mercury-Induced Cerebellar Toxicity

The cerebellum, essential for motor coordination, balance, and fine motor control, is highly vulnerable to environmental toxicants, including mercury. Among mercury's chemical forms, inorganic mercury such as mercuric chloride ($HgCl_2$) has been strongly implicated in cerebellar dysfunction due to its ability to cross the blood–brain barrier and accumulate in neuronal tissue. A growing body of evidence demonstrates that mercury-induced cerebellar toxicity arises through a complex interplay of oxidative stress, neuroinflammation, apoptosis, and structural degeneration. A detailed review of these mechanisms follows, with an emphasis on experimental findings from animal and cellular models providing insights into cerebellar vulnerability.

2.2.2.1 Oxidative Stress Activity

Oxidative stress is one of the earliest and most consistently reported consequences of mercury exposure in cerebellar tissue. Following systemic absorption, Hg^{2+} ions bind to thiol and selenol groups in antioxidant enzymes, impairing the activity of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase, thereby weakening redox homeostasis. Studies in Wistar rats chronically exposed to $HgCl_2$ demonstrated increased malondialdehyde (MDA), a lipid peroxidation marker, alongside significant reductions in SOD and GPx activity in cerebellar homogenates (Teixeira *et al*., 2018). Similar findings were reported in proteomic studies showing that long-term inorganic mercury intoxication induces oxidative damage and downregulation of antioxidant proteins in cerebellar neurons, ultimately compromising mitochondrial function (Bittencourt *et al*., 2021). The accumulation of reactive oxygen species (ROS) not only damages lipids and proteins but also initiates signalling cascades that propagate

inflammatory and apoptotic responses, establishing oxidative stress as a central initiating mechanism in mercury-induced cerebellar neurotoxicity.

2.2.2.2 Inflammatory Activity

Neuroinflammation plays a synergistic role with oxidative stress in mediating mercury's neurotoxicity. Reactive oxygen species generated by Hg^{2+} ions activate nuclear factor kappa B (NF-κB), which in turn upregulates pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. This cytokine surge promotes microglial activation, further amplifying local inflammatory responses. Cariccio *et al*. (2019) reported that mercury exposure initiates a feed-forward cycle of oxidative stress and inflammation, which contributes to progressive cerebellar degeneration. In vivo studies in rats confirmed that mercuric chloride exposure increases cerebellar cytokine expression and enhances Iba1-positive microglial activation, corroborating the role of inflammation in histological damage (Enogieru & Abhelemhen, 2025). Chronic inflammation sensitizes Purkinje cells to oxidative injury and primes apoptotic pathways, linking inflammatory and degenerative processes.

2.2.2.3 Apoptotic Activity

Mercury-induced apoptosis is a downstream effect of combined oxidative and inflammatory insults. Apoptotic cell death is triggered through mitochondrial dysfunction, characterized by Bax upregulation, Bcl-2 downregulation, and caspase-3 activation. Moneim (2015) demonstrated that mercury exposure induces caspase-dependent apoptosis in cerebellar neurons, leading to neuronal loss and motor deficits. Zahed *et al*. (2024) expanded on these findings, identifying proteomic alterations in apoptosis-regulating proteins following inorganic mercury exposure. Histological investigations confirm a significant reduction in Purkinje cell density, nuclear pyknosis, and structural disorganization of cerebellar cortical layers following $HgCl_2$ intoxication (Bittencourt *et al*., 2021; Enogieru & Abhelemhen, 2025). These findings

emphasize apoptosis as a critical pathway through which cerebellar architecture is disrupted, translating molecular events into functional deficits.

2.2.2.4 Histological Alterations

The culmination of oxidative, inflammatory, and apoptotic mechanisms manifests as marked histological changes in cerebellar architecture. In rat models, $HgCl_2$ exposure produces vacuolations in the molecular and Purkinje cell layers, shrunken Purkinje neurons with pyknotic nuclei, and overall cerebellar atrophy (Enogieru & Abhelemhen, 2025). These structural alterations correlate with motor coordination deficits and reductions in body weight, indicating systemic and neurological consequences. Studies of methylmercury exposure further highlight the cerebellum's vulnerability, where granule cell apoptosis and Purkinje cell dysfunction result in pronounced motor impairments (El-Azab *et al*., 2018). Such findings illustrate that while different mercury species vary in potency, the cerebellum remains a shared target of neurotoxicity. Importantly, advanced proteomic and transcriptomic analyses now reveal that mercury alters neurotransmission pathways, mitochondrial bioenergetics, and cytoskeletal integrity, offering mechanistic insights into long-term neurodegenerative processes (Zahed *et al*., 2024).

Mercury-induced cerebellar toxicity is mediated by a cascade of interconnected mechanisms. Oxidative stress initiates the toxic process by impairing antioxidant defences (Zahed *et al.*, 2024), while neuroinflammation amplifies neuronal vulnerability (Novo *et al.*, 2021). These insults converge on apoptotic pathways, leading to Purkinje cell death and disruption of cerebellar layers (Bittencourt *et al.*, 2021; Enogieru & Abhelemhen, 2025). Histological damage translates into functional impairments such as motor deficits and weight loss, underscoring the cerebellum's sensitivity to mercury exposure (Teixeira *et al.*, 2018). Collectively, these findings highlight the importance of mechanistic studies in shaping our

understanding of cerebellar vulnerability and guiding therapeutic interventions aimed at mitigating mercury-induced neurotoxicity (Abubakar, 2019).

Given the multifactorial nature of mercury-induced cerebellar toxicity, therapeutic interventions have focused on targeting oxidative stress, inflammation, and apoptosis simultaneously. Natural antioxidants, particularly polyphenolic compounds, have emerged as promising agents in this context. Catechin, a flavonoid abundant in green tea, demonstrates potent radical-scavenging and anti-inflammatory properties. In rodent models, administration of green tea extracts rich in catechin restored antioxidant enzyme activity, reduced lipid peroxidation, and preserved cerebellar architecture (Mousa & Ibrahim, 2023). Molecular docking studies further support catechin's ability to activate the Nrf2 pathway, thereby upregulating endogenous antioxidant defences and attenuating oxidative damage (Hussain *et al*., 2017; Clifford *et al*., 2021).

Other natural compounds have also shown efficacy. Folic acid supplementation significantly ameliorated cerebellar alterations in Wistar rats exposed to $HgCl_2$, reducing microglial activation and improving histological outcomes (Enogieru & Abhelemhen, 2025). Similarly, berberine has been reported to counteract mercury-induced apoptosis by downregulating Bax expression and inhibiting caspase-3 activation, thereby protecting neuronal populations (Moneim, 2015). Collectively, these findings highlight the therapeutic relevance of dietary antioxidants and plant-derived compounds as accessible interventions. However, questions remain regarding their bioavailability, dose-response relationships, and long-term efficacy, underscoring the need for focused mechanistic and translational studies.

Mercury-induced cerebellar toxicity arises from an interplay of oxidative stress, neuroinflammation, apoptosis, and structural degeneration, with Purkinje cells being especially vulnerable. These mechanisms converge to impair cerebellar function and produce motor

deficits, as documented across animal, cellular, and molecular studies. While natural antioxidants such as catechin, folic acid, and berberine provide compelling evidence of neuroprotection, the literature reveals critical gaps in defining their precise mechanisms, optimal dosing, and long-term safety. Addressing these gaps through controlled animal studies and molecular analyses is essential for translating preclinical findings into therapeutic strategies. This review therefore establishes the mechanistic foundation of $HgCl_2$-induced cerebellar dysfunction and situates antioxidant-based interventions as promising, yet underexplored, avenues for future research.

2.2.4 Current Treatment Options for Mercury Chloride Cerebellar Dysfunction

Treatment for mercury-induced cerebellar dysfunction focuses on minimizing exposure, enhancing excretion, and mitigating oxidative damage, with strategies varying by mercury form. For elemental mercury, immediate removal from the source and supportive care, such as ventilation for inhalation exposure, are primary, as no specific antidote exists; chelation is rarely effective due to its rapid oxidation and distribution (Clarkson and Magos, 2006). Organic mercury poisoning, like methylmercury, relies on supportive therapy for symptoms (e.g., ataxia, tremors) and chelation with dimercaptosuccinic acid (DMSA) or dimercaptopropane sulfonate (DMPS) to promote urinary excretion, though efficacy is limited for established cerebellar damage (Farina *et al*., 2011). Inorganic mercury, including $HgCl_2$, benefits from chelation therapy with DMSA or British Anti-Lewisite (BAL), which bind Hg^{2+} ions and facilitate renal elimination, reducing systemic levels by 50–70% in acute cases (Bjørklund *et al*., 2021). However, chelating agents such as DMSA and DMPS have limited ability to cross the blood–brain barrier, reducing their effectiveness in mitigating cerebellar damage, and may exacerbate symptoms if initiated late (Kosnett, 2013; ATSDR, 2024).

Emerging treatments emphasize antioxidants to counteract oxidative stress, a key driver of $HgCl_2$ cerebellar toxicity. N-acetylcysteine (NAC), a glutathione precursor, scavenges ROS

and replenishes GSH, reducing MDA levels and inflammation in rat models of $HgCl_2$ exposure (Egba *et al*., 2022). In Wistar rats treated with 0.4 mg/kg $HgCl_2$, NAC (200 mg/kg) restored SOD and GPx activities by 40–60% and attenuated TNF-α upregulation, preserving cerebellar function (Asuku *et al*., 2024). Selenium supplementation, often combined with chelators, forms insoluble Hg-selenide complexes, enhancing excretion and neuroprotection; studies show it mitigates Purkinje cell loss in $HgCl_2$-exposed rats (Branco *et al*., 2021). For $HgCl_2$-specific cerebellar dysfunction, a combined approach of DMSA (30 mg/kg/day) and NAC has shown promise in reducing caspase-3 activation and histological damage, with 30–50% improvement in motor coordination in rodent models (Bjørklund *et al*., 2021). In human cases of occupational $HgCl_2$ poisoning, chelation with DMSA lowered blood Hg levels from 50–100 μg/L to <20 μg/L within weeks, alleviating ataxia, though residual cerebellar symptoms persist due to irreversible neuronal loss (Rooney, 2007; Kosnett, 2013; ATSDR, 2024).

Supportive therapies address cerebellar symptoms, including physical rehabilitation for ataxia and occupational therapy for motor deficits. Recent 2020–2025 studies explore novel agents like β-caryophyllene, a cannabinoid agonist, which ameliorates $HgCl_2$-induced oxidative stress and microglial activation in rat cerebellum, increasing Purkinje cell counts by 20–30% (Yahyazadeh & Gur, 2024; Orheruata *et al*., 2025; Bhat *et al*., 2025). In-silico docking supports these, showing β-caryophyllene binding to CB2 receptors (-7.5 kcal/mol), modulating inflammation (Branco *et al*., 2021). For chronic $HgCl_2$ exposure, EDTA chelation combined with antioxidants reduces neuroinflammation, as seen in preliminary trials where EDTA lowered oxidative markers by 25–40% (Vezzoli *et al*., 2023). Limitations include chelators' potential to mobilize Hg from tissues, risking redistribution to the brain, and the need for early intervention; no treatment fully reverses advanced cerebellar atrophy (Farina *et al*., 2013). Future directions include targeted BBB-penetrating chelators and Nrf2 activators like catechin, which docking studies show bind with -7.1 kcal/mol, upregulating antioxidants (Branco *et al*.,

2021). Overall, for $HgCl_2$ cerebellar dysfunction, a multimodal strategy, chelation with DMSA, antioxidants like NAC, and supportive care, offers the best outcomes, emphasizing prevention through exposure reduction.

2.3 CATECHIN

Catechin, a naturally occurring flavonoid, is a secondary plant metabolite abundant in dietary sources such as green tea (Camellia sinensis), cocoa, apples, berries, and red wine (Reygaert, 2018). In green tea, catechins comprise 30–40% of dry leaf weight, with epigallocatechin gallate (EGCG) as the predominant subtype, followed by epicatechin, catechin, and epicatechin gallate (Ricci *et al*., 2018). These compounds are also present in dark chocolate and grapes, contributing to their widespread consumption and nutritional significance. Synthesized via the phenylpropanoid pathway in plants, catechin serves as antioxidants to counter environmental stressors, enhancing plant resilience (Bernatoniene and Kopustinskiene, 2018). Catechin's bioavailability in humans, influenced by gut metabolism and dietary intake, positions it as key candidates for health-focused research, particularly in mitigating oxidative and inflammatory damage associated with toxic exposures (Rudrapal *et al*., 2024; Li *et al*., 2024; Ferrari & Naponelli, 2025).

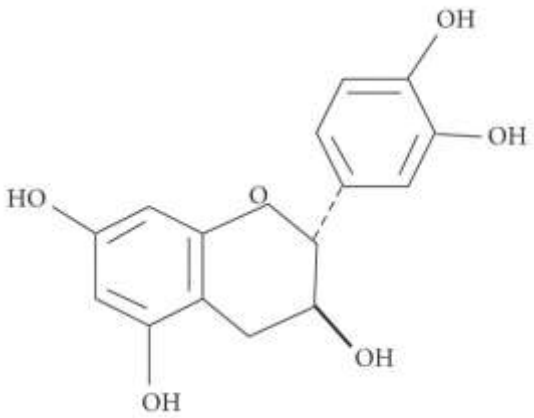

Figure 2.1. Molecular Structure of Catechin. Source: Dey *et al.* (2022)

Catechin is a polyphenolic compound classified within the flavan-3-ol subgroup of flavonoids, defined by its C6–C3–C6 backbone consisting of two aromatic rings linked via a heterocyclic ring (Fraga *et al*., 2010). The abundance of hydroxyl groups, particularly on the B-ring, underlies its potent antioxidant properties, enabling efficient neutralization of reactive oxygen species and chelation of transition metals such as iron and mercury (Ostolski *et al*., 2021). Catechin occurs in stereoisomeric forms, notably (+)-catechin and (−)-epicatechin, which differ in configuration and exert distinct influences on absorption, metabolism, and pharmacological activity (Shikha *et al*., 2024; Rehan *et al*., 2024). In recent years, advanced methodologies including molecular docking and in silico modelling have been employed to predict catechin's interactions with redox-sensitive proteins such as Nrf2 and caspases, while proteomic and metabolomic profiling in exposed animal models has elucidated its systemic effects on oxidative stress pathways (Singh *et al*., 2021). Moreover, innovations in nano delivery systems have been developed to enhance catechin's bioavailability and blood–brain barrier penetration, thereby strengthening its translational potential in neurological applications (Chen *et al*., 2022). Collectively, these approaches have reinforced interest in catechin's anti-inflammatory, antioxidant, and neuroprotective capacities, particularly in mitigating heavy metal-induced neurotoxicity such as that caused by mercuric chloride ($HgCl_2$). This highlights its promise as a candidate therapeutic molecule for protecting cerebellar integrity against toxic insults and justifies further investigation in both experimental and clinical contexts.

2.3.1 Mechanism of Action in the Body

Catechin exerts diverse pharmacological effects through antioxidant, anti-inflammatory, anti-apoptotic, and enzyme-modulating mechanisms (Manach *et al*., 2004). Its chemical structure, featuring a C6-C3-C6 backbone with hydroxyl groups, enables interaction with cellular pathways, making it a potent agent against oxidative stress, inflammation, and toxicant-induced damage, particularly in the context of heavy metal neurotoxicity like that caused by mercury

chloride ($HgCl_2$) (Fraga *et al*., 2010). The following details the mechanisms of action for catechin, specifically how its antioxidant capacity, modulation of enzymes and cellular signalling, and anti-inflammatory/anti-apoptotic effects contribute to its neuroprotective potential, with supporting evidence drawn from current literature and computational docking studies.

2.3.1.1 Antioxidant Mechanism

Catechin neutralizes reactive oxygen species (ROS) via its hydroxyl group, particularly on the B-ring, which donate electrons to scavenge free radicals like superoxide and hydroxyl radicals (Bernatoniene and Kopustinskiene, 2018). It also upregulates nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor that induces antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx). In neuronal cell cultures, it increased Nrf2 activity by 40–60%, enhancing GSH levels and reducing lipid peroxidation by 30–50% (Isemura *et al*., 2019). Catechin also chelates pro-oxidant metal ions (e.g., Fe^{2+}, Hg^{2+}), preventing Fenton reactions; in-silico docking shows it binds to Hg^{2+} with a binding energy of -5.8 kcal/mol, reducing its oxidative potential (Branco *et al*., 2021). This mechanism is critical for mitigating $HgCl_2$-induced cerebellar oxidative stress.

2.3.1.2 Anti-Inflammatory and Anti-Apoptotic Effects

Catechin suppresses inflammation by inhibiting nuclear factor-kappa B (NF-κB), a key regulator of pro-inflammatory cytokines like TNF-α and IL-6. In microglial cells, it reduced NF-κB activation by 50%, lowering TNF-α expression by 30–40% (Reygaert, 2018). In-silico studies indicate catechin binds to NF-κB's p65 subunit (-7.0 kcal/mol), stabilizing its inactive state (Isemura *et al*., 2019). Catechin also inhibits apoptosis by modulating caspase-3 and Bax/Bcl-2 pathways. In neuronal cultures exposed to oxidative stress, it decreased caspase-3 activity by 35% and increased Bcl-2 expression, preventing cell death (Xu *et al*., 2019; Nan *et al*., 2018). Docking data show catechin binding to caspase-3's active site (-6.5 kcal/mol),

inhibiting its function (Branco *et al*., 2021). These effects suggest catechin's potential to counteract $HgCl_2$-induced cerebellar inflammation and neuronal loss.

2.3.1.3 Enzyme Modulation

Catechin modulates enzymes involved in detoxification and metabolism, enhancing cellular resilience. They inhibit cytochrome P450 enzymes, reducing the bioactivation of xenobiotics, and induce phase II enzymes like UDP-glucuronosyltransferase, facilitating toxicant excretion (Manach *et al*., 2004). In rat liver models, catechin increased GST activity by 20–30%, aiding heavy metal detoxification (Egba *et al*., 2022). Catechin has been shown to suppress the activity of matrix metalloproteinases (MMPs), such as MMP-2 and MMP-9, thereby mitigating tissue injury associated with toxicant exposure (Tanabe *et al*., 2023). This modulation supports catechin's protective role against $HgCl_2$-induced cerebellar damage by limiting toxicant bioaccumulation and secondary injury.

2.3.1.4 Cellular Signalling and Neuroprotection

Catechin influences cellular signalling pathways, enhancing neuroprotection. They activate PI3K/Akt and MAPK pathways, promoting neuronal survival. In rat cerebellar neurons, catechin upregulated Akt phosphorylation by 30%, counteracting oxidative stress-induced cell death (Isemura *et al*., 2019). Catechin also inhibited JNK and p38 MAPK, reducing stress-induced apoptosis; in-vitro studies showed that it decreased JNK activation by 40% (Bernatoniene and Kopustinskiene, 2018). In the context of $HgCl_2$, catechin's modulation of Nrf2 and NF-κB pathways mitigates oxidative and inflammatory damage, while Akt activation supports Purkinje cell survival (Egba *et al*., 2022).

CHAPTER THREE

MATERIALS AND METHODS

3.1 ETHICAL APPROVAL

This study was submitted for review to the Research Ethics Committee of the College of Medical Sciences, University of Benin, Benin City, Nigeria and approved with the REC Approval Number: CMS/REC/2025/784.

3.2 REAGENTS AND CHEMICALS

Catechin was manufactured by Central Drug House (P) Ltd., New-Delhi, India while mercury chloride and vitamin E were manufactured by Loba Chemie PVT Ltd, Mumbai, India.

3.3 DETERMINATION OF DOSAGE

10 mg/kg bw of Catechin and 20 mg/bw of Catechin was used for this study, this is the dosage used by Ahmed *et al*., (2013). 5 mg/kg bw of mercury chloride was used for this study, this is the dosage used by Said *et al*., (2021). 200 mg/kg bw of vitamin E was used for this study, this is the dosage used by Enogieru & Idemudia, (2025)

3.4 PROCUREMENT AND CARE OF EXPERIMENTAL ANIMALS

Wistar rats used in this study were bred at the animal house of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria. The rats were fed with standard rat chow (Bendel livestock feed, Edo state, Nigeria) and water throughout the entire study period. They were weighed before commencement and throughout the experiment using a digital weighing scale calibrated in grams and were recorded to the nearest whole number. Protocols for these experiments were in line with the guide for the care and use of laboratory animals (National Research Council of the National Academies, 2011).

3.5 RESEARCH DESIGN

A total of sixty-four (64) Wistar rats weighing between 170 – 180 g were used for this study. They were randomised into eight groups (A, B, C, D, E, F, G and H) with eight rats in each group. All administrations were given orally, alongside the rat's feed, throughout the entire study period of twenty-eight days

Table 3.1. Experimental Design

GROUPS	DOSAGE
GROUP A (CONTROL)	1 ml of Distilled water
GROUP B ($HgCl_2$)	5 mg/kg body weight (bw) of Mercury Chloride ($HgCl_2$)
GROUP C (CA + $HgCl_2$)	10 mg/kg bw of Catechin (CA) + 5 mg/kg bw of $HgCl_2$
GROUP D (CA + $HgCl_2$)	20 mg/kg bw of CA + 5 mg/kg bw of $HgCl_2$
GROUP E (Vit. E + $HgCl_2$)	200 mg/kg bw of CA + 5 mg/kg bw of $HgCl_2$
GROUP F (CA)	10 mg/kg bw of CA
GROUP G (CA)	20 mg/kg bw of CA
GROUP H (Vit. E)	200 mg/kg bw of Vitamin E

3.6 NEUROBEHAVIOURAL ASSESSMENT

3.6.1 Open Field Test

The open field test (OFT) was conducted to assess locomotor activity, exploratory behaviour, and anxiety-related responses, as described in recent protocols (Enogieru & Abhelemhen, 2025; Enogieru & Idemudia, 2025). The apparatus consisted of a square wooden arena (100 × 100 × 40 cm) with marked grids. Rats were individually placed at the centre of the field and observed for 5 minutes. Parameters measured included ambulation (line crossings), rearing, grooming, immobility, thigmotaxis (time near the walls), sniffing, and central square entries. The arena was cleaned with 70% ethanol between trials to remove olfactory cues. This test is

widely used to measure the effects of neurotoxins and antioxidants on motor and emotional behaviours (Seibenhener and Wooten, 2015).

Figure 3.1. Open Field Test Apparatus

3.6.2 String Test

The string test was used to evaluate forelimb strength, coordination, and balance in Wistar rats. The apparatus consisted of a horizontal string (approximately 2 mm in diameter and 50 cm in length) suspended between two supports, 40 cm above a padded surface to avoid injury. Rats were placed at the midpoint of the string, ensuring that both forepaws gripped it securely before release. The latency to grip loss (time to fall) was recorded, with a maximum cut-off of 60 seconds. Each rat underwent three trials separated by 5-minute intervals, and the average latency was calculated for analysis. This test is widely recognized for detecting neuromuscular deficits and cerebellar dysfunction (Miedel *et al*., 2017; Sandner *et al*., 2018).

Figure 3.2. String Test Apparatus

3.6.3 Movement Initiation Test

The movement initiation test was used to measure akinesia by evaluating the latency required for rats to initiate movement after being placed at the centre of a square arena. Each rat was gently positioned and the time taken to take the first step with either forelimb was recorded, with a maximum cut-off of 60 seconds. The test was repeated three times with inter-trial intervals of 5 minutes, and the mean latency was used for analysis. Longer initiation times reflect motor deficits commonly associated with neurotoxicity and oxidative stress (Enogieru and Idemudia, 2025; Ben-Azu *et al*., 2022).

3.6.4 Step Test

The step test was performed to assess bradykinesia and forelimb akinesia. Each rat was gently held so that only one forepaw touched the surface of the testing platform while the body was supported. The platform was then slowly moved backward (about 90 cm in 5 seconds), and the number of adjusting steps made by the free forelimb was counted. Each paw was tested separately across three trials, and the mean value was calculated. Reduced stepping responses are indicative of motor initiation deficits and nigrostriatal dysfunction (Sandner *et al*., 2018).

3.7 COLLECTION OF BRAIN AND BODY WEIGHTS

Upon completion of the neurobehavioral tests, the rats were euthanized by cervical dislocation. The rats were weighed then the skull was opened and the brains of the rats were harvested, blotted free of blood and weighed immediately using an electronic weighing balance calibrated in milligrams, with the results recorded to the nearest two decimal places. The relative brain weights were calculated as follows:

$$Relative\ brain\ weight\ = \frac{(\text{absolute brain weight}\ (g))}{(\text{Body weight of rat}\ (g))\ \times 100}$$

3.8 HISTOLOGY

The harvested cerebellar tissues were preserved in 10% phosphate-buffered formalin for histopathology. The tissues were processed via the paraffin wax-embedded method by Drury and Wallington (1980). They were dehydrated for one hour each at room temperature through ascending grades of ethanol: 50% ethanol, 70% ethanol, 90% ethanol, absolute ethanol I, and Absolute ethanol II. Dehydrated tissues were cleared at room temperature in two changes of xylene for 1 hour in each change. The tissues were infiltrated in two changes of molten paraffin wax at 60°C for one hour in each change, and finally embedded in paraffin wax multi-block plastic embedded moulds. The paraffin-blocked tissues were trimmed and mounted on a wooden chuck for sectioning on a rotary microtome. Sections of 7 µm thickness were produced from the tissue blocks using a rotary microtome. The sections were transferred into a water bath (40°C) to allow separation of the folded ribbons of sections. The sections were mounted on new, clean glass slides and were dried at 40°C on a slide drier to enhance adherence of the sections to the slides. Sections were then stained with haematoxylin and eosin, cleared in xylene, mounted in DPX, for examination and interpretation using a light microscope.

3.9 HAEMATOXYLIN AND EOSIN STAINING PROCEDURES

Tissue sections were deparaffinized in two changes of xylene for two minutes in each change and passed through two changes of absolute alcohol for four minutes each. They were hydrated using a series of descending grades of alcohol until water was used. Procedures of Haematoxylin and Eosin adopted on the sections was described by Drury and Wallington (1980). The sections were:

1. Dewaxed in two changes of xylene for two minutes in each change;

2. Rehydrated in descending grades of alcohol (absolute II, absolute I, 95%, 90%, 70% and 50% ethanol) for two minutes each;

3. Rinsed in distilled water for three minutes and stained in haematoxylin for 15-20 minutes;

4. Excess haematoxylin stain was removed by rinsing well in running tap water for two to three minutes (sections were examined microscopically at this stage to confirm sufficient degree of staining);

5. Differentiated in acid alcohol (0.5% HCL in 70% ethanol) for two to three minutes;

6. Rinsed well in running water for 10-15 minutes;

7. Counterstained in 1% aqueous eosin for two to four minutes;

8. Excess stain was washed off in running water and examined under a microscope;

9. Dehydrated rapidly in ascending grades of ethanol (50% through absolute ethanol), cleared in xylene and mounted in a synthetic resin medium (DPX).

3.10 PHOTOMICROGRAPHY

The processed slides were captured with a LABO® trinocular microscope (Labo Microsystems GmbH, Germany), which was mounted on an Omax 9.0MP USB Digital Microscope Camera (made in Korea). The camera features a 9 megapixel (3488 × 2616 pixel) high resolution colour digital camera and a 0.5X reduction lens. It was connected to a laptop which had installed ToUpView software (version ×64, 3.7.71.49; built in 2016). A panoramic view of the slides was captured using a 40× objective lens.

3.11 EVALUATION OF OXIDATIVE STRESS PARAMETERS

Reagents

The chemicals and reagents used in this study were of analytical grade. The 5, 5-dithio-bis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH) standard, thiobarbituric acid (TBA),

trichloroacetic acid (TCA), were products of Merck (Germany). Ethylene diamine tetra acetic acid (EDTA), epinephrine (adrenaline), hydrogen peroxide (H_2O_2), potassium hydroxide (KOH), sodium carbonate (Na_2CO_3), sodium citrate, sodium chloride (NaCl), sodium dihydrogen phosphate (NaH_2PO_4), sodium hydrogen carbonate ($NaHCO_3$), sodium hydrogen phosphate ($NaHPO_4$), sodium hydroxide (NaOH), and sulphuric acid (H_2SO_4) were manufactured by British Drug House (BDH) (England). Hydrochloric acid (HCl), potassium permanganate ($KMnO_4$), and pyrogallol were manufactured by May and Bayer (England).

3.11.1 Determination of Concentration of Total Protein

Principle

Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex.

Assay Procedure

Biuret reagent (2.5 mL) was added to 0.05 mL of sample and 0.05 mL of standard. The blank contained 2.5 mL of Biuret and 0.05 mL of distilled water. The solution in each tube was incubated for 10 min at 37 °C, and the absorbance was read at 546 nm against the reagent blank.

Calculations

When measurements are taken at 546 nm, total protein concentration may be calculated as follows:

$$Total\ Protein\ (g/L) = 190 \times\ A_{Sample}$$

$$Total\ Protein\ (g/dL) = 19 \times\ A_{Sample}$$

When using a standard

$$Total\ Protein\ Concentration = \frac{A_{sample} \times Standard\ Concentration}{A_{standard}}$$

3.11.2 Determination of MDA Concentration

The concentration of MDA was determined according to the method of Buege and Aust (1978). The principle that underlies this assay is that MDA – a product of lipid peroxidation when heated with thiobarbituric acid (TBA), in the presence of an acid, forms a pink or reddish complex that is measured spectrophotometrically at 532 nm. The table below clearly illustrates the procedure adopted in the determination of the level of malondialdehyde.

Assay Procedure

An aliquot of the cerebellum homogenate was added to 3.0 mL of TCA – TBA – HCl reagent and mixed thoroughly by swirling. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed via centrifugation at 1000 g for 10 min. The absorbance of the clear supernatant was measured against a reference blank at 535 nm.

Calculation

The MDA concentration of each sample was calculated as follows:

$$\frac{O.D \times V_t \times 1000}{a \times V \times L \times Y}$$

Where,

O.D = Absorbance of sample test at 535 nm

V_t = Total volume of the reaction mixture, 3.6 mL

a = Molar extinction coefficient of product, $1.56 \times 10^5\ M^{-1}cm^{-1}$

L = Light path, 1.0 cm.

V = Volume of sample homogenate used, 0.6 mL

Y = mg of tissue in the sample used

The unit of MDA is moles/mg wet tissue

3.11.3 Determination of Superoxide Dismutase (SOD) Activity

Principle

The activity of SOD was assessed based on the method of Misra and Fridovich (1972). Adrenaline auto-oxidizes rapidly in aqueous solution to adrenochrome whose concentration can be determined spectrophotometrically at 420 nm. The auto-oxidation depends on the presence of superoxide anions (O^{2-}). Superoxide dismutase (SOD) inhibits this auto-oxidation by catalysing the breakdown of superoxide anions. The degree of inhibition is thus a measure of SOD activity. The amount of enzyme producing 50% inhibition is defined as one unit of the enzyme activity.

Assay Procedure

Sample homogenate (0.2 mL) was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) and allowed to equilibrate. The reaction was initiated by the addition of 0.3 mL of freshly prepared 0.03 mM adrenaline as substrate. The solution was mixed by inversion. The reference tube contained 2.7 mL of carbonate buffer and 0.3 mL of adrenaline, while the blank contained 2.5 mL of carbonate buffer, 0.2 mL of distilled water and 0.3 mL of 0.03 mM adrenaline. The increase in absorbance at 420 nm due to the formation of adrenochrome was monitored every 30 sec for 120 sec. One unit of SOD activity was taken as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome within 120 sec.

Calculation

$$\%\ Inhibition = \frac{O.D_{test} - O.D_{reference}}{O.D_{test}} \times \frac{100}{1}$$

$$Enzyme\ Activity\ (units/mg\ protein) = \frac{\%\ inhibition}{50\ \times Y}$$

Where

Y = mg of protein in the volume of sample.

A unit of SOD activity was taken as the amount of SOD required to cause 50% inhibition of the auto-oxidation of adrenaline to adrenochrome per minute.

3.11.4 Determination of Catalase Activity

Principle

This is based on the method of Cohen, *et al.,* (1970). This estimation is based on the measurement of the rate of decomposition of hydrogen peroxide (H_2O_2), after the addition of the material containing the enzyme.

Catalase catalyses the reaction: $2H_2O_2 \longrightarrow 2H_2O + O_2$

The quantity of hydrogen peroxide decomposed is directly proportional to the concentration of the enzyme in the sample. The hydrogen peroxide produced in tissues is measured by reacting it with excess potassium permanganate ($KMNO_4$) and then measuring the residual $KMNO_4$ spectrophotometrically at 480 nm.

Assay Procedure

Sample homogenate (0.5 mL) was placed in ice – cold test tubes, the blank contained 0.5 mL distilled water. Cold phosphate-buffered H_2O_2 (30 mM, 5 mL) was added to both blank and sample tubes at fixed intervals, and were mixed by inversion. After 3 min, the reaction was stopped by rapid addition of 1 mL of 6 M H_2SO_4. The tubes were mixed thoroughly by inversion after which 7 mL of 0.01 M $KMNO_4$ was added. Absorbance was read at 480 nm within 3 min.

Calculation

The activity of catalase in each sample is calculated thus:

$$\frac{O.D_{min} \times V_t \times 1000}{M \times V \times L \times Y}$$

Where,

O.D = Absorbance of sample test at 480 nm

Vt = Total volume of the reaction mixture, 13.5 mL

M = Molar extinction coefficient of H_2O_2, 43.6 $M^{-1}cm^{-1}$

L = Light path, 1.0 cm

V = Volume of sample homogenate used, 0.5 mL

Y = mg of protein in tissue used

3.11.5 Determination of Glutathione Peroxidase Activity

Glutathione peroxidase (GPx) activity was measured according to the method described by Nyman (1959).

Principle

This is based on the oxidation of pyrogallol to purpurogallin by peroxidase, resulting to a deep brown colouration, which is read at 430 nm.

Procedure

To an aliquot of sample (0.2 mL), 5 mL of phosphate-buffered H_2O_2, and 1.5 mL of pyrogallol were added. The reaction mixture was allowed to stand for 30 min at room temperature. A deep colour was formed, which was read at 430 nm.

Calculation

$$Enzyme\ Activity = \frac{O.D_{min} \times V_t \times D_f}{E \times V_s \times Y}$$

Where,

OD = Absorbance of test

V_t = Total volume of reaction mixture

D_f = Dilution factor

E = Molar extinction coefficient, 12 $M^{-1}cm^{-1}$

V_s = Volume of sample

Y = mg of protein used

3.11.6 Determination of Concentration of Reduced Glutathione

The concentration of reduced glutathione (GSH) was determined using the method described by Ellman (1959).

Reagents

5, 5^1-dithiobis-2-nitrobenzoic acid (DTNB), sodium citrate, and trichloroacetic acid (TCA)

Procedure

To 1.0 mL of sample, 2.5 mL of 10 % TCA was added and centrifuged at 3000 g for 10 min. Then, 1.0 mL of the supernatant was treated with 0.5 mL of Ellman's reagent (0.0189 % DTNB and 1% sodium citrate) and 3.0 mL of 0.3 M phosphate buffer (pH 8.0). The yellow colour developed was read immediately at 412 nm and expressed as µM GSH/g plasma.

Calculation

$$Concentration\ of\ GSH = \frac{A_{test} \times Standard\ Concentration}{A_{standard}}$$

3.12 *IN-SILICO* MOLECULAR DOCKING TECHNIQUES

3.12.1 Preparation of Crystal Protein

The crystal structure of TNF-α (PDB ID-2AZ5), NRF2 (PDB ID-7OFE), Caspase 3 (PDB ID-3KJF), NF-κB (PDB ID-1IKN) and Interleukin-6 (PDB ID-4YW7) were downloaded via the protein preparation wizard of Maestro v11.8. The crystal structure of the protein was prepared using the protocol described by Elekofehinti *et al*. (2020) and Elekofehinti *et al*. (2021). The protein was pre-processed by creating zero bonds to metals, deleting waters from 5.0 Å of het groups, adjusting bond orders and setting the het states at pH 7.0 ± 2.0 (Schrödinger Suite 2012). The protein was refined by optimizing the H-bond network using PROPKA and removing water molecules with less than 3 H-bonds to non-waters. The retrained minimization was carried out using the OPLS3 force field to avoid steric clashes that may exist in the structure. The minimization was terminated while the RMSD of non-hydrogen atoms reached 0.30 Å.

3.12.2 Preparation of the Chemical Compound

The compound (Catechin PDB ID-9068) was retrieved from National Center for Biotechnology Information (PubChem). The compound was prepared using LigPrep. The LigPrep panel enables the conversion of structures; generate variations of structures and elimination of unwanted structures. After, stereoisomer computation was left to generate at most 32 per ligand and the output format was left as maestro, the OPLS3 force field was left at pH 7.0 ± 2.0 using epic.

3.12.3 Receptor Grid Generation

The receptor grid file was generated using a receptor grid generation panel, which represents the active sites of the receptor for glide ligand docking jobs. The ligand-binding site was defined by picking the co-crystallized ligand of the protein structure on the workspace. The

van der Waals radii of the receptor atoms with partial atomic charge was set to a scaling factor of 1.0 and partial cutoff of 0.25 to soften the potential for non-polar parts of the receptor.

3.12.4 Glide Extra Precision Docking

The compounds were prepared by LigPrep and docked into the active site of the protein crystal using extra precision with the ligand sampling set generated as flexible. The choice of the best-docked structure for each ligand was made using model energy score (emodel) that combines glide score, the non-bonded interaction energy and the excess internal energy of the generated ligand conformation.

3.12.5 ADME Profiles

The absorption, distribution, metabolism, excretion and molecular properties of the compounds were predicted using QikProp panel (Release, 2018).

3.13 STATISTICAL ANALYSIS

The obtained data were analysed, and graph production was carried out, using GraphPad Prism software, version 9.0 (GraphPad Software, San Diego, CA, USA). All values were analysed for normality of data using the Shapiro-Wilk test and homogeneity of variance with the Levene test, as previously reported (Enogieru and Idemudia, 2024). The data demonstrated normal distribution and homogeneity of variance. Statistical significance was then determined by one-way analysis of variance (ANOVA) for all parameters. Tukey's *post hoc* test was performed to determine groups involved in statistical differences and results were expressed as mean ± S.E.M. A p-value of < 0.05 was defined as statistically significant.

CHAPTER FOUR

RESULTS

4.1 ACUTE TOXICITY STUDY

Table 4.1 shows the acute toxicity study observations after 72 hours. Results showed that there was no observable morbidity, mortality or diarrhoea across the experimental groups following oral administration of Catechin at doses from 10 mg/kg to 5000 mg/kg body weight.

Table 4.1. Acute toxicity study and observations after 72 hours

Observation	Group A (10 mg/kg)	Group B (100 mg/kg)	Group C (1000 mg/kg)	Group D (1600 mg/kg)	Group E (2900 mg/kg)	Group F (5000 mg/kg)
Morbidity	None	None	None	None	None	None
Mortality	None	None	None	None	None	None
Diarrhoea	None	None	None	None	None	None

n=3/group

4.2 EFFECT OF TREATMENT ON BRAIN AND BODY WEIGHT

Figures 4.1-4.8 shows brain and body weights changes across all experimental groups. For weight change (Figure 4.3), $HgCl_2$-only group showed a significant decrease ($p<0.05$) when compared to the control group while pretreatment groups showed a significant increase ($p<0.05$) when compared to the $HgCl_2$-only group. CA- and vitamin E-only groups showed no significant difference ($p>0.05$) when compared to control.

For initial (Figure 4.1) and final body weight (Figure 4.2), brain weight (Figure 4.4), relative brain weight (Figure 4.5), cerebellum weight (Figure 4.6), relative cerebellum weight (Figure 4.7) and cerebellum-brain weight ratio (Figure 4.8) no significant differences ($p>0.05$) were observed across experimental groups.

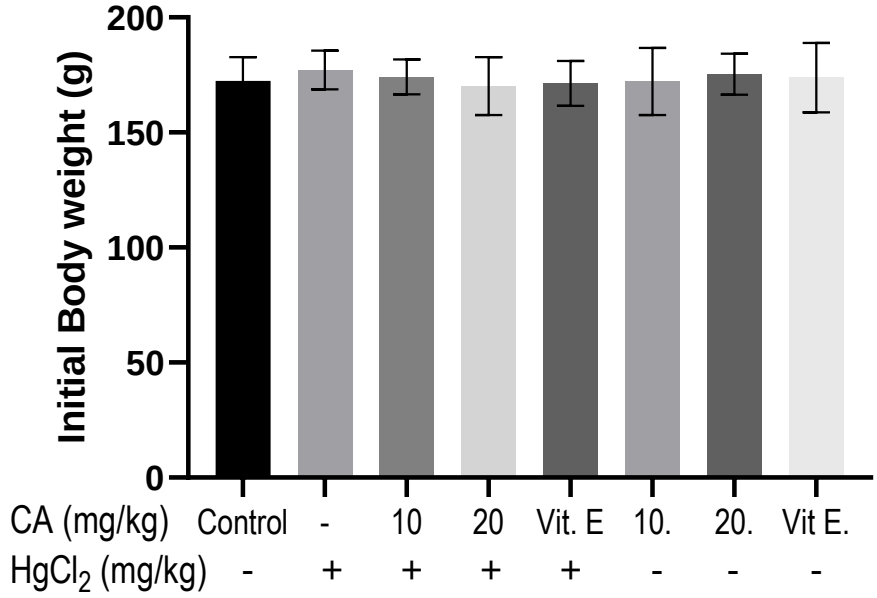

Figure 4.1. Initial body weight across experimental groups

and * represent $p<0.05$ following comparison to the control and $HgCl_2$-only group II respectively.

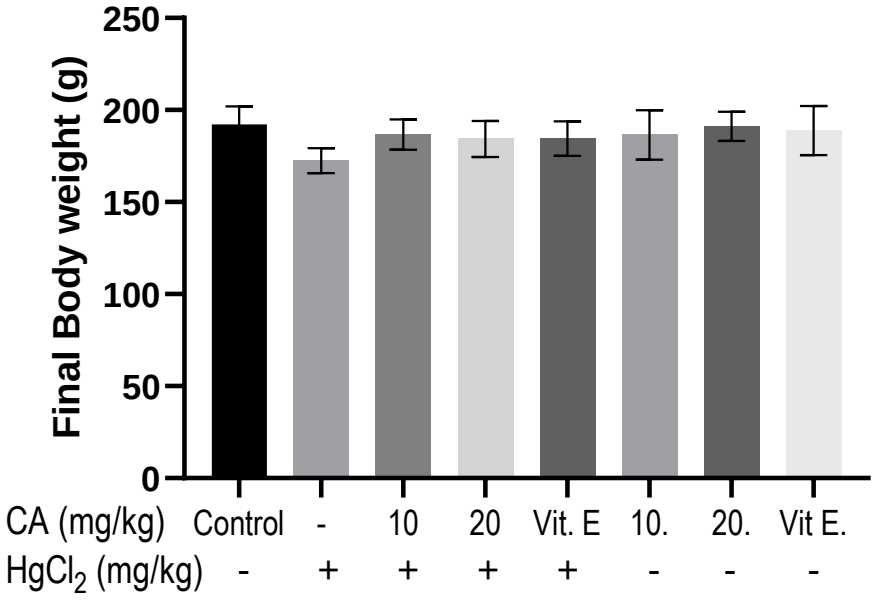

Figure 4.2. Final body weights across experimental groups

and * represent $p<0.05$ following comparison to the control and $HgCl_2$-only group II respectively.

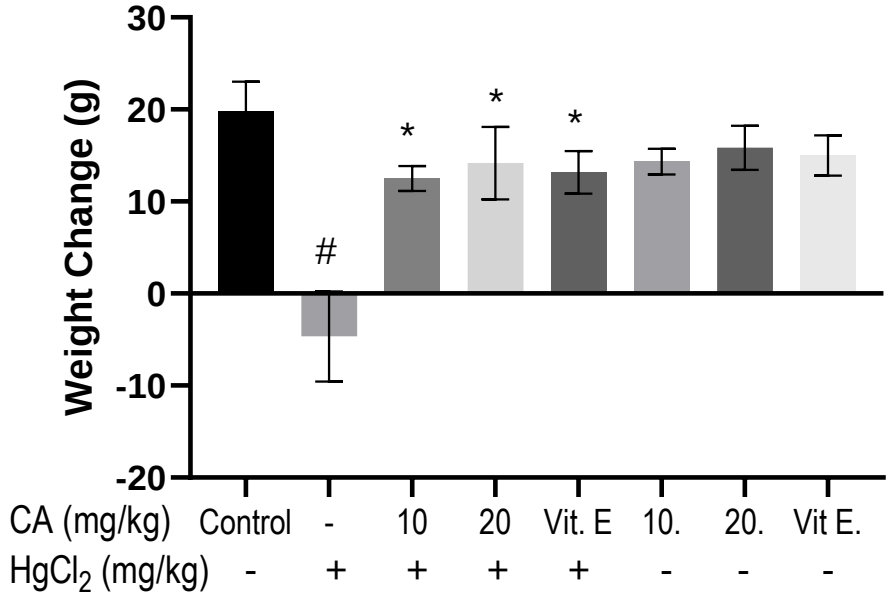

Figure 4.3. Weight change across experimental groups

and * represent $p<0.05$ following comparison to the control and $HgCl_2$-only group II respectively.

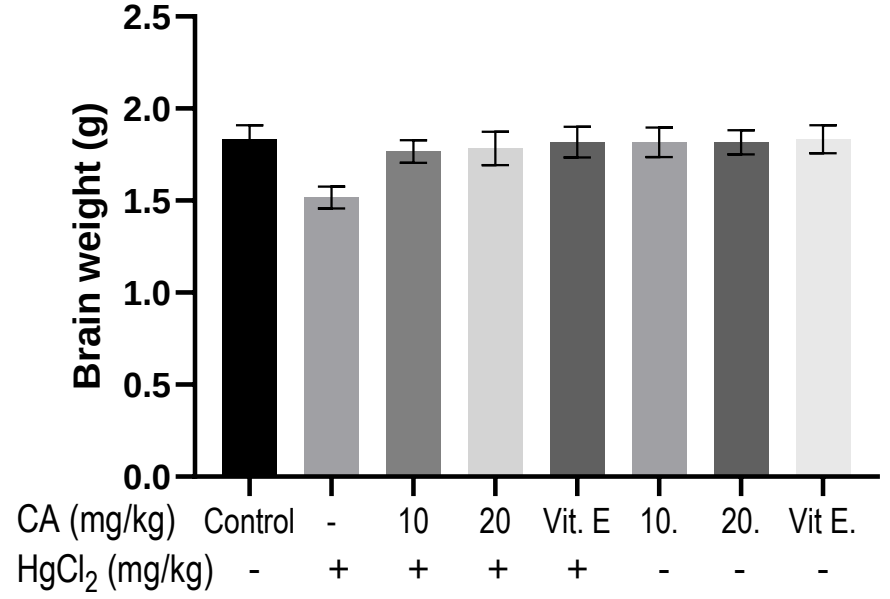

Figure 4.4. Brain weight across experimental groups

and * represent $p<0.05$ following comparison to the control and $HgCl_2$-only group II respectively.

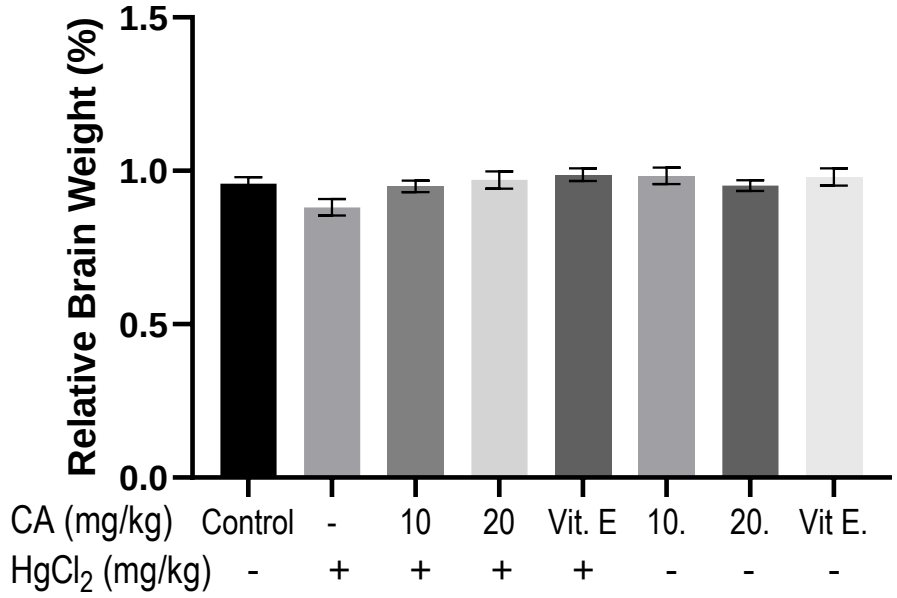

Figure 4.5. Relative brain weight across experimental groups

and * represent $p<0.05$ following comparison to the control and $HgCl_2$-only group II respectively.

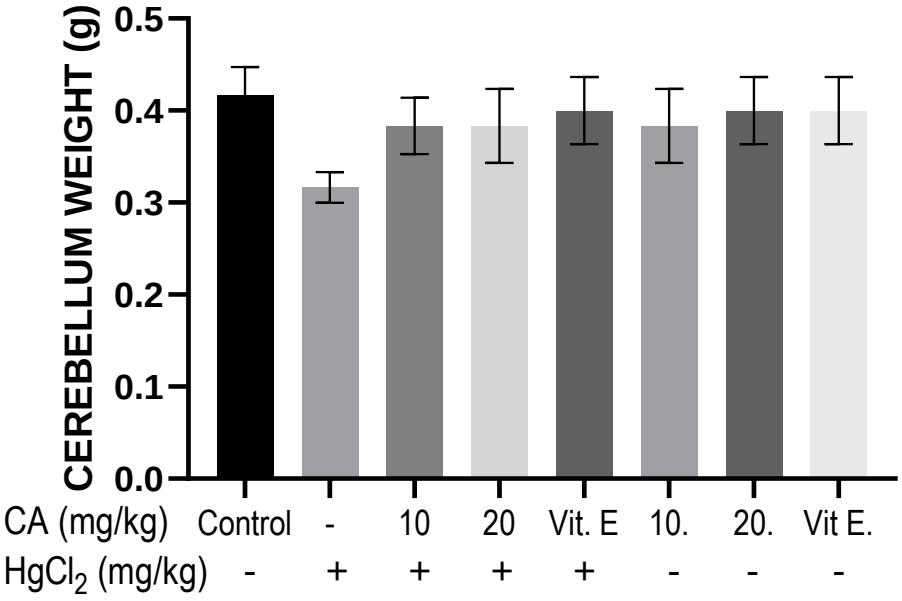

Figure 4.6. Cerebellum weight across experimental groups

and * represent $p<0.05$ following comparison to the control and $HgCl_2$-only group II respectively.

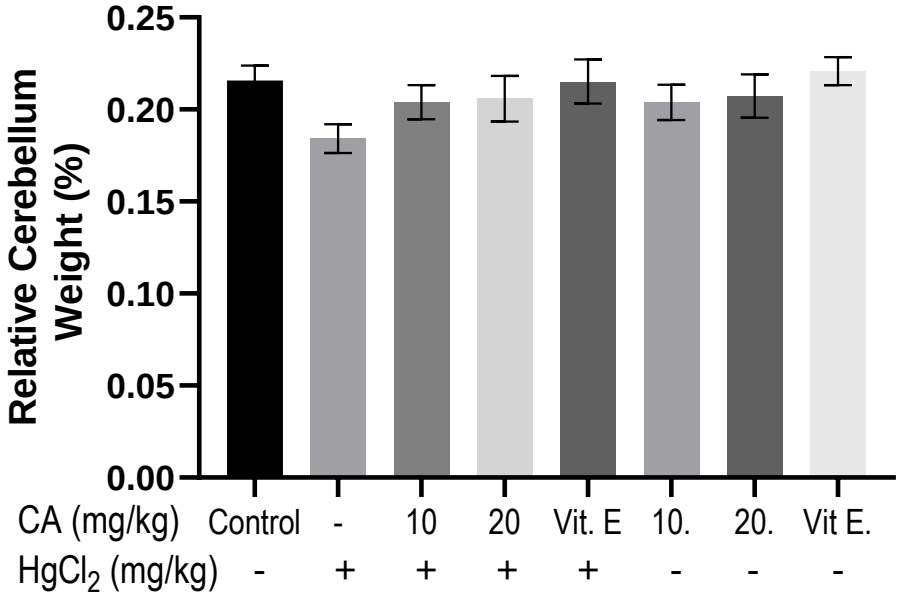

Figure 4.7. Relative Cerebellum weight across experimental groups

and * represent $p<0.05$ following comparison to the control and $HgCl_2$-only group II respectively.

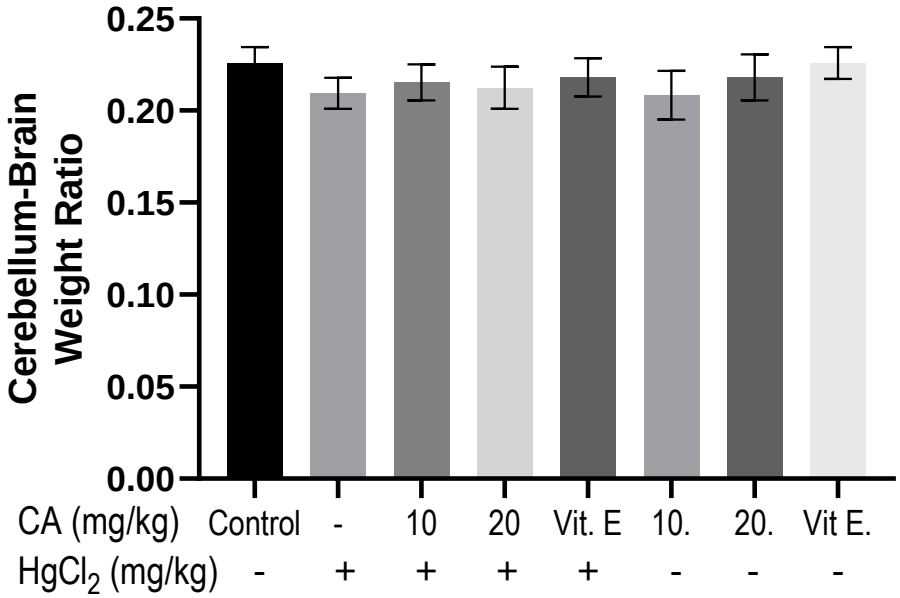

Figure 4.8. Cerebellum-Brain weight ratio across experimental groups

and * represent $p<0.05$ following comparison to the control and $HgCl_2$-only group II respectively.

4.3 EFFECT OF TREATMENT ON NEUROBEHAVIOURAL ACTIVITY

4.3.1 Open Field Test (OFT)

Figure 4.9-4.16 shows open field test parameters of control and treatment groups. When compared to the control group, $HgCl_2$-only group displayed a significant decrease ($p<0.05$) in rearing, ambulation, central square entry and line crossing while a significant increase ($p<0.05$) was observed in grooming, immobility, thigmotaxis and sniffing. In addition, when compared to the $HgCl_2$-only group, pretreatment groups showed a significant decrease ($p<0.05$) in grooming, immobility, thigmotaxis, and sniffing while a significant increase ($p<0.05$) was observed in rearing, ambulation, and central square entry (20 mg/kg bw CA and 200 mg/kg bw vitamin E). For all parameters (Figures 4.9-4.16), no significant difference ($p>0.05$) was observed in CA- and vitamin E-only groups when compared to control.

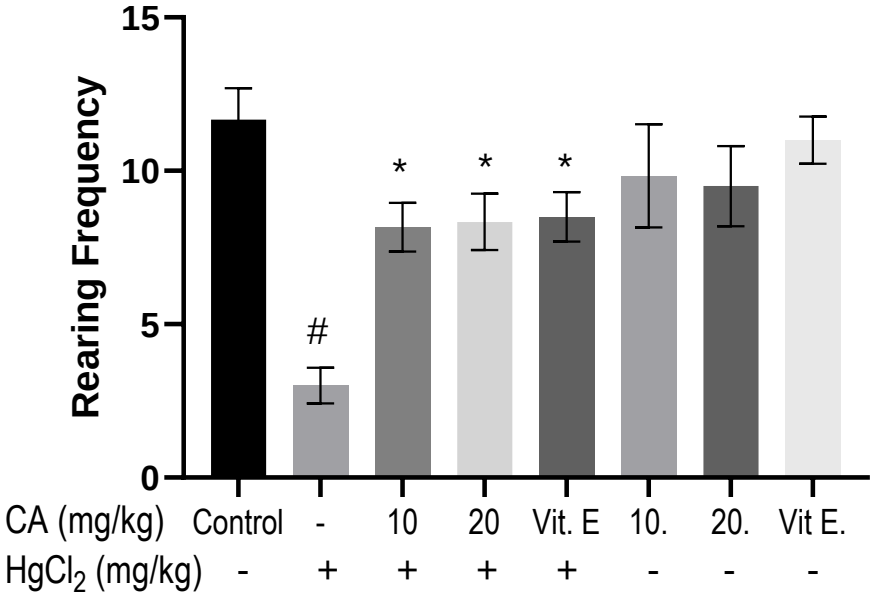

Figure 4.9. Rearing Frequency of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

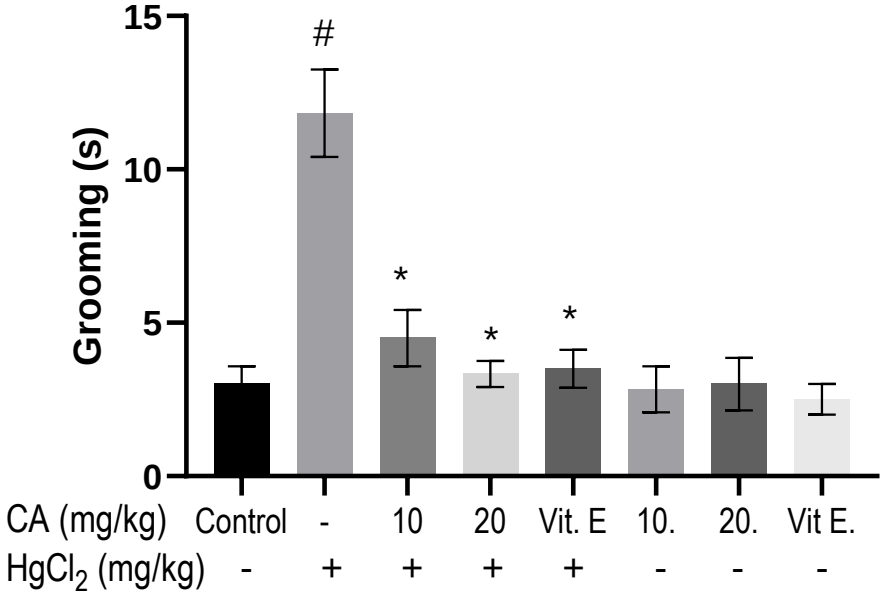

Figure 4.10. Grooming of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

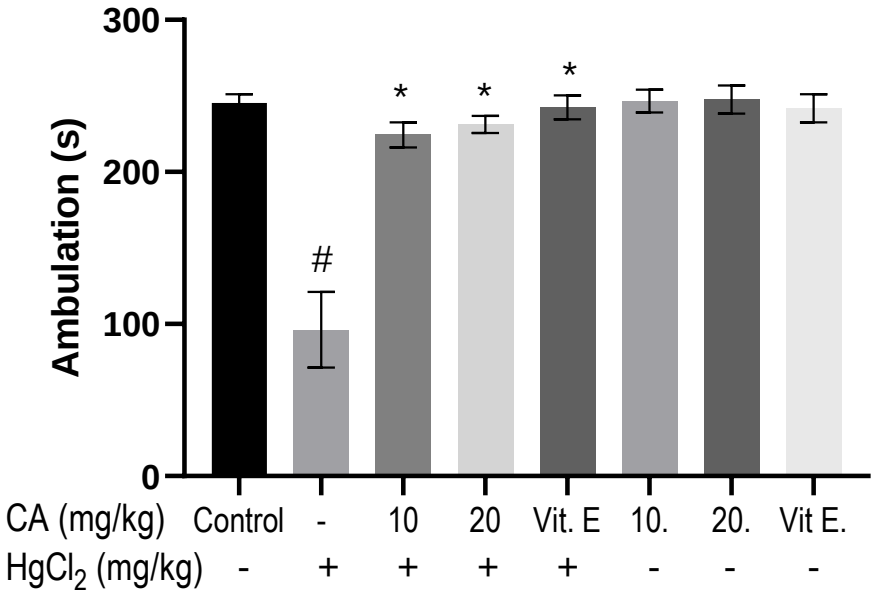

Figure 4.11. Ambulation of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

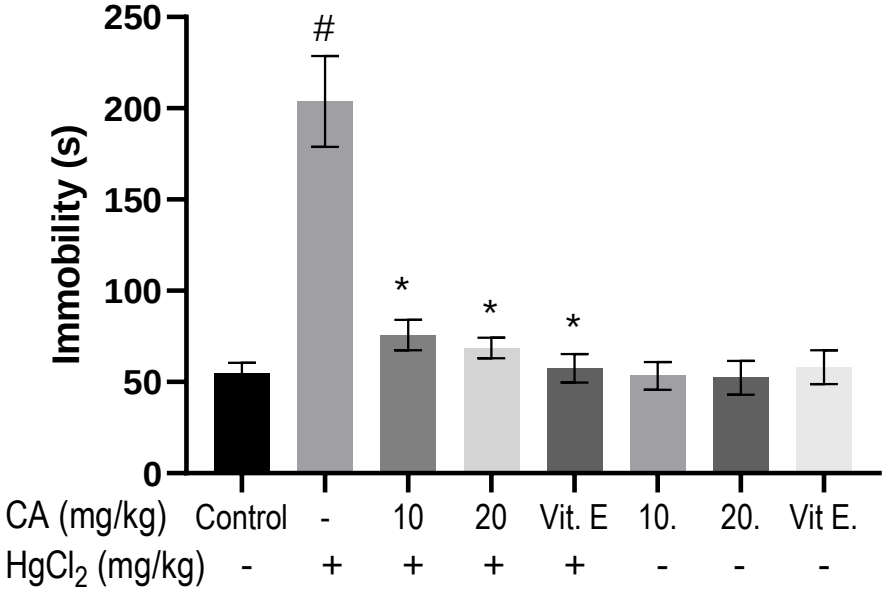

Figure 4.12. Immobility of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

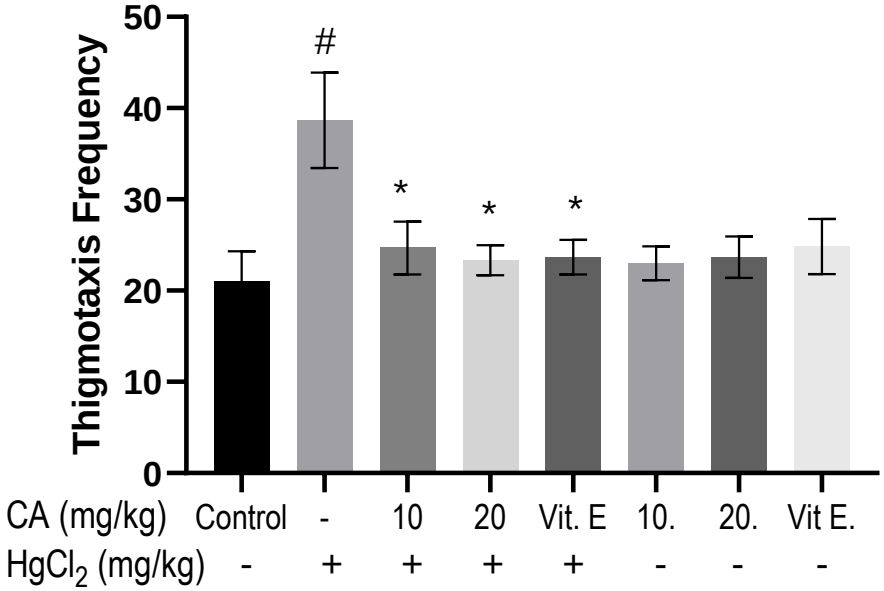

Figure 4.13. Thigmotaxis frequency of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

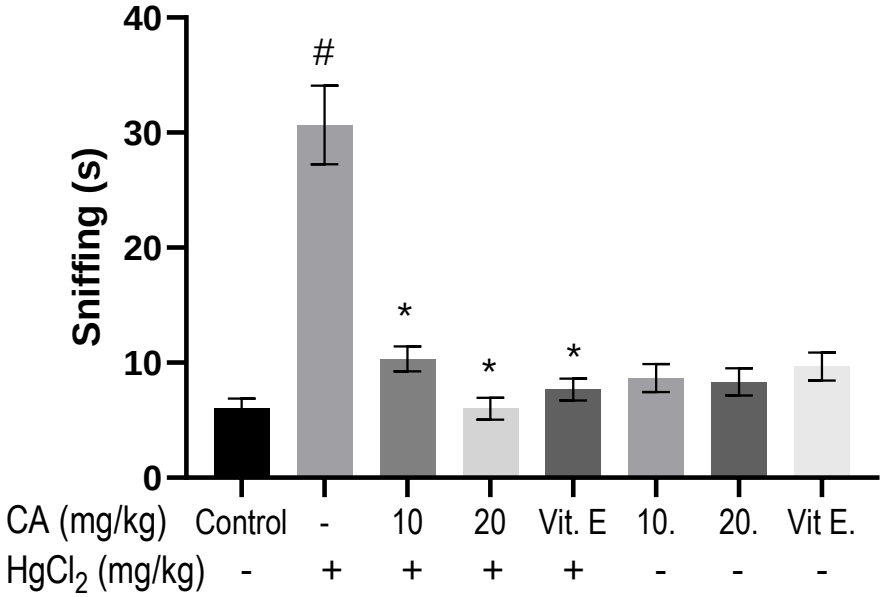

Figure 4.14. Sniffing of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

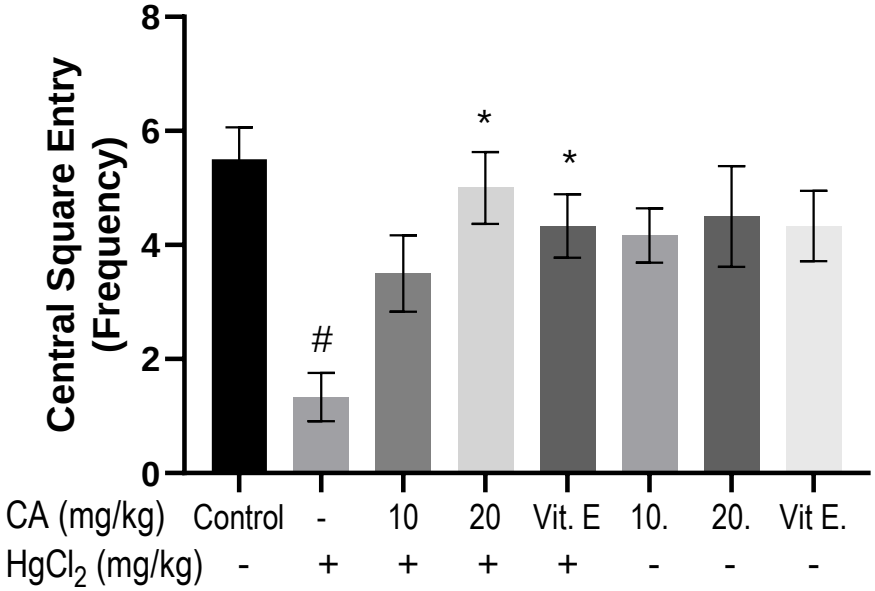

Figure 4.15. Central Square Entry frequency of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

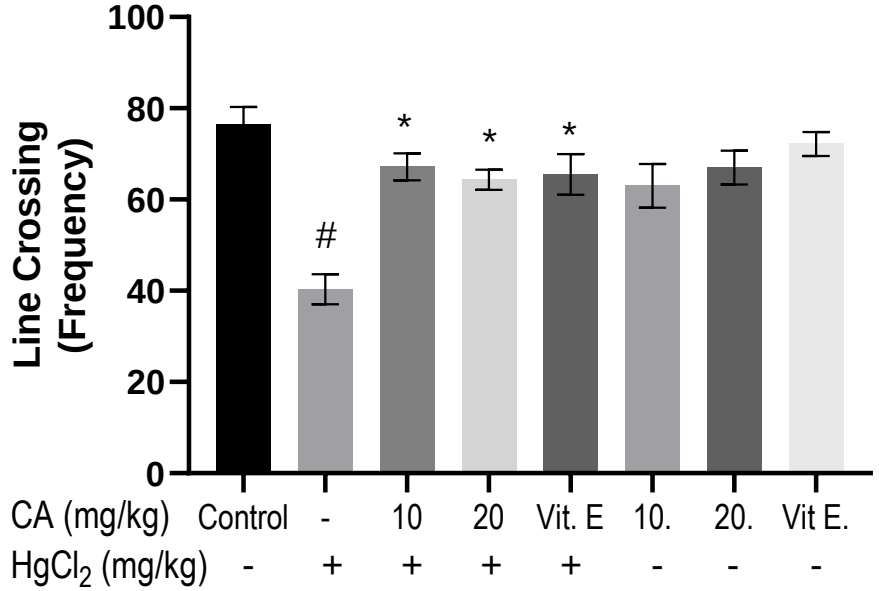

Figure 4.16. Line Crossing frequency of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

4.3.2 String Test, Movement Initiation and Step Test

Figure 4.17-4.19 shows parameters of control and treatment groups of string test, movement initiation test and step test after 28 days. When compared to the control group, the $HgCl_2$-only group showed a significant decrease ($p<0.05$) in latency to grip loss and step test while a significant increase ($p<0.05$) was observed in movement initiation. When compared to $HgCl_2$-only group, pretreatment groups showed a significant increase ($p<0.05$) in latency to grip loss and a significant decrease ($p<0.05$) in movement initiation. However, for step test a significant increase was observed in the 20 mg/kg bw CA and 200 mg/kg bw vitamin E groups only. No significant difference ($p>0.05$) was observed in the CA- and vitamin E-only groups when compared to control.

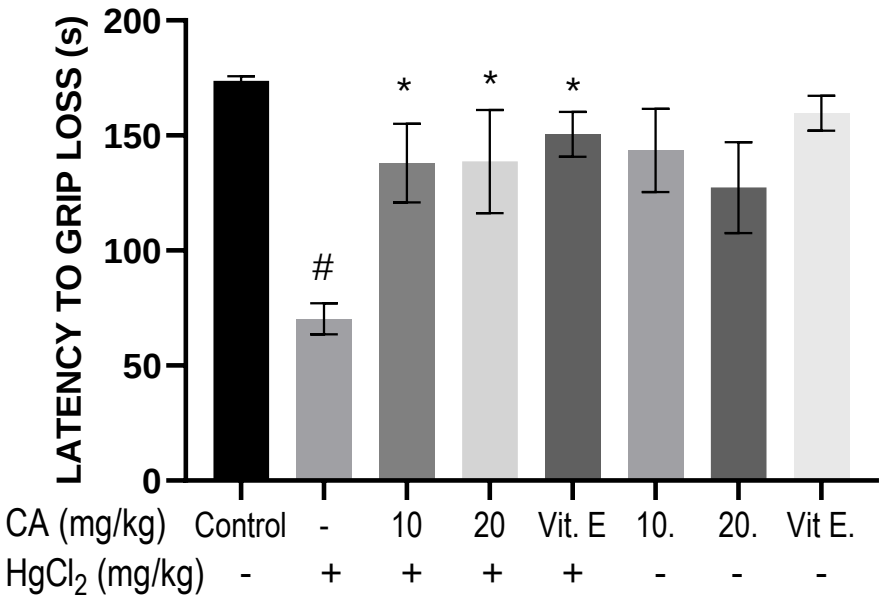

Figure 4.17. Latency to Grip Loss of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

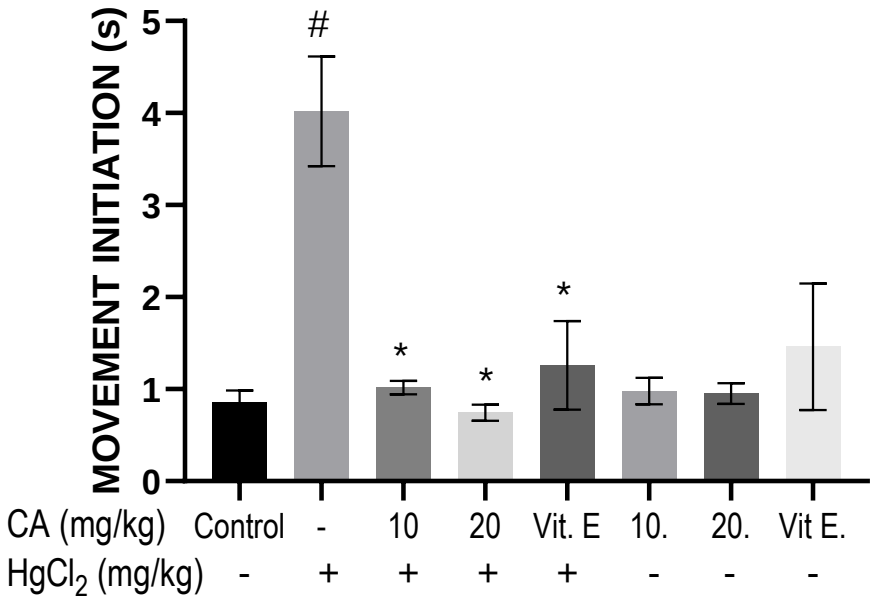

Figure 4.18. Movement Initiation of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

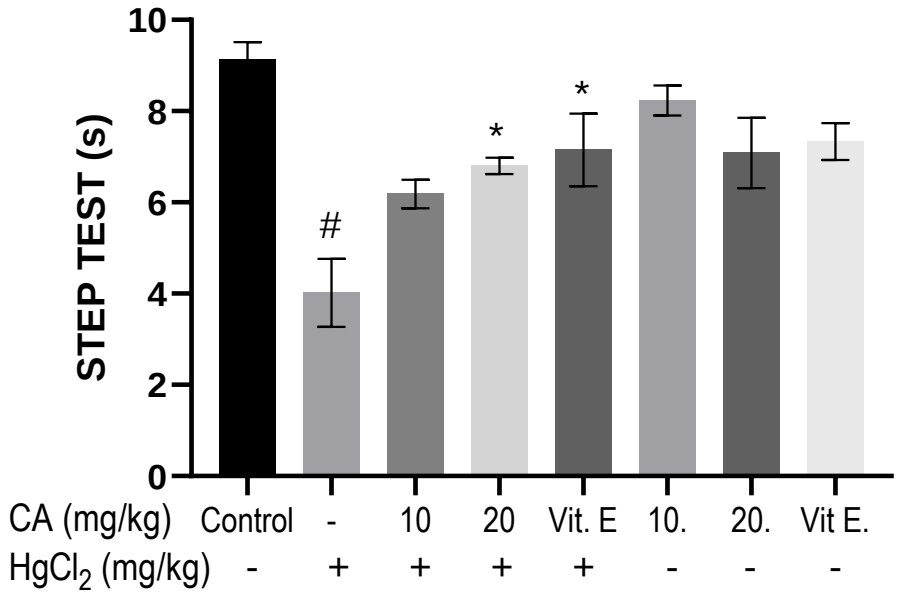

Figure 4.19. Step Test of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

4.4 Effect of Treatment on Oxidative Stress

Figures 4.20-4.24 show the oxidative stress parameters in the cerebellum of the control and treatment groups after 28 days. When compared to the control, $HgCl_2$-only group showed a significant decrease ($p<0.05$) in SOD, CAT, GPx, and GSH (Figure 4.21-4.24) while a significant increase ($p<0.05$) was recorded in cerebellar MDA concentration (Figure 4.20). However, for SOD rats pretreated with CA and vitamin E showed a significant increase ($p<0.05$) when compared to $HgCl_2$-only group. For GPx (Figure 4.23) and GSH (Figure 4.24), pretreatment groups (10 and 20 mg/kg of CA) showed a significant increase ($p<0.05$) when compared to the $HgCl_2$-only group. Similarly, for CAT (Figure 4.22), the 20 mg/kg bw CA pretreatment group showed a significant increase ($p<0.05$) when compared with the $HgCl_2$-only group. For MDA (Figure 4.20), the pretreatment groups showed a significant decrease ($p<0.05$) when compared with the $HgCl_2$-only group. No significant difference ($p>0.05$) were observed in the CA and vitamin E treated groups when compared to the control.

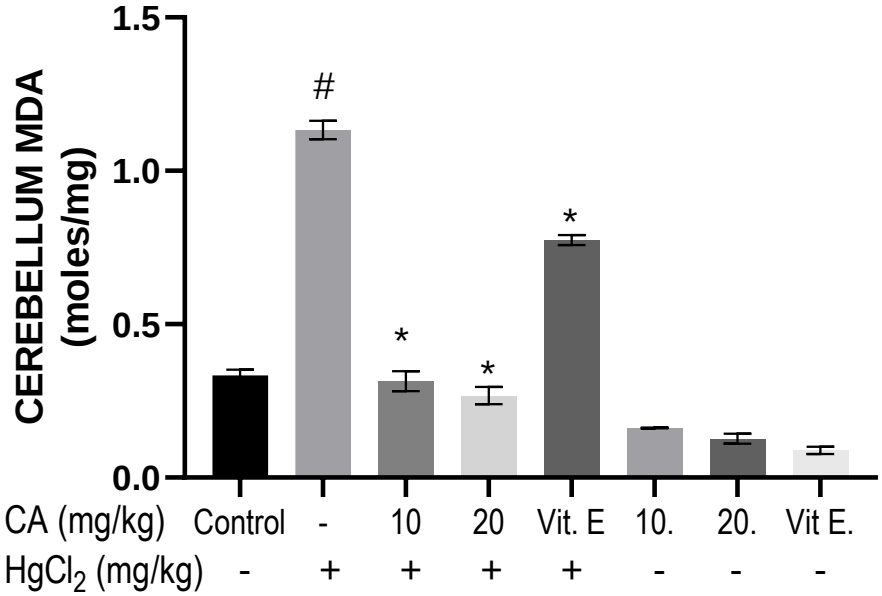

Figure 4.20. MDA in the cerebellum of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

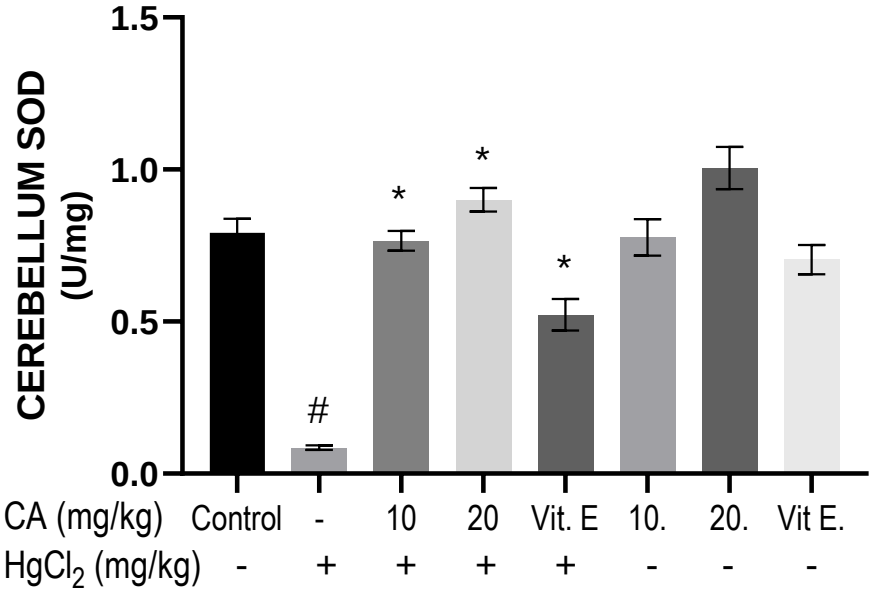

Figure 4.21. SOD in the cerebellum of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

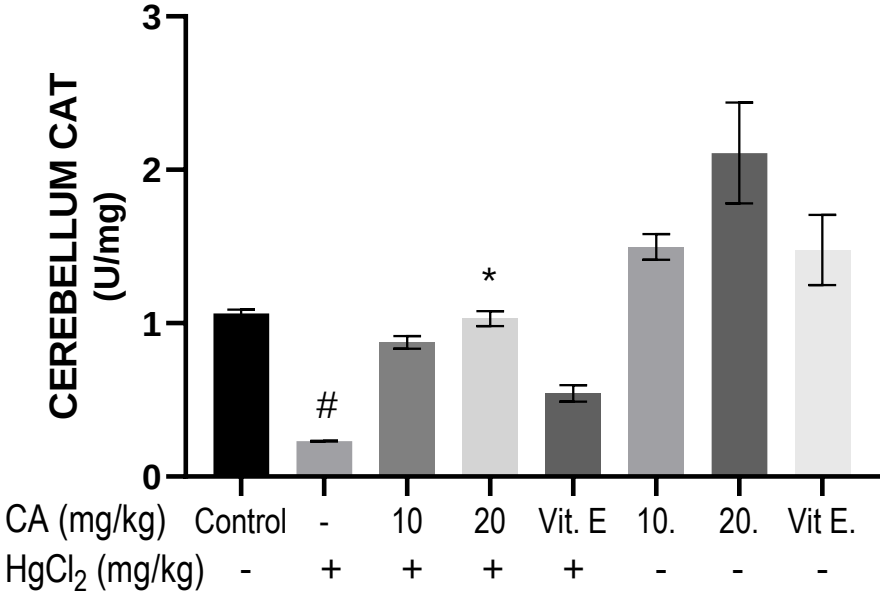

Figure 4.22. CAT in the cerebellum of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

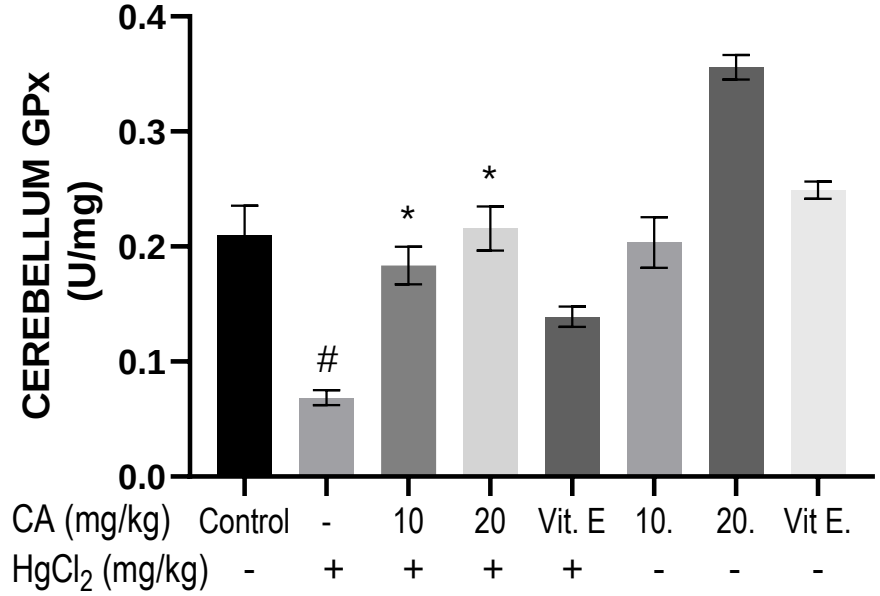

Figure 4.23. GPx in the cerebellum of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

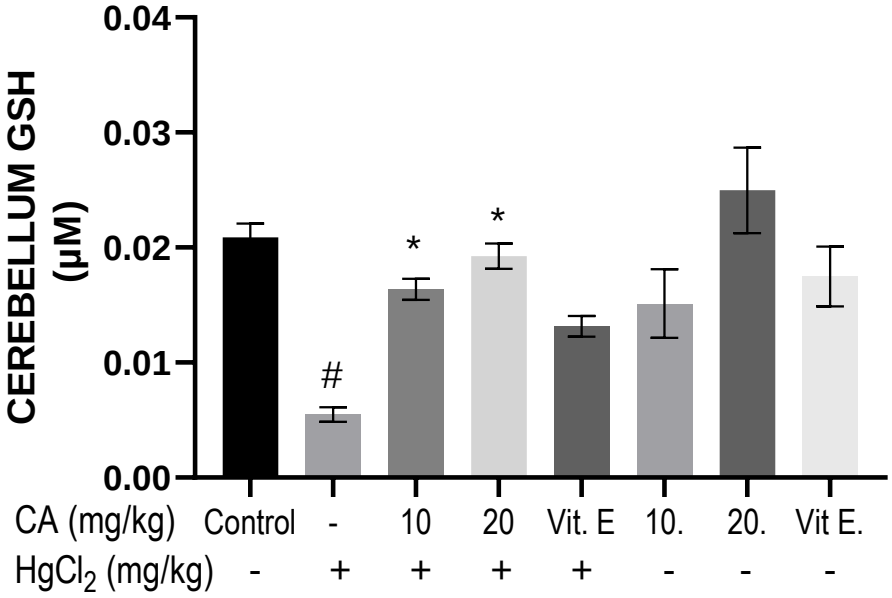

Figure 4.24. GSH in the cerebellum of control and treatment groups after 28 days.

# $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group

4.5 CEREBELLAR Hg CONCENTRATION

Figure 4.5 shows Hg accumulation in the cerebellum of control and treatment groups after 28 days. There was a significant increase ($p<0.05$) in the $HgCl_2$-only group when compared to the control. Pretreatment with CA or vitamin E caused a significant decrease ($p<0.05$) in cerebellar Hg concentration when compared to the $HgCl_2$-only group.

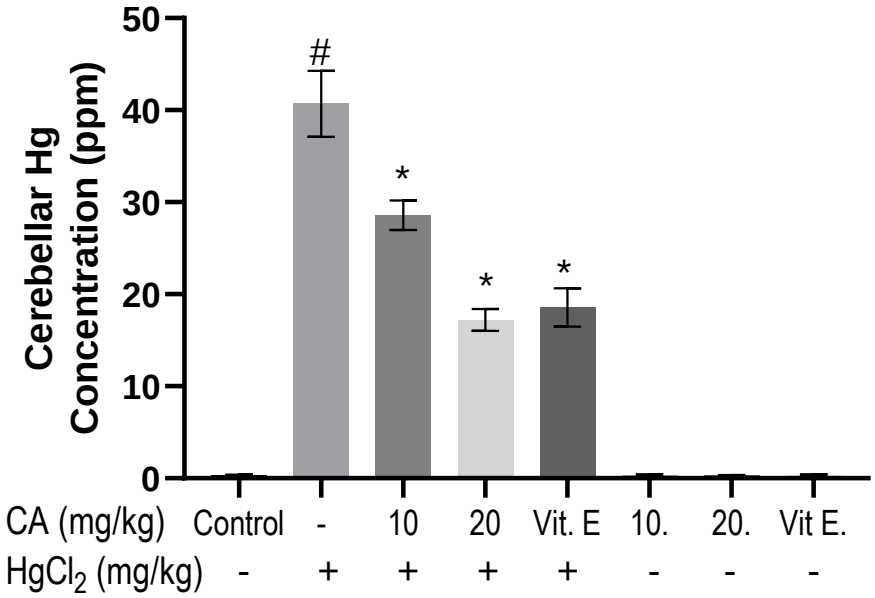

Figure 4.25. Hg accumulation in the cerebellum across experimental groups after 28 days. # $p<0.05$ compared with the control group; * $p<0.05$ compared with $HgCl_2$ group.

4.6 EFFECT OF TREATMENT ON THE HISTOLOGY OF THE CEREBELLUM

Plate 4.1 shows normal histology of the cerebellum in control group presenting normal histoarchitecture of cerebellum layers – Molecular layer (ML); Purkinje Cell layer (PCL); Granular Cell layer (GCL). Plate 4.2 represents $HgCl_2$-only exposed group showing degenerating Purkinje cells (big arrows), with nuclei appearing irregular, darkly stained and pyknotic. Also observed are vacuolations in the Molecular and Purkinje cell layers (arrows). Plate 4.3-4.5 shows representative histology of the cerebellum across $HgCl_2$-treated groups. Plate 4.6-4.8 shows the histological structure of the cerebellum in groups treated with CA- and vitamin E-only.

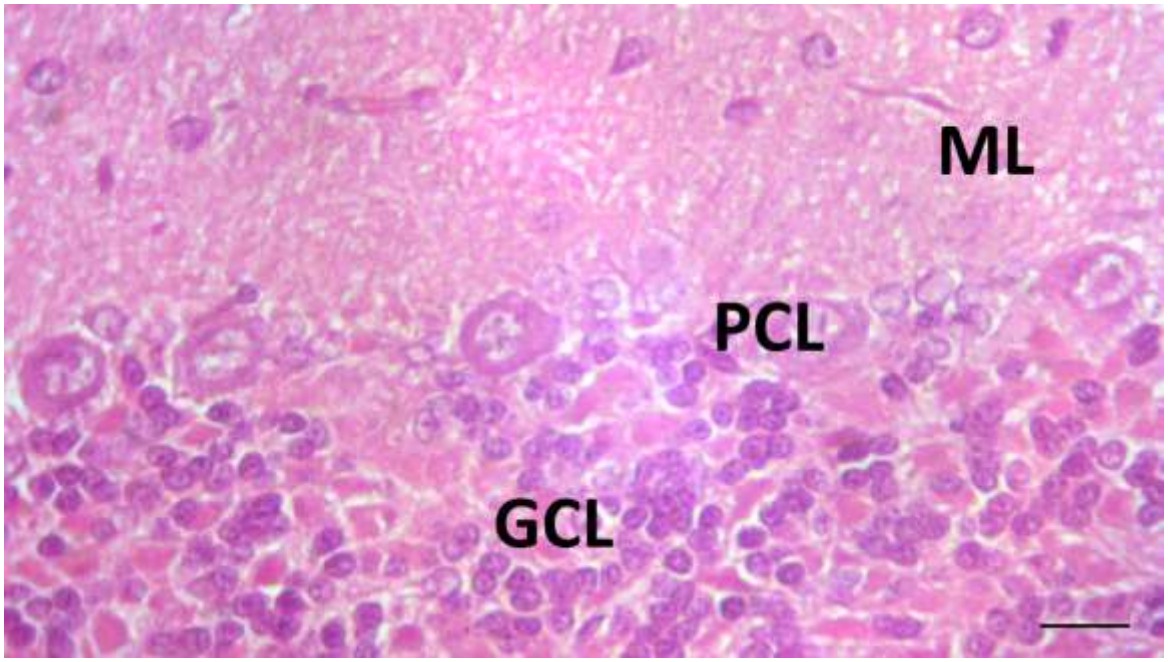

Plate 4.1. Representative histology of the cerebellum in Control group revealing normal histology in the Molecular layer (ML), Purkinje Cell layer (PCL), and Granular Cell layer (GCL) (H&E - 400x; Scale bar: 25µm).

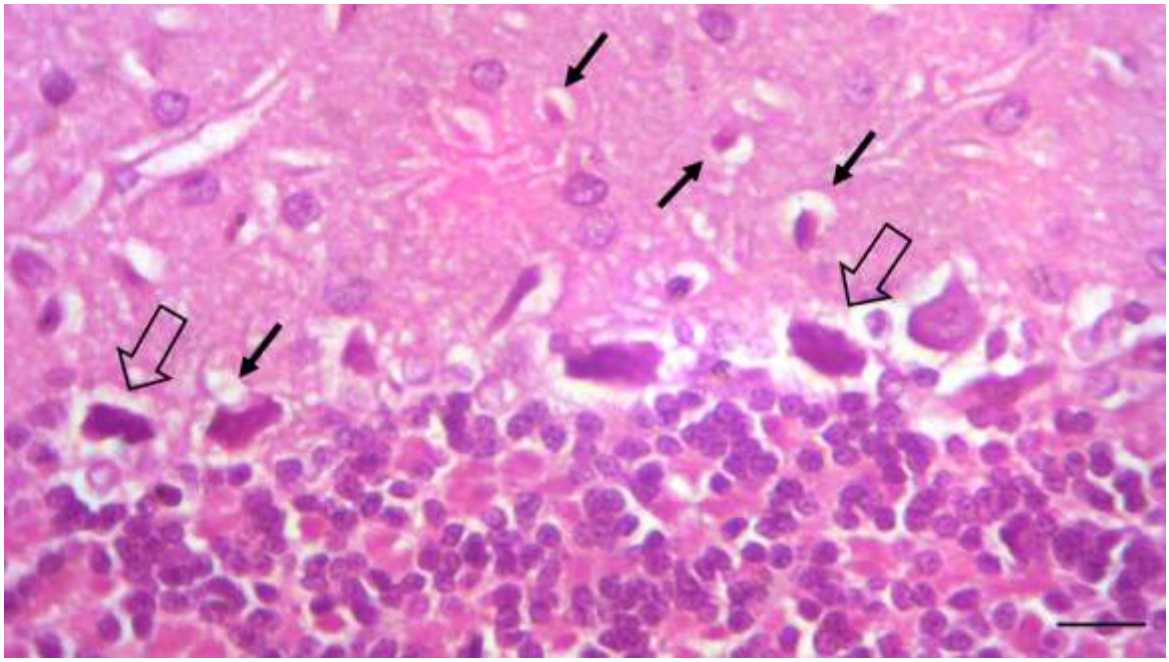

Plate 4.2. Representative histology of the cerebellum in $HgCl_2$-treated group showing degenerating Purkinje cells (big arrows), with nuclei appearing irregular, darkly stained and pyknotic. Also observed are vacuolations in the Molecular and Purkinje cell layers (arrows). (H&E - 400x; Scale bar: 25µm).

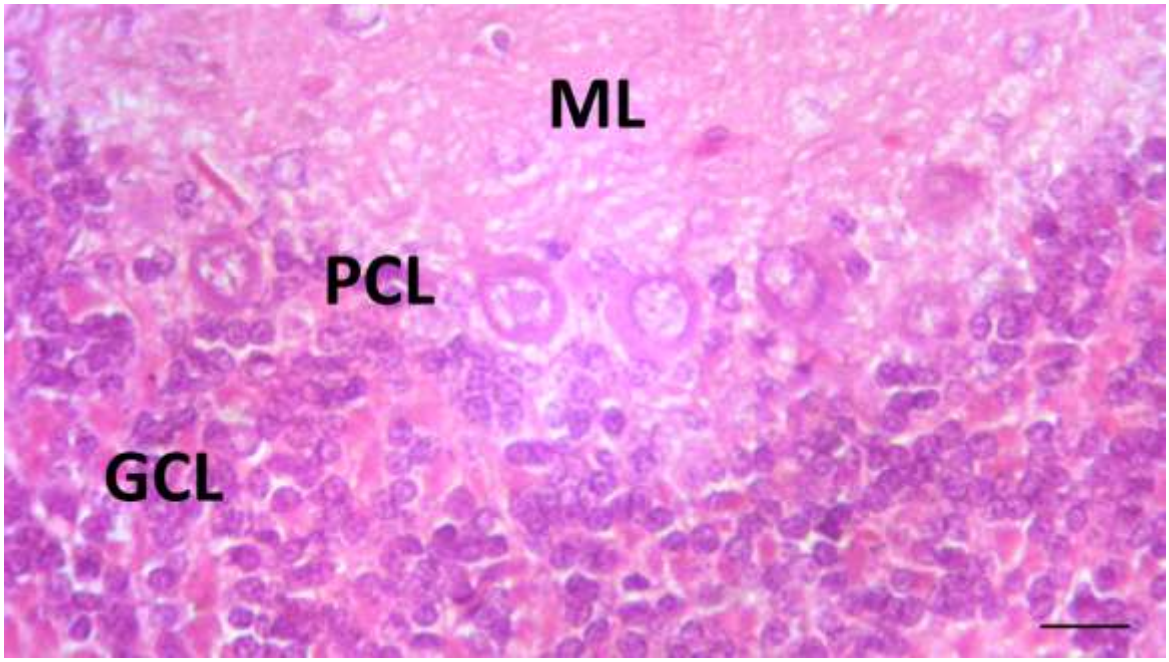

Plate 4.3. Representative histology of the cerebellum in rats pretreated with 10 mg/kg Catechin showing relatively normal histological structure in the Molecular layer (ML), Purkinje Cell layer (PCL), and Granular Cell layer (GCL). (H&E - 400x; Scale bar: 25µm)

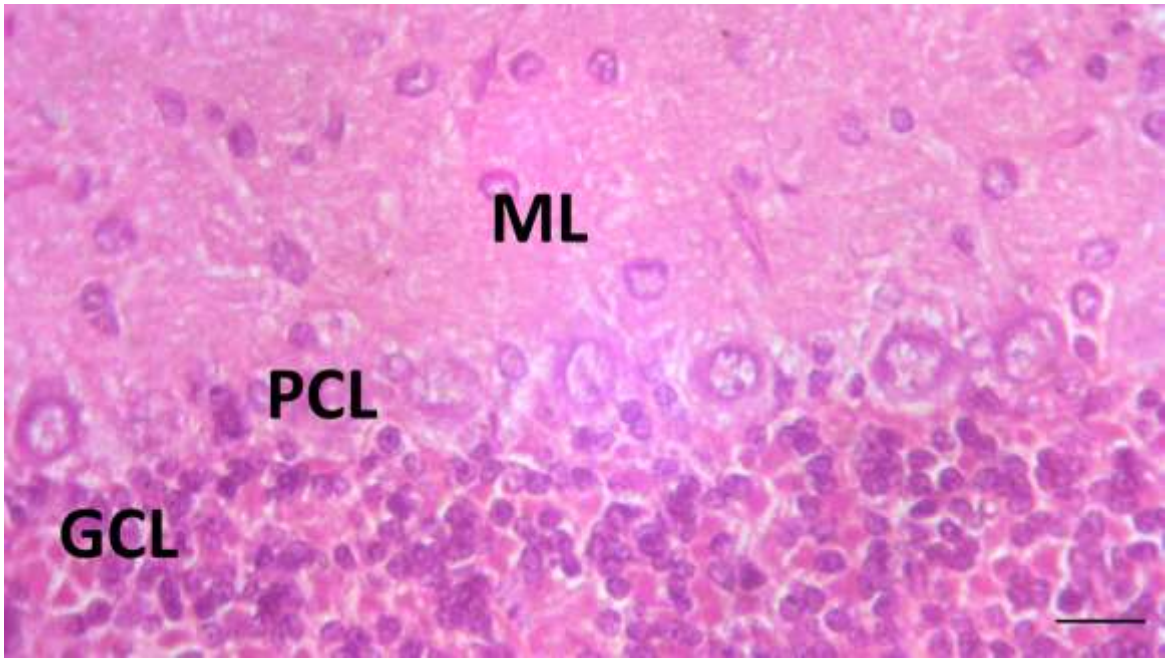

Plate 4.4. Representative histology of the cerebellum in rats pretreated with 20 mg/kg Catechin showing relatively normal histological structure in the Molecular layer (ML), Purkinje Cell layer (PCL), and Granular Cell layer (GCL). (H&E - 400x; Scale bar: 25µm)

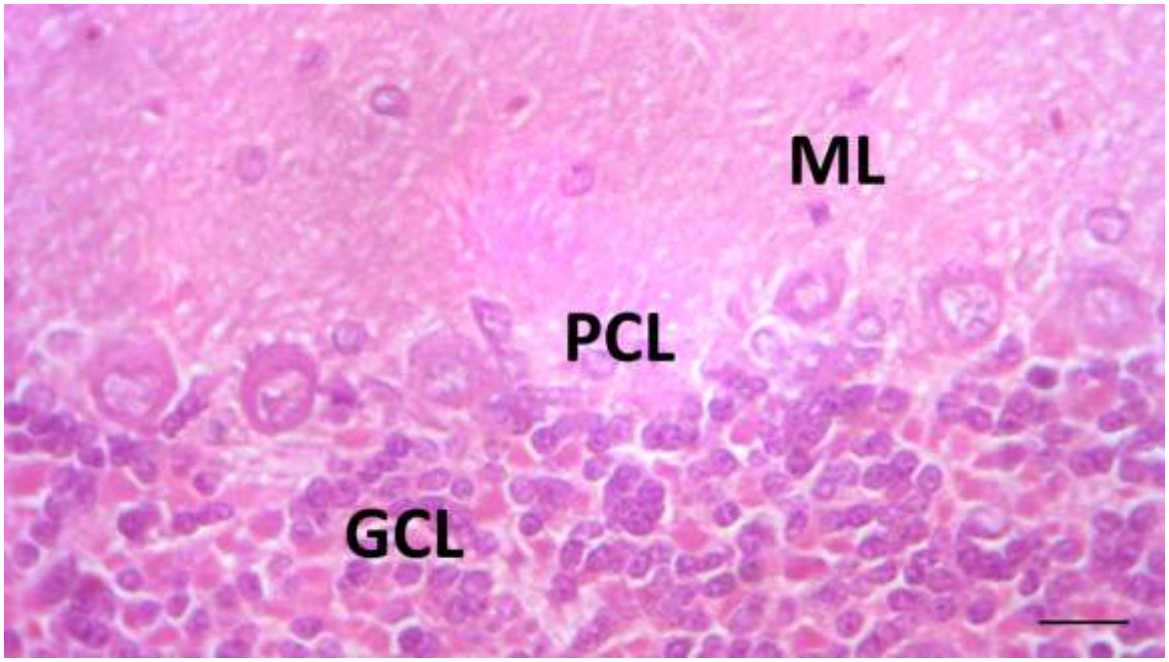

Plate 4.5. Representative histology of the cerebellum in rats pretreated with 200 mg/kg of vitamin E showing relatively normal histological structure in Molecular layer (ML), Purkinje Cell layer (PCL), and Granular Cell layer (GCL). (H&E - 400x; Scale bar: 25µm)

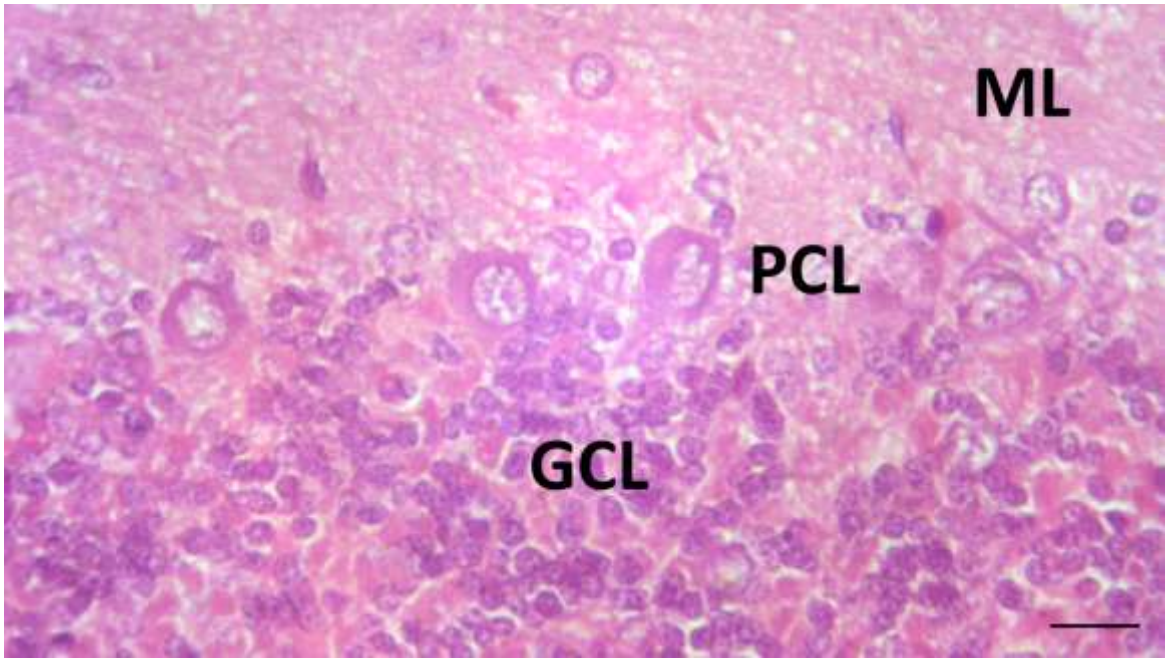

Plate 4.6. Representative histology of the cerebellum in rats treated with 10 mg/kg CA showing relatively normal histological structure in the Molecular layer (ML), Purkinje Cell layer (PCL), and Granular Cell layer (GCL). (H&E - 400x; Scale bar: 25µm)

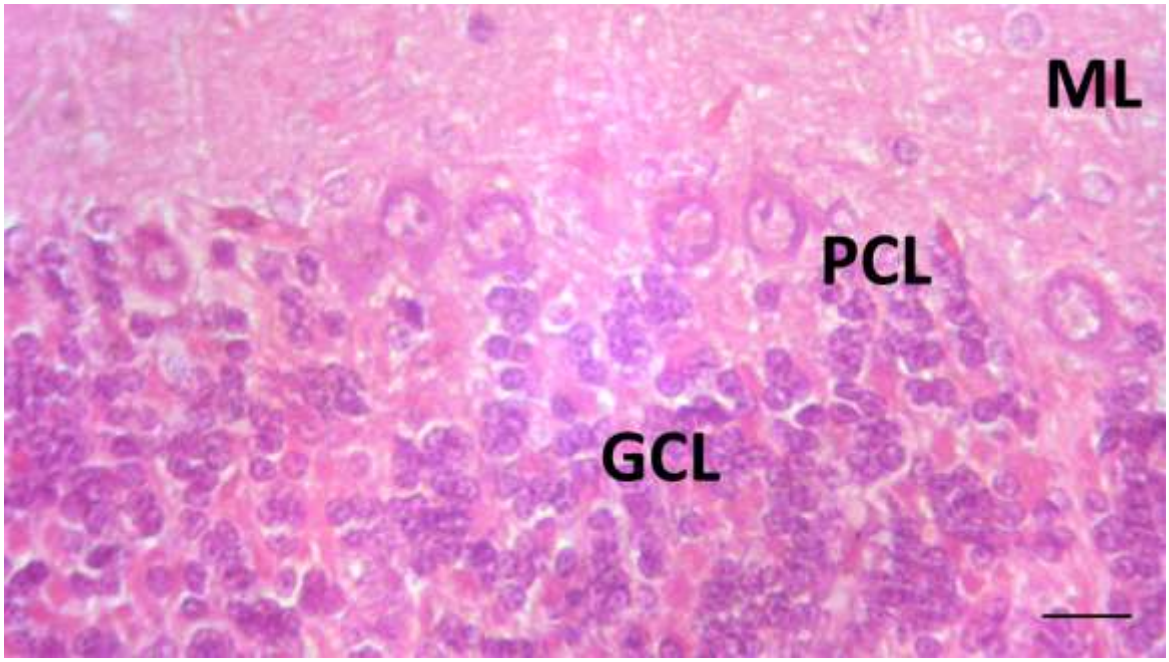

Plate 4.7. Representative histology of the cerebellum in rats treated with 20 mg/kg Catechin showing relatively normal histological structure in the Molecular layer (ML), Purkinje Cell layer (PCL), and Granular Cell layer (GCL). (H&E - 400x; Scale bar: 25µm)

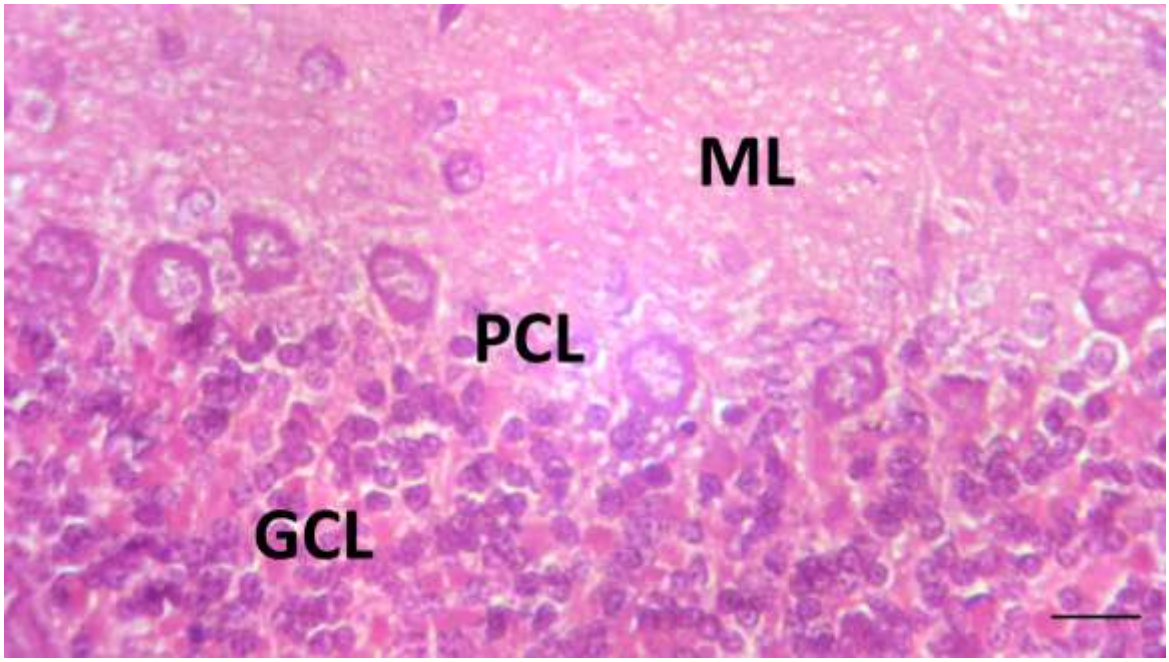

Plate 4.8. Representative histology of the cerebellum in rats treated with 200 mg/kg vitamin E showing relatively normal histological structure in the Molecular layer (ML), Purkinje Cell layer (PCL), and Granular Cell layer (GCL). (H&E - 400x; Scale bar: 25µm)

4.7 *IN-SILICO* MOLECULAR DOCKING RESULTS

Table 4.2 presents the molecular docking scores of Catechin compared with two standard reference ligands, Amantadine and Riluzole, against selected protein targets (NRF2, TNF-α, IL-6, NF-κB, and Caspase 3). The binding affinities are expressed in docking scores, where more negative values indicate stronger binding interactions between the ligand and the target protein. Catechin demonstrated the most favourable binding affinities across all the protein targets when compared to the standard ligands, Amantadine and Riluzole.

Figures 4.26-4.30 illustrates the 2D molecular interaction diagrams of Catechin docked with NRF2, TNF-α, IL-6, NF-κB, and Caspase-3, highlighting the specific amino acid residues involved within the binding pockets of each protein target. The diagrams reveal a network of hydrogen bonding, hydrophobic interactions, van der Waals forces, and pi-pi (π–π) stacking that contribute to the overall stability of ligand–protein complexes.

Table 4.2. Molecular docking scores

	DOCKING SCORES				
LIGANDS PROTIENS	**NRF2**	**TNF-α**	**IL-6**	**NF-κB**	**Caspase 3**
Catechin	-4.854	-6.446	-3.063	-4.817	-5.371
Amantadine (Standard)	-4.39	-2.692	-0.247	-2.078	0.061
Riluzole (Standard)	-4.11	-4.478	-2.462	-2.798	-3.965

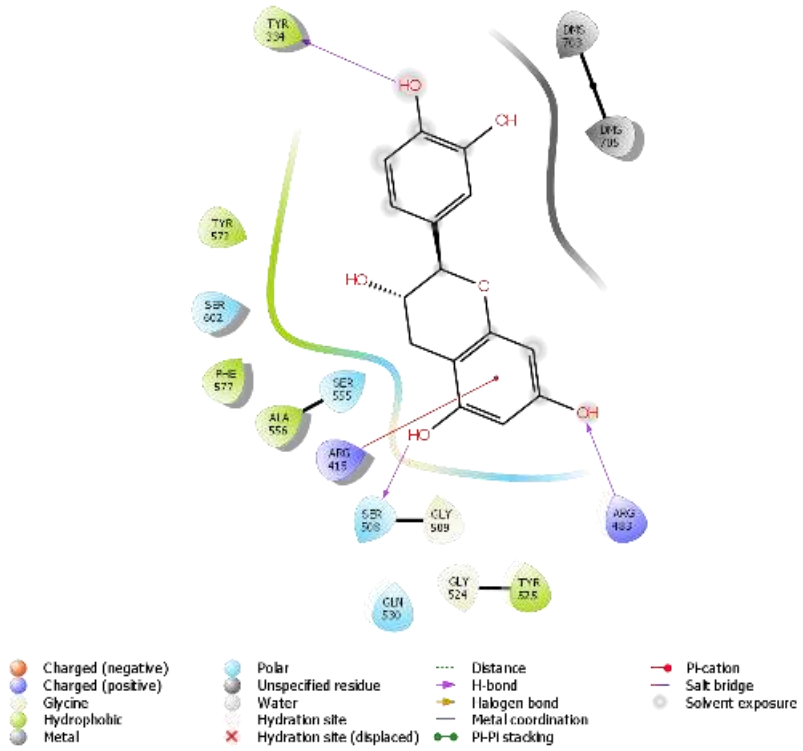

Figure 4.26. 2D interaction of Catechin with NRF2 showing interacting amino acid residues within the binding pocket

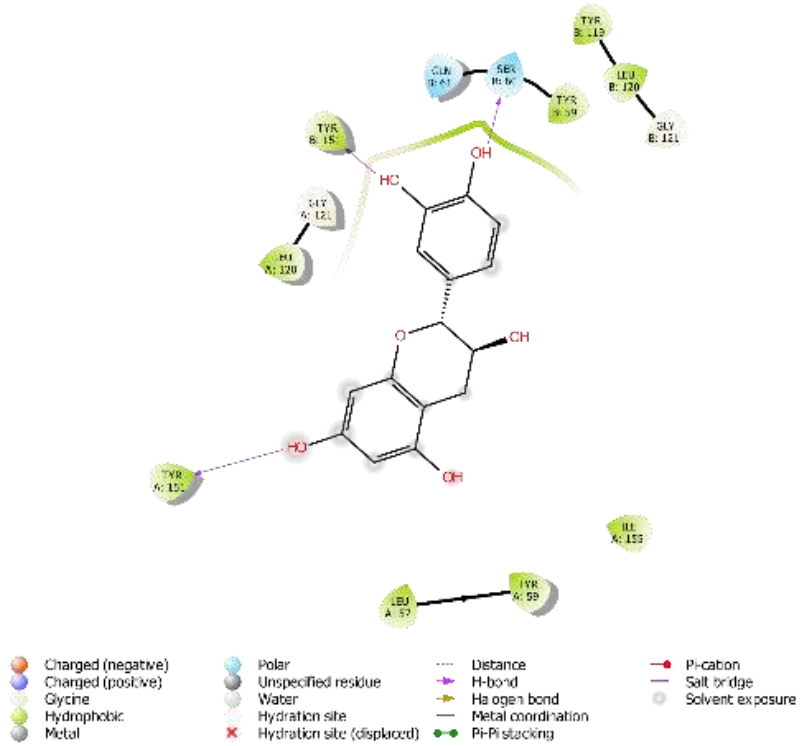

Figure 4.27. 2D interaction of Catechin with TNF-α showing interacting amino acid residues within the binding pocket

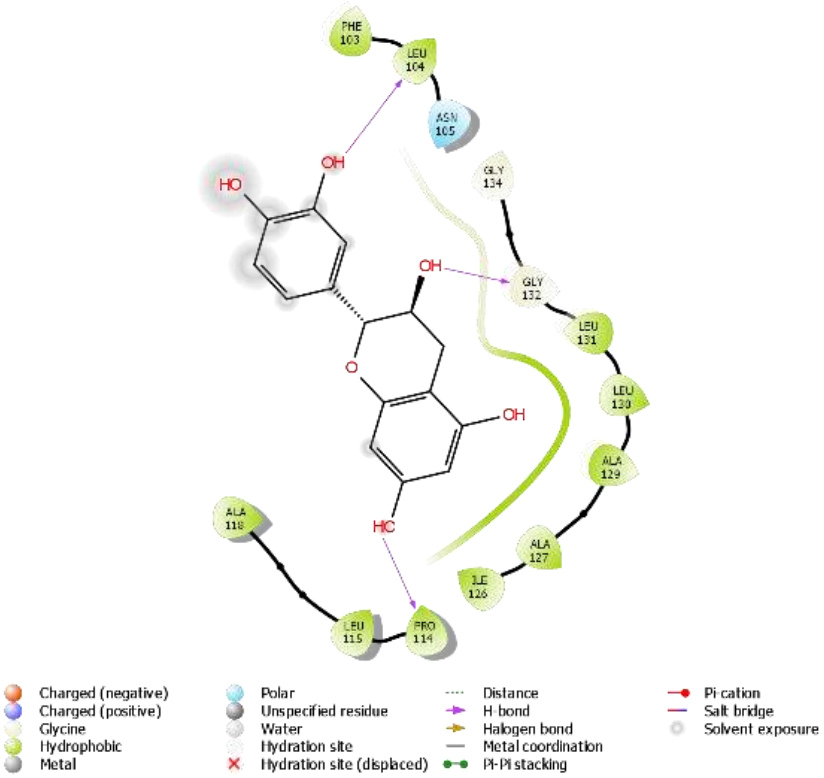

Figure 4.28. 2D interaction of Catechin with NF-κB showing interacting amino acid residues within the binding pocket

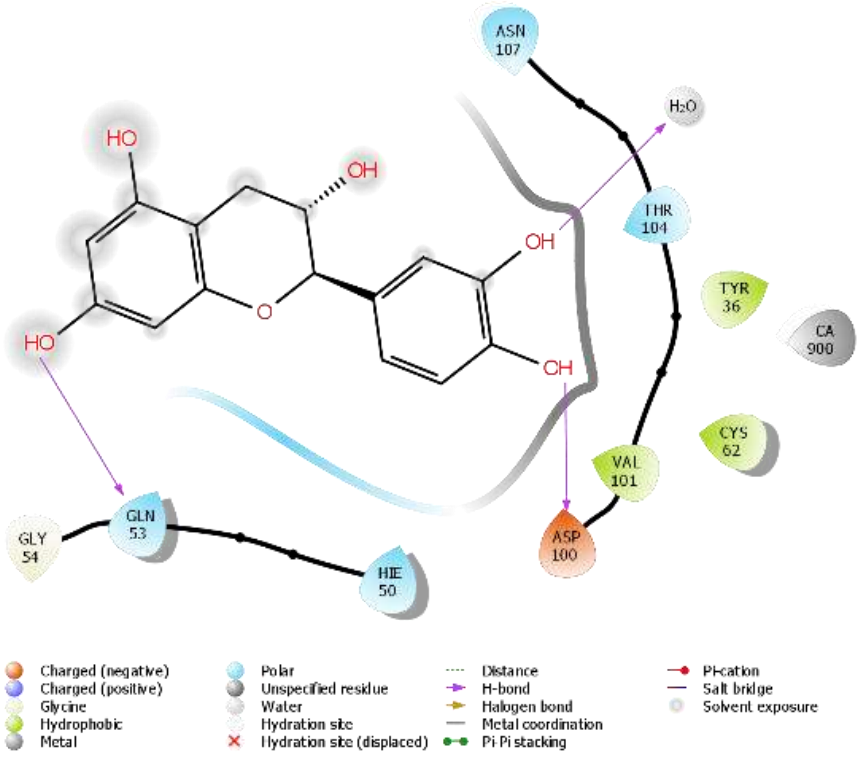

Figure 4.29. 2D interaction of Catechin with IL-6 showing interacting amino acid residues within the binding pocket

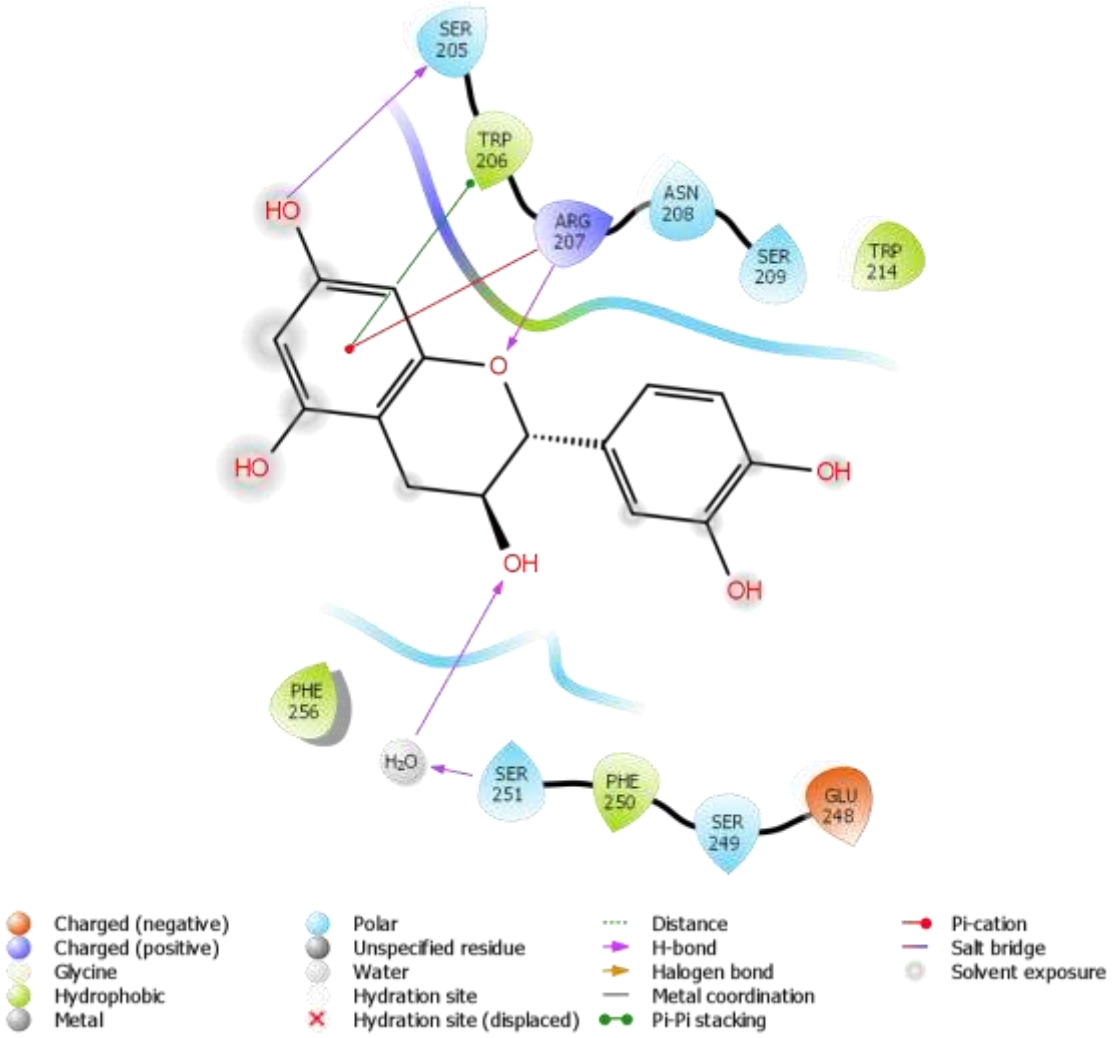

Figure 4.30. 2D interaction of Catechin with Caspase-3 showing interacting amino acid residues within the binding pocket

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

Mercury, a widespread environmental toxicant, poses significant risks to global public health due to its persistence and bioaccumulation in ecosystems. Exposure to its inorganic form, mercury (II) chloride ($HgCl_2$), is particularly concerning because of its high toxicity and ability to accumulate in various organ systems, including the brain. As a potent neurotoxin, $HgCl_2$ readily crosses the blood-brain barrier, inducing oxidative stress, mitochondrial dysfunction, and neuronal damage, particularly in sensitive regions like the cerebellum and cerebral cortex (Farina *et al*., 2013; Ijomone *et al*., 2020). These mechanisms can trigger reactive gliosis and widespread neural injury, underscoring the need for interventions to mitigate mercury's toxic effects on the central nervous system. This study aims to investigate the activity of catechin in cerebellum of mercury chloride induced toxicity.

5.1.1 Catechin is a Safe Dietary Substance

The acute toxicity study demonstrated that oral administration of Catechin at doses ranging from 10 mg/kg to 5000 mg/kg body weight did not produce morbidity, mortality, or diarrhoea within 72 hours. This outcome suggests that Catechin is well tolerated even at high doses, consistent with previous findings where green and black tea extracts, rich in catechin, showed no signs of acute toxicity at comparable levels (Chachiyo *et al*., 2020; Ardiana *et al*., 2017). The lack of gastrointestinal disturbances in this study also aligns with evidence that catechin and related polyphenols generally exhibit good gastrointestinal tolerance even at elevated concentrations (Chengelis *et al*., 2008; Dekant *et al*., 2017).

These findings place Catechin within the lowest hazard category under OECD acute toxicity guidelines, as compounds with oral LD_{50} values above 5000 mg/kg are considered practically non-toxic (Rudrapal *et al* 2024; Cladis *et al*., 2022). Collectively, the results confirm that

Catechin possesses a wide margin of safety under acute exposure conditions, supporting its potential use as a therapeutic or nutraceutical agent, while highlighting the importance of longer-term toxicity assessments to validate its safety in chronic use.

5.1.2 Catechin Maintained Brain and Body Weight in $HgCl_2$-Exposed Rats.

Alterations in body weight are widely regarded as key markers of overall health and physiological integrity, often signalling systemic toxicity or underlying disruptions in metabolic balance. In the present study, no significant difference was observed in the final body weight of the $HgCl_2$-only group when compared with the control, suggesting that the administered dose (5 mg/kg) did not elicit marked systemic wasting. This finding is consistent with reports where similar dosing regimens failed to produce significant weight changes despite evidence of tissue-specific toxicity (Atkinson *et al*., 2001; Sampada & David, 2025). Likewise, although brain and cerebellum weights showed no significant alterations, previous studies confirm that $HgCl_2$ at comparable doses can still induce histological and ultrastructural changes in sensitive brain regions, including the cerebellum and hippocampus (Enogieru & Abhelemhen, 2025; Said *et al*., 2021). These findings highlight that the absence of gross weight changes does not necessarily preclude the presence of mercury-induced neurotoxicity.

Interestingly, rats pretreated with CA and Vitamin E did not show significant differences in body weight compared with the $HgCl_2$-only group. This observation suggests that while both agents possess well-documented antioxidant and cytoprotective properties, their impact may be more pronounced at the level of biochemical and cellular protection rather than in altering gross measures such as body mass. Prior studies have highlighted that Vitamin E and catechin reduce oxidative stress and prevent histological alterations in $HgCl_2$ toxicity, but changes in body weight are not consistently observed as endpoints (Ahmed *et al*., 2020; Kumari & Anuradha, 2016). This aligns with reports where antioxidants primarily mitigated cellular and organ-level damage rather than significantly influencing general growth metrics.

In contrast, weight change outcomes provide a more sensitive reflection of toxicological impact. The present study demonstrated a significant decrease in weight change in the $HgCl_2$-only group compared to the control, a finding consistent with the known cytotoxic and degenerative effects of mercury on vital organs. Importantly, pretreatment with CA and Vitamin E resulted in a significant increase in weight change relative to $HgCl_2$-only, indicating protective effects against mercury-induced atrophy. These findings are supported by previous work showing that both Vitamin E and catechin can preserve organ integrity and prevent mercury-induced degeneration in brain and peripheral tissues (Owoeye *et al*., 2018a; Mousa & Ibrahim, 2023). Furthermore, no significant differences in brain weight, cerebellar weight, or relative cerebellum-brain weight ratios across all experimental groups suggest that gross neuroanatomical mass was maintained, with mercury's neurotoxic effects likely manifesting more subtly at the histological and biochemical level rather than through measurable weight changes (Borowska *et al*., 2018).

5.1.3 Catechin Mitigates $HgCl_2$ Induced Neurobehavioural Defects

The present study demonstrated that $HgCl_2$ exposure caused significant alterations in both exploratory and motor behaviours, reflecting widespread neurotoxicity. In the open field test, rats exposed to $HgCl_2$ displayed reduced rearing, ambulation, line crossing, and central square entries, alongside elevated immobility, grooming, and sniffing. These behavioural deficits indicate reduced exploratory drive and heightened anxiety-like responses, consistent with previous findings that mercury disrupts hippocampal and cerebellar function through oxidative stress and neurotransmitter imbalance (Enogieru & Ezennia, 2024; Mitra *et al*., 2022). Similarly, performance in the string, movement initiation, and step tests revealed motor impairments in $HgCl_2$-treated animals, with reduced grip strength, delayed movement initiation, and impaired stepping. These results align with prior evidence that mercury exposure

damages Purkinje neurons and motor circuits, leading to impaired coordination and neuromuscular control (Owoeye *et al*., 2018b).

Taken together, these behavioural outcomes underscore the breadth of $HgCl_2$ neurotoxicity, which manifests across both locomotor and affective domains. The deficits observed in exploratory activity and motor performance may share common underlying mechanisms, particularly oxidative damage to the cerebellum and basal ganglia. Reports indicate that mercury disrupts calcium homeostasis, mitochondrial function, and dopaminergic neurotransmission, leading to both reduced exploratory behaviours and impaired fine motor control (Tams *et al*., 2018). The increased grooming and sniffing behaviours observed in the $HgCl_2$-only group may further reflect compensatory or stress-related responses to neurochemical imbalance. Thus, the combined open field and motor coordination tests confirm that $HgCl_2$ toxicity extends beyond gross motor impairment to include anxiety-like phenotypes and deficits in behavioural adaptability.

Pretreatment with Catechin (CA) or Vitamin E markedly improved both exploratory and motor behaviours, restoring locomotor activity, reducing anxiety-related indices, and enhancing grip strength and stepping ability. These improvements highlight the protective capacity of antioxidants to buffer against mercury-induced oxidative stress. Vitamin E has been shown to reduce Purkinje cell damage and restore behavioural performance in mercury-exposed animals (Ahmed *et al*., 2020), while catechin is recognized for its radical-scavenging, metal-chelating, and neuroprotective properties in models of heavy metal neurotoxicity (Sárközi, 2015). The ability of CA and Vitamin E to restore normal function in both anxiety-related and motor parameters suggests that their benefits extend across multiple neurobehavioural domains.

Importantly, animals treated with CA or Vitamin E alone showed no significant deviations from controls in either open field or motor tests, underscoring their behavioural safety. This

observation supports the conclusion that the neuroprotective benefits of these compounds are specific to counteracting mercury-induced deficits rather than reflecting intrinsic behavioural stimulation. Collectively, the results confirm that $HgCl_2$ produces broad-spectrum neurobehavioral impairments encompassing locomotor, motor coordination, and anxiety-related domains, while antioxidant pretreatments with CA and Vitamin E confer significant protection. These findings reinforce the therapeutic potential of dietary antioxidants in mitigating heavy metal neurotoxicity, though further studies linking behavioural outcomes with histological and biochemical analyses are warranted.

5.1.4 Catechin Attenuates $HgCl_2$ Induced Oxidative Stress

The oxidative stress results highlight the vulnerability of the cerebellum to $HgCl_2$ toxicity. Elevated malondialdehyde (MDA) levels in the $HgCl_2$-only group confirm enhanced lipid peroxidation, a hallmark of oxidative damage. Concurrently, the significant decreases in antioxidant enzymes, SOD, CAT, GPx, and GSH, reflect impaired cellular defence mechanisms against reactive oxygen species. These findings are consistent with prior reports showing that $HgCl_2$ disrupts the redox balance in cerebellar tissue, leading to oxidative damage and neuronal dysfunction (Enogieru & Abhelemhen, 2025; Imosemi & Oladejo, 2023). Such reductions in endogenous antioxidants are particularly critical in the cerebellum, which is highly sensitive to oxidative stress during toxicant exposure (Enogieru & Momodu, 2021; Auza *et al*., 2024).

Pretreatment with catechin (CA) and vitamin E showed significant protective effects, with reductions in MDA and improvements in SOD, CAT, GPx, and GSH activity. Catechin is well documented for its radical-scavenging and metal-chelating abilities, which restore antioxidant enzyme function and limit lipid peroxidation (Kumari & Anuradha, 2016). Similarly, vitamin E is a lipid-soluble antioxidant that stabilizes cell membranes and interrupts lipid peroxidation chains, providing neuroprotection against mercury-induced oxidative damage (Mousa &

Ibrahim, 2023). Interestingly, while CA consistently improved all oxidative stress markers, vitamin E showed selective efficacy, with weaker effects on GPx and GSH restoration. This suggests possible mechanistic differences in their antioxidant activity, where CA's polyphenolic structure may offer broader redox regulation compared to vitamin E's primary role in lipid membrane stabilization (Winiarska-Mieczan *et al*., 2020).

The absence of significant oxidative stress changes in groups treated with CA or vitamin E alone indicates that these compounds do not disrupt redox homeostasis under normal conditions. This is consistent with prior studies where dietary antioxidants remained behaviourally and biochemically neutral in healthy models but effectively mitigated toxin-induced oxidative imbalance (Akinmoladun *et al*., 2020; Kalender *et al*., 2012). Collectively, these findings emphasize that $HgCl_2$ induces severe cerebellar oxidative stress, while CA and vitamin E act as protective agents, with catechin showing a more consistent capacity to restore enzymatic and non-enzymatic antioxidant defences.

5.1.5 Catechin Inhibits Hg Accumulation and Histological Alterations in the Cerebellum of $HgCl_2$-Exposed Rats

The findings of cerebellar mercury accumulation confirm the neurotoxic potential of $HgCl_2$. Rats in the $HgCl_2$-only group exhibited significantly elevated cerebellar mercury concentrations compared to controls, consistent with the ability of inorganic mercury to cross the blood–brain barrier and accumulate in neural tissues. Such accumulation is strongly associated with neuronal degeneration, oxidative damage, and functional deficits in the cerebellum (Enogieru & Abhelemhen, 2025; Animoku *et al*., 2016). Previous studies have similarly shown that mercury preferentially accumulates in Purkinje cells of the cerebellar cortex, leading to motor impairments and histopathological alterations (Animoku *et al*., 2019; Ibegbu *et al*., 2014). Pretreatment with catechin (CA) or vitamin E prior to $HgCl_2$ exposure significantly reduced cerebellar mercury concentrations, suggesting a protective role in

limiting mercury uptake or enhancing detoxification pathways. Catechin has been reported to exert metal-chelating effects and enhance the elimination of toxic metals, while vitamin E stabilizes neuronal membranes and prevents mercury-induced oxidative stress, thereby reducing tissue retention of mercury (Owoeye *et al*., 2018; Mousa & Ibrahim, 2023). The fact that groups treated with CA or vitamin E alone showed no significant differences relative to controls further supports their safety. Collectively, these results demonstrate that CA and vitamin E not only mitigate oxidative and functional cerebellar damage but also play a role in reducing mercury accumulation, underscoring their therapeutic potential against heavy metal neurotoxicity.

The histological findings reveal significant differences between control and $HgCl_2$-treated cerebellum. In the control group, the cerebellar layers appear intact, with a clearly defined molecular layer (ML), Purkinje cell layer (PCL), and granular cell layer (GCL). The Purkinje cells exhibit normal morphology, characterized by prominent nuclei and preserved cytoplasmic structure, consistent with healthy cerebellar tissue. In contrast, the $HgCl_2$-only group displayed marked pathological changes. The Purkinje cells appeared degenerative, with pyknotic, darkly stained nuclei and shrunken cytoplasm (large arrows). Vacuolations were also observed within the ML and PCL (small arrows), features indicative of neurodegeneration and neuronal stress. Such alterations are consistent with previous reports describing Purkinje cell degeneration, vacuolation, and general cytoarchitectural disruption following mercury exposure (Enogieru & Abhelemhen, 2025; Mousa & Ibrahim, 2023).

Further confirmation is provided in the $HgCl_2$-only group showing the most severe damage, while groups pretreated with catechin (CA) or vitamin E preserved near-normal cerebellar architecture. Similarly, the CA- and vitamin E-only groups closely resembled the control group, highlighting their safety and absence of structural toxicity. These observations reinforce

the biochemical results and behavioural findings, which also demonstrated that CA and vitamin E effectively protect against $HgCl_2$-induced damage. Collectively, the histological evidence underscores the cerebellum's vulnerability to mercury toxicity and the neuroprotective potential of antioxidant supplementation.

5.1.6 Catechin Shows High Binding Affinity to Molecular Targets

The molecular docking results presented in Table 4.2 and Figure 4.6–4.10 highlight the strong interaction potential of catechin compared to the reference ligands amantadine and riluzole. Catechin showed the most favourable binding affinities across NRF2, TNF-α, IL-6, NF-κB, and caspase-3, suggesting a broader capacity to modulate oxidative stress and inflammatory signalling pathways. This aligns with earlier findings that flavonoids, including catechin, exert protective effects by activating NRF2 and suppressing NF-κB signalling, thereby reducing pro-inflammatory cytokine release such as TNF-α and IL-6 (Ahmad *et al*., 2024; Mishra *et al*., 2024). The ability of catechin to strongly interact with caspase-3 further indicates potential anti-apoptotic activity, consistent with its reported role in attenuating programmed neuronal death in oxidative stress-mediated conditions (Thiruvengadam *et al*., 2021).

The 2D molecular interaction diagrams in Figure 4.6–4.10 provide mechanistic insight into these results. Catechin forms a complex network of hydrogen bonds, hydrophobic interactions, and pi-pi (π–π) stacking within the active sites of the protein targets, enhancing stability and specificity. Similar molecular docking studies on catechin has demonstrated comparable binding patterns, reinforcing their roles as multitarget modulators of oxidative stress and inflammatory responses (Bellavite, 2023; Chen *et al*., 2018). These findings collectively suggest that catechin could provide a mechanistic basis for neuroprotection by attenuating ROS accumulation, blocking NF-κB-driven inflammation, and reducing apoptotic signalling through caspase-3 inhibition.

Extending the molecular docking results into the context of the previous histological and biochemical findings strengthens the mechanistic narrative. The demonstrated ability of catechin to bind tightly to NRF2, TNF-α, IL-6, NF-κB, and caspase-3 complements the observed neuroprotective outcomes in $HgCl_2$-exposed rats. For example, activation of NRF2 and inhibition of NF-κB at the docking level mechanistically explain the reductions in oxidative stress markers (MDA, SOD, CAT, GPx, and GSH) observed in the cerebellum following catechin supplementation. Similarly, strong predicted binding to TNF-α and IL-6 provides a molecular rationale for catechin's modulation of inflammatory cascades that contribute to Purkinje cell degeneration, as confirmed in the histological findings. Furthermore, catechin's potential interaction with caspase-3 supports its role in reducing apoptotic cell death, which likely underlies improvements in motor coordination and exploratory behaviour observed in open field and string tests. Together, the convergence of computational and experimental results highlights catechin's multitarget therapeutic potential against mercury-induced neurotoxicity.

5.2 CONCLUSION

This study demonstrates that mercuric chloride ($HgCl_2$) exposure induces neurotoxicity characterized by impaired behaviour, oxidative stress, and cerebellar histological alterations, while catechin (CA) and vitamin E supplementation significantly mitigated these effects by restoring antioxidant balance, reducing mercury accumulation, and preserving neuronal integrity. Importantly, both antioxidants showed no adverse effects when administered alone, confirming their safety. Molecular docking results further revealed catechin's strong interactions with NRF2, NF-κB, TNF-α, IL-6, and caspase-3, providing mechanistic support for its multitarget neuroprotective role. Collectively, these findings highlight the therapeutic potential of catechin and vitamin E in counteracting mercury-induced cerebellar dysfunction.

5.3 CONTRIBUTIONS TO KNOWLEDGE

The study has contributed to knowledge in the following ways:

1. It revealed that catechin attenuated mercury chloride-induced neurobehavioral deficits, mitigated oxidative stress, and inhibited elevated mercury levels in the cerebellum of experimental rats.

2. It revealed that catechin protected against mercury chloride-treated histological alterations in the cerebellum of experimental rats.

3. It demonstrated that catechin exhibited higher binding affinity to NRF-2, TNF-α, IL-6, NF-κB, and Caspase-3 than standard drugs, Riluzole and Amantadine.

5.4 RECOMMENDATIONS

Further research should investigate the synergistic effects of combined catechin and vitamin E supplementation, optimize dosing strategies for maximal neuroprotection, and extend evaluations to other brain regions affected by mercury. Translational studies in higher animal models and eventually clinical settings are also recommended to establish their potential as affordable and effective interventions against mercury and other heavy metal-induced cerebellar dysfunction.

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