

**ACTIVITY OF AQUEOUS *Tetrapleura tetraptera* FRUIT
EXTRACT ON THE CEREBRUM OF LEAD ACETATE-
EXPOSED RATS**

**BY
DANIEL ODIANOSEN ESEINE
PG/BMS0700909**

**UNIVERSITY OF BENIN
BENIN CITY**

DECEMBER, 2025.

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DANIEL ODIANOSEN ESEINE

PG/BMS0700909

B.Sc. (UNIBEN, 2012)

**A THESIS WRITTEN IN THE DEPARTMENT OF ANATOMY,
SCHOOL OF BASIC MEDICAL SCIENCES AND
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REQUIREMENTS FOR THE AWARD OF MASTER'S
DEGREE (M.Sc.) IN ANATOMY OF THE UNIVERSITY OF
BENIN.**

DECEMBER, 2025.

CERTIFICATION

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

.....
ADAZE B. ENOGIERU (PhD)
Associate Professor
Supervisor

.....
DATE

.....
ADAZE B. ENOGIERU (PhD)
Head of Department
Head of Department

.....
DATE

.....
EXTERNAL EXAMINER

.....
DATE

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DEDICATION

To God almighty, for His never-ending mercies and favour.

To Daniel O. Eseine Esq, I wish you were here Dad.

To Ns C. O. Eseine, who moves mountains for me.

To Christabel Eseine-Aloja, for the push.

To Ns Juliet Eloebhose-Eseine, for Efua.

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ABSTRACT

Cerebral dysfunction, a hallmark of various neurocognitive disorders, may result from congenital anomalies, progressive neurodegeneration, or exposure to neurotoxic agents. Lead (Pb), a highly toxic heavy metal, readily crosses the blood–brain barrier and accumulates in the cerebrum, where it disrupts calcium homeostasis and promotes oxidative stress. This cascade contributes to neuronal injury and cognitive decline. Emerging evidence suggests that dietary antioxidants can counteract lead-induced oxidative damage and help preserve cerebral integrity. This study evaluated the neuroprotective effects of aqueous *Tetrapleura tetraptera* (TT) fruit extract on lead acetate induced cerebral toxicity. Sixty-four adult Wistar rats were randomly divided into eight treatment groups (n=8) and treated for 28 days as follows: Group A (control – 1 mL distilled water), Group B (Pb only, 100 mg/kg [bw]), Group C (TT - 500 mg/kg [bw] + Pb - 100mg/kg [bw]), Group D (TT - 1000 mg/kg [bw] + Pb – 100 mg/kg [bw]), Group E (Vitamin E – 200 mg/kg [bw] + Pb - 100mg/kg [bw]), Group F (TT only - 500 mg/kg [bw]), Group G (TT only - 1000 mg/kg [bw]), and Group H (Vitamin E only – 200 mg/kg [bw]). High Performance Liquid Chromatography (HPLC) was carried out to identify the phytochemicals contained in the extract. Molecular docking results showed the polyphenols, catechol and phloroglucinol, displayed a higher binding affinity with Caspase 3, IL-6 and comparable binding affinity with NRF2 against standard drugs like Levodopa and Clonazepam. Pre-sacrifice, neurobehavioral assessments were conducted to evaluate cerebral dysfunction. Lead only exposed rats showed significant decrease in rearing frequency, and increase in grooming, thigmotaxis, sniffing, immobility time respectively. Post-sacrifice, cerebral tissues were analysed for lead concentration, antioxidant enzymes activity, lipid peroxidation and histopathological changes. Lead only exposed rats showed significant impaired ($p<0.05$) weight gain and antioxidant enzymes function, elevated lipid peroxidation, and increased cerebral lead levels. Histological analysis revealed vacuolation of granular cells and presence of pyknotic nuclei in the prefrontal cortex. However, pretreatment with *Tetrapleura tetraptera* significantly ($p<0.05$) mitigated these effects in lead-exposed rats suggesting strong neuroprotective properties. The study identifies *Tetrapleura tetraptera* potential as a natural, neuroprotective and therapeutic agent against lead-induced cerebral dysfunction. Further studies exploring the application of *Tetrapleura tetraptera* in other models of cerebral dysfunction are recommended.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Cerebral dysfunction encompasses a wide spectrum of neurological impairments that affect the brain's ability to regulate cognition, behaviour, and motor functions (Boss and Huether, 2011). It also refers to any disturbance in the normal physiological function of the cerebrum or the brain as a whole (American Psychological Association, 2018). Cerebral dysfunction can be classified as either traumatic or acquired. Traumatic causes involve external physical forces, such as those from road traffic accidents, sports injuries, and falls, and can lead to either focal or diffuse brain damage depending on the severity of the injury (Arciniegas *et al.*, 2013). Acquired (non-traumatic) causes arise from internal factors like strokes, tumours, infections (e.g., meningitis), neurodegenerative diseases (e.g., Alzheimer's, Parkinson's), and toxic exposure (e.g., alcohol, lead) (Jacobson & Gerner, 2021). Common underlying mechanisms, including oxidative stress, neuroinflammation, and impaired cerebral perfusion, disrupt neuronal signalling and synaptic integrity. These pathological processes ultimately cause long term deficits in attention, cognition, behaviour, memory, and motor control (Hinkle & Cheever, 2018; Zhang *et al.*, 2023).

Lead (Pb) exposure remains a pressing global public health issue due to its widespread environmental presence and well-established toxicity across multiple organ systems (Fatima *et al.*, 2025). As a non-biodegradable heavy metal, lead persists in ecological systems, accumulating in soil, water, and air primarily as a result of anthropogenic activities such as mining, industrial processing, fossil fuel combustion, and the use of lead containing materials (Niede and Benbi, 2022; Howard *et al.*, 2024). Despite regulatory restrictions, particularly in high-income countries, lead exposure continues to pose a significant threat in low- and middle-income regions, where environmental surveillance and policy enforcement are often

inadequate. According to the Institute for Health Metrics and Evaluation (IHME), lead exposure contributed to an estimated 1.5 million deaths worldwide in 2021, with the majority occurring in economically disadvantaged populations (IHME, 2024). Once inside the body, lead circulates via the bloodstream to soft tissues and is eventually stored in bones, from where it can be gradually released, leading to prolonged toxic effects (Srivastava *et al.*, 2024). The central nervous system (CNS) is especially vulnerable, with the brain as the principal target organ (Wu *et al.*, 2023).

Chronic exposure to lead has been shown to cause histopathological alterations and oxidative stress in the cerebral cortex, leading to neuronal degeneration, disrupted cytoarchitecture, and impaired antioxidant defence mechanisms (Borda, 2024). These changes can compromise higher cognitive functions such as attention, decision-making, and memory consolidation, which are core responsibilities of the cerebrum. Neuroimaging and neuropathological studies have also demonstrated that prolonged lead exposure results in white matter abnormalities, cerebral atrophy, and in some cases, intracranial calcifications (Maheshwari *et al.*, 2021). These structural changes are often associated with clinical manifestations like behavioural disturbances, tremors, and long-term cognitive decline (Liu *et al.*, 2020). Thus, while the hippocampus often receives focused attention in discussions of lead induced neurotoxicity, the cerebrum is significantly impacted as well, especially through mechanisms involving oxidative damage and neuronal apoptosis (Gudadhe *et al.*, 2021). Given the largely irreversible nature of lead induced damage to the nervous system, especially the brain, it is critical to explore affordable and sustainable therapeutic options, particularly for populations at heightened risk. In this regard, plant derived antioxidants have shown considerable promise. These compounds are known for their ability to neutralize reactive oxygen species (ROS), enhance the activity of the body's own antioxidant enzymes, and regulate inflammatory pathways (Abeyrathne *et al.*, 2022; Akbari *et al.*, 2022; Enogieru & Momodu, 2022).

Tetrapleura tetraptera is a flowering-medicinal plant belonging to the *Leguminosae* family. It is a deciduous tree mostly found in the western part of Africa and is usually referred to as Gum tree in English. Other names include Prekese (Ghana), Oshosho (Igbo), Aidan (Yoruba), Eseyese and Ighimiaka (Edo) (Mensah *et al.*, 2024). *Tetrapleura tetraptera* has a variety of chemical compositions that translate into its nutritive value. Significant amounts of various nutrients such as ash, fibre, proteins, carbohydrates, vitamins, and fats are present in *Tetrapleura tetraptera* (Mensah *et al.*, 2024). Similar to the nutritional composition, *Tetrapleura tetraptera* possesses an essential concentration of phytochemicals such as phenols, tannins, alkaloids, steroids, flavonoids, triterpenoids, and phytate (Adusei *et al.*, 2019, Akin-Idowu *et al.*, 2011). The plant's abundant phytochemicals account for its antioxidant, antimicrobial, anti-inflammatory, anti-diabetic, anti-parasitic, and anti-proliferative activities. These biological properties in turn translate to health benefits including lower blood pressure, enhanced immune system, malaria treatment, diabetes and hypertension management, and cancer prevention (Mensah *et al.*, 2024).

1.2 STATEMENT OF RESEARCH PROBLEM

The cerebrum, which governs complex cognitive functions such as reasoning, attention, and voluntary motor control, is notably vulnerable to lead induced neurotoxicity (Li *et al.*, 2021). Lead crosses the blood-brain barrier by mimicking calcium ions and accumulates in cerebral tissue, where it disrupts synaptic signalling, impairs neurogenesis, and promotes oxidative stress (Toscano and Guilarte, 2005; Gudadhe *et al.*, 2024). These pathological processes often result in cortical atrophy, synaptic degeneration, and deficits in executive functioning; indicators of cerebral neurodegeneration, and ultimately cerebral dysfunction. (Maheshwari *et al.*, 2021). It continues to be a powerful environmental neurotoxin, presenting serious health risks even at levels once deemed harmless. The CDC's updated blood lead reference value is 3.5 µg/dL (Centres for Disease Control and Prevention, 2021). Recent guidelines for adults

recommend focusing on exposure reduction, as chronic low-level lead exposure has adverse health effects and no safe threshold has been identified (Council of State and Territorial Epidemiologists, 2021). Nonetheless, growing research indicates that blood lead concentrations under 3.5 µg/dL may still cause biological damage (Centres for Disease Control and Prevention, 2024; World Health Organization, 2024). Studies have shown a link between low-level environmental lead exposure and diminished intellectual function in children, with no known safe threshold (Lanphear *et al.*, 2005; National Institute of Environmental Health Sciences, 2022).

Lead and other toxic heavy metals pose a significant public health threat because of their environmental ubiquity, persistence, and well-known ability to cause cerebral dysfunction; mediated by neuroinflammation, oxidative stress, and programmed cell death (apoptosis). (Santa Maria *et al.*, 2019; Halabicky, *et al.*, 2022). The devastating real-world impact of lead neurotoxicity is exemplified by the Zamfara lead poisoning outbreak in Nigeria (Acharya, 2024). Since 2010, severe lead pollution from artisanal gold mining has resulted in the deaths of more than 400 children and left thousands with chronic exposure requiring urgent medical care. This public health crisis highlights the critical need for long-term, effective neuroprotective measures especially in vulnerable, underserved communities (Akinwumi *et al.*, 2023). In addition, the sustained bioaccumulation of lead complicates treatment approaches and underscores the importance of early preventive and therapeutic strategies (Singh *et al.*, 2024).

1.3 AIM OF STUDY

The aim of the study was to investigate the activity of aqueous *Tetrapleura tetraptera* fruit extract on lead acetate induced cerebral toxicity in adult Wistar rats.

1.4 SPECIFIC OBJECTIVES

The specific objectives of the study were to:

- i. Investigate the qualitative phytochemical constituents of the aqueous *Tetrapleura tetraptera* fruit extract.
- ii. Investigate the activity of aqueous *Tetrapleura tetraptera* fruit extract on the brain and body weight changes in rats treated with or without lead acetate.
- iii. Investigate the activity of aqueous *Tetrapleura tetraptera* fruit extract on the neurobehavioral activities (Open Field Test (OFT), Tail Suspension Test (TST)) in rats treated with or without lead acetate.
- iv. Evaluate the activity of aqueous *Tetrapleura tetraptera* fruit extract on; the antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) activity, lipid peroxidation (malondialdehyde concentration), and concentration of lead in the cerebrum of rats treated with or without lead acetate.
- v. Investigate the activity of aqueous *Tetrapleura tetraptera* fruit extract on the histology of the cerebrum in rats treated with or without lead acetate.
- vi. Ascertain the *in-silico* activity of aqueous *Tetrapleura tetraptera* fruit extract on apoptotic, oxidative and inflammatory markers.

1.5 JUSTIFICATION OF STUDY

Given that oxidative stress is a well-established mechanism through which various neurotoxic agents induce cerebral damage, the therapeutic exploration of antioxidant-rich compounds has become increasingly vital (Li *et al.*, 2021). Extensive research underscores the essential role of antioxidants in neutralizing reactive oxygen species (ROS) and preserving neuronal integrity (Akbari *et al.*, 2022). While the body naturally generates its own antioxidants, introducing additional sources from outside can strengthen its overall antioxidant defence and bolster the

function of internal (endogenous) compounds (Engwa, *et al*, 2022). Medicinal plants have emerged as prominent external sources of antioxidants, attracting significant interest for their potent free radical scavenging abilities (Nwozo *et al.*, 2023). Their natural composition, affordability, and typically low incidence of side effects make them particularly attractive for therapeutic use and as candidates in pharmaceutical research and drug development (Singh and Gohil, 2024).

Tetrapleura tetraptera, a medicinal plant traditionally used across West Africa, has garnered increasing scientific interest due to its abundance of bioactive compounds including flavonoids, phenols, and alkaloids (Sakodie *et al.*, 2020; Mbatchou *et al.*, 2022). These constituents exhibit potent antioxidant, anti-inflammatory, and anti-apoptotic properties, which are particularly relevant to the protection of the cerebrum. Some studies propose that *Tetrapleura tetraptera* can mitigate oxidative stress and neuroinflammation in cerebral tissues, thereby supporting cognitive function and offering neuroprotection against degenerative changes (Lawal *et al.*, 2025). Despite promising evidence, there remains a significant gap in the literature concerning the specific protective effects of *Tetrapleura tetraptera* against lead induced damage in the cerebrum. Limited research has investigated its impact on neurobehavioral outcomes, such as memory and learning, in animal models exposed to lead. By addressing this gap in the literature, this study can offer new insights into the therapeutic potential of *Tetrapleura tetraptera*, supporting its possible use as a natural intervention to prevent or mitigate the effects of lead induced damage in the cerebrum. Such findings could inform future public health strategies and clinical interventions, especially in regions where lead exposure remains a pressing concern and access to conventional neuroprotective drugs is limited.

CHAPTER TWO

LITERATURE REVIEW

2.1 THE BRAIN

The brain is a vital and complex organ that serves as a central component of the central nervous system (CNS), securely housed and shielded within the bony structure of the skull, also referred to as the neurocranium (Bhushan *et al.*, 2022). Structurally, the brain is organized into three major and functionally distinct regions: the cerebrum, which is responsible for higher cognitive functions; the cerebellum, which coordinates motor control and balance; and the brainstem, which governs many of the body's automatic and life sustaining processes (Bhushan *et al.*, 2022). Embryologically, these three major brain regions originate from the development and differentiation of three primary brain vesicles during early neural development:

- i. The Forebrain (prosencephalon), which consists of the;
 - Telencephalon: becomes the paired cerebral hemispheres (cerebrum) and the neocortex.
 - Diencephalon: the “in-between” region of the brain forming the thalamus, hypothalamus, and metathalamus (Carstens and Sarnat, 2023).
- ii. The Midbrain (Mesencephalon), differentiates into the tectum, tegmentum, and cerebral peduncles (Carstens and Sarnat, 2023; Chakravarthi *et al.*, 2025).
- iii. The Hindbrain (Rhombencephalon), includes;
 - The Metencephalon: gives rise to the pons and cerebellum.
 - The Myelencephalon: becomes the medulla oblongata.

These vesicles are responsible for functions like cognition, sensory relay, motor coordination, and vital autonomic functions (Bhushan *et al.*, 2022; Carstens and Sarnat, 2023).

2.2 THE CEREBRUM

The cerebrum is part of the central nervous system situated in the cranial cavity and connected to the cerebellum through the brainstem (Ekinici *et al.*, 2008). The median longitudinal fissure separates the cerebrum into two cerebral hemispheres attached with each other through a bundle of white fibres called the corpus callosum (Bui, 2019). Each cerebral hemisphere has a cortical grey matter, a white matter beneath the grey matter, and a collection of subcortical neuronal masses within the white matter basal ganglia (Ekinici *et al.*, 2008; Bui, 2019). Each cerebral hemisphere has a cavity which is called the lateral ventricle. The lateral ventricles communicate with the third ventricle through the anterior interventricular foramen (Ekinici *et al.*, 2008). The ventricles contain cerebrospinal fluid, a nutrient material for the brain tissue (Bui, 2019).

2.2.1 External Features

Each cerebral hemisphere has three borders, three surfaces, three poles, and six lobes. External surfaces of the cerebrum show elevations known as gyri and depressions known as sulci (Bui, 2019; Jin *et al.*, 2016; Ribas, 2010)

2.2.1.1 Borders

The superomedial border extends from the frontal pole to the occipital pole; it separates the superomedial surface from the medial surface (Jin *et al.*, 2016). The inferolateral margin of cerebral hemisphere separates the superolateral surface of the cerebral hemisphere from the inferior surface. The part of the inferolateral border anterior to the stem of the lateral sulcus is named the superciliary border (Singh, 2014). The inferomedial border separates the medial surface from the inferior surface. It is interrupted by the brainstem and the diencephalon in the anterior medial orbital border and posterior medial occipital border (Singh, 2014; Jin *et al.*, 2016).

2.2.1.2 Surfaces

Each cerebral hemisphere presents three principal surfaces: the superolateral, medial, and inferior surfaces (Bui, 2019). The superolateral surface is convex, directed outward toward the cranial vault, and is the most expansive of the three (Singh, 2014). The medial surface appears flattened and faces the opposing hemisphere. The intervening space between the two medial surfaces is termed the longitudinal cerebral fissure and houses the falx cerebri. A defining feature of this surface is the C-shaped section of the corpus callosum (Jin *et al.*, 2016). The inferior surface is oriented toward the base of the cranial cavity. It is bisected by the stem of the lateral sulcus into two regions: the orbital and tentorial parts. The orbital part, situated anteriorly, overlays the orbital plate and anterior cranial fossa. In contrast, the tentorial part occupies the posterior region, resting atop the tentorium cerebelli (Ribas, 2010).

2.2.1.3 Poles

Each cerebral hemisphere comprises three distinct poles: the frontal, temporal, and occipital poles (Bui, 2019). The frontal pole, positioned at the anterior extremity of the hemisphere, aligns with the superciliary arch (Bui, 2019). The occipital pole marks the posterior boundary of the hemisphere, whereas the temporal pole represents the foremost part of the temporal lobe. Notably, the temporal pole resides in the anterior region of the middle cranial fossa (Singh, 2014; Jin *et al.*, 2016)

2.2.1.4 Lobes

The cerebral hemisphere is anatomically divided into five lobes; the frontal, parietal, temporal, occipital, and insular lobes (Bui, 2019). This division is established using three consistent sulci namely the central, lateral, and parieto-occipital sulci; and two conceptual reference lines. The first imaginary line runs vertically from the parieto-occipital sulcus to the pre-occipital notch (Bui, 2019). The second is a horizontal line that extends from the posterior ramus of the lateral sulcus and intersects with the first vertical line (Bui, 2019). Together, these sulci and reference

lines demarcate the five lobes of the hemisphere (Bui, 2019; Jin *et al.*, 2016; Nishikuni and Ribas, 2013).

The frontal lobe lies anterior to the central sulcus and superior to the lateral sulcus (Singh, 2014). The parietal lobe is bordered anteriorly by the central sulcus, posteriorly by the vertical imaginary line connecting the parieto-occipital sulcus with the pre-occipital notch, and inferiorly by the horizontal imaginary line drawn from the posterior ramus of the lateral sulcus (Bui, 2019; Jin *et al.*, 2016; Nishikuni and Ribas, 2013).

The temporal lobe is bounded superiorly by the posterior ramus of the lateral sulcus and the second imaginary line, while its inferior border is defined by the inferolateral margin of the cerebral hemisphere (Bui, 2019; Jin *et al.*, 2016; Nishikuni and Ribas, 2013). The occipital lobe occupies the region posterior to the vertical line joining the parieto-occipital sulcus to the pre-occipital notch (Javed *et al.*, 2023; Rehman and Al Khalili, 2019).

The insula, also referred to as the central lobe or the island of Reil, is a concealed segment of the cerebral cortex that lies within the floor of the lateral sulcus (Jin *et al.*, 2016). It is covered by expanded cortical regions of the frontal, parietal, and temporal lobes, which collectively form the frontal, parietal, and temporal opercula respectively (Javed *et al.*, 2023). The limen insulae represents its apex and maintains continuity with the anterior perforated substance (Guenot *et al.*, 2019). The circular sulcus surrounds the insula on all sides except at the limen (Javed *et al.*, 2023; Rehman and Al Khalili, 2019). Internally, the sulcus centralis divides the insula into anterior and posterior segments. The anterior region contains 3 - 4 short gyri known as gyri brevia, while the posterior region holds 1 - 2 elongated gyri referred to as gyri longa (Jin *et al.*, 2016).

The flat medial surface of each cerebral hemisphere presents the C-shaped bundle of white fibres known as the corpus callosum (Ribas, 2010). It presents from anterior to posterior the

rostrum, genu, body, and splenium (Ribas, 2010). Genu is the sharp anterior bend of the corpus callosum that is present 4cm posterior to the frontal pole (Nishikuni and Ribas, 2013). Splenium is a comparatively larger part positioned 6cm anterior to the occipital pole (Ribas, 2010). The rostrum continues downward as lamina terminalis that makes the anterior border of the third ventricle (Rehman and Al Khalili, 2019). Lamina terminalis is a thin sheet of grey matter which extends from the rostrum to the upper surface of the optic chiasma; in addition, it presents the cranial end of the primitive neural tube (Rehman and Al Khalili, 2019). The convex superior surface of the corpus callosum forms the floor of the median longitudinal fissure; the surface is covered by a thin sheet of grey matter known as indusium griseum (Ribas, 2010; Rehman and Al Khalili, 2019)

2.2.1.5 Histological Structure

The cerebral cortex contains several distinct types of neurons, including pyramidal cells, stellate (granule) cells, horizontal cells of Cajal, fusiform cells, and Martinotti cells (Garcia-Lopez, *et al.*, 2010; García-Cabezas *et al.*, 2020).

Pyramidal cells are characterized by their pyramid-shaped bodies, with apices directed toward the external cortical surface. Their axons emerge from the centre of the basal surface and extend into the white matter as projection fibres, while dendrites arise from the angular points of the cell body (Chauhan *et al.*, 2021; Amunts and Zilles, 2015). Based on their dimensions, pyramidal cells are further classified into small, medium, and large categories (Garcia-Lopez *et al.*, 2010; García-Cabezas *et al.*, 2020).

Stellate or granule cells are star-shaped and measure approximately 8 µm in diameter (Chauhan *et al.*, 2021). Due to their appearance under Nissl staining, they are referred to as granule cells. These neurons possess short axons and multiple dendritic processes. In certain cortical regions,

dense aggregations of granule cells produce a cloudy appearance referred to as the koniocortex (Garcia-Lopez *et al.*, 2010; García-Cabezas *et al.*, 2020; Chauhan *et al.*, 2021).

The horizontal cells of Cajal are fusiform in shape and oriented horizontally within the most superficial cortical layer. Fusiform cells, also spindle-shaped, are arranged vertically and typically reside in the deeper layers of the cortex (Garcia-Lopez *et al.*, 2010; García-Cabezas *et al.*, 2020; Chauhan *et al.*, 2021).

Martinotti cells are small, multipolar neurons found throughout all layers of the cerebral cortex. They play a role in modulating cortical circuit activity and synaptic integration (Garcia-Lopez *et al.*, 2010; Chauhan *et al.*, 2021).

2.2.2 Laminar Organization in the Cerebral Cortex

Microscopically, the following horizontal organizations of cellular layers (laminae) are observed (Ribas, 2010; Chauhan *et al.*, 2021):

- Molecular (Plexiform) layer I; consists of apical dendritic tufts of the pyramidal neurons, horizontally organized axons, Cajal-Retzius cells and glial cells.
- External granular layer II; consist of mainly granular cells and small pyramidal cells.
- External pyramidal layer III; consists of small and medium-sized pyramidal cells. Layer III can be divided into most superficial IIIa, intermediate layer IIIb and deep layer IIIc. Horizontally running myelinated fibres makes the band of Baillarger stripe that can be appreciated between layer IIIc and IV in some isocortical regions.
- Internal granular layer IV; contains small round granular cells (spiny stellate cells, spiny interneurons) and small pyramidal cells. Layer IV shows the band of Baillarger on the outer side conspicuous in the visual cortex; the band is named as the stripe of Gennari or of Vicq d'Azur.

- Internal pyramidal layer V; contains the largest pyramidal cells. Axons of the pyramidal cells leave the cortex and reach subcortical areas. Layer V is subdivided into Va, Vb and Vc sublayers. The inner band of Baillarger is observed in layer V in myelin-stained sections.
- Multiform (Polymorphous) layer VI: Excitatory cells like spiny stellate cells, pyramidal cells, inverted pyramidal neurons, bipolar/fusiform cells, and odd-shaped cells are present in layer VI. It also contains inhibitory interneurons. This layer is also divided into sublayers VIa and VIb.

The homotypical cortex is the part of the cerebral cortex where all six layers are well defined. The heterotypic cortex is the part of the cerebral cortex where all six layers are not well defined. It is further subdivided into the granular and agranular cortex. In the granular cortex, granular layers are well defined while pyramidal layers are poorly defined; granular cortex is mostly seen in sensory areas. In the agranular cortex, granular layers are poorly defined while pyramidal layers are well defined. The agranular cortex is mostly seen in motor areas (Kandel *et al.*, 2021)

2.2.3 Cerebral Dysfunction

A cerebral dysfunction is a broad term that encompasses a wide range of conditions affecting the brain's ability to function normally which may be focal or global (NINDS, 2025). Focal and global processes can manifest as deficits or become foci for seizure activity. These processes may also affect subcortical systems, altering arousal (e.g., causing stupor or coma) or integration of thought (e.g., causing delirium) (NINDS, 2025).

Focal dysfunction usually results from structural abnormalities (e.g. tumours, abscesses, stroke, trauma, malformations, gliosis, demyelination). Manifestations depend on the lesion's location, size, and development rate. Lesions that are < 2 cm in diameter or that develop very slowly

may be asymptomatic. Larger lesions, rapidly developing lesions (over weeks or months rather than years), and lesions that simultaneously affect both hemispheres are more likely to become symptomatic. Focal lesions in white matter can interrupt the connectivity between brain areas and cause the disconnection syndrome (inability to do a task that requires coordinated activity of ≥ 2 brain regions, despite retention of basic functions of each region) (Boes & Thiebaut de Schotten, 2022).

Global dysfunction is caused by exposure to toxins such as lead, metabolic disorders (including hypoxia and ischemia (commonly), diffuse inflammation, vasculopathy, major trauma, disseminated cancer, degenerative disorders. It may also result from disorders that occur in a specific area of the brain (e.g., abscesses, tumours, trauma) if they increase intracranial pressure or cause herniation. These disorders affect multiple dimensions of cerebral function. Recovery from brain injury depends on the plasticity of the remaining cerebrum and redundancy (Huang, 2023).

2.3 LEAD

Lead (Pb) is a dense, bluish-grey heavy metal with an atomic weight of 207.2u and a density of 11.34 g/cm³. It melts at 621.43°F and is known for its malleability, ductility, and ability to form both inorganic salts and organo-lead compounds. Lead is chemically stable and nondegradable, making it a persistent environmental contaminant (Khalef *et al.*, 2022). Inorganic lead, commonly found in old paint, soil, dust, and plumbing, accumulates in the environment and the human body (WHO, 2023). Organic forms, such as tetraethyllead (formerly used in gasoline), are more bioavailable and neurotoxic due to their lipophilicity. Lead constitutes about 0.002% of the Earth's crust but is now widespread due to industrial activities like mining, smelting, and battery production (Raj and Das, 2023).

Lead exposure typically occurs through inhalation or ingestion, with workers in high-risk industries facing occupational hazards, and the general population vulnerable to environmental exposure from polluted air, contaminated food, household dust or certain products (World Health Organization, 2024). Once inside the body, lead distributes across the bloodstream, soft tissues, and bones where it can reside for decades. Although the half-life of lead in blood is approximately 30 days, bones act as long-term storage sites. Biologically, lead interferes with multiple physiological systems (Collin *et al.*, 2022). It disrupts heme production, damages kidney function, and significantly impacts the nervous system, posing heightened risks to children due to the sensitivity of their developing brains. Long-term exposure has been linked to cognitive decline, anaemia, high blood pressure, and reproductive health issues. Because of its persistence and far-reaching biological effects, lead continues to be a central concern in both environmental science and neurotoxicology research (Schneider, 2023).

2.3.1 Human Exposure and Metabolism

Lead enters the human body primarily through three pathways;

- Ingestion of contaminated food, water, or soil
- Inhalation of lead-laden air;
- Absorption through the skin of organo-lead compounds (Collin *et al.*, 2022).

Among these, ingestion is the dominant route, with lead contaminated dust, soil, water, and food contributing to gastrointestinal exposure. According to a pivotal 2012 European Food Safety Authority survey, grain products contributed 16.1% of dietary lead intake, followed by dairy at 10.4%, non-alcoholic beverages at 10.2%, and vegetables at 8.4% (European Food Safety Authority [EFSA] & Contam Panel, 2012).

Occupational exposure remains a major concern. Workers in industries like battery recycling, smelting, pigment or glass production, and paint removal risk inhaling lead fumes or contacting

contaminated dust, especially when safety measures are inadequate (Kitui, 2022). Reports show these environments can elevate blood lead levels well beyond those seen in the general population. Additionally, coal combustion by power stations and industrial boilers releases lead particles into the air, which can settle on crops and soil, contributing further to environmental exposure (Odubo & Kosoe, 2024).

Once ingested, lead is primarily absorbed in the small intestine, particularly the duodenum, through both DMT1-dependent and independent mechanisms (Patwa *et al.*, 2022). Uptake efficiency depends on particle solubility, age, nutritional state, and competing minerals like calcium and iron. Children and pregnant women absorb as much as 40 - 70% of ingested lead, compared to just 5 - 15% in well-nourished adults (Wapnir, 2024). In the bloodstream, about 99% of lead binds to red blood cells, distributing to critical organs including the brain, kidneys, liver, and aorta. Meanwhile, over 90% of the body's lead is stored in bones, where it can remain for decades, slowly leaching back into circulation, especially during physiological changes like pregnancy or osteoporosis (Collin *et al.*, 2022).

Unlike many other toxins, lead isn't metabolized. It causes harm by displacing essential metals (e.g., calcium, zinc, iron) at enzyme sites and membrane transporters (Aslam *et al.*, 2021). It also binds to sulfhydryl groups, disrupting antioxidant defences, mitochondrial function, and triggering oxidative stress (Witkowska *et al.*, 2021). This leads to widespread biological effects: inhibited neurotransmitter release, impaired heme synthesis, and reduced kidney filtration (Penticoff and Fortin, 2023).

Lead is eliminated slowly, mainly through urine (70 - 80%), with smaller amounts excreted via faeces, sweat, hair, and nails (Collin *et al.*, 2022). Because detoxification is inefficient and most lead is sequestered in bone, even a single high exposure event can have lasting health consequences.

2.3.2 Mechanism of Lead Induced Neurotoxicity

Lead's neurotoxic effects arise from its ability to cross the blood–brain barrier, primarily by hijacking calcium transport channels, allowing it to accumulate in neurons and glial cells (Gudadhe *et al.*, 2024). This accumulation disrupts synapse development and plasticity, contributing to cognitive impairments and behavioural problems, especially in children (Ramírez Ortega *et al.*, 2021). Additionally, lead can interact directly with DNA, interfering with enzymes responsible for replication and repair. This disruption can result in DNA strand breaks and mutations, playing a role in its long-term carcinogenic and developmental toxicity. These overlapping mechanisms illustrate the complex web of lead induced damage, reinforcing why even minimal exposure remains a major concern in environmental health and a central issue in public policy discussions (Cuomo *et al.*, 2022).

2.3.2.1 Oxidative Stress

Lead's primary mechanism of toxicity involves the induction of oxidative stress, which occurs when the production of reactive oxygen species (ROS) surpasses the body's antioxidant defences (Borda, 2024). This imbalance is driven by two interconnected processes; the increased formation of ROS including superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals; and the simultaneous depletion or inactivation of internal antioxidants like glutathione and protective enzymes (Huchzermeyer *et al.*, 2022).

At the molecular level, lead's tendency to bind covalently to sulfhydryl ($-SH$) groups renders antioxidant enzymes particularly vulnerable (Jomova *et al.*, 2025). By attaching to cysteine residues in critical enzymes such as glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT), lead effectively disables their active sites crippling the body's key defences against ROS (Sule *et al.*, 2022).

In addition, lead inhibits δ -aminolaevulinic acid dehydratase (δ -ALAD), a zinc-dependent enzyme essential to heme biosynthesis (Zeeshan *et al.*, 2022). This not only disrupts haemoglobin production but also causes the build-up of δ -aminolaevulinic acid, a pro-oxidant that further enhances ROS generation (Huang *et al.*, 2020).

Lead also replaces essential metal cofactors, particularly zinc, from their native sites on antioxidant enzymes, further destabilizing their structure and impairing their function (Witkowska *et al.*, 2021). This interference hampers the conversion of superoxide to hydrogen peroxide via superoxide dismutase (SOD), and the subsequent detoxification of hydrogen peroxide (H_2O_2) to water by catalase (CAT) and glutathione peroxidase (GPx), fuelling a cycle of unchecked oxidative damage to lipids, proteins, and DNA (Jomova *et al.*, 2025).

Over time, this persistent oxidative stress impairs cellular function and survival, especially in red blood cells, neurons, and kidney cells; tissues highly sensitive to disruptions in redox balance (Gwozdzinski *et al.*, 2021).

2.3.2.2 Apoptosis

Lead triggers programmed cell death primarily by disrupting mitochondrial integrity and activating the intrinsic apoptotic cascade (Han *et al.*, 2021). When lead accumulates within cells, it promotes the opening of the mitochondrial permeability transition pore (mPTP), collapsing the inner membrane potential and allowing cytochrome C to escape into the cytosol (Asadi *et al.*, 2022). Once in the cytoplasm, cytochrome C binds to apoptotic protease activating factor-1 (APAF-1) and recruits procaspase-9 to form the apoptosome, which in turn cleaves and activates executioner caspases such as caspase-3 (Asadi *et al.*, 2022). Activated caspase-3 then cleaves a host of downstream substrates including structural proteins, DNA repair enzymes, and cell-cycle regulators leading to the characteristic morphological and biochemical hallmarks of apoptosis (Asadi *et al.*, 2022). A sustained rise in intracellular Ca^{2+}

concentration is another critical trigger of lead mediated apoptosis. Lead disrupts calcium homeostasis by inducing both extracellular Ca^{2+} influx and mitochondrial Ca^{2+} release, thereby overloading the cell's buffering capacity (Panda *et al.*, 2021). This calcium surge further promotes mPTP opening and amplifies reactive oxygen species (ROS) production, creating a vicious cycle of oxidative damage and mitochondrial injury. In neuronal cells, excessive glutamate release exacerbated by mitochondrial dysfunction adds an excitotoxic component that accelerates calcium-driven apoptotic signalling. Beyond mitochondria, lead directly inactivates key antioxidant enzymes by binding to their sulfhydryl groups, depleting cellular glutathione reserves and impairing detoxification pathways (Jomova *et al.*, 2025). This loss of antioxidant defence allows lipid peroxidation, protein carbonylation, and DNA strand breaks to proceed unchecked, sensitizing cells to apoptotic stimuli (Di Carlo and Sorrentino, 2024). In rodent models, lead exposure upregulates pro-apoptotic BAX and down-regulates anti-apoptotic BCL-2, tipping the balance toward mitochondrial outer membrane permeabilization and cell death. Finally, chronic lead accumulation in tissues ensures a persistent apoptotic threat (Qu and Zheng, 2024). Even after external exposure ends, lead stored in mitochondria and other organelles continues to disrupt ionic gradients and redox status, sporadically triggering apoptosis in susceptible cell populations such as renal tubular cells, neurons, and hematopoietic precursors. Clinically, this underlies the slow, progressive organ damage seen in lead poisoned individuals and highlights why interventions must both remove circulating lead and address its long-term cellular reservoirs (Jomova *et al.*, 2025; Qu and Zheng, 2024).

2.3.2.3 The Ionic Mimicry Mechanism

Calcium ions (Ca^{2+}) are indispensable for neuronal communication, entering presynaptic and postsynaptic compartments through voltage-gated calcium channels upon membrane depolarization and triggering neurotransmitter release and downstream signalling cascades (Dolphin, 2021). One of the critical outcomes of Ca^{2+} influx is the activation of the cAMP

response element-binding protein (CREB) family of transcription factors, which promotes the expression of brain-derived neurotrophic factor (BDNF), a neurotrophin essential for dendritic growth, synaptogenesis, and long-term potentiation underlying learning and memory (Virgolini and Aschner, 2021; Gudadhe *et al.*, 2024). However, lead subverts this process through “molecular mimicry,” exploiting its similar ionic radius and charge to masquerade as Ca^{2+} and gain entry into neurons via the same L-type and other voltage-sensitive calcium channels (Florea *et al.*, 2013). Once inside the synaptic terminal, lead ions bind preferentially to calcium sensor domains and effector proteins, such as those regulating vesicle fusion, thereby displacing Ca^{2+} and effectively “locking out” the genuine ion (Tamagno & Freeman, 2025). This deceptive blockade prevents the Ca^{2+} -dependent phosphorylation cascades necessary for CREB activation, leading to significant downregulation of BDNF gene transcription and reduced secretion of mature BDNF protein (Dolphin, 2021; Gudadhe *et al.*, 2024). As extracellular BDNF levels fall, Tropomyosin receptor kinase B (TrkB) receptor signalling is compromised, undermining both acute modulation of synaptic efficacy and the long-term structural adaptations that sustain cognitive functions (Bazzari and Bazzari, 2022). In animal models, developmental lead exposure correlates with impaired long-term potentiation, diminished dendritic arborization, and behavioural deficits in memory tasks, reflecting the synaptic degeneration wrought by chronic interference with Ca^{2+} -BDNF pathways (Dolphin, 2021). Moreover, when the severity or duration of lead infiltration outpaces the cell’s capacity for repair through mitochondrial calcium buffering or endoplasmic-reticulum release regulation, irreversible damage ensues, including activation of apoptotic pathways and cytoskeletal breakdown that mature neurons cannot readily replace (Gudadhe *et al.*, 2024). Consequently, the “Trojan horse” strategy by which lead hijacks calcium signalling at synapses not only disrupts moment-to-moment neurotransmission but also erodes the neurotrophic support system critical for lifelong brain health (Collin *et al.*, 2022; Gudadhe *et al.*, 2024).

2.3.3 Effects of Lead on the Cerebrum

Historical records show that the toxic effects of lead in adults were recognized long before childhood lead poisoning was first described, tracing back to as early as 2000 BC (Needleman, 1999). In adults, lead exposure can damage nerves associated with sensory function and motor control, contributing to the development of neurodegenerative conditions such as Alzheimer's and Parkinson's diseases (Monnet-Tschudi *et al.*, 2006). It has also been linked to impaired hearing and vision, schizophrenia, and a decline in cognitive abilities, although the specific affected areas of the brain are still being studied (Shi *et al.*, 2025).

The absorption of lead through the gastrointestinal tract varies depending on both individual physiological traits and the chemical nature of the ingested substance. Children face a notably higher risk due to their frequent hand-to-mouth behaviours and tendency to play in contaminated soil as they can absorb up to 50% of an ingested dose of water-soluble lead (Ziegler *et al.*, 1978; World Health Organization, 2010). In contrast, the uptake of lead from soil is significantly lower than that from dissolved forms (Dignam *et al.*, 2019; Drexler and Brattin, 2007). Experimental studies reinforce this age-related difference: juvenile Rhesus monkeys absorbed 38% of ingested lead compared to just 26% in adult females (Pounds *et al.*, 1978). Similarly, rat pups have been shown to absorb approximately 40 - 50 times more lead than their adult counterparts highlighting the heightened vulnerability of younger organisms to gastrointestinal lead uptake (Aungst and Fung, 1981; Forbes and Reina, 1972; Kostial *et al.*, 1978). According to Centres for Disease Control and Prevention (CDC, 2005), between 1999 and 2002, an estimated 310,000 children aged 1 - 5 were at risk of harmful lead exposure.

A strong body of both experimental and clinical evidence shows that lead exerts lasting negative effects on brain function, forming the basis for cognitive deficits observed in affected children (Marshall *et al.*, 2021). Of special concern is lead's impact on glutamatergic neurotransmission, which plays a vital role in brain development, synaptic plasticity, learning,

memory, and mood regulation (Tamagno and Freeman, 2025). Furthermore, disruptions to dopaminergic signalling, essential for motor coordination, attention, memory, and executive processing (Brown, 1997) may lead to a wide range of behavioural disorders and cognitive impairments. Sciarillo *et al* (1992) investigated the effects of early lead exposure on socio-emotional development in children aged 4 to 5 years. Their findings indicated a rise in various behavioural issues among lead-exposed children, with aggression notably increasing at blood lead levels of 15 µg/dL. In a separate study, Mendelsohn *et al* (1998) examined children between 12 and 36 months of age, an age group considered too young for academic failure. Using the Bayley Scales of Infant Development (BSID) to assess emotional and social functioning, the study revealed that children with blood lead levels of 25 µg/dL scored significantly lower than non-exposed peers, particularly in emotional regulation and orientation/engagement domains.

Recent analyses of cross-sectional and mixed study designs have found that blood lead levels are linked to measurable declines in intelligence; specifically, for every 10 µg/dL increase in blood lead levels, an average IQ reduction of 1 to 5 points has been observed (Dignam *et al.*, 2019). Thus far, researchers have been unable to identify a definitive exposure level beneath which lead's effects on cognition are negligible (Pocock *et al*, 1994). In fact, pooled data from additional studies provide compelling evidence that cognitive impairment in children may occur even at blood lead levels below 10 µg/dL, and potentially as low as 5 µg/dL (Hornung, *et al.*, 2019). In a study by Trope *et al* (1998), two male cousins residing in the same household were assessed to compare the cognitive effects of lead exposure. One subject, a 10-year-old boy with elevated blood lead levels, demonstrated significant neuropsychological impairments, including challenges in reading, writing, and arithmetic. Additionally, he exhibited difficulties with language processing and attention control. In contrast, his 9-year-old cousin, who had not been exposed to lead, showed no such deficits. Interestingly, while high-resolution magnetic

resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) revealed structurally normal brains in both children, the lead-exposed child displayed pronounced abnormalities in cerebral metabolite profiles.

Early longitudinal studies on children exposed to lead have demonstrated that neuropsychological deficits, particularly declines in IQ can persist well into adulthood. One notable investigation by Stokes *et al* (1998) followed young adults (average age 24.3) who, as children, lived near a lead smelter that lacked emission control systems. In 1974, the average blood lead level for children in that area was 50 µg/dL, and 39.6 µg/dL in 1975. Although detailed blood lead levels were available for only 25% of participants (mean: 49.3 µg/dL), evaluation in adulthood showed that even after levels had normalized, the exposed group performed significantly worse than controls on cognitive tests, as well as tasks assessing fine motor skills and postural stability. Weisskopf *et al* (2007) found that low-level cumulative exposure to lead in nonoccupational settings can adversely affect cognitive function, particularly in the visuospatial/visuomotor domain.

In a separate long-term follow-up, White *et al* (1993) assessed neuropsychological outcomes in adults who had been hospitalized for lead poisoning before the age of 4. Testing approximately 50 years after the initial exposure, the study found that individuals in the lead-exposed group exhibited persistent impairments in abstract reasoning, cognitive flexibility, verbal memory and fluency, and fine motor speed, highlighting the enduring legacy of early-life lead toxicity.

Numerous studies have demonstrated that the cerebrum is highly vulnerable to toxicants such as lead. For example, Al-Khafaf and colleagues investigated the effects of lead exposure in rats administered 30 mg/kg daily for thirty days. Their findings revealed significant histopathological damage, including perivascular cuffing by mononuclear inflammatory cells,

vascular congestion, and degeneration of cortical neurons, accompanied by multifocal encephalomalacia. Additional alterations included the formation of glial nodules, neuronophagia, and haemorrhaging within cerebral tissue. Biochemical analyses showed a notable decline in the activity of key antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), alongside elevated levels of malondialdehyde (MDA), a marker of lipid peroxidation. These findings collectively suggest that the observed cerebral damage was primarily driven by oxidative stress (Al-Khafaf, 2021).

Studies also report marked histological damage in the cerebrum of rats administered lead acetate at a dosage of 100 mg/kg over a 28-day period was reported. The findings reveal pronounced degeneration of pyramidal neurons within the cerebral cortex. Biochemical analysis links these structural abnormalities to a reduction in antioxidant enzyme activity including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) alongside an elevation in malondialdehyde (MDA) levels, a marker of lipid peroxidation. Together, these changes point to oxidative stress as the primary mechanism underlying the observed neurodegeneration (Enogieru & Egbon, 2022; Enogieru, & Momodu, 2022)

Ramesh and colleagues reported significant histopathological changes in the cerebrum of rats exposed to lead at a dosage of 30 mg/kg over a 28-day period. Observations included severe vascular congestion, morphological alterations to neuronal cell bodies, and localized areas of haemorrhage. The researchers attributed these pathological findings to lead-induced oxidative stress, suggesting that this mechanism may underlie the observed cerebral damage in the exposed animals (Ramesh *et al*, 2019)

Sidhu and Nehru (2004) reported significant histological abnormalities in the cerebrum of rats exposed to lead at a dose of 50 mg/kg over an eight-week period. The observed changes included neuronal degeneration, disorganization of the cerebral cortical layers, and cellular

hypertrophy. These alterations were attributed to an elevated generation of reactive oxygen species (ROS), which disrupted the balance between oxidants and antioxidants, ultimately resulting in oxidative stress within cerebral tissues.

Enogieru and Williams reported a disorganized and reduced number of cells in the outer pyramidal layer with vacuolations in the cerebral cortex of rats exposed to lead at a dose of 100 mg/kg for twenty-eight days. The authors attributed these histological alterations to oxidative stress (decreased SOD, CAT, GPx, and increased MDA, NO) as the mechanism of action (Enogieru & Williams, 2024).

Nuhu and co-authors documented notable histopathological changes in the cerebral cortex of rats exposed to lead at a concentration of 500 ppm over a 21-day period. Their findings revealed degeneration of both cortical and pyramidal neurons, which appeared shrunken and irregularly dispersed throughout the cortical tissue. The authors attributed these neurotoxic effects to lead's capacity to displace calcium ions within the cortex, thereby disrupting calcium-dependent cellular processes essential for neuronal structure and function (Nuhu *et al.*, 2021)

Huang and his team reported notable histological changes in the cerebral cortex of guinea pigs subjected to six months of lead exposure through drinking water containing 0.2% lead. Their analysis revealed a loss of Doublecortin-immunoreactive (DCX⁺) cells within the cortical regions, a finding indicative of impaired neurogenesis and disrupted neuronal maturation (Huang, 2012).

2.3.4 Current Treatment Options for Lead Induced Cerebral Dysfunction

Acute poisoning, especially with encephalopathy, requires immediate treatment. Chelation therapy is recommended if blood lead level is 45µg/dL or higher. For blood levels between 20µg/dL and 45µg/dL, treatment is indicated if the child is symptomatic. Asymptomatic children with blood levels below 20µg/dL require long-term neurodevelopmental follow-up,

and counselling is required. In all cases, immediate removal of the source of lead exposure is essential (Hon *et al.*, 2017).

In the case of foreign body ingestion (e.g., a child who has swallowed a fishing weight too large to exit the stomach), approximately three days should be allowed to see if the object will pass through. However, in children the absorption of lead through the gut is considerably greater than it is for adults, and toxic levels can be achieved very quickly. If gastroscopic or colonoscopic removal is possible, this should be performed sooner rather than later (Gustavsson & Gerhardsson, 2005)

Severe lead poisoning (levels $>60\mu\text{g/dL}$) due to acute ingestion may require (Schaidler, 2012)

- Airway maintenance.
- Management of coma and seizures.
- Intravenous (IV) drip of normal saline.
- Orogastric or nasogastric catheter and irrigation

Parenteral chelators such as calcium disodium edetate given intramuscularly (IM) or IV. There is a growing trend to administer it by slow IV drip. Oral chelation therapy is an option sometimes used for mild-to-moderate poisoning. Chelators work by forming a tight chemical bond with heavy metals, enabling them to be excreted. It is often employed at levels of 45-60 $\mu\text{g/dL}$. For mild lead poisoning ($<45\ \mu\text{g/dL}$) it may be sufficient to detect the source of the exposure, remove the patient from it and monitor the clinical status (Needleman, 2004).

The use of herbs and medicinal plants in the treatment of lead poisoning has gained increasing attention due to their natural antioxidant properties, low toxicity, chelating ability, and potential to mitigate the neurotoxic effects of heavy metals (Shivhare *et al.*, 2024). Conventional chelation therapies such as EDTA (ethylenediaminetetraacetic acid) and DMSA (dimercaptosuccinic acid) are effective in reducing systemic lead levels but may carry adverse

effects and are often inaccessible in low-resource settings (Mukherjee *et al.*, 2023). As a result, plant-based therapies offer a promising complementary or alternative approach via their protective effects through multiple mechanisms. First, many herbs contain bioactive compounds such as flavonoids, phenols, and sulphur-containing molecules which act as natural chelators, binding lead ions and facilitating their excretion (Shivhare *et al.*, 2024). Second, these compounds enhance the activity of endogenous antioxidant enzymes, including catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx), which are typically suppressed in lead-exposed cerebral tissue. Third, certain herbs exhibit neuroprotective properties by stabilizing neuronal membranes, reducing lipid peroxidation, and preserving neurotransmitter balance.

Several plants have demonstrated efficacy in experimental models of lead toxicity. *Allium sativum* (garlic) has been shown to increase glutathione levels and reduce lead accumulation in brain tissue (Shivhare *et al.*, 2024). *Coriandrum sativum* (coriander) exhibits both chelating and antioxidant effects, while *Curcuma longa* (turmeric) reduces neuroinflammation and oxidative damage. *Silybum marianum* (milk thistle) supports hepatic detoxification and indirectly protects cerebral function. *Ginkgo biloba* enhances cerebral blood flow and mitigates cognitive deficits associated with lead exposure (Hore *et al.*, 2022). These findings suggest that herbal interventions may not only reduce systemic lead burden but also directly counteract the biochemical and functional impairments in the brain.

Plant-based therapies offer a multifaceted approach to cerebral dysfunction by restoring antioxidant balance, reducing oxidative stress, and protecting neural integrity (Bains *et al.*, 2025; Choudhury *et al.*, 2024). While further clinical validation is needed (Al-Ani *et al.*, 2022), the integration of herbal medicine into lead poisoning management protocols, particularly in regions with high environmental exposure and limited access to conventional treatment, represents a valuable and culturally relevant strategy (Mulugeta & Jabeen, 2022).

2.4 *Tetrapleura tetraptera*

Tetrapleura tetraptera, commonly known as Aidan fruit, is a flowering medicinal plant from the Leguminosae family. It is a deciduous tree native to West Africa and is widely cultivated in countries such as Ghana and Nigeria. Locally, it is referred to as “Prekese” in Twi (Ghana) and “Aridan” in Yoruba (Nigeria) (Mensah *et al.*, 2024; Okpala, 2016). The plant is valued for its rich nutritional profile, containing notable levels of ash, fibre, protein, carbohydrates, vitamins, and fats (Mensah *et al.*, 2024). Interestingly, the chemical composition of its fruit varies by part; seeds, pulp, and woody shells show significant differences in their carbohydrate and mineral content (Korang *et al.*, 2023).

In addition to its nutritional value, *Tetrapleura tetraptera* contains a diverse array of phytochemicals, including alkaloids, phenols, tannins, steroids, flavonoids, triterpenoids, and phytate (Okpala, 2016; Onda *et al.*, 2017). These naturally occurring bioactive compounds contribute to human health in ways that often surpass the effects of traditional micro- and macronutrients (Okpala, 2016). The abundance of these phytochemicals in *Tetrapleura tetraptera* underpins its broad pharmacological properties, which include antimicrobial, hypoglycaemic, neuromuscular, molluscicidal, trypanocidal, and anti-ulcer. Thanks to its distinctive aroma and wide-ranging medicinal properties, *Tetrapleura tetraptera* is commonly incorporated into a variety of culinary preparations. Beyond the kitchen, it serves as a flavouring agent in products such as perfumes, pomades, alcoholic beverages, and biscuits (Okpala, 2016). Extracts from *Tetrapleura tetraptera* exhibit notable antioxidant and anti-inflammatory activities, which contribute to their pathogen-inhibiting potential. The plant has been traditionally used in managing numerous health conditions, including convulsions, asthma, hypertension, rheumatic pain, leprosy, inflammation, and malaria fever relief (Onda *et al.*, 2017).

According to the World Health Organization, approximately 80% of the global population continues to rely on traditional remedies, including herbal medicine, for treating various illnesses (World Health Organization, 2019; Amo, 2013). This underscores the importance of understanding the chemical composition and therapeutic potential of medicinal plants like *Tetrapleura tetraptera*, and their role in promoting public health activities (Mensah *et al.*, 2024; Kuate *et al.*, 2015)

Given its rich chemical composition and diverse biological activities, *Tetrapleura tetraptera* shows considerable promise for application in ethnomedicine, pharmaceuticals, and industrial food production, particularly in the development of functional food products. Functional foods play a crucial role in enhancing human health by addressing malnutrition and mitigating the risks associated with both acute and chronic conditions, such as cardiovascular disease, cancer, and diabetes (Fekete *et al.*, 2025; Hossain *et al.*, 2025). These foods can act as dietary supplements, supplying essential nutrients that support overall well-being. With its established profile of bioactive phytochemicals, nutrient-dense constituents, and therapeutic properties, *Tetrapleura tetraptera* holds significant potential as a potent functional food, capable of eliciting targeted physiological effects within the human body.

2.4.1 Common Names of *Tetrapleura tetraptera*

Tetrapleura tetraptera is widely recognized across Nigeria, West Africa and beyond under various common or vernacular names, which reflect its botanical characteristics, culinary uses, and traditional medicinal applications in different cultures (Mensah *et al.*, 2024). These names are often derived from the plant's distinctive four-winged fruit pods, its rich aroma, and traditional use in soups, tonics, and herbal treatments. (Akinmoladun *et al.*, 2020).

Tetrapleura tetraptera naturally thrives in West African rainforests, attaining heights of 20 - 25 meters and canopy spreads ranging from 1.2 to 3 meters (Adesina *et al.*, 2016). The leaves are

sessile, sparsely pubescent, and borne on a common rachis measuring 15 - 30 cm, with a mild dorsal groove near the apex (Nwoba, 2015). The plant produces pinkish-cream flowers that transition to orange and are arranged in spikelike panicles measuring 5 - 20 cm, typically emerging from the upper leaf axils. Each flower is supported on a slender stalk and contains ten short stamens with glandular-tipped anthers (Nwoba, 2015)

Table 2.1: Common names for *Tetrapleura tetraptera* (Akinmoladun *et al.*, 2020)

Language/Region	Common Name
English	Aidan fruit/Four-sided fruit
Yoruba (Nigeria)	Aridan
Igbo (Nigeria)	Osakirisa / Oshosho
Twi (Ghana)	Prekese
Ga (Ghana)	Soup stick
Hausa (Nigeria/Ghana)	Daddawa-Baki
Efik (Nigeria)	Edeminang

2.4.2 Taxonomy and Description

Table 2.2: Taxonomic tree of *Tetrapleura tetraptera* (N'zebo *et al.*, 2018)

Rank	Classification
Domain	Eukaryota
Kingdom	Viridiplantae (green plants)
Phylum	Streptophyta
Subphylum	Streptophytina
Class	Magnoliopsida

Subclass	Rosids
Order	Fabales
Family	Fabaceae (Legume family)
Subfamily	Caesalpinioideae
Clade	Mimosoid clade
Tribe	Mimoseae
Genus	<i>Tetrapleura</i>
Species	<i>Tetrapleura tetraptera</i>

The fruits are distinctive, hanging persistently from sturdy 25 cm peduncles at the branch tips. They are 15 - 25 cm long, about 5 cm wide, and lined with four prominent, wing-like ridges each approximately 3 cm wide. The pods are dark purple-brown, glabrous, shiny, and slightly curved (Adesina *et al.*, 2016). Encased within are small, hard, black seeds, about 8mm long, which produce a characteristic rattling sound when shaken. These seeds are tightly embedded within the pod structure, lacking any natural dehiscence for dispersal (Adesina *et al.*, 2016).

The distinct anatomical parts of *Tetrapleura tetraptera* particularly the pod, seed, pulp, and woody shell, serve various culinary, medicinal, and industrial purposes (Mensah *et al*, 2024).

2.4.3 Conventional Uses of *Tetrapleura tetraptera*

Tetrapleura tetraptera is a rich source of antioxidants with a high concentration of vitamins, minerals, and phytochemicals. It also has anti-inflammatory properties. Its leaves, bark, and, above all, fruits contain medicinal properties (Mensah *et al*, 2024).

Tetrapleura tetraptera has a rich history in traditional medicine. It is often used for its potential therapeutic benefits in managing conditions like diabetes and hypertension and helps in inflammation and feverish ailments. Its antioxidant and presumptive anti-inflammatory

properties are getting recognition. However, research in these areas is still in progress (Tsala *et al.*, 2014).

Around West Africa it is used in soups and stews for its medicinal benefits. In Ghana, it is used for postpartum care for new mothers while in Nigeria it is used to treat fever and inflammation. Sierra Leoneans utilise it in traditional medicines to treat high blood pressures while certain tribes in Ivory coast incorporate it in herbal mixtures for detoxification (Mensah *et al.*, 2024) and also aid wound healing (Tsala *et al.*, 2014).

2.4.4 Phytochemical Composition of *Tetrapleura tetraptera*

The presence of phytochemicals and other bioactive compounds of *Tetrapleura tetraptera* gives the extracts of the plant essential biological properties that play a crucial role in ethnomedicine. Reported biological properties of *Tetrapleura tetraptera* extracts are antimicrobial properties (e.g. antifungal, and antibacterial) (Lin, 2019), antioxidant properties, anti-inflammatory properties (Aladesanmi, 2006; Irondi *et al.*, 2015), anti-diabetic properties (Ojewole & Adewunmi, 2004), antiparasitic properties (e.g. anti-trypanosomal, anti-helminthic, molluscicidal activity) (Adusei *et al.*, 2019), antiprotozoal (e.g. antimalaria) and antiviral (Moukette *et al.*, 2015), gastrointestinal disorders (Atchan *et al.*, 2023)

2.4.5 Pharmacological Activities of *Tetrapleura tetraptera*

2.4.5.1 Antimicrobial Activity

The use of medicinal plants such as *Tetrapleura tetraptera* in the treatment of human diseases including respiratory infections, convulsions, and rheumatism has drawn increasing scientific interest in recent years, particularly across Africa (Koma *et al.*, 2016; Onda *et al.*, 2017). This rising attention is largely fuelled by the growing cost of conventional antibiotics and the widespread emergence of antimicrobial resistance (Ebana *et al.* 2016). Various parts of *Tetrapleura tetraptera* notably the leaves, stem bark, and fruit pods, have demonstrated potent

antimicrobial activities, attributed to their phytochemical constituents, such as flavonoids, phenols, alkaloids, tannins, and saponins (Ebana *et al* 2016; Koma *et al* 2016; Larbie, 2020).

The leaf extract of *Tetrapleura tetraptera* has shown efficacy against bacterial and fungal pathogens including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (Larbie, C., 2020). Comparatively, the stem bark extract appears to possess stronger antimicrobial potency, especially against *S. aureus*, *Streptococcus pneumoniae*, and *Candida* species (Koma, O. S., *et al* 2016). These findings support the selective and complementary use of different plant parts in managing diverse infections (Koma, O. S., *et al* 2016). Research also suggests that the solvent used for extraction significantly influences antimicrobial efficacy. For instance, Enabulele and Ugha (2019) reported that aqueous extracts of *Tetrapleura tetraptera* fruit and seeds were more effective against *Klebsiella pneumoniae* and *S. aureus* than ethanolic extracts. Similarly, antifungal activity is observed to vary depending on plant part and extraction medium. Anyamele *et al.* (2023) found that aqueous fruit extracts effectively inhibited *Aspergillus* and *Mucor* but not *Rhizopus*, while petroleum ether and ethanol extract successfully suppressed *Rhizopus* growth (Ebana *et al.*, 2016). These outcomes underscore the diverse antimicrobial spectrum of *Tetrapleura tetraptera* and highlight its potential role in ethnomedicine as an accessible, plant-based antimicrobial agent.

2.4.5.2 Antioxidant Activity

Antioxidant activity represents one of the most prominent biological attributes of *Tetrapleura tetraptera*, underpinning its widespread application in ethnomedicine. As a reducing agent, it functions by neutralizing free radicals and inhibiting oxidative chain reactions, thereby preventing cellular damage (Adusei, 2019). This therapeutic effect is contributed by various parts of the plant, including the stem bark, fruit pulp, and leaves.

Studies by Famobuwa *et al* (2016) confirmed that the stem bark possesses robust antioxidant properties. Similarly, Koma *et al* (2016) found that the stem bark demonstrated significantly higher free radical scavenging capacity than the leaves, reinforcing the notion that the bioactivity of the plant is closely tied to its antioxidant profile.

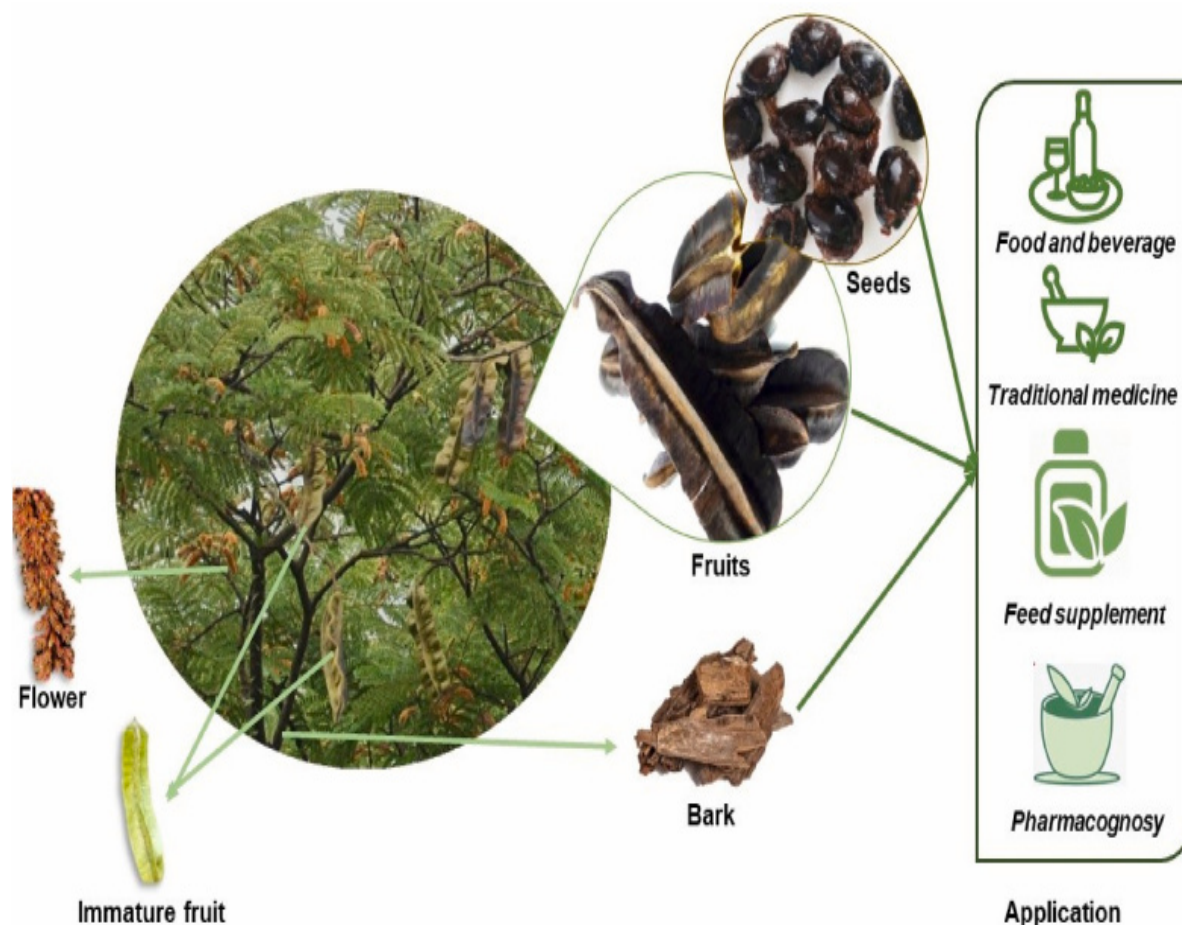


Figure 2.1: Parts of *Tetrapleura tetraptera* and their applications (Mensah *et al*, 2024)

Interestingly, Adusei *et al.* (2019) observed that the fruit pulp exhibited stronger antioxidant activity compared to the whole fruit and seeds. These differences have been largely attributed to varying concentrations of phenolic compounds, a key class of bioactive molecules known for their antioxidant effects.

Using HPLC/LC-MS analysis, Dzah (2022) identified 24 phenolic compounds in ultrasound-assisted extracts of *Tetrapleura tetraptera* dry fruit. Among the most abundant were:

- Epigallocatechin – 366.00 µg/g
- Orientin – 310.22 µg/g
- Caffeic acid – 344.91 µg/g
- Ferulic acid 4-O-glucuronide – 493.02 µg/g
- Ferulic acid 4-O-glucoside – 370.58 µg/g

These compounds collectively contributed to strong cellular antioxidant capacity in HepG2 liver cells, outperforming the reference standard gallic acid. The minimum inhibitory concentration (MIC) of the *Tetrapleura tetraptera* extract was 171.79 µg/mL, compared to 242.70 µg/mL for gallic acid (Dzah, 2022). The synergistic effect of multiple phenolics in the extract is likely responsible for its superior efficacy.

2.4.5.3 Anti-Diabetic Activity

Diabetes mellitus (DM) is a metabolic disorder that results from a deficiency in insulin secretion or function (Omonkhua, 2014). According to the International Diabetes Federation (IDF, 2019), approximately 600 million people worldwide currently live with diabetes, and this number is projected to increase significantly. In response to this growing health concern, diabetes treatment has received considerable global attention in recent years.

Although allopathic medications remain the mainstay of diabetes management, emerging research has highlighted the therapeutic potential of *Tetrapleura tetraptera* in addressing hyperglycaemia (Omonkhua, 2014). Several studies have linked diabetes mellitus to increased production of free radicals and a corresponding reduction in antioxidant defence mechanisms. In diabetic individuals, decreased levels of antioxidant parameters have been consistently observed (Bacanli *et al.*, 2019).

Phytochemicals, particularly those with antioxidant capabilities, have been suggested as promising agents in diabetes management. Among these, polyphenols have shown the ability

to mitigate the complications associated with diabetes (Bacanli, M., *et al* 2019). Given the polyphenol content of *Tetrapleura tetraptera*, the plant presents potential antidiabetic properties (Dzah, 2022),

Furthermore, extracts from the stem and leaves of *Tetrapleura tetraptera* contain limonene, a cyclic monoterpene known for its antihyperglycemic and antioxidant effects (Anyamele *et al* 2023; Bacanli, 2019). These properties support its ability to reduce oxidative stress and improve metabolic control in diabetic conditions. In a study conducted by Adesina *et al.* (2016), the fruit extract of *Tetrapleura tetraptera* exhibited superior glucose-lowering activity compared to glibenclamide, a standard antidiabetic drug. Additionally, the root bark extract of the plant has been recommended as a natural remedy for diabetes management (Omonkhua *et al.*, 2014).

2.4.5.4 Anti-inflammatory Activity

One other important biological property of *Tetrapleura tetraptera* is its anti-inflammatory activities since its use in the management of arthritis, inflammation, and rheumatic pains. Different solvent fraction of the leaf carries varying anti-inflammatory activity (Ayoola, 2018). In addition, the anti-arthritis property of *Tetrapleura tetraptera* fruit is owed to its anti-inflammatory activity (Onda *et al.*, 2017). The composition of carvacrol in *Tetrapleura tetraptera* may be a crucial factor for the anti-inflammatory features displayed by the plant extract (Anyamele, 2023). Carvacrol is a monoterpene that is available in the essential oil of different plants (de Carvalho *et al.*, 2022). Because of the therapeutic effect of carvacrol, it is used in traditional medicine. Carvacrol has a positive effect in a dose-dependent manner in the reduction of IL-1 β and IL-8 when assessed against the aforementioned pro-inflammatory cytokines (de Carvalho *et al.*, 2022). Studies have also shown that carvacrol inhibits the expression and inflammatory cytokine levels of cyclooxygenase-2 and nitric oxide synthase (Cicalău, 2021). Other compounds available in *Tetrapleura tetraptera* such as menthol, methyl

eugenol, α -copaene, and octadecanoic acid are known to possess anti-inflammatory activities (Anyamele, 2023). Ojewole and Adewunmi (2004) examined the anti-inflammatory effects of *Tetrapleura tetraptera* fruit extract on egg albumin-induced inflammation of hind paw oedema in rats with results indicating a 50–800mg/kg dose-dependent reduction of the inflammation induced. These findings substantiate *Tetrapleura tetraptera* as a potent medicinal plant that could be exploited in ethno-health care services.

2.4.5.5 Antiparasitic Activity

Tetrapleura tetraptera has emerged as a promising alternative to synthetic insecticides, which are often associated with environmental toxicity and negative impacts on non-target species. Its extract has demonstrated potent larvicidal effects against *Anopheles gambiae* larvae, making it a useful candidate in malaria vector control strategies (Seiyaboh, 2020). Additionally, fruit extracts of *Tetrapleura tetraptera* have exhibited schizonticidal activity during early-stage infections of *Plasmodium falciparum*, alongside strong anti-plasmodial properties (Adesina, 2016). Beyond malaria, *Tetrapleura tetraptera* has shown therapeutic potential against other parasitic diseases. African trypanosomiasis and helminthiasis, both classified as neglected tropical diseases (NTDs) affecting remote communities in sub-Saharan Africa, remain difficult to control. However, Obeng *et al.* (2021) reported that both the fruits and stem bark of *Tetrapleura tetraptera* possess significant anti-trypanosomal and anti-helminthic activities, reinforcing its ethnomedicinal relevance in managing parasitic infections.

Moreover, extracts from *Tetrapleura tetraptera* have demonstrated superior molluscicidal activity compared to other indigenous plants, particularly against freshwater snails, the intermediate hosts responsible for transmitting *Schistosoma* species, the causative agents of schistosomiasis (bilharzia) (Joseph, 2019). According to Adesina *et al.* (2016), the stem bark, fruit, and root of the plant all exhibit anti-molluscicidal properties, reinforcing the plant's

potential utility in schistosomiasis control programs. Nonetheless, further scientific investigation is warranted to fully validate and optimize its parasitic control applications.

2.4.5.6 Anti-Proliferative (Anti-Cancer) Properties

Chemotherapeutic substances that have been involved in the management and treatment of cancer have been developed from medicinal plants since recent cancer therapeutics have unbearable side effects like hair loss, toxicity, and chemo-resistance (Ozaslan, 2016). The effects of cancer drugs have called for less toxic and more effective ways of managing cancer. As a medicinal plant, *Tetrapleura tetraptera* has been reported to be cytotoxic to carcinoma cells and reduce tumour burden. In addition, the methanolic extract of the fruits of the plant has been reported to have an anti-proliferative ability against leukaemia and human breast cancer (Aikins *et al.*, 2021). Dzah (2022) studied the cytotoxicity of various concentrations of *Tetrapleura tetraptera* extract on the human liver cancer cell line HepG2. In all the concentrations of the extract used (50 µg/mL – 350 µg/mL), *Tetrapleura tetraptera* extract had a high cytotoxic effect relative to the same concentrations of gallic acid (Dzah, 2022). The concentration of the extract was proportional to the anti-cancer effect, with a minimum inhibition concentration of 168.06 µg/mL. Polyphenols have been associated with the induction of cellular apoptosis through various mechanisms. Polyphenols may become prooxidants under oxidative stress and produce significant concentrations of free radicals which can induce cancer cell apoptosis (Tian, 2022). In different cancer cells, aqueous extract of *Tetrapleura tetraptera* displayed prooxidant action to induce changes in mitochondrial membrane potential (Mbaveng, 2021). Plant polyphenols can also induce conditions to mimic caloric restriction mimetics in cells, thus inducing molecular mechanisms governing mitochondrial biogenesis and mitophagy (Davinelli *et al.*, 2020). Such effects are some reasons behind the antiproliferative and antitumor characteristics of polyphenols and their potential in cancer management and treatment. This property of *Tetrapleura tetraptera* fruit extract is encouraging

and calls for further isolation and categorizing of potential anti-cancer agents from the plant to enhance its use in ethnomedicine (Aikins *et al.*, 2021).

2.4.5.7 Antiviral Properties

Tetrapleura tetraptera contains notable concentrations of bioactive compounds such as piperazine, 2-hydroxy-gamma-butyrolactone, 6-octadecenoic acid, octadecanoic acid, and thymol (Erukainure, 2017; Ma, 2014). These compounds have been reported to exhibit antiviral properties against various viruses. For instance, studies have demonstrated that piperazine effectively inhibits the replication of the chikungunya virus; specifically, treatment with 6 mM piperazine significantly suppressed viral growth within 72 hours (Aggarwal, 2017). Thymol (2-isopropyl-5-methylphenol), another compound present in *Tetrapleura tetraptera*, has long been used in traditional medicine for its antiviral capabilities (Mensah *et al.*, 2024). It has shown considerable binding affinity to the spike glycoprotein of SARS-CoV-2, although it does not engage in hydrogen bonding with the protein (Nagoor *et al.*, 2017). Thymol was also listed as an active ingredient in disinfectants approved for COVID-19 control by both the United States Environmental Protection Agency and the Canadian Government (Kowalczyk *et al.*, 2020)

Furthermore, octadecanoic acid has been found to exert synergistic antiviral effects when used in combination with antiviral drugs like ribavirin, while maintaining low cytotoxicity (Linton, 2013). Given that *Tetrapleura tetraptera* contains measurable levels of octadecanoic acid (Anyamele, 2023), its extracts may offer enhanced antiviral efficacy when used alongside other therapeutic agents. These findings suggest that the plant holds promising potential in the development of antiviral interventions, warranting further investigation.

2.4.6 Summary of Literature Review

The brain is a complex organ housed in the neurocranium, divided into three regions: the cerebrum (cognitive function), the cerebellum (motor control), and the brainstem (life-

sustaining processes). The cerebrum consists of two hemispheres separated by the median longitudinal fissure and connected by the corpus callosum. Each hemisphere is divided into five lobes: the frontal, parietal, temporal, occipital, and insular lobes. The cerebral cortex contains distinct neuron types, including pyramidal cells, stellate cells, and Martinotti cells; organized into six horizontal cellular layers (laminae), ranging from the molecular layer (I) to the multiform layer (VI). Cerebral dysfunction can be focal (due to structural abnormalities like tumours) or global (caused by toxins like lead or metabolic disorders).

Lead is a persistent, nondegradable heavy metal that serves as a significant environmental contaminant which enters the body primarily through ingestion and inhalation. Children are vulnerable, absorbing 40–70% of ingested lead compared to 5–15% in adults. Once absorbed, 90% of lead is stored in bones, where it can remain for decades. Lead's mechanism of toxicity includes oxidative stress, apoptosis and ionic mimicry. Lead exposure is linked to cognitive deficits, behavioural disorders, and reduced IQ. Animal studies show lead causes neuronal degeneration in the cerebral cortex, a hallmark of cerebral dysfunction.

Tetrapleura tetraptera is a medicinal plant native to West Africa, valued for its nutritional content and diverse pharmacological properties. It contains bioactive compounds including phenols, flavonoids, tannins, alkaloids, and saponins. Activities of these compounds are exhibited through its antioxidant, antimicrobial, anti-diabetic, anti-inflammatory characteristics.

While lead exposure causes cerebral dysfunction through oxidative stress and ionic mimicry, *Tetrapleura tetraptera* offers a potential therapeutic approach. Its rich antioxidant profile allows it to mitigate oxidative damage and potentially chelate heavy metals, making it a valuable candidate for managing lead-induced neurotoxicity.

CHAPTER THREE

MATERIALS AND METHOD

3.1 ETHICAL APPROVAL

Before the commencement of this study, ethical approval was obtained from the Research Ethics Committee, College of Medical Sciences, University of Benin, Benin City, Nigeria with REC Approval Number: CMS/REC/2025/796

3.2 REAGENTS / CHEMICALS

Lead Acetate (LOBA Chemie – UN NO.: 1616) was purchased and used for the study. A dose of 100 mg/Kg body weight was used to induce neurotoxicity as previously reported by El-Tantawy (2016); Zargar *et al.* (2022); Enogieru and Momodu (2022); Enogieru and Iyoha (2023).

3.3 COLLECTION OF PLANT MATERIALS

Tetrapleura tetraptera fruit pods were purchased from New Benin market, Benin City and authenticated (UBH- T472). in the Herbarium unit of the Department of Plant Biology and Biotechnology, University of Benin, Benin City.

3.4 EXTRACTION OF PLANT MATERIAL

Plant extraction was performed as previously described by Enogieru and Iyoha (2023). A total of 5kg of dried fruit was pulverized into a fine powder using a blender. Aliquots of 10g of the powdered sample were extracted with distilled water using a single decoction step (1 × 50 ml, 10 minutes at 95°C). Following extraction, the mixtures were filtered through Whatman No. 1 filter paper, and the resulting aqueous extracts were frozen and freeze dried. The extracts were stored in the dark at 40°C until further use. Prior to experimentation, the freeze-dried extracts were reconstituted in distilled water.

3.5 PHYTOCHEMICAL SCREENING

Phytochemical screening entails a comprehensive analysis of plant extracts to identify and characterize the various biologically active compounds they contain.

3.5.1 Qualitative Phytochemical Screening

Qualitative screening is used to identify the presence or absence of classes of compounds (Singh *et al.*, 2022). Qualitative screening of *Tetrapleura tetraptera* fruit extract was carried out in the Pharmacognosy Department, University of Benin. Saponins, flavonoids, tannins, phenols, steroids, and alkaloids were screened for their presence in the plant material.

3.5.1.1 Test for Phenols

2mls of 5% neutral FeCl_3 solution were added to 1 ml of aqueous extract, the dark blue colour showed the presence of phenolic compounds (Trease & Evans, 2002).

3.5.1.2 Test for Flavonoids

To 1ml of aqueous extract, a few drops of concentrated sulphuric acid (H_2SO_4) was added along the sides of the test tube. The formation of a yellow colour indicated the presence of flavonoids (Trease & Evans, 2002).

3.5.1.3 Test for Tannins

1ml of freshly prepared 10% KOH was added to 1 ml of aqueous extract. Dirty white precipitates indicated the presence of tannins (Trease & Evans, 2002).

3.5.1.4 Test for Alkaloids (Mayer's Reagent Test)

Small quantities of each portion were stirred with 5ml of 1% aqueous HCl in a water bath and then filtered. Of the filtrate, 1ml was placed into 2 test tubes. To the first portion, a few drops of Dragendorff's reagent were added; the occurrence of orange-red precipitate was taken as positive. To the second 1ml, Mayer's reagent was added and the appearance of a buff-coloured precipitate was an indication of the presence of alkaloids (Santhi and Sengottuvel, 2016).

3.5.1.5 Test for Steroids

Aqueous extract was shaken with chloroform and conc. H₂SO₄ was added along the walls of the test tube; a red colour showed the presence of steroids (Trease & Evans, 2002).

3.5.1.6 Test for Saponins

2 ml of aqueous extract was taken in a test tube, shaken vigorously, and observed for froth formation (Trease & Evans, 2002).

3.6 PROCUREMENT AND CARE OF EXPERIMENTAL ANIMALS

For this study, sixty-four (64) adult Wistar rats weighing between 160g to 180g were procured and bred in the Animal House, Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City. The rats were kept in clean polypropylene cages at normal room temperature and were allowed access to Chikun Feed Grower Mash (Olam Agri Holdings Pte Ltd., Lagos State, Nigeria) and water *ad libitum* throughout the experimental period. The rats were weighed weekly before commencement and throughout the duration of the experiment using a digital weighing scale (manufactured by William Jiang Appliances, China) calibrated in grams and values were recorded to the nearest whole number. Protocols for this experiment were in accordance with the guide for the care and use of laboratory animals (National Research Council of the National Academics, 2011).

3.7 TREATMENT REGIMEN

After the period of acclimatization, the animals were randomly assigned into eight (8) groups (A, B, C, D, E, F, G and H) of eight (8) rats each (n=8).

Administration lasted for twenty-eight (28) days. All administrations were carried out via oral route using an orogastric tube (manufactured by KETU, China). Treatment groups were pre-treated with *Tetrapleura tetraptera* extract.

Table 3.1: Summary of experimental design.

GROUP	TREATMENT
Group A	Control
Group B	100mg/kg body weight of lead acetate only
Group C	500mg/kg body weight <i>Tetrapleura tetraptera</i> + 100mg/kg body weight of lead acetate
Group D	1000mg/kg body weight <i>Tetrapleura tetraptera</i> + 100mg/kg body weight of lead acetate
Group E	Vitamin E 200mg/kg body weight + 100mg/kg body weight of lead acetate
Group F	500mg/kg body weight of <i>Tetrapleura tetraptera</i> only
Group G	1000mg/kg body weight of <i>Tetrapleura tetraptera</i> only
Group H	Vitamin E only

3.8 NEUROBEHAVIOURAL ASSESSMENTS

After administration on the 28th day, neurobehavioral activities were assessed. Neurobehavioral assessment is a clinical study of behavior that emphasizes the role of brain dysfunction (Zasler *et al.*, 2013). It is an essential component of rehabilitation and is used to discern behaviors that are organically based from those that are reactive to the onset of disability (Woods *et al.*, 2012). To evaluate the effects of treatments on neurobehavioural activities, Open Field Test (OFT) and Tail Suspensions Tests (TST) were carried out.

3.8.1 Open Field Test

The Open Field Test (OFT) is a widely used neurobehavioural assay to evaluate locomotor activity, exploratory behaviour, and anxiety-related responses in test animals (Walsh & Cummins, 1976).

The rats were individually placed at the centre of the box (72cm x 72cm x 30cm) and allowed to explore freely for 5 minutes. The test was conducted under daylight and low noise conditions to reduce external influences. After each session, the box was cleaned with 70% ethanol to remove residual odour.

Behavioural parameters recorded include:

- Rearing frequency; provide insights into emotionality and stress.
- Grooming; provide insights into emotionality and stress.
- Thigmotaxis; time spent in centre vs. periphery, used as an index of anxiety.
- Sniffing
- Immobility time; Indicates general locomotor activity.

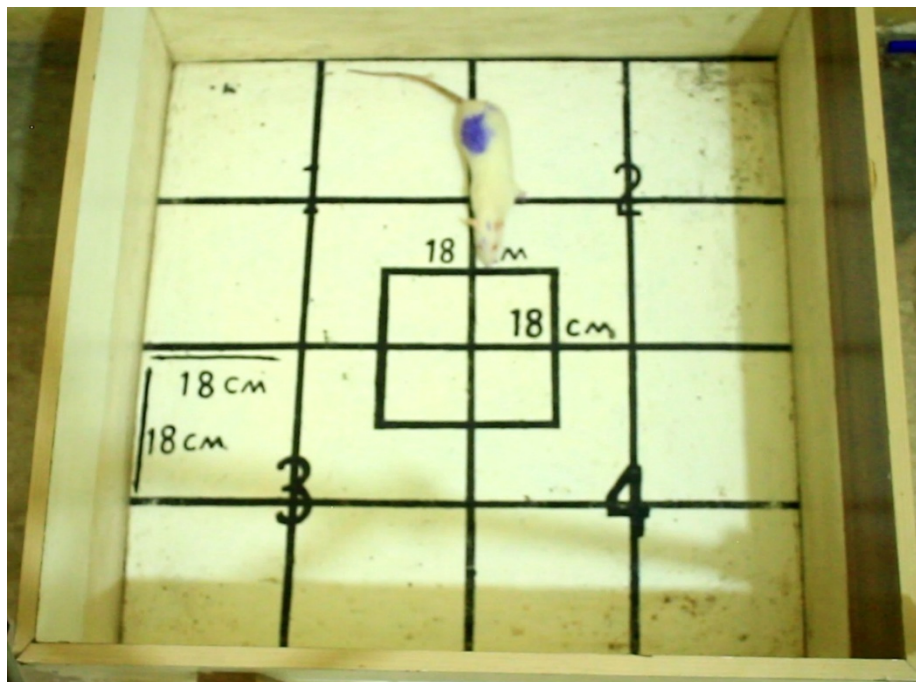


Fig 3.1; Open Field Test (OFT) apparatus

3.8.2 Tail Suspension Test

The Tail Suspension Test (TST) is a behavioural assay used for evaluating antidepressant-like activity in rodents, particularly mice. The test is based on the principle that rodents subjected

to an acute, inescapable stressor, being suspended by the tail, will exhibit immobility, which is interpreted as behavioural despair (Steru *et al.*, 1985).

The rat was hung by the tail from 50 cm above the ground for 5 minutes. A non-reflective wood board was used to enhance video visibility, and the environment was kept quiet to minimize external stimuli.

The variable measured is:

- Duration of immobility: Defined as the absence of limb movement and passive hanging posture.



Fig 3.2; Tail Suspension Test apparatus

3.9 EVALUATION OF BODY AND BRAIN WEIGHTS

After the neurobehavioral assessments were completed, the rats were euthanized with low level anaesthesia, followed by cervical dislocation. Rats' brains were excised from their skulls,

blotted clean of blood, and instantly weighed using an electronic balance calibrated in milligrams and recorded to the nearest two decimal places. The relative brain weights were calculated as follows:

$$\text{Relative brain weight} = [\text{absolute brain weight (g)} / \text{body weight (g)}] \times 100$$

3.10 CEREBRAL OXIDATIVE STRESS PARAMETERS

The cerebrum was removed from the brain after being harvested, blotted of blood, and weighed immediately with an electronic balance calibrated in milligrams and recorded to the nearest two decimal places. The harvested and weighed cerebrum was washed twice in cold phosphate buffered saline (PBS), homogenized using acid-washed sand and PBS in porcelain mortar and pestle. The homogenate was centrifuged at $10,000 \times g$ for 15 minutes at 4°C . The supernatants were collected for the estimation of the various biochemical assays.

3.10.1 Estimation of Catalase (CAT) Activity

This was determined by the method of Cohen *et al.* (1970).

▪ Principle

Catalase is present in nearly all animal, plant, and bacteria cells. It acts to prevent the accumulation of noxious H_2O_2 which is converted to O_2 and H_2O .

▪ Preparation of reagent

- i. 0.01M KMnO_4 was prepared by dissolving 0.158g of KMnO_4 in 100ml of distilled water.
- ii. Phosphate buffer (pH 7.4); 0.426 of NaH_2PO_4 was weighed and dissolved in 100ml of distilled water.
- iii. 6M H_2SO_4 : 32.3ml of conc. H_2SO_4 was added to 66.7 ml of distilled water.
- iv. 30Mm H_2O_2 solution was prepared by measuring 0.34ml of 30% of H_2O_2 in 100ml of phosphate buffer.

- **Procedure**

To a known volume of supernatant (0.5ml), 5.0ml of H₂O₂ was added. This was then mixed by inversion and allowed to stand for 30 minutes. The reaction was stopped by adding 6M H₂SO₄. The absorbance was taken at 480nm within 30-60 seconds against distilled water.

- **Calculation**

$$\text{Activity} = [\text{OD} \times \text{min} \times V_t] / [M \times V \times L \times Y]$$

Where, OD = absorbance

L = light path = 1cm

V₁ = total volume of the reaction sample

M = molar extinction co-efficient of H₂O₂ (40/M/cm).

3.10.2 Estimation of Glutathione (GSH) Concentration

This was determined using the method described by Ellman (1959).

- **Reagents**

5, 51-dithiobis-2-nitrobenzoic acid (DTNB), sodium citrate, and trichloroacetic acid (TCA)

- **Procedure**

To 1.0 ml of the tissue homogenate, 2.5 mL of 10 % TCA was added and centrifuged at 3000g for 10 min. Then, 1.0 mL of the supernatant was treated with 0.5ml of Ellman's reagent (0.0189% DTNB and 1% sodium citrate) and 3.0ml 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**

$$\text{Concentration of GSH} = [(A_0 - A_1) \times 100] / A_0$$

$$[\text{A}_{\text{test}} \times \text{Conc. of Standard}] / \text{A}_{\text{stand}} \% \text{ Glutathione Reduced}$$

Where, A₀ = Absorbance of reference sample

A₁ = Absorbance of sample

3.10.3 Estimation of Superoxide Dismutase (SOD)

This was determined according to the method of Misra and Fridovich (1972).

▪ Principle

Adrenaline undergoes autoxidation rapidly to adrenochrome whose concentration can be determined at 420 nm with the aid of a spectrophotometer. The auto-oxidation of adrenaline depends on the presence of superoxide anions. Superoxide dismutase inhibits the auto oxidation of adrenaline by catalysing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD which is determined at 420nm.

▪ Preparation of reagents

Carbonate buffer (0.05M) pH 10.2: this was prepared by dissolving 0.2014 g of Na₂CO₃.

0.2604 g of NaHCO₃ and 0.0372g of EDTA in 100ml of distilled water.

Hydrochloric acid (0.005M): this was prepared by adding 0.044ml concentrated HCL to 99.96ml of distilled water.

Adrenaline solution (0.3mM): this was prepared by dissolving 0.01098g of adrenaline in 100ml of 0.005 M HCl solution.

▪ Procedure

A supernatant volume of 0.2ml was mixed with 2.5 ml of carbonate buffer and 0.3ml of adrenaline solution, and 0.2ml of distilled water were mixed with 2.5ml of carbonate buffer and 0.3 ml adrenaline as reference sample. These were mixed and absorbance read at 420 nm.

$$\% \text{ inhibition} = [(O.D \text{ test} - O.D \text{ ref}) \times 100] / O.D \text{ test}$$

Enzyme activity was calculated thus:

$$\text{SOD activity (Unit/ mg protein)} = \% \text{inhibition} / (50 \times Y)$$

Where, Y = mg of protein in the volume of sample used.

3.10.4 Estimation of Glutathione Peroxidase (GPx) Activity

This was determined by the method of Rotruck *et al.*, (1973).

▪ **Principle**

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

▪ **Preparation of reagent**

Pyrogallol (20mM): 0.2552g of pyrogallol was dissolved in 100 ml of distilled water.

▪ **Procedure**

To an aliquot of supernatant (0.2ml), 2.5ml of phosphate buffer, 2.5ml of H₂O₂, 1.5ml of distilled water and 2.5ml of pyrogallol was added. The reaction was allowed to stand for 30 minutes at room temperature. A deep brown colour was formed which was read at 420nm.

▪ **Calculation**

$$\text{Activity} = [(\text{OD/Min}) \times (\text{VtDf})] / [\text{E} \times \text{Vs} \times \text{Y}]$$

OD = Absorbance of test

Vt = Total volume of reaction mixture

Df = Dilution factor = 1

E = Molar extinction coefficient (12/M/cm)

Vs = Volume of sample

Y = mg of protein used

3.10.5 Estimation of Malondialdehyde (MDA) Concentration

This was determined using the thiobarbituric acid assay as described by Buege and Aust (1978).

▪ Principle

Malondialdehyde is a product of lipid peroxidation which reacts with thiobarbituric acid to give a red species.

▪ Preparation of reagent

Stock TCA-TBA-HCL was prepared by mixing 15g of trichloroacetic acid, 0.375 g of thiobarbituric acid and 0.25 N hydrochloric acid. This solution was mildly heated to assist in the dissolution of the thiobarbituric acid.

▪ Procedure

A volume of supernatant (1.0ml) was added to 2.0ml of TCA-TBA-HCL and mixed thoroughly. The solution was heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifuging at 1000g for 10 minutes. The absorbance was determined at 535nm against a blank.

The concentration of MDA was determined using the formula:

$$\text{MDA (unit/mg protein)} = (A \times V_t \times 1000) / (M \times V \times l \times Y)$$

A = absorbance of sample test at 535nm

V_t = total volume of the reaction = 3ml

M = molar extinction co-efficient of product = $1.56 \times 10^5 \text{ m}^{-1} \text{ cm}^{-1}$

l = light path = 1cm

V = volume of tissue extract used = 1ml

Y = mg of tissue in the volume of sample used

3.11 HISTOLOGY OF THE CEREBRUM

The excised cerebrum tissues were fixed in 10% buffered formal saline and processed by paraffin embedding with sections cut at 5µm thickness. The paraffin wax embedding method of Carleton *et al.*, (1980) was used to prepare the tissues. They were each dehydrated for an hour at room temperature using ethanol concentrations of 70%, 90%, absolute ethanol I, and absolute ethanol II. Two xylene changes at room temperature, lasting an hour each, was used to clear dehydrated tissue. The tissues were soaked in two separate batches of molten paraffin wax for one hour each at 60 °C before being embedded in multi-block paraffin wax moulds. The paraffin blocked tissues were cut into smaller pieces and put on a wooden chuck for rotary microtome sectioning. A rotary microtome was used to slice the tissue blocks into sections that were 5µm thick. To spread the folded ribbons, the sections were placed in a water bath at 40°C. These pieces were fixed to a fresh, spotless glass slide. To increase the adherence of the sections to the slides, these were dried at 40°C using a slide dryer.

3.12 HEMATOXYLIN 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 Carleton *et al.*, (1980). The sections were:

- i. Dewaxed in two changes of xylene for two minutes in each change;
- ii. Rehydrated in descending grades of alcohol (absolute II, absolute I, 95%, 90%, 70% and 50% ethanol) for two minutes each;
- iii. Rinsed in distilled water for three minutes and stained in haematoxylin for 15-20 minutes

- iv. 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);
- v. Differentiated in acid alcohol (0.5% HCL in 70% ethanol) for two to three minutes;
- vi. Rinsed well in running water for 10-15 minutes;
- vii. Counterstained in 1% aqueous eosin for two to four minutes;
- viii. Excess stain was washed off in running water and examined under a microscope;
- ix. Dehydrated rapidly in ascending grades of ethanol (50% through absolute ethanol), cleared in xylene and mounted in a synthetic resin medium (DPX).

3.13 PHOTOMICROGRAPHY

The sections of the prefrontal cortex of the cerebrum were obtained and examined under a Lasec UB 200i microscope equipped with Eakins Microscope Camera (2307su, UK). The camera features 9 megapixels (3488 × 2616 pixel) high resolution colour digital camera and 0.5X reduction lens. It was then connected to a computer. A panoramic view of the slides was captured using ×4 and ×10 objective lenses.

3.14 MOLECULAR DOCKING

3.14.1 High Performance Liquid Chromatography (HPLC)

HPLC is a method used to purify and separate mixture of complex samples. HPLC is an active process in the sample is injected into a liquid stream at high pressure known as the mobile phase across a separation column to achieve the separation into individual components. The separation column contains a stationary phase usually made up of beads or particles that separate the compound mixtures. After this separation, the samples are exposed to a detector system that identify and quantify the individual compounds.

Programming

Model; Shimadzu (Nexera MS)

Column; Ubondupak C18
Length = 100mm
ID = 4.6mm
Thickness = 7um

Mobile phase; Methanol / water

Flow rate; 1ml/min

Sample injection; 10ul

Detector; UV e 254nm (Diode Array Detector DAD)

Pressure; 15mpa

Extraction

10g of sample was measured into the amber bottle. 20mls of acetonitrile was then added. The mixture was shaken vigorously for thirty (30) minutes. The aqueous end was then run off while the organic solvent end was collected into 25ml standard flask, made up to the mark and then ready for analysis.

Analysis

The standard form of analytes profile was first injected into the HPLC and this generated a chromatogram, with a given peak area and peak profile. These were used to create a window in the HPLC in preparation of the test sample analysis. The aliquot of the extracted test samples was injected into the HPLC also to obtain a corresponding peak area and peak profile in a chromatogram. The peak area of the sample is compared with that of the standard, relative to the concentration of the standard to obtain the concentration of the sample.

Calculation

$$\text{Sample concentration} = \frac{\text{Sample peak area (Tetrapleura tetraptera)} \times \text{Standard concentration}}{\text{Standard peak area}}$$

Standard concentration; 10.29mg/g

Standard peak area; 9998.8566

$$\begin{aligned}\text{Sample concentration} &= \frac{14818.9575 \times 10.29}{9998.8566} \\ &= 15.25\text{mg/g}\end{aligned}$$

3.14.2 Preparation of Crystal Protein

The crystal structure of Nrf2 (PDB ID-7OFE), Caspase 3 (PDB ID-3KJF), and Interleukin 6 (PDB ID-4YW7) were downloaded via the protein preparation wizard of maestro v11.8. The protocol described in previous studies was used to prepare crystal structure of the protein (Elekofehinti *et al.* 2020a, b). The protein was preprocessed 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, 2025). 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.

3.14.3 Preparation of the phyto-compounds

The compounds were retrieved from National Center for Biotechnology Information (Pubchem). The compounds were 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.

3.14.4 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 scaling factor of 1.0 and partial cutoff of 0.25 to soften the potential for non-polar parts of the receptor.

3.14.5 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.

3.14.6 ADME Profiles

The absorption, distribution, metabolism, excretion and molecular properties of the compounds were predicted using Qikprop panel (Schrödinger, 2025).

3.15 STATISTICAL ANALYSIS

The obtained data was 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 posthoc test was performed to determine groups involved in statistical differences and results were expressed as mean $\pm$ S.E.M. A *p*-value of < 0.05 was defined as statistically significant.

CHAPTER FOUR

RESULTS

4.1 PHYTOCHEMICAL SCREENING

Table 4.1 shows the preliminary phytochemical constituents in *Tetrapleura tetraptera*. Results showed the presence of alkaloids, phenols, saponins, flavonoids, and steroids, while tannins were absent.

Table 4.1: Preliminary phytochemical findings of *Tetrapleura tetraptera*

PHYTOCHEMICALS	
Alkaloids	+
Tannins	-
Phenols	+
Saponins	+
Flavonoids	+
Steroids	+

+: Present

-: Absent

4.2 EVALUATION OF SAFETY PROFILE; DETERMINATION OF LD₅₀

Table 4.2 shows the LD₅₀ study observations after 72 hours. Results showed that there was no observable morbidity, mortality or diarrhea across the experimental groups following oral administration of *Tetrapleura tetraptera* at doses from 10mg/kg to 5000 mg/kg body weight.

The rats used in this LD₅₀ determination were not part of the treatment or control groups.

Table 4.2: LD₅₀ 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

4.3 EFFECT OF TREATMENT ON BRAIN AND BODY WEIGHT

Figures 4.1 – 4.5 show the weights findings across the experimental groups. Results showed a significant decrease ($p<0.05$) in weight change in lead exposed rats when compared to control. However, a significant increase ($p<0.05$) was observed in lead exposed rats pre-treated with *Tetrapleura tetraptera* when compared to lead only. Also, no significant difference ($p>0.05$) was observed in the *Tetrapleura tetraptera* only treated groups when compared to control. For whole brain weight, lead only exposed rats showed significant decrease ($p<0.05$) when compared to control. However, there was a significant increase ($p<0.05$) in the lead exposed rats pretreated with 1000 mg/kg body weight of *Tetrapleura tetraptera* when compared with the lead only exposed group. There was also no significant difference ($p>0.05$) observed in the *Tetrapleura tetraptera* only treated groups when compared to control. No significant differences ($p>0.05$) were observed in relative brain weight across the experimental groups.

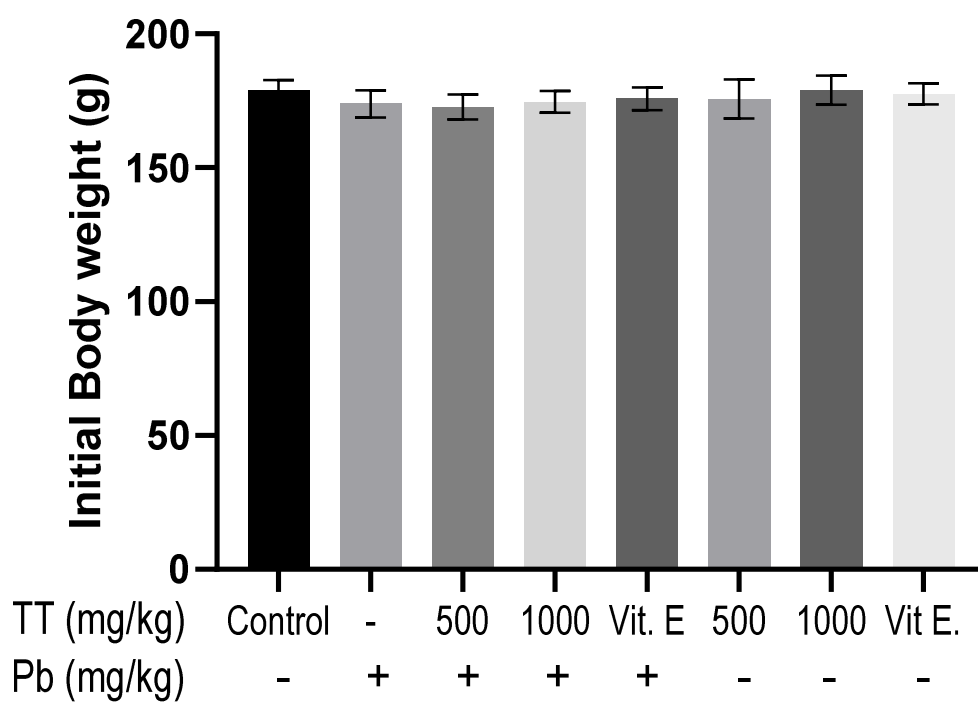


Figure 4.1: Initial body weight across experimental groups.

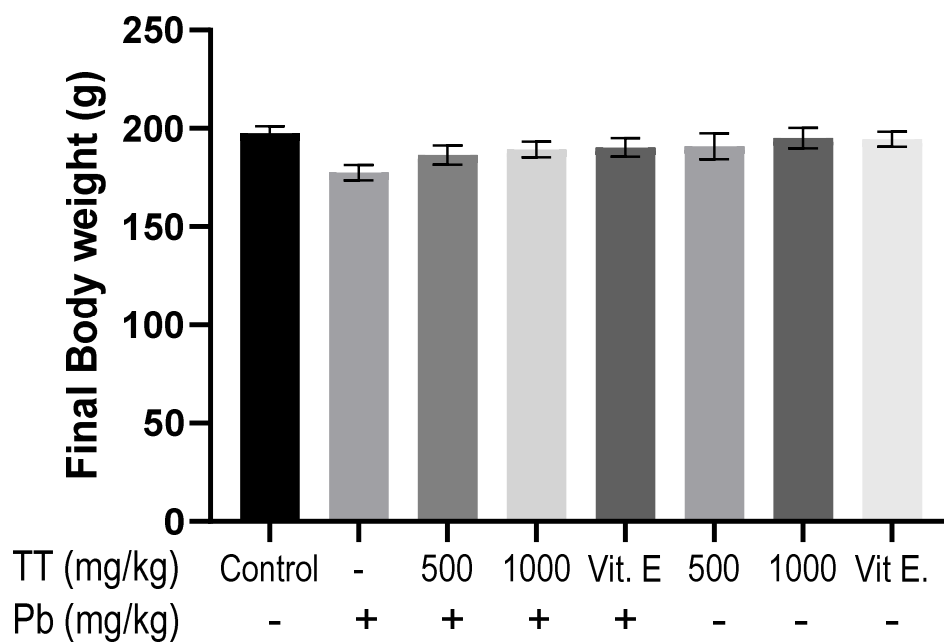


Figure 4.2: Final body weight across experimental groups.

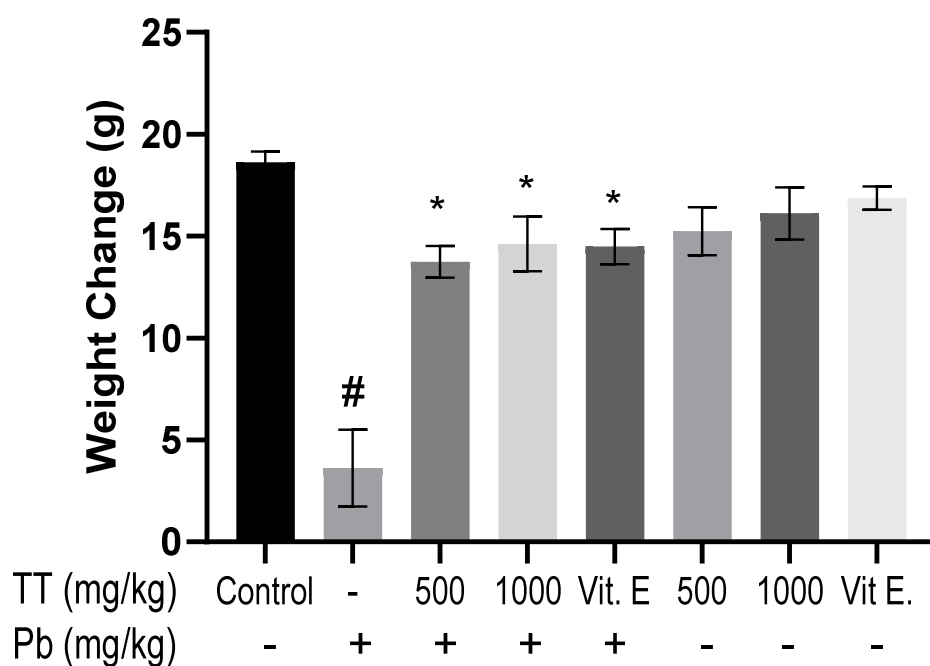


Figure 4.3: Weight change across experimental groups.

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

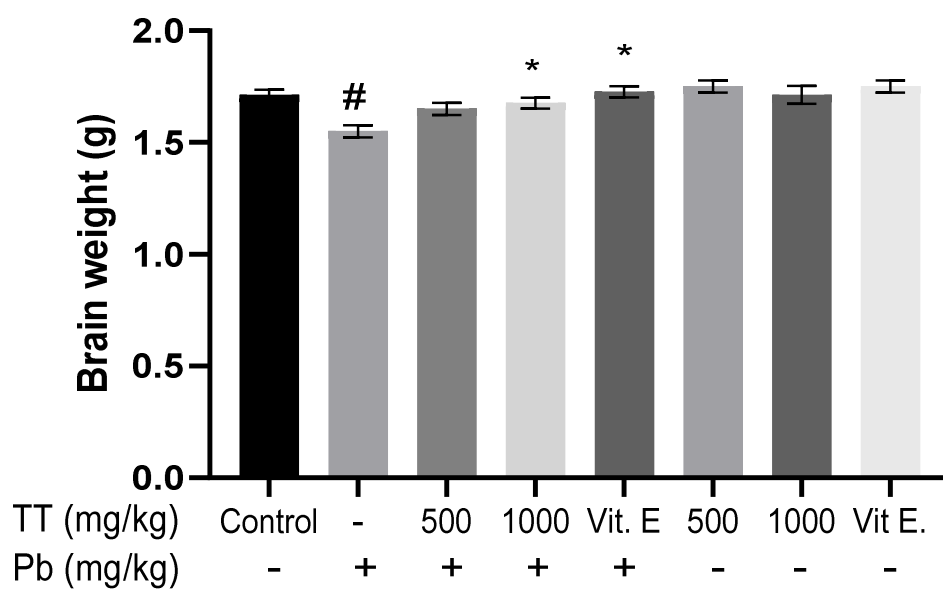


Figure 4.4: Brain weight across the experimental groups.

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

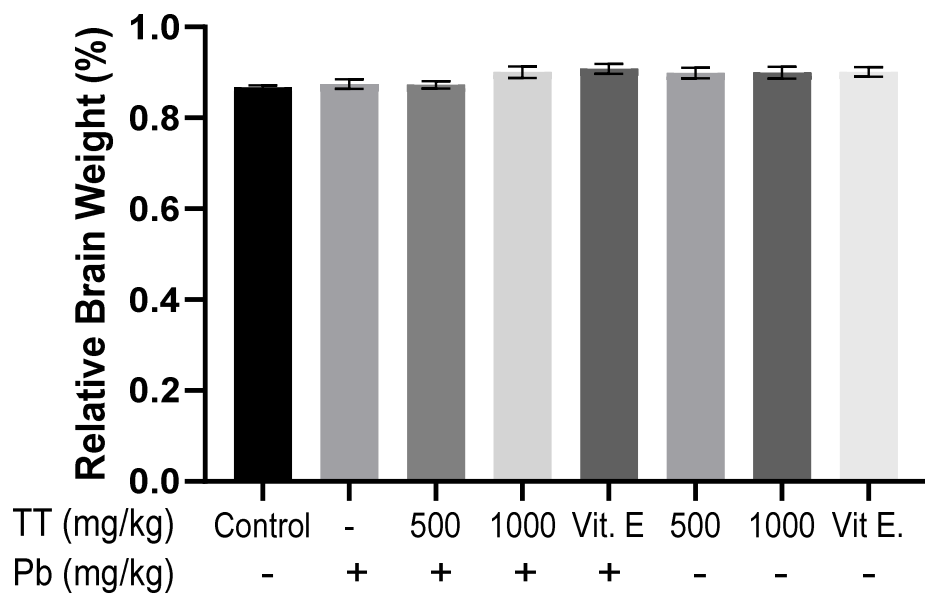


Figure 4.5: Relative brain weight across the experimental groups.

4.4 EFFECT OF TREATMENT ON NEUROBEHAVIOURAL ACTIVITY

Figure 4.6 shows the rearing frequency across the experimental groups. For rearing frequency, there was a significant decrease ($p<0.05$) in the lead only treated groups when compared to the control group. However, there was a significant increase ($p<0.05$) in lead exposed rats pretreated with 500mg/kg body weight and 1000 mg/kg body weight of *Tetrapleura tetraptera* when compared to the lead only exposed group. There was also no significant difference ($p>0.05$) observed in the *Tetrapleura tetraptera* only treated groups when compared to control.

Figures 4.7 – 4.10 show the grooming, thigmotaxis, sniffing and immobility activities across the experimental groups. There was a significant increase ($p<0.05$) in the lead only treated groups when compared to the control group. However, there was a significant decrease ($p<0.05$) in the lead exposed rats pretreated with 500mg/kg body weight and 1000 mg/kg body weight of *Tetrapleura tetraptera* when compared to the lead only exposed group. There was also no significant difference ($p>0.05$) was observed in the *Tetrapleura. tetraptera* only treated groups.

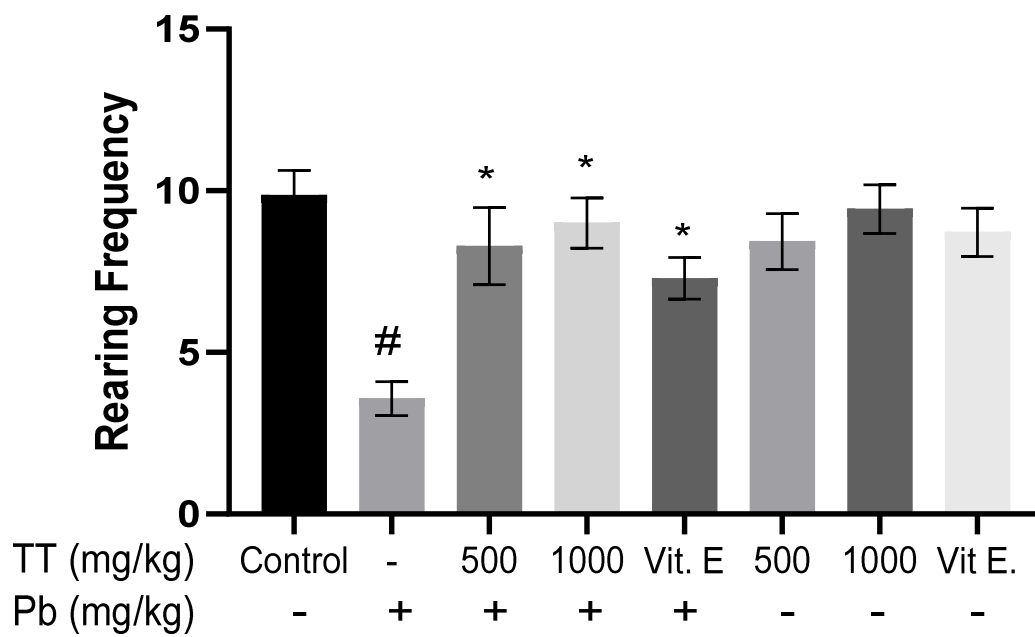


Figure 4.6: Rearing frequency across the experimental groups after 28 days.

and * represent $p < 0.05$ following comparison to the control and lead only group B respectively.

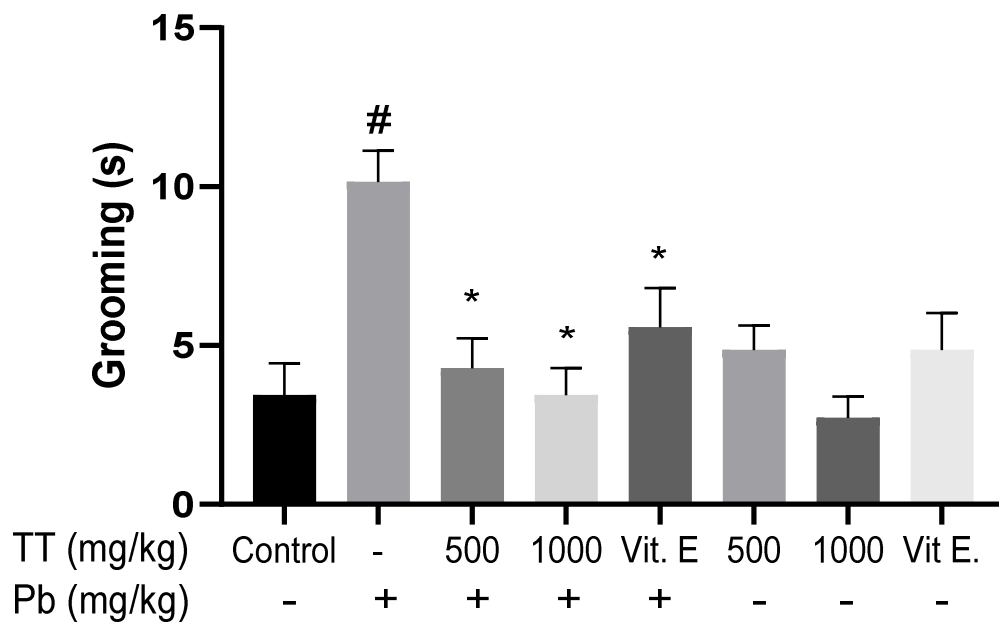


Figure 4.7: Grooming across the experimental groups after 28 days.

and * represent $p < 0.05$ following comparison to the control and lead only group B respectively.

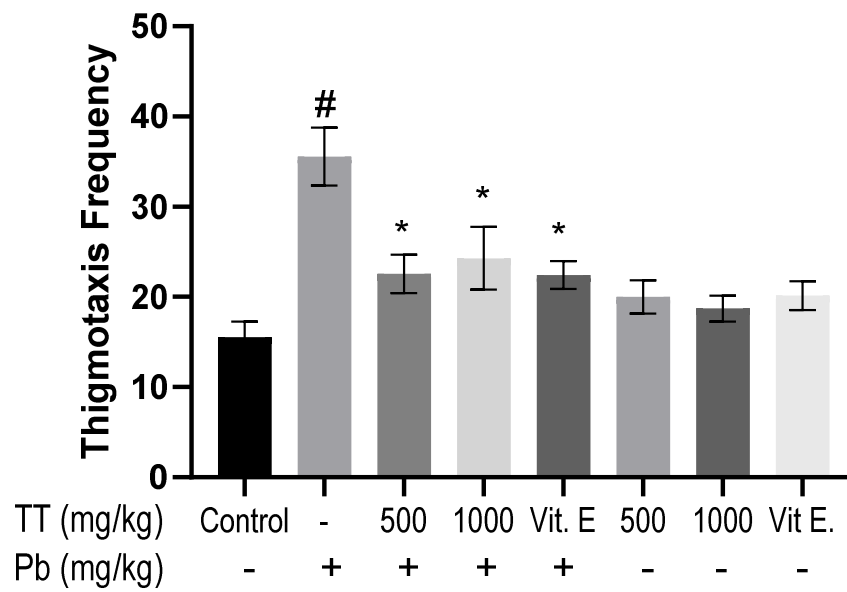


Figure 4.8: Thigmotaxis frequency across the experimental groups after 28 days.

and * represent $p < 0.05$ following comparison to the control and lead only group B respectively.

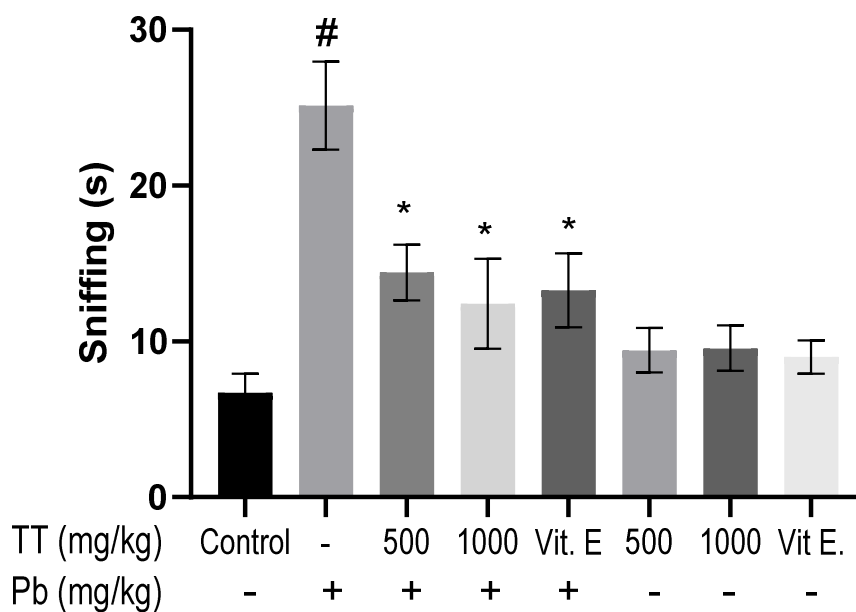


Figure 4.9: Sniffing across the experimental groups after 28 days.

and * represent $p < 0.05$ following comparison to the control and lead only group B respectively.

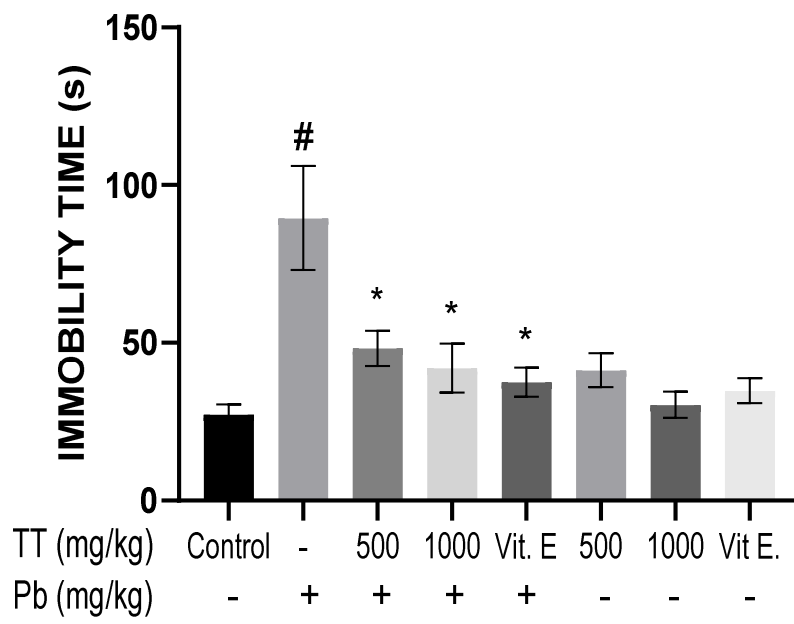


Figure 4.10: Immobility time across the experimental groups after 28 days

and * represent $p < 0.05$ following comparison to the control and lead only group B respectively.

4.5 EFFECT OF TREATMENT ON ANTIOXIDANT ENZYMES ACTIVITY

Figures 4.11- 4.12 show the cerebral superoxide dismutase (SOD) and catalase (CAT) activity across the experimental groups respectively. Rats exposed to lead only showed a significant decrease ($p<0.05$) in both SOD and CAT activity when compared to control. However, there was a significant increase ($p<0.05$) in both SOD and CAT activity in lead exposed rats pretreated with *Tetrapleura tetraptera* compared to the lead only group. There was also a significant decrease ($p<0.05$) in both SOD and CAT concentration in the cerebrum of lead exposed rats pre-treated with Vit E.

Figure 4.13 shows the cerebral glutathione peroxidase (GPx) activity across experimental groups. There was a significant decrease ($p<0.05$) in the glutathione activity in rats treated with lead only when compared to control. Conversely, there was a significant increase ($p<0.05$) in GPx activity in lead exposed rats pretreated with 1000 mg/kg of *Tetrapleura tetraptera*, when compared to lead only group.

Figure 4.14 shows the glutathione (GSH) concentration in the cerebrum across experimental groups. Rats treated with lead only showed a significant decrease ($p<0.05$) in GSH concentration when compared to control. However, there was a significant increase ($p<0.05$) in GSH concentration in rats pretreated with 500 mg/kg and 1000 mg/kg *Tetrapleura tetraptera* when compared to lead only groups.

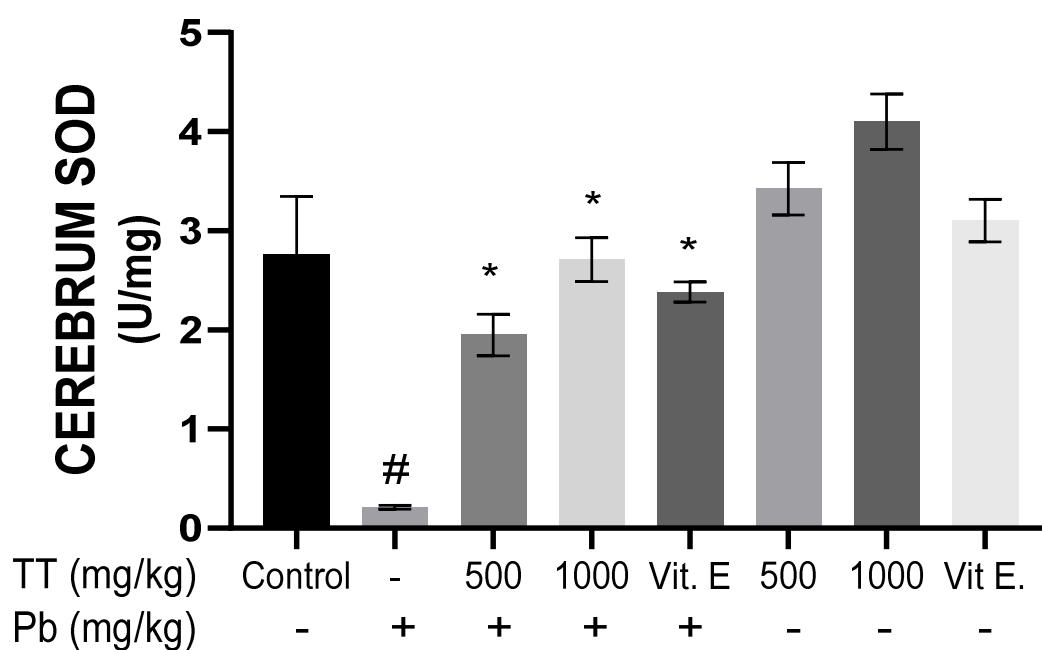


Figure 4.11: SOD activity in the cerebrum of rats across experimental groups.

$p < 0.05$ compared with control group; * $p < 0.05$ compared with lead only group

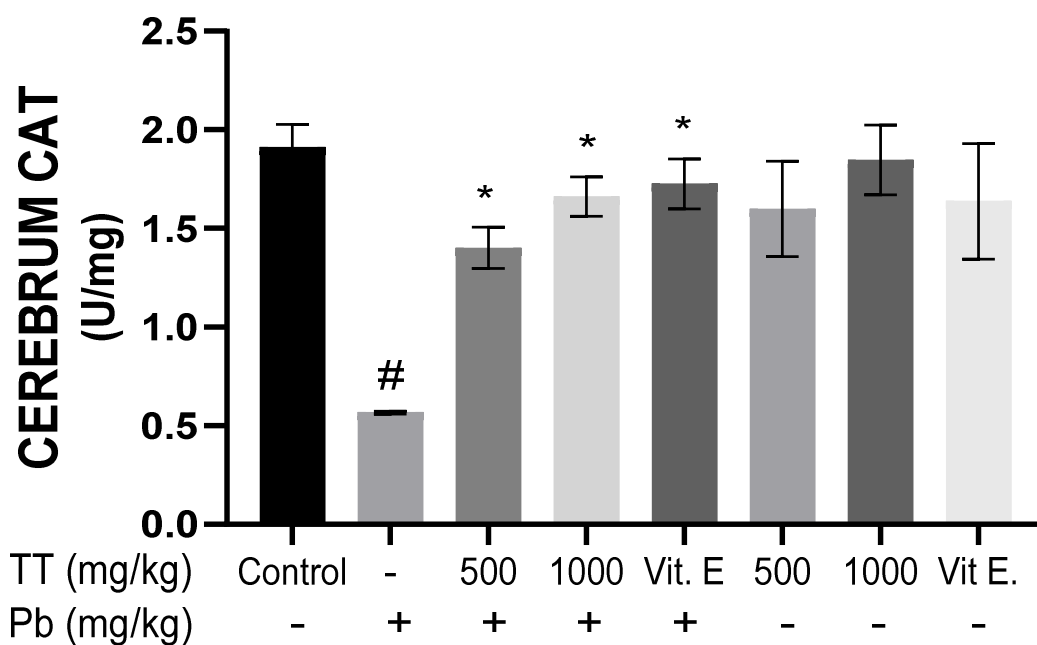


Figure 4.12: CAT activity in the cerebrum of rats across experimental groups.

$p < 0.05$ compared with control group; * $p < 0.05$ compared with lead only group

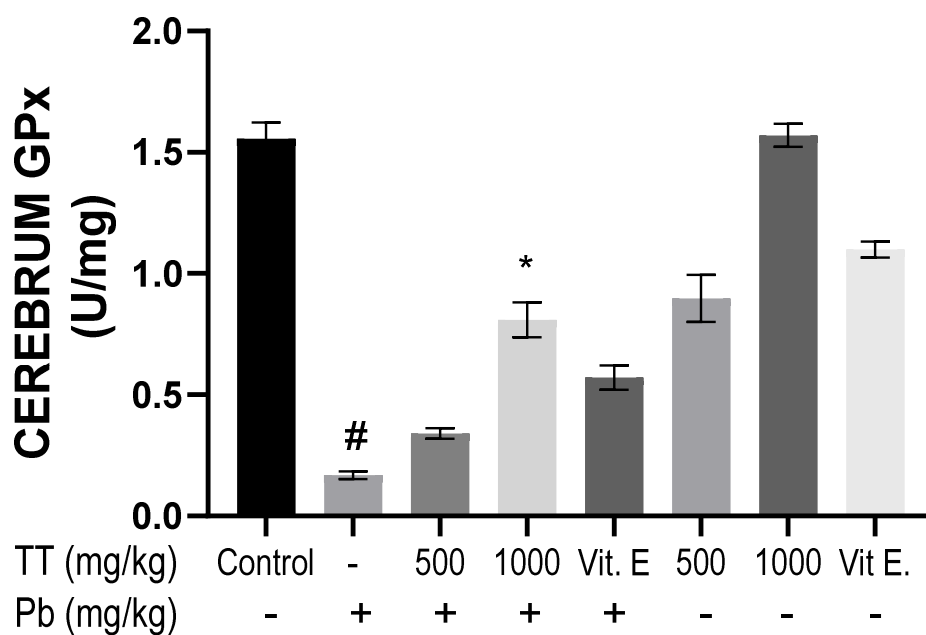


Figure 4.13: GPx activity in the cerebrum of rats across experimental groups.

$p < 0.05$ compared with control group; * $p < 0.05$ compared with lead only group.

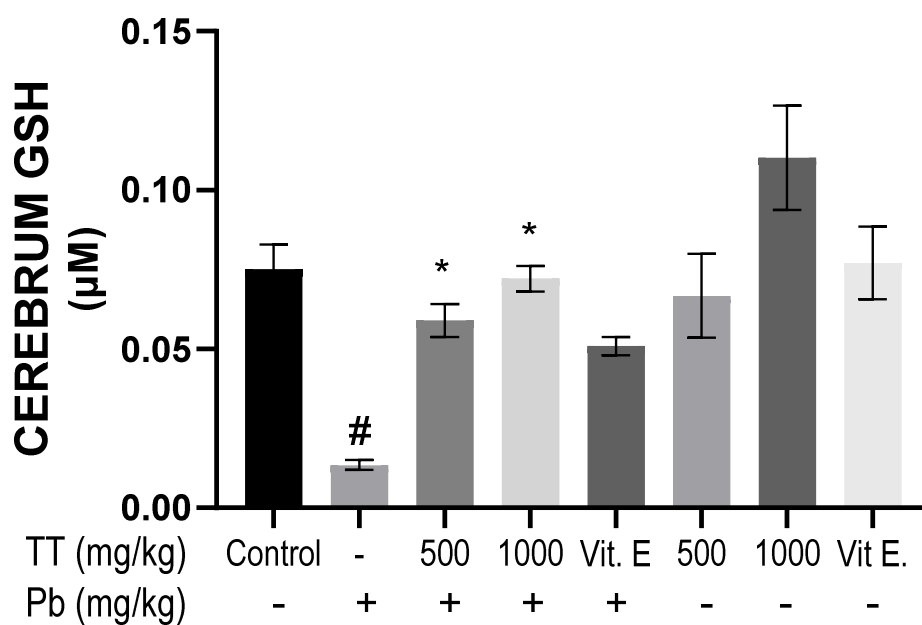


Figure 4.14: GSH activity in the cerebrum of rats across experimental groups.

$p < 0.05$ compared with control group; * $p < 0.05$ compared with lead only group

4.6 EFFECT OF TREATMENT ON LIPID PEROXIDATION

Figure 4.15 shows malondialdehyde (MDA) concentration in the cerebrum across experimental groups. Rats treated with lead only showed a significant increase ($p<0.05$) in MDA concentration when compared to the control. However, there was a significant decrease ($p<0.05$) in MDA concentration in rats pretreated with 500 mg/kg and 1000 mg/kg of *Tetrapleura tetraptera* when compared to lead only treated group. There was also a significant decrease ($p<0.05$) in MDA concentration in the cerebrum of lead exposed rats pre-treated with Vit E when compared to the lead only treated group.

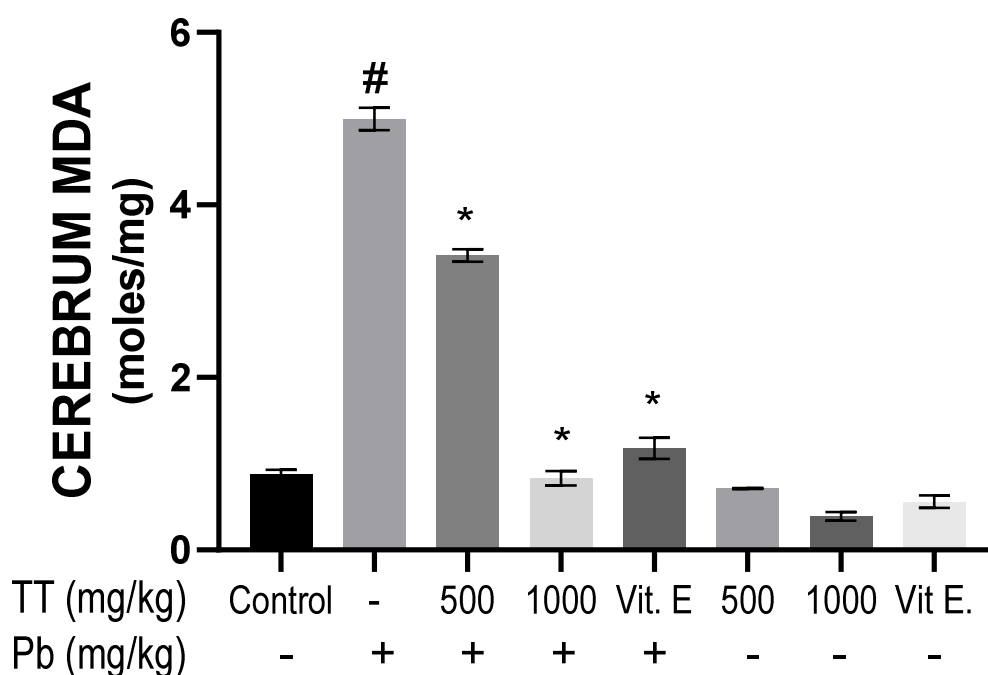


Figure 4.15: MDA activity in the cerebrum of rats across experimental groups.

$p<0.05$ compared with control group; * $p<0.05$ compared with lead only group

4.7 EFFECT OF TREATMENT ON LEAD ACCUMULATION

Figure 4.16 shows lead concentration in the cerebrum across experimental groups. There was a significant increase ($p<0.05$) of lead concentration in the cerebrum of rats treated with lead only when compared to control. However, there was a significant decrease ($p<0.05$) in lead exposed rats pre-treated with *Tetrapleura tetraptera*, when compared to lead only exposed rats. There was also a significant decrease ($p<0.05$) in lead concentration in the cerebrum of lead exposed rats pre-treated with Vit E when compared to the lead only treated group.

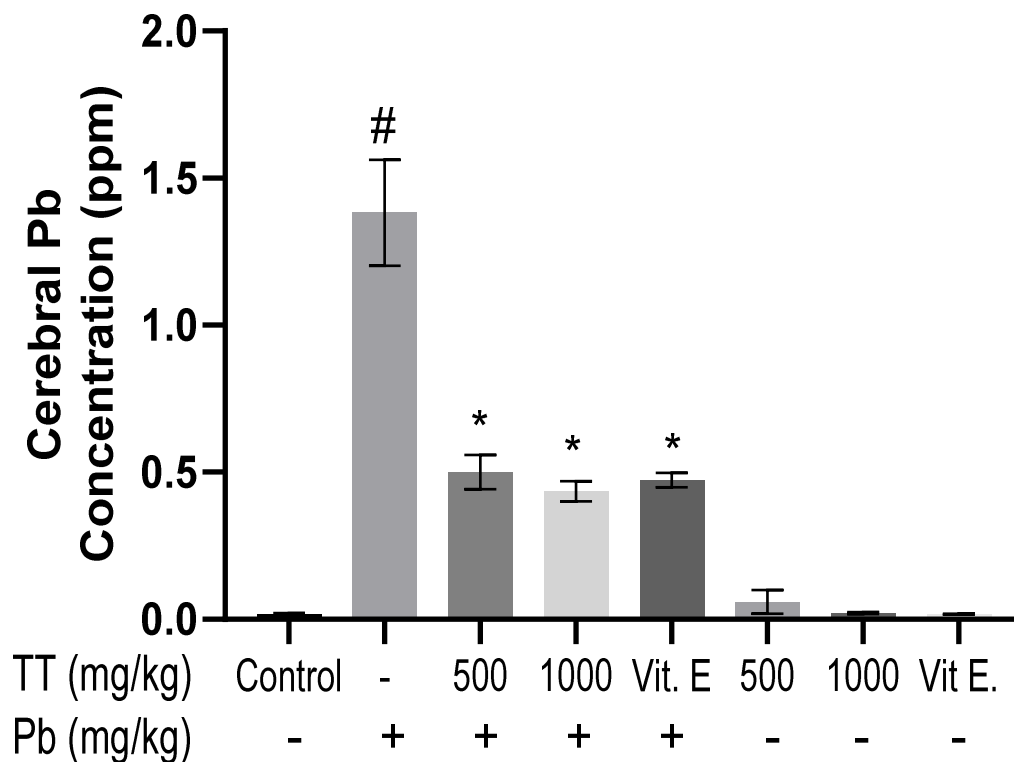


Figure 4.16: Lead concentration in the cerebrum of rats across experimental groups.

$p<0.05$ compared with control group; * $p<0.05$ compared with lead only group.

4.8 EFFECT OF TREATMENT ON HISTOLOGY OF THE CEREBRUM

Plate 4.1 shows the histology of rats in the control group with normal granular structure. Plate 4.2 shows the cerebral histology of the lead only exposed rats with vacuolisation and pyknotic nuclei indicative of stress or cellular degeneration. Plates 4.3 and 4.4 show the cerebral histology of lead exposed rats pretreated with *Tetrapleura tetraptera* with normal granular layer observed suggesting the neuroprotection of the cells by the extract. Plate 4.5 show the cerebral histology of lead exposed rats pretreated with Vit E. possibly validating its antioxidant activities. Plates 4.6 and 4.7 show the cerebral histology of rats treated with *Tetrapleura tetraptera* only. Plate 4.8 shows the histology of a rats treated with Vit E only.

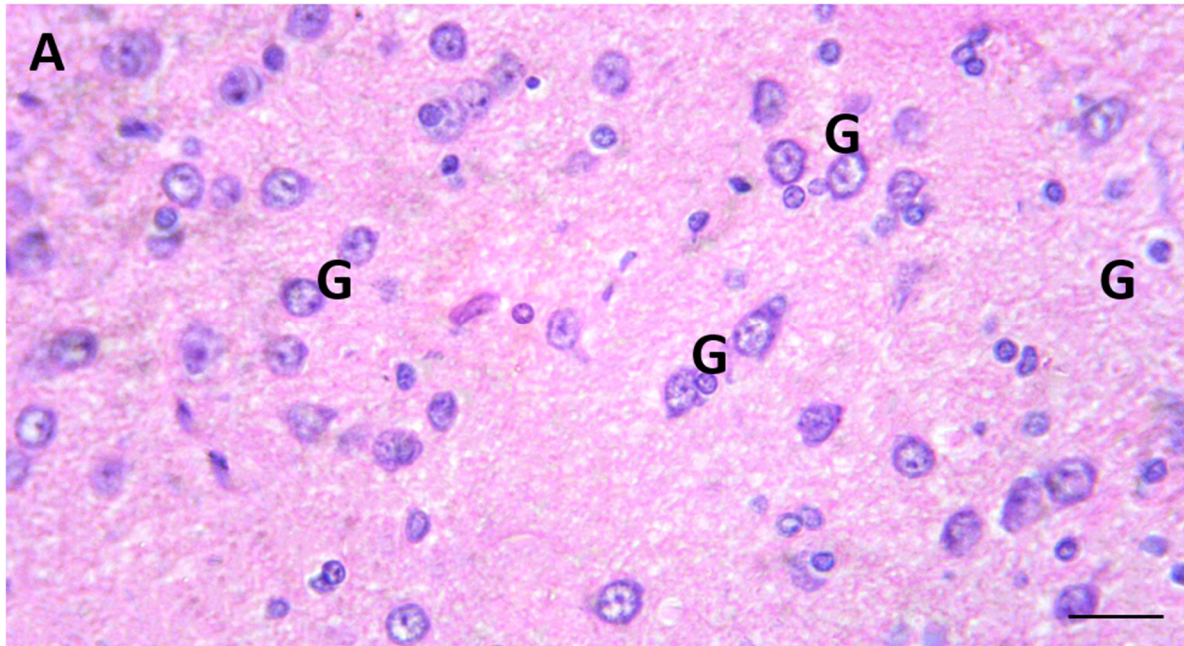


Plate 4.1: Representative histology of the cerebral cortex (internal granular layer IV) in control Group A showing normal granular (G) cells. (H&E; 400x - Scale bar: 25 μ m)

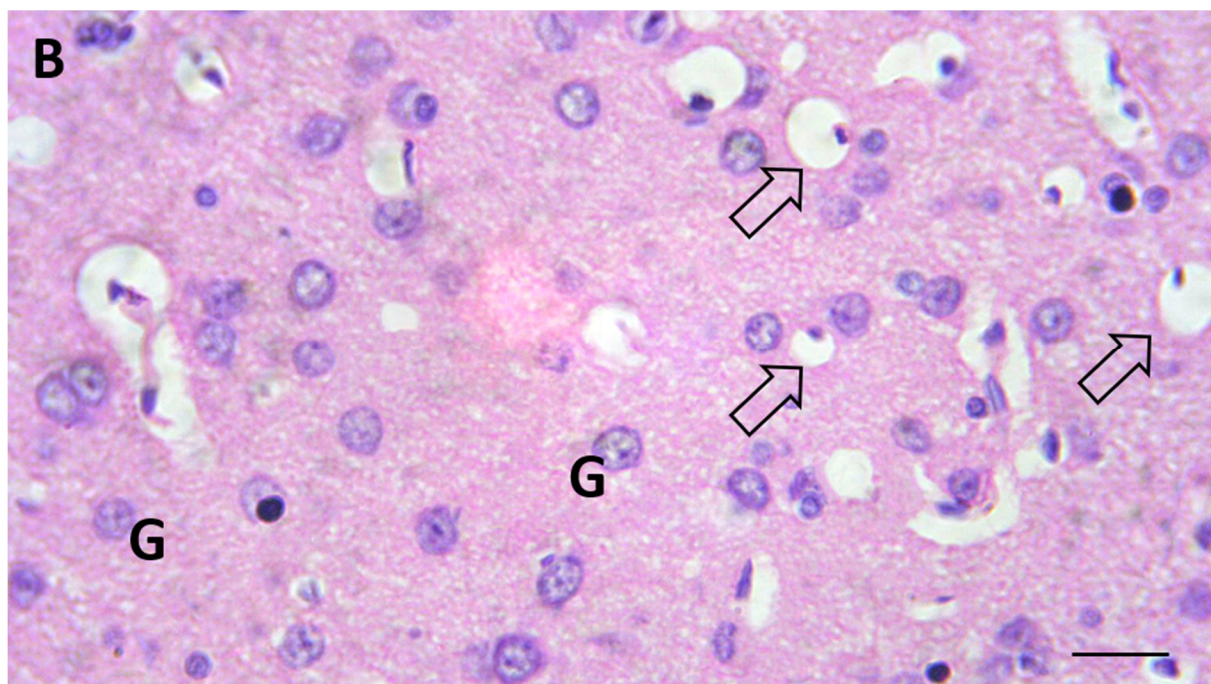


Plate 4.2: Representative histology of the cerebral cortex (internal granular layer IV) in treatment Group B. Vacuolisation (arrows) and darkly stained pyknotic nuclei observed. Granular cells also seen (G). (H&E; 400x - Scale bar: 25 μ m)

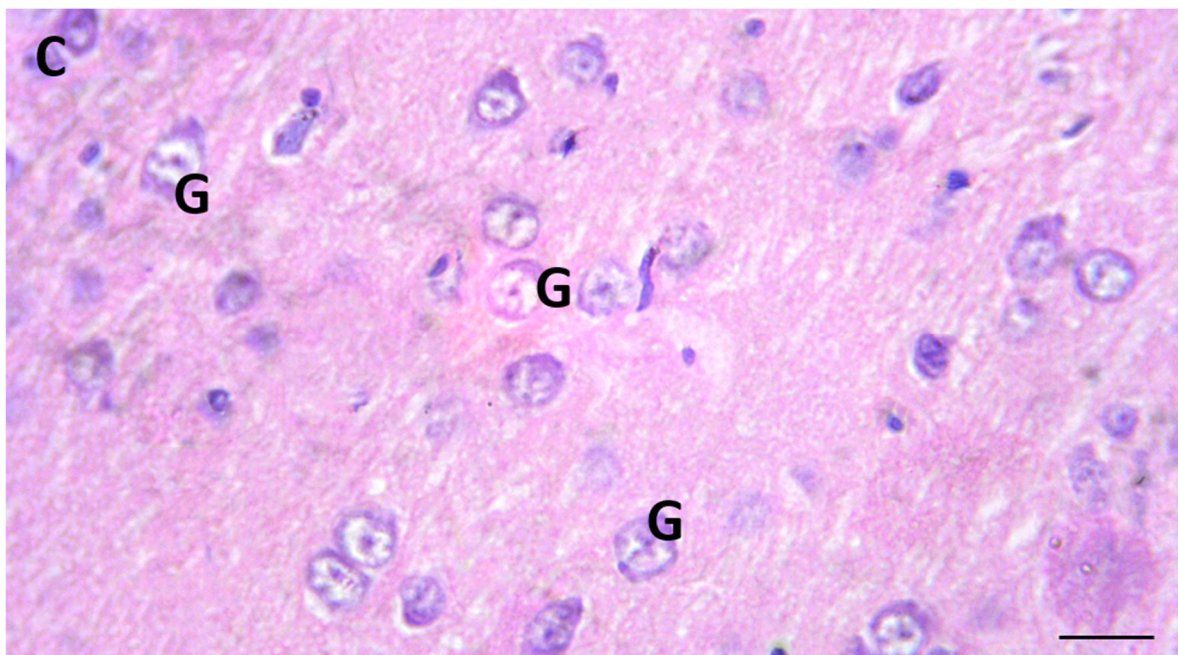


Plate 4.3: Representative histology of the cerebral cortex (internal granular layer, IV) in treatment group C. Normal features of the internal granular layer observed. (H&E; 400x - Scale bar: 25 μ m)

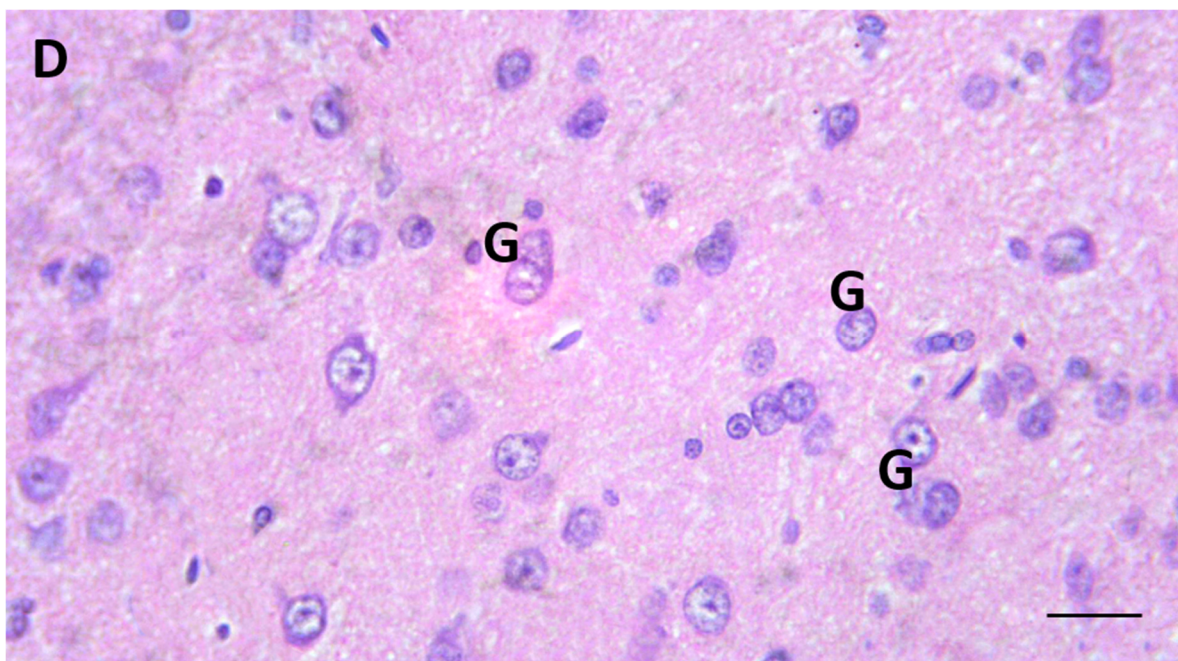


Plate 4.4: Representative histology of the cerebral cortex (internal granular layer, IV) in treatment group D. Normal features of the internal granular layer observed. (H&E; 400x - Scale bar: 25 μ m)

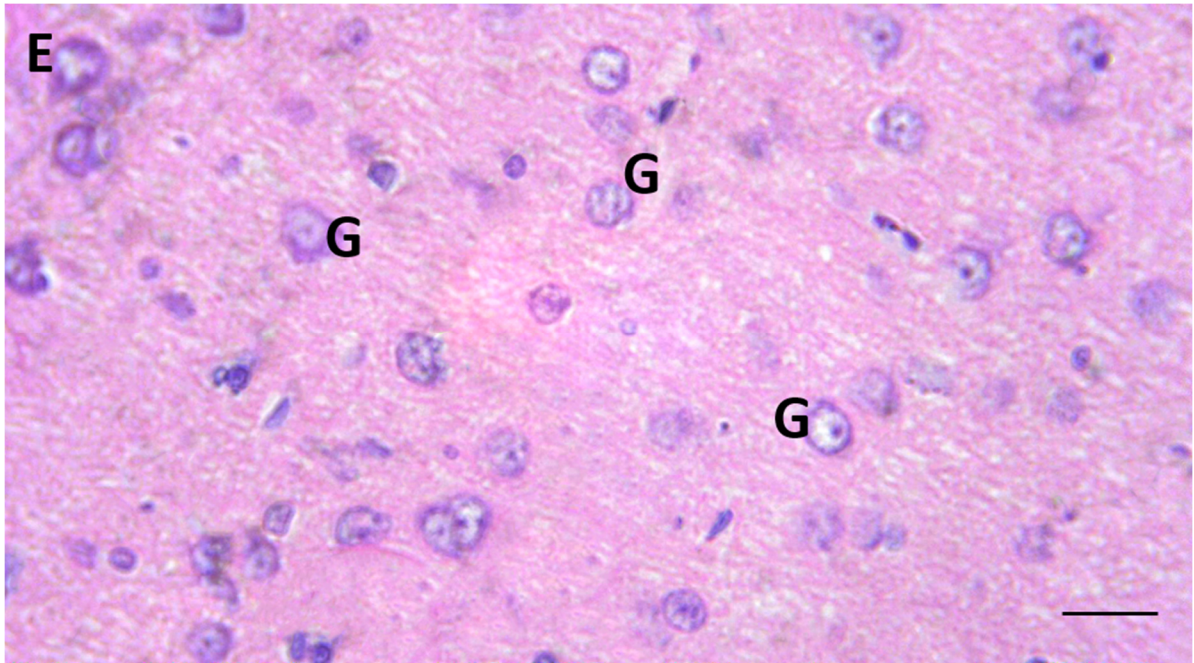


Plate 4.5: Representative histology of the cerebral cortex (internal granular layer, IV) in treatment group E. Normal features of the internal granular layer observed. (H&E; 400x - Scale bar: 25 μ m)

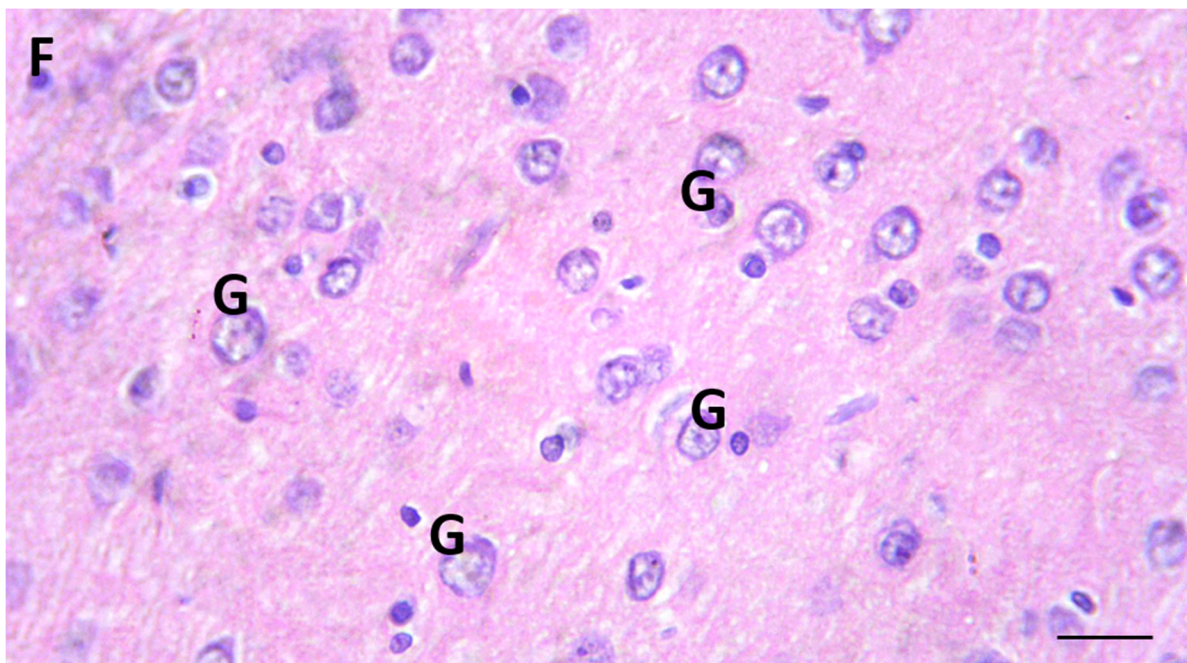


Plate 4.6: Representative histology of the cerebral cortex (internal granular layer, IV) in treatment group F. Normal features of the internal granular layer observed. (H&E; 400x - Scale bar: 25 μ m)

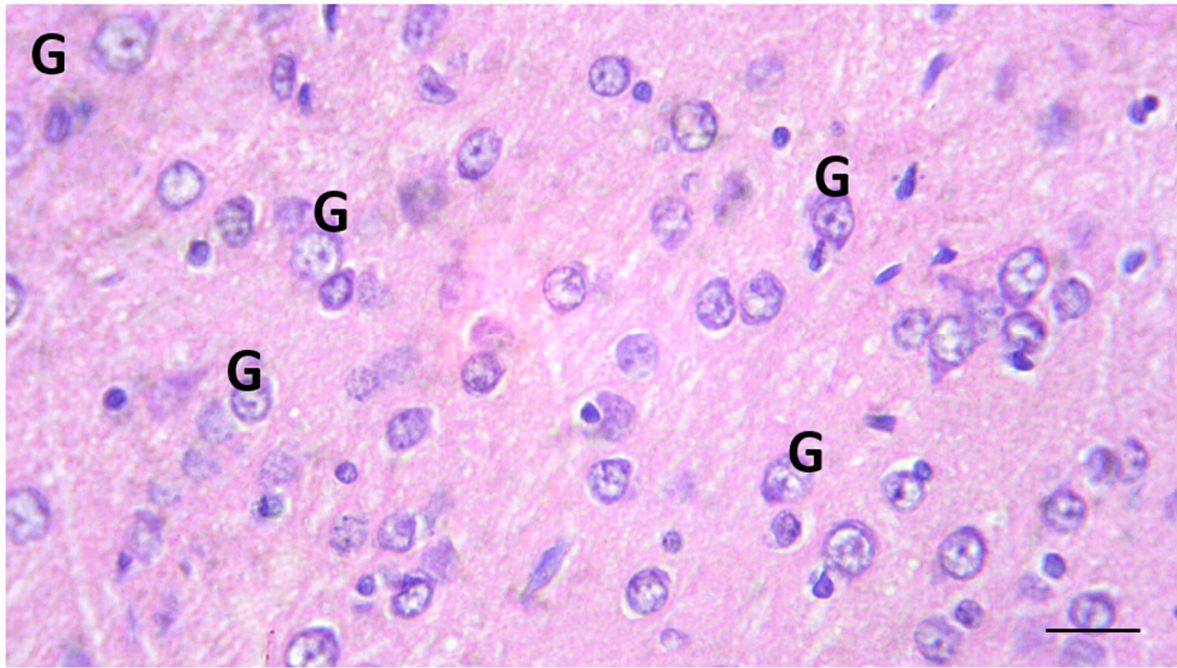


Plate 4.7: Representative histology of the cerebral cortex (internal granular layer, IV) in treatment group G. Normal features of the internal granular layer observed. (H&E; 400x - Scale bar: 25 μ m)

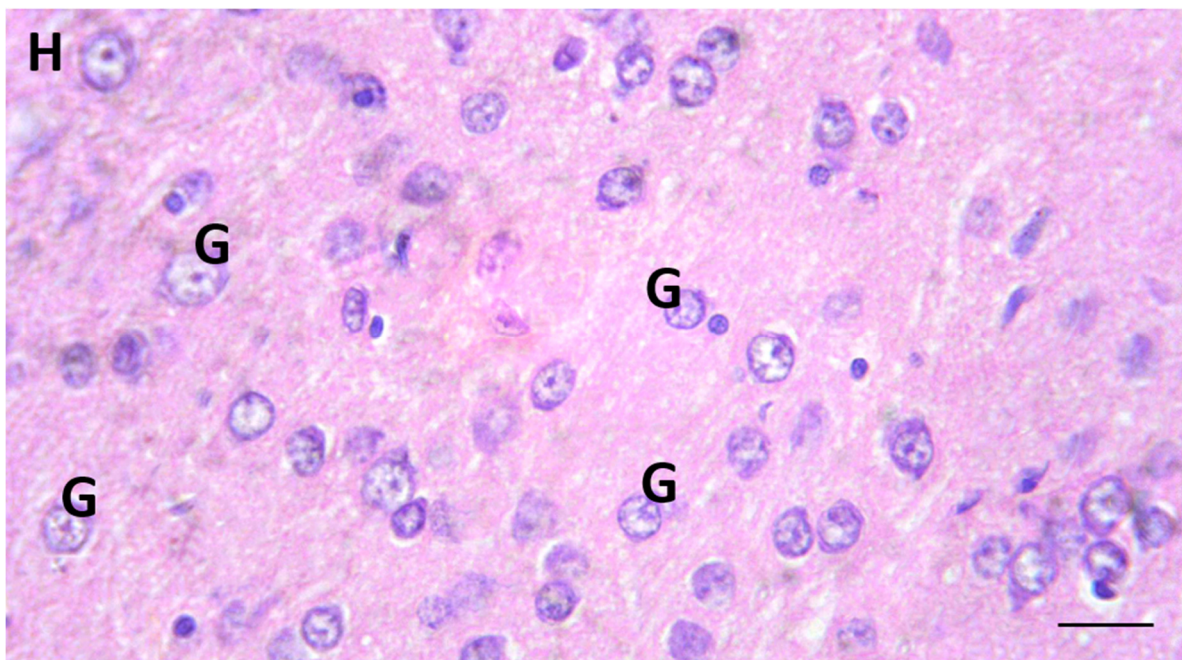


Plate 4.8: Representative histology of the cerebral cortex (internal granular layer, IV) in treatment group H. Normal features of the internal granular layer observed. (H&E; 400x - Scale bar: 25 μ m)

4.9 *Tetrapleura tetraptera* LIGAND–RECEPTOR INTERACTIONS

Figure 2 shows the High-Performance Liquid Chromatography (HPLC) result with identified polyphenols; catechol and phloroglucinol. Figures 4.18 – 4.20 show 2D interaction of Phloroglucinol and Catechol with IL-6, Nrf2 and Caspase 3 interacting amino acid residues within the binding pocket.

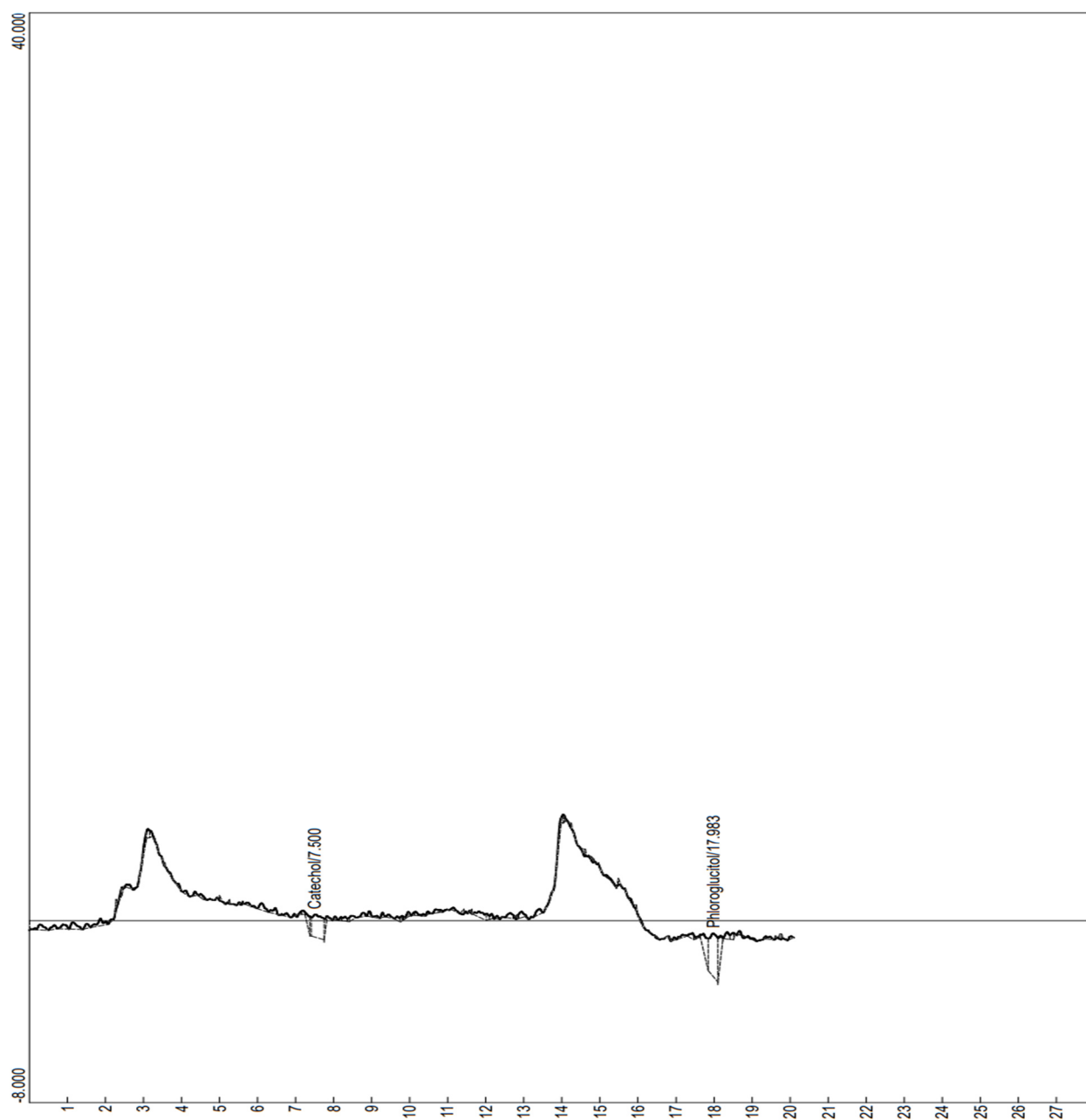


Fig 4.17: High Performance Liquid Chromatography (HPLC) result showing identified polyphenols; catechol and phloroglucinol

Interleukin 6 (IL-6)

Table 4.3: Identified compounds and their docking scores with Interleukin 6

Compound	Docking score (kcal/mol)
Phloroglucinol	-3.634
Catechol	-3.402
Levodopa (Standard)	-3.012
Clonazepam (Standard)	-2.517

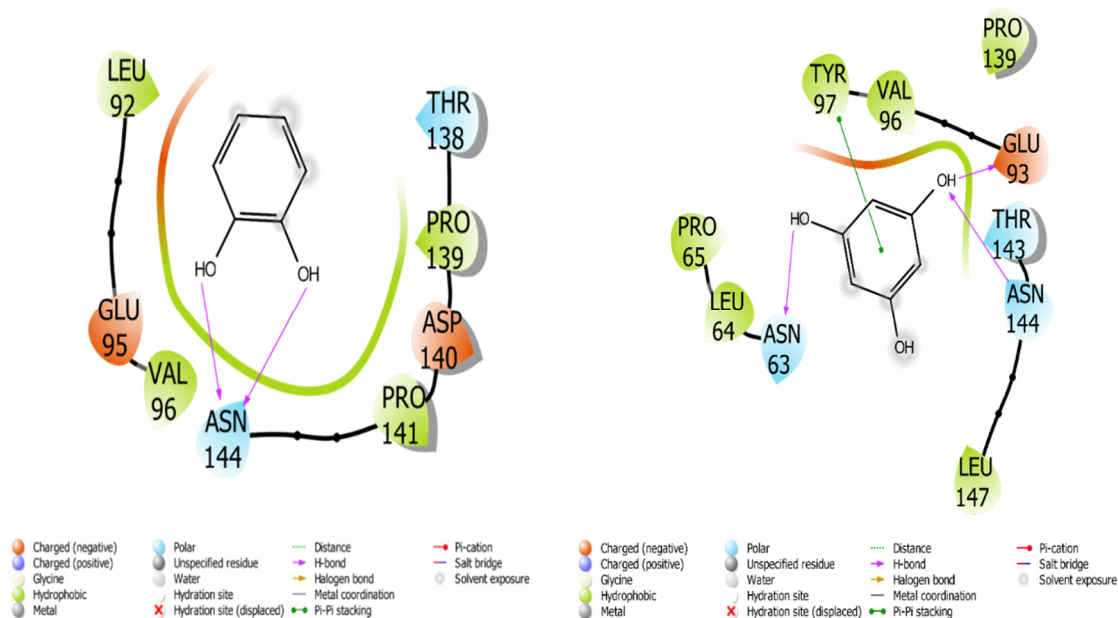


Figure 4.18: 2D interaction of Phloroglucinol and Catechol with IL-6 showing interacting amino acid residues within the binding pocket

Nrf2

Table 4.4: Identified compounds and their docking scores with Nrf2

Compound	Docking score (kcal/mol)
Levodopa (Standard)	-4.488
Catechol	-4.167
Phloroglucinol	-3.707
Clonazepam (Standard)	-2.263

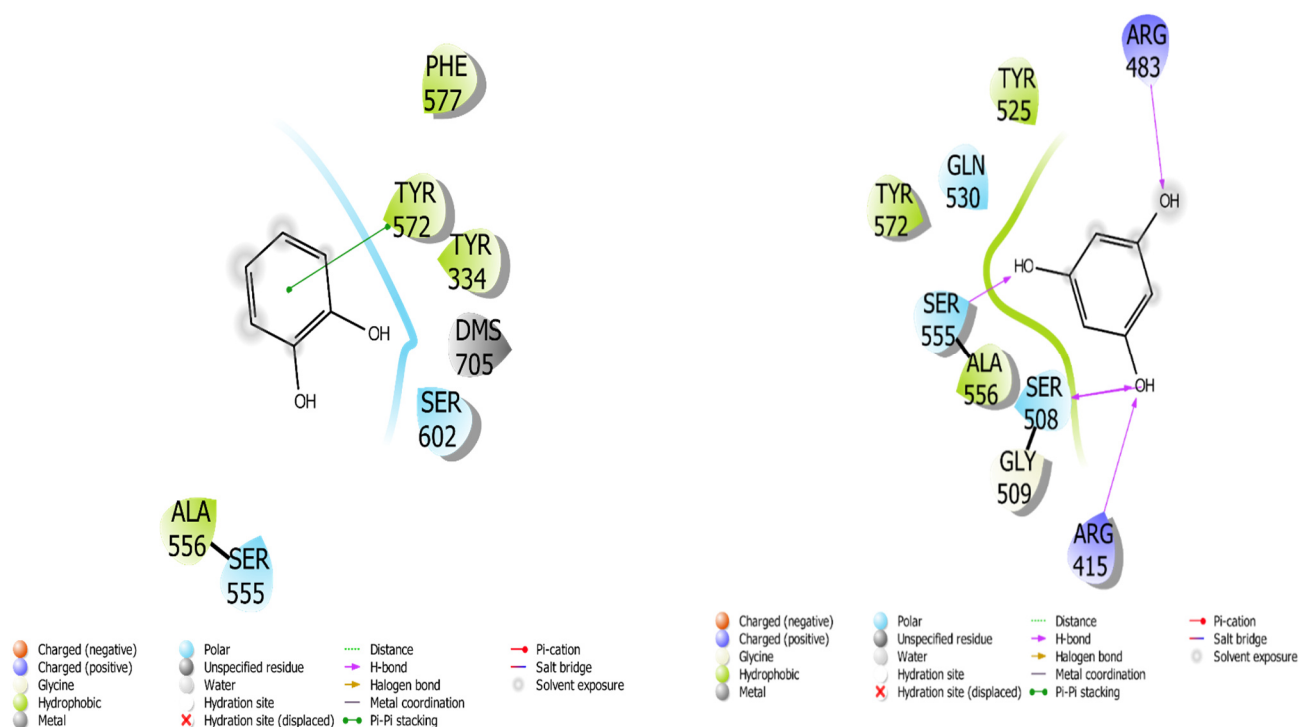


Figure 4.19: 2D interaction of Phloroglucinol and Catechol with Nrf2 showing interacting amino acid residues within the binding pocket

Caspase-3

Table 4.5: Identified compounds and their docking scores with Caspase-3.

Compound	Docking score (kcal/mol)
Levodopa (Standard)	-5.385
Clonazepam (Standard)	-5.282
Phloroglucinol	-3.753
Catechol	-3.503

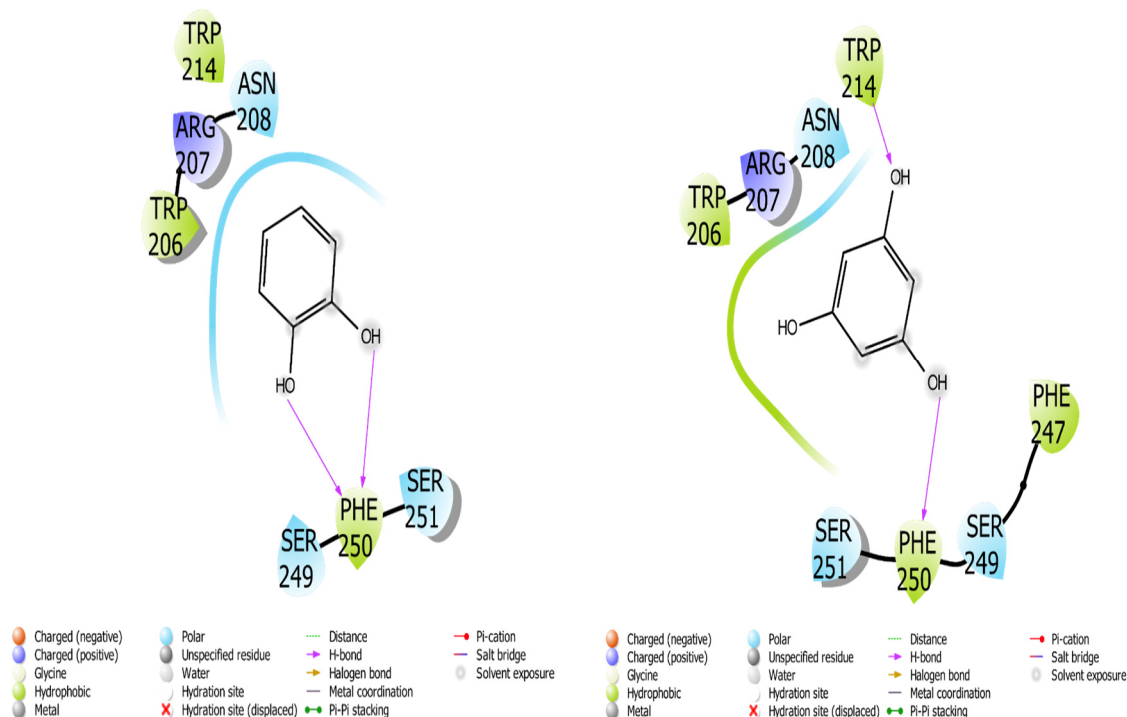


Figure 4.20: 2D interaction of Phloroglucinol and Catechol with Caspase-3 showing interacting amino acid residues within the binding pocket

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 *Tetrapleura tetraptera* Attenuates Weight Loss in Lead Exposed Rats

Variations in body weight serve as critical indicators of overall health and physiological status (Wang and Wu, 2022). Significant changes may reflect systemic disturbances and often act as early warning signs of underlying medical conditions (Wang and Wu, 2022). Exposure to pharmacological agents and toxicants particularly lead has been documented to affect body weight in animal models (Collin *et al.*, 2022; Enogieru and Momodu, 2022). Such alterations, especially reductions in body mass, are considered reliable markers of the toxic impact of these substances (Montoro-Huguet *et al.*, 2021). Lead toxicity disrupts normal growth and developmental pathways, frequently leading to developmental delays, and in more severe cases, resulting in stunted growth (Ortega *et al.*, 2021). Therefore, lead exposure represents a substantial health hazard due to its capacity to compromise both somatic and organ development, ultimately affecting overall physiological outcomes. The results of this study revealed a marked reduction in both body and brain weights among lead exposed rats compared to the control group. This decline may be attributed to several physiological disruptions commonly linked to heavy metal toxicity. Notably, lead ingestion has been associated with anorexia, characterized by diminished food intake in experimental animals (Wang *et al.*, 2022). Furthermore, lead exposure has been shown to reduce muscle mass and induce cachexia, a metabolic syndrome defined by persistently elevated basal metabolic rates without a corresponding increase in caloric or protein consumption (Rausch *et al.*, 2021). These factors likely contribute to the impaired weight gain observed in lead only exposed rats. Interestingly, rats pretreated with *Tetrapleura tetraptera* exhibited a significant increase in body weight, suggesting that the extract may counteract lead induced weight loss (Oumar *et al.*, 2025). This improvement may be linked to the nutritional and therapeutic attributes of *Tetrapleura*

tetraptera, which contains bioactive constituents such as saponins and alkaloids known to stimulate appetite and enhance nutrient absorption (Meziane *et al.*, 2025). Additionally, its antioxidant properties may promote metabolic efficiency and safeguard against muscle wasting, thereby supporting overall physiological resilience (Lommelli *et al.*, 2025). Also, rats pretreated with Vit E exhibited a significant increase in body weight suggesting its improvement of appetite in agreement with studies reporting that vitamin E pretreatment or co-treatment attenuates weight loss and neurobehavioral deficits and is associated improved antioxidant markers, changes consistent with restored appetite and food intake (Enogieru & Idemudia, 2024).

5.2 *Tetrapleura Tetraptera* Inhibits Neurobehavioural Changes in Lead Exposed Rats

Previous research underscores the critical role of behavioural assessments in evaluating the neurotoxic effects of environmental exposures, as they offer functional insights into how neurotoxins impact the central nervous system (Roberts and Stout, 2023). Panina and colleagues in 2023 highlighted neurobehavioral evaluation as the primary approach for systematically monitoring neurotoxic exposure, noting its capacity to detect changes in mood, emotional regulation, social interaction, and both cognitive and motor functions (Panna *et al.*, 2023). Empirical evidence further demonstrates that exposure to neurotoxins, particularly lead, can impair working memory, reduce processing speed, and disrupt visuospatial and motor abilities, as revealed through neurobehavioral testing (Ortega *et al.*, 2021).

The Open Field Test (OFT) is a well-established behavioural paradigm used to evaluate cerebral dysfunction through general locomotor activity, exploratory behaviour, and anxiety-like responses in animal models (Kraeuter *et al.*, 2019). By monitoring movement patterns (locomotor activity); thigmotaxis and sniffing (anxiety); frequency of rearing (exploration) and grooming (emotion), the OFT provides insight into the functional status of neural circuits (Sturman *et al.*, 2018). Its sensitivity to changes in emotional reactivity and motor function

makes it a valuable tool for assessing the impact of neurotoxic agents and for screening potential anxiolytic or stimulant compounds (Deshpande *et al.*, 2020, Belzung & Griebel, 2001; Wu, 2025; Lee, 2025). The Tail Suspension Test (TST) is a widely accepted behavioural assay used to evaluate depressive-like states and the efficacy of antidepressant treatments in animal models, particularly mice (Cryan *et al.*, 2005). By suspending the animal by its tail and recording the duration of immobility, researchers assess behavioural despair; an indicator for mood-related dysfunction (Stéru *et al.*, 1985). Immobility is interpreted as a passive coping strategy, and reductions in this behaviour following pharmacological intervention suggest antidepressant activity. Increased immobility in the TST correlates with dysfunction in neurotransmitter systems, which are central to mood regulation and cognitive function (Berrocoso *et al.*, 2013). The test reflects activity in the prefrontal cortex of the cerebrum, hippocampus, and limbic system, areas commonly affected in depression and other cerebral disorders (Gao, 2025, Mendelson, 2019). It is a valuable tool for screening novel therapeutics and exploring the neurobiology of affective disorders (Can *et al.*, 2012).

Results from this study indicate a significant decrease in rearing frequency for the lead only treated rats, which may reflect reduced exploratory drive and increased anxiety levels, indicative of overall lower cognitive engagement, a characteristic of lead neurotoxicity (Wang *et al.*, 2022). These findings align with previous research demonstrating that lead exposure significantly reduces rearing frequency and other exploratory behaviours in Wistar rats, likely due to disrupted neurotransmitter signalling, calcium dysregulation, and mitochondrial dysfunction. (Tartaglione *et al.*, 2020, Ramírez Ortega *et al.*, 2021; Singh *et al.*, 2024). In contrast, lead exposed rats that received pre-treatment with *Tetrapleura tetraptera* exhibited a marked increase in rearing frequency, indicating a mitigation of lead induced neurobehavioral deficits and reflecting enhanced exploratory behaviour alongside reduced anxiety-like responses. Rats pretreated with Vit E. also showed significant increase in rearing times

concurring with studies by Enogieru and Idemudia (2024). Grooming, an index of neurological health and stress response, was found to have significantly increased in the lead only exposed groups indicating anxiety. This is consistent with the findings of Zafar *et al.*, (2025) and Khudhair *et al.*, (2024) who reported increased grooming behaviour in rats, reflecting heightened emotional arousal and neurobehavioral dysregulation in lead exposed rats. However, lead exposed rats pretreated with *Tetrapleura tetraptera* demonstrated a significant decrease in grooming suggesting an attenuation of lead induced deficit. Rats pretreated with Vit E. as well showed significant decrease in grooming behaviour suggesting amelioration of the neurobehavioral effects of lead (Salehi *et al.*, 2015). Thigmotaxis, characterised by a preference for wall-hugging in unfamiliar environments, is a well-established behavioural marker of emotional distress and heightened anxiety in rodents (Kalueff *et al.*, 2023). It was observed to have increased significantly in the lead only exposed groups. There was however, a significant decrease in lead exposed rats pretreated with *Tetrapleura tetraptera* indicative of lower anxiety levels and increased exploratory behaviour. Sniffing behaviour in rats serves as an indicator of sensory engagement, emotional arousal, and cognitive processing. A significant increase in sniffing patterns was seen among lead only exposed rats suggesting significant disruption in olfactory-driven exploration neurotransmitter systems in the hippocampus, olfactory bulb and pre-frontal cortex (Rojas-Líbano & Kay, 2012). A significant decrease of sniffing behaviour in lead exposed *Tetrapleura tetraptera* pretreated rats may therefore suggest a restoration of olfactory function and emotional stability. Immobility time is a sensitive behavioural marker for emotional suppression, passive coping, and depressive-like states in rodents (Can *et al.*, 2012; Cao *et al.*, 2021). Lead only exposed rats showed significant prolonged immobility, suggesting heightened emotional blunting and reduced motivational drive, indicative of neurobehavioral toxicity due to lead which disrupts monoaminergic neurotransmission, particularly serotonin and dopamine pathways (Erçan *et al.*, 2023).

However, the significant reduction in immobility time among *Tetrapleura tetraptera* pretreated rats may reflect a reversal of lead induced neurochemical deficits. Rats pretreated with Vit E. also displayed significant reductions in thigmotaxis, sniffing and immobility time in agreement with studies carried out by Olatunde *et al* (2024); Onitsha and Okutu (2021), Enogieru and Idemudia (2024).

5.3 *Tetrapleura tetraptera* Inhibits Oxidative Stress in Lead Exposed Rats and Mitigates Lead Accumulation

Oxidative stress is widely acknowledged as a key mechanism by which heavy metals exert neurotoxic effects (Pyatha *et al.*, 2022). Metals like lead contribute to this process by promoting the generation of reactive oxygen species (ROS); chemically unstable molecules capable of initiating cellular damage (Nowicka, 2022). This imbalance arises when the production of ROS exceeds the capacity of the body's antioxidant defence systems to neutralize them or repair the resulting damage (Nowicka, 2022; Liu *et al.*, 2022; Pyatha *et al.*, 2022). Such an imbalance may result from excessive production of reactive oxygen species (ROS), diminished antioxidant defences, or a combination of both (Liu *et al.*, 2022). ROS are particularly harmful due to their unpaired electrons, which drive them to react destructively with cellular components such as proteins, lipids, and DNA (Juan *et al.*, 2021). Lead possesses the ability to cross the blood–brain barrier where it interferes with enzymatic systems vital for maintaining cerebral homeostasis (Collin *et al.*, 2022; Li *et al.*, 2021). The brain is especially vulnerable to oxidative damage because of its high oxygen consumption and lipid-rich composition, making robust antioxidant protection essential for preserving neural integrity (Collin *et al.*, 2022).

To reduce oxidative stress, the body depends on antioxidants which are molecules capable of safely donating electrons to reactive oxygen species (ROS), thereby neutralizing their reactivity and stabilizing cellular environments (Shaw *et al.*, 2022). Among the most vital components of this defence system are the enzymatic antioxidants superoxide dismutase (SOD), catalase

(CAT), glutathione peroxidase (GPx) and the tripeptide glutathione (GSH), which collectively prevent or repair oxidative damage (Rao *et al.*, 2025). These enzymes are particularly susceptible to lead toxicity, as their structural integrity and catalytic function rely on essential trace elements such as zinc, copper, and selenium (Jomova *et al.*, 2022). Superoxide dismutase (SOD) acts as the first line of defence by catalysing the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen, thereby reducing the potential for cellular injury (Saxena *et al.*, 2022).

Catalase (CAT) and glutathione peroxidase (GPx) also play a key role in this defence by decomposing hydrogen peroxide, a potent reactive oxygen species (ROS) into water and alcohols, thereby preventing its accumulation and the exacerbation of oxidative stress which may lead to membrane lipid peroxidation (Anwar *et al.*, 2024; Lana *et al.*, 2024; Zhair *et al.*, 2023). In doing so, catalase (CAT) and glutathione peroxidase (GPx) also helps preserve the activity of superoxide dismutase (SOD), which may be inhibited in the presence of excess hydrogen peroxide (Jomova, 2024). Impaired catalase (CAT) and glutathione peroxidase (GPx) function can lead to elevated hydrogen peroxide levels, posing a threat to sensitive brain regions such as the cerebrum (Anwar *et al.*, 2024; Jomova, 2024). Glutathione (GSH) complements these enzymatic defences by directly scavenging free radicals, protecting cellular macromolecules from oxidative damage (Lana *et al.*, 2024). In contrast, malondialdehyde (MDA), a by-product of lipid peroxidation, serves as a reliable biomarker for oxidative stress. Formed during the degradation of polyunsaturated fatty acids, malondialdehyde (MDA) reflects the extent of membrane damage caused by reactive oxygen species (ROS) and is widely used to assess oxidative injury in tissues (Zengin, 2021; Zhang *et al.*, 2025). Oxidative defence mechanisms are especially critical in the brain, where high metabolic activity and oxygen consumption make neural tissue particularly vulnerable to damage from reactive oxygen species (ROS) (Houldsworth, 2024; Bhatt *et al.*, 2021).

Findings from this study revealed a significant reduction in the activity of antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as decreased glutathione (GSH) levels, accompanied by a significant increase in malondialdehyde (MDA) concentration in lead only exposed rats. These results are consistent with previous reports indicating that the neurotoxic effects of lead are primarily mediated through oxidative stress pathways (Ramírez Ortega *et al.*, 2021; Pyatha *et al.*, 2022). Earlier studies have demonstrated that lead exposure induces oxidative damage in brain tissue by suppressing key antioxidant defences and promoting lipid peroxidation, as evidenced by increased malondialdehyde (MDA) levels in experimental models (Li *et al.*, 2023; Zhang *et al.*, 2025). However, pretreatment with *Tetrapleura tetraptera* effectively mitigated the neurotoxic effects of lead by enhancing the activity of these enzymatic and non-enzymatic antioxidants, and reducing malondialdehyde (MDA) concentration, thereby demonstrating its potent antioxidant capacity (Moukette *et al.*, 2015). Rats pretreated with Vit E also showed a significant increase in the antioxidant enzyme activity of superoxide dismutase (SOD), catalase (CAT) as well as a significant decrease in malondialdehyde (MDA) levels; concurring with previous studies by Almarzougi & Al-Sowayan (2024).

5.4 *Tetrapleura Tetraptera* Mitigates Lead Accumulation and Histological Alterations in The Cerebrum of Lead-Exposed Rats

Lead readily accumulates in the cerebrum following exposure, where it disrupts neural function and cellular architecture (Maheshwari *et al.*, 2021). It enters the cerebrum through the bloodstream predominantly bound to erythrocytes, with only a small fraction in plasma available to cross the blood–brain barrier (BBB) (Kathuria & Ramachandran, 2020). It exploits physiological uptake pathways, particularly divalent metal transporter 1 (DMT1) and voltage-dependent calcium channels, to traverse the neurovascular endothelium and gain access to the brain parenchyma (Kathuria & Ramachandran, 2020). Once in the cerebral tissue, Lead avidly

binds to sulfhydryl groups on neuronal proteins and to phospholipid membranes, concentrating especially in regions such as the prefrontal cortex and hippocampus (Sidhu & Nehru, 2004). Its affinity for these sites, coupled with a markedly slower washout rate from brain compared to blood, results in sustained retention and progressive accumulation within neurons and glial cells (Sidhu & Nehru, 2004). It causes degeneration of neurons particularly in the prefrontal cortex of the cerebrum and hippocampus, affecting cognition, memory and emotional regulation. The cerebrum is particularly susceptible to the toxic effects of lead exposure due to its intricate neural architecture and high metabolic activity (Gudadhe *et al.*, 2024). As the primary centre for higher-order cognitive functions including attention, memory, decision-making, and sensory integration, the cerebrum relies heavily on the structural and functional integrity of its neurons (Abed, 2023). Lead neurotoxicity disrupts cerebral homeostasis by impairing synaptic transmission, altering neurotransmitter dynamics, and inducing oxidative stress, all of which compromise cognitive performance (Ortega *et al.*, 2021). Studies have shown that cortical neurons, especially in regions such as the prefrontal cortex, are highly vulnerable to lead induced degeneration, leading to deficits in executive function and behavioural regulation (Gudadhe *et al.*, 2024). Chronic lead levels are visible in histological sections as shrinkage of neurons, disruption of normal cell layering, vacuolation, and perineuronal space enlargement (Sidhu, and Nehru, 2004; Nwakanma, *et al*, 2014; Naqi, 2015). Increased doses of lead result in chromatolysis (breakdown of Nissl substance) and neuronal hypertrophy, with many cells showing signs of apoptosis or necrosis (Borda *et al.*, 2024). Histological damage intensifies with higher concentrations of lead. Mild exposure may cause subtle changes, while high doses lead to widespread cell death and structural disorganization. Results from this study show a significant increase in lead accumulation in the cerebrum of rats exposed to lead only compared with the control group. This agrees with studies by Sallu and Kumar (2025), Wong *et al.*, (2024) and Xu *et al.*, (2024) establishing lead as a potent neurotoxin

capable of crossing the blood-brain barrier and accumulating in brain tissues, and also suggesting a direct mechanism of neurotoxic action, forming the basis for the neurological and cognitive deficits.

However, there was a reduction in cerebral lead concentration observed in rats pre-treated with *Tetrapleura tetraptera* extract, when compared to the lead-only group. This is likely attributable to the plant's rich phytochemical profile, particularly its high content of polyphenols and flavonoids (Sarkodie *et al.*, 2020), compounds known for their metal-chelating abilities which may have enabled them to bind with lead ions in the bloodstream and prevent their transport across the BBB into the brain as well as its antioxidant activity ((Saliu *et al.*, 2021; Tsala *et al.*, 2014). There was also a significant decrease in cerebral lead concentration in the Vitamin E-treated group further support the role of antioxidant defence in combating lead neurotoxicity through the neutralisation of free radicals, thereby maintaining the structural and functional integrity of the neurons and blood-brain barrier (Salehi *et al.*, 2015).

In the present study, histological analysis of the cerebrum in lead only exposed rats revealed pronounced cellular abnormalities, including vacuolated granular cells and pyknotic nuclei. These changes suggest lead exposure induces substantial neurotoxic damage within the cerebrum, particularly targeting granule cells that are essential for sensory processing, signal integration and intracortical communication (Ramírez Ortega *et al.*, 2021; Gudadhe *et al.*, 2024). These results agree with Enogieru and Egbon (2022) who reported that lead acetate treated Wistar rats developed pyramidal neurons with cytoplasmic shrinkage, pyknotic nuclei, and chromatolysis in the cerebral cortex. Gudadhe *et al.* (2024), also in their review of lead neurotoxicity, highlight vacuolation of cortical neurons and nuclear pyknosis as reproducible hallmarks of lead-induced damage in the cerebrum of exposed rats. The observed vacuolations reflect compromised cellular integrity and may indicate disrupted synaptic architecture, which

could contribute to the cognitive and behavioural deficits commonly associated with lead neurotoxicity (Carmona *et al.*, 2021).

Conversely, rats pre-treated with *Tetrapleura tetraptera* exhibited relatively preserved cerebral histology, with granular cells appearing structurally intact and showing minimal signs of degeneration. This neuroprotective effect is consistent with prior research indicating that plant-derived antioxidants can attenuate heavy metal-induced neurodegenerative changes (Enogieru & Momodu, 2022; Enogieru & Egbon, 2022). The therapeutic potential of *Tetrapleura tetraptera* is largely attributed to its abundance of phytochemicals, which have demonstrated efficacy in mitigating oxidative stress and neuroinflammation, key contributors to neuronal damage (Kölliker-Frers *et al.*, 2021; Bellavite, 2023; Tavan *et al.*, 2024)

5.5 *Tetrapleura tetraptera* Ligand–Receptor Interactions

High Performance Liquid Chromatography of the extract identified polyphenols; catechol and phloroglucinol, which may form stable complexes with lead ions (Pb^{2+}) and facilitate their renal and biliary excretion, agreeing with the studies of Wang *et al* (2024) who reported phenolic compounds coordinating with metal ions such as lead ions through their hydroxyl groups, forming stable complexes that influence bioavailability and detoxification pathways. Anti-inflammatory and neuroprotective effects come via inhibition of NF- κ B and MAPK pathways in microglia and astrocytes; reduction of glial scar formation and preservation of synaptic proteins and dendritic spines (Bobe and Linder, 2023; Calixto *et al.*, 2024; Singh *et al.*, 2025). Phenols modulate blood-brain barrier transport system by inhibiting efflux pumps (P-glycoprotein), stabilising tight junction proteins and preserving barrier integrity (Regdon *et al.*, 2021; Lv *et al.*, 2019; Velásquez-Jiménez *et al.*, 2021; Han *et al.*, 2012; Borda *et al*, 2024; Ferreira *et al.*, 2017); and also exert its antioxidant activity by activating Nrf2 signalling, directly scavenging ROS and recycling other antioxidants e.g. vitamin E (Bains *et al.*, 2025, Lande *et al*; 2025; Zhai *et al*; 2023)

Nrf2 (Nuclear factor erythroid 2-related factor 2) is a regulator of antioxidant response. It controls the expression of genes involved in detoxification, redox balance, and cellular defence (Vomund *et al.*, 2017; Mayer *et al.*, 2024). Activation of Nrf2 is protective in conditions involving oxidative stress, such as lead-induced neurotoxicity, Parkinson's disease, and inflammatory disorders (Parga *et al.*, 2021; Yang *et al.*, 2022). Catechol demonstrates strong binding (-4.167 kcal/mol) to Nrf2, suggesting a highly stable interaction and potential activation or modulation, indicating it may effectively engage Nrf2 and contribute to antioxidant defence mechanisms. Also, phloroglucinol shows binding (-3.707 kcal/mol), validating its known antioxidant properties. The strong binding of phloroglucinol and catechol suggests they may enhance Nrf2-mediated transcription of antioxidant enzymes like glutathione peroxidase, superoxide dismutase, and Heme Oxygenase-1 (HO-1), potentially reducing cellular damage (Mayer *et al.*, 2024; Parga *et al.*, 2021). This agrees with previous studies by Ryu *et al* (2023) and Oh *et al.*, (2009).

Interleukin-6 (IL-6) is a key cytokine involved in inflammation, immune regulation, and neurodegeneration (Tanaka *et al.*, 2014). Elevated IL-6 levels are implicated in conditions such as Alzheimer's disease, multiple sclerosis, and lead-induced neurotoxicity (Erta *et al.*, 2012; Gudadhe *et al.*, 2024). Compounds that bind effectively to IL-6 may help modulate its activity, potentially reducing inflammatory cascades and protecting neural tissue (Hunter & Jones, 2015). Results from molecular docking indicate that phloroglucinol displays the strongest predicted binding to IL-6, with a docking score of -3.634 kcal/mol, suggesting a relatively stable complex that may interfere with IL-6's pro-inflammatory signalling. Catechol also demonstrates good binding affinity (-3.402 kcal/mol), indicating potential biological relevance. Levodopa and clonazepam, used as reference standards, show weaker binding scores, suggesting lower direct interaction with IL-6 compared to the test compounds. The strong binding of phloroglucinol and catechol suggests it possesses anti-inflammatory

potential, which could be responsible for the neuroprotective activity of *Tetrapleura tetraptera* observed in this study. This agrees with previous studies indicating that *Tetrapleura tetraptera* possesses anti-inflammatory effects (Carmona *et al.*, 2021; Elekofehinti *et al.*, 2020).

Caspase-3 is a central executioner enzyme in the apoptotic cascade, responsible for cleaving cellular substrates during programmed cell death. In neurotoxicity models, especially those involving lead exposure, Caspase-3 activation is a hallmark of neuronal apoptosis. Compounds that bind to Caspase-3 may either inhibit its activity (preventing cell death) or enhance its activation (promoting apoptosis), depending on the context and binding dynamics. Phloroglucinol shows the strongest predicted binding to Caspase-3 (-3.753 kcal/mol), this suggesting a highly stable interaction and potential modulation of apoptotic pathways. Catechol binds closely to Caspase 3 (-3.503 kcal/mol), indicating it may also interact significantly with Caspase-3, possibly influencing cell survival or death. The binding of phloroglucinol and catechol suggests they may influence apoptotic signalling, potentially offering neuroprotective or regulatory effects in models of heavy metal toxicity or neurodegeneration. This aligns with previous studies indicating potent anti-apoptotic effects such as that of Lawal *et al.*, (2025) which reported suppression of the apoptotic cascade and boosted BCL-2 expression in *Tetrapleura tetraptera* pretreated rats.

5.6 FINDINGS

The findings from this study are as follows:

1. Aqueous *Tetrapleura tetraptera* fruit extract mitigated lead acetate-induced decrease in body and whole brain weights.
2. Aqueous *Tetrapleura tetraptera* fruit extract attenuated lead acetate-induced cognitive deficits.

3. Aqueous *Tetrapleura tetraptera* fruit extract inhibited lead acetate-induced dysregulation of antioxidant enzymes in the cerebrum of experimental rats.
4. Aqueous *Tetrapleura tetraptera* fruit extract protected against lead acetate-induced lipid peroxidation in the cerebrum of experimental rats.
5. Aqueous *Tetrapleura tetraptera* fruit extract inhibited accumulation of lead in the cerebrum of experimental rats.
6. Aqueous *Tetrapleura tetraptera* fruit extract mitigated lead acetate-induced histological alterations in the cerebrum of experimental rats.
7. Identified compounds in aqueous *Tetrapleura tetraptera* fruit extract exhibited potent in-silico antioxidant, anti-inflammatory anti-apoptotic activity.
8. Aqueous *Tetrapleura tetraptera* fruit extract contains phytochemicals such as, flavonoids, alkaloids, phenols, tannins, saponins and glycosides.

5.7 CONTRIBUTION TO KNOWLEDGE

The study has contributed to knowledge in the following ways:

1. Revealed that aqueous *Tetrapleura tetraptera* fruit extract attenuates lead acetate-induced neurobehavioral deficits, mitigates oxidative stress, and inhibits elevated lead levels in the cerebrum of experimental rats.
2. Showed that aqueous *Tetrapleura tetraptera* fruit extract protects against lead acetate-induced histological alterations in the cerebrum of experimental rats.
3. Revealed that aqueous *Tetrapleura tetraptera* fruit extract inhibits lead acetate-induced apoptosis in the cerebrum of experimental rats.

5.8 CONCLUSION

The molecular docking analysis revealed that bioactive compounds from aqueous *Tetrapleura tetraptera* fruit extract exhibit potent binding affinities to key neuroinflammatory, antioxidant and apoptotic targets, including IL-6, Nrf-2, and Caspase-3. Notably, the compounds, Phloroglucinol and Catechol, demonstrated strong interactions, suggesting potential to modulate oxidative stress, suppress inflammatory signalling, and regulate apoptotic pathways.

Taken together, these findings suggest that *Tetrapleura tetraptera* confers neuroprotection through multi-target mechanisms via attenuation of inflammation, enhancement of antioxidant activity, and inhibition of apoptosis. This integrative evidence underscores its therapeutic promise in mitigating lead-induced cerebral dysfunction and supports its continued exploration as a neuroprotective agent.

5.9 RECOMMENDATION

Further investigations on *Tetrapleura tetraptera* should focus on isolating specific bioactive compounds responsible for its neuroprotective effects against lead-induced cerebral dysfunction. Mechanistic studies using diverse models of neurodegeneration are warranted to elucidate other antioxidant, anti-inflammatory, and anti-apoptotic pathways. These efforts will advance *Tetrapleura tetraptera* as a candidate for clinical evaluation. Eventually, this could support its development as a plant-based therapeutic agent targeting cerebral dysfunction and cognitive impairments associated with heavy metal exposure and related neurological disorders.

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APPENDIX



RESEARCH ETHICS COMMITTEE
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.



Chairman: Prof. F. A Imarhiagbe
MBChb, FMCP
Cert Clin Res and ethics (NIH), MD.
0803449092

Email: researchethics.cms@gmail.com

P.M.B 1154, BENIN CITY

Our Ref: CMS/REC/01/VOL.2/796

Date: 18th September, 2025

Re: EVALUATION OF THE PROTECTIVE EFFECT OF AQUEOUS EXTRACT OF *tetrapleura tetrapter* FRUIT ON LEAD ACETAYTE-INDUCED NEUROTOXICITY IN ADULT WISTAR RATS

Name of Principal Investigator: OSUCHUKWU IKECHUKWU WILLIAM

Department Of Anatomy,
School of Basic Medical Science
College of Medical Sciences,
University of Benin

REC Approval No: CMS/REC/2025/796

This is to inform you that the research described in the submitted proposal, the Informed Consent Forms and other participant information materials have been reviewed and approved by the College Research Ethics Committee, University of Benin.

This approval dates from **18th September, 2025 to 19th September, 2026**. In multi-year research, Endeavour to submit your annual report to the REC early in order to obtain renewal of your approval and avoid disruption of your research.

The National Code of Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the code including ensuring that all adverse events are reported promptly to the REC. No, changes are permitted in the research without prior approval by REC except in circumstances outlined in the code. REC reserves the right to conduct compliance visit to your research site without prior notice. Thank you.

PROF. F.A IMARHIAGBE
Chairman, REC

Promoting best ethical & scientific standard for research in Nigeria