ACTIVITY OF *Rosmarinus officinalis* IN THE HIPPOCAMPUS OF LEAD ACETATE-EXPOSED RATS

BY

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UNIVERSITY OF BENIN
BENIN CITY

JUNE, 2025

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B.Sc. (DELSU, 2021)

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

JUNE, 2025

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

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DEDICATION

To God almighty, for His never ending mercies and favour, my parents, Mr. and Mrs. K.O. Onyesom, and my wonderful siblings.

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ABSTRACT

Hippocampal dysfunction is a key feature of several neurocognitive disorders and may arise from factors such as congenital defects, neurodegeneration, or exposure to neurotoxicants. Lead (Pb), a potent heavy metal, crosses the blood-brain barrier and accumulates in the hippocampus, where it disrupts calcium signaling and induces oxidative stress, thus contributing to neuronal damage and cognitive deficits. Evidence suggests that dietary antioxidants may help mitigate Pb-induced oxidative damage and preserve hippocampal function. Accordingly, this study investigated the protective activity of aqueous *Rosmarinus officinalis* leaf extract (*R. officinalis*) against lead acetate (PbA) induced hippocampal toxicity. Forty-eight (48) adult Wistar rats were randomly assigned into six groups (A-F). Group A served as control; Group B received 100 mg/kg body weight [bw] of PbA only; Group C received 100 mg/kg bw of *R. officinalis* extract and PbA; Group D received 200 mg/kg bw of *R. officinalis* extract and PbA; Group E received 100 mg/kg bw of *R. officinalis* extract only and Group F received 200 mg/kg bw of *R. officinalis* extract only. All administrations, via an orogastric tube, lasted for twenty-eight (28) days. Thereafter, neurobehavioral activities were evaluated using the Novel object recognition, Y-maze and Elevated plus maze tests. Following the sacrifice of the experimental rats, the hippocampi were collected for Pb concentration, antioxidant enzymes activity, lipid peroxidation, acetylcholinesterase activity, nitric oxide levels, and histological assessments as well as apoptosis. The findings showed that PbA-exposed rats exhibited significant ($p<0.05$) weight loss, cognitive and memory impairments, dysregulated antioxidant enzymes activity, and increased lipid peroxidation, nitric oxide, Pb and AChE levels, along with atrophy and vacuolation of pyramidal cells and astrocytes in the CA1 region of the hippocampus. Also, there was an upregulation of Caspase-3 expression in the hippocampus of experimental rats exposed to PbA, indicating apoptosis as a possible mechanism of action. However, pretreatment with *R. officinalis* significantly ($p<0.05$) mitigated the adverse effects induced by PbA in the hippocampus of experimental rats suggesting strong metal-chelating, anti-cholinesterase, and NO-scavenging effects. Similarly, the downregulation of caspase-3 expression in the hippocampus of PbA-exposed rats following pretreatment with *R. officinalis* supports its anti-apoptotic potential. Overall, these findings suggest that *R. officinalis* exhibits potent antioxidant, metal-chelating, nitric oxide-scavenging, anti-cholinesterase and anti-apoptotic properties, thus providing novel evidence supporting *R. officinalis* as a promising neuroprotective agent with potential for drug development against hippocampal dysfunction.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Lead (Pb) exposure and contamination continues to be a global public health concern, largely due to its pervasive presence in the environment and its well-documented toxic effects on multiple physiological systems (Fatima *et al*., 2025). As a non-biodegradable heavy metal, Pb persists in ecosystems, accumulating in soil, water, and air as a result of anthropogenic activities such as mining, industrial processing, combustion of fossil fuels, and the use of Pb-containing products (Niede and Benbi, 2022; Howard *et al*., 2024). Despite regulatory efforts aimed at curbing its use, especially in developed countries, Pb exposure remains alarmingly high in low and middle income regions where environmental monitoring and policy enforcement are limited. According to estimates by the Institute for Health Metrics and Evaluation (IHME), Pb exposure was responsible for approximately 1.5 million deaths globally in 2021, with a disproportionate burden carried by economically disadvantaged populations (World Health Organization, 2023; IHME, 2024).

Once absorbed into the body, Pb is distributed via the bloodstream to soft tissues and eventually deposited in bones, where it can be mobilized over time, leading to chronic toxicity (Srivastava *et al*., 2024). The central nervous system (CNS) is particularly susceptible, with the brain being the primary target organ (Wu *et al*., 2023). Among the brain structures affected, the hippocampus—a region integral to learning, memory formation, and emotional regulation—is notably vulnerable due to its high metabolic rate, rich vascular supply, and role in synaptic plasticity (Song, 2023; Popa *et al*., 2023). Pb exerts its neurotoxic effects in the hippocampus by mimicking calcium ions, thereby crossing the blood-brain barrier and interfering with calcium-mediated neuronal signaling, neurotransmitter release, and intracellular second messenger systems (Penticoff and Fortin, 2023; Gudadhe *et al*., 2024).

Exposure to Pb is strongly associated with oxidative stress, a pathological condition marked by an overproduction of reactive oxygen species (ROS) and a concurrent depletion of endogenous antioxidant defenses. Oxidative damage to lipids, proteins, and DNA underlies much of the neurodegenerative pathology induced by Pb, including apoptotic neuronal death, neuroinflammation, and mitochondrial dysfunction. These effects are implicated in the pathogenesis of major neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, where the hippocampus often shows early and profound degenerative changes (Jing *et al*., 2020; Chen *et al*., 2021; Pyatha *et al*., 2022). Given the irreversible nature of many Pb-induced neurotoxic effects, there is an urgent need to identify sustainable and cost-effective interventions to mitigate its impact on the brain, particularly in vulnerable populations. In this context, plant-based antioxidants have emerged as promising therapeutic agents due to their capacity to scavenge ROS, upregulate endogenous antioxidant enzymes, and modulate inflammatory responses (Abeyrathne *et al*., 2022; Akbari *et al*., 2022; Enogieru and Momodu, 2022).

Rosmarinus officinalis L., commonly known as rosemary, is a hardy, aromatic shrub from the Mediterranean region that belongs to the Lamiaceae family. Traditionally valued in both culinary practices and folk medicine, it has attracted considerable attention for its diverse bioactive properties (Allegra *et al*., 2020; Pappachan *et al*., 2023; Adepoju *et al*., 2024). Its bioactive profile is characterized by a high concentration of phenolic compounds such as rosmarinic acid, carnosic acid, and carnosol, which are recognized for their strong antioxidant properties (Mehta *et al*., 2023; Adepoju *et al*., 2024). These compounds exert their protective actions by directly neutralizing free radicals, inhibiting lipid peroxidation, and enhancing the activity of endogenous antioxidant enzymes. Beyond its antioxidant properties, *R. officinalis* has demonstrated a broad spectrum of pharmacological activities including anti-inflammatory, antinociceptive, antiulcerogenic, antidepressant, anxiolytic, and hepatoprotective effects (Lawal *et al*., 2022; Kamli *et al*., 2022; Jeevalatha *et al*., 2022).

1.2 STATEMENT OF RESEARCH PROBLEM

Pb remains a potent environmental neurotoxin, posing substantial risks to human health even at concentrations previously considered safe. The U.S. Centers for Disease Control and Prevention (CDC) currently recommends a blood lead reference value of less than 5 µg/dL for children and less than 25 µg/dL for adults in the absence of significant environmental exposure (Swaringen *et al*., 2022; Ericson *et al*., 2021). However, mounting evidence suggests that blood Pb levels below 5 µg/dL may still exert biological harm (Collin *et al*., 2022). Low Pb levels have also been linked to a wide spectrum of neurodevelopmental deficits, including diminished cognitive performance, learning difficulties, lower IQ, and behavioural disorders—particularly in children whose developing brains are highly susceptible to neurotoxic insults (Albores-Garcia *et al*., 2021; Bjørklund *et al*., 2024).

The hippocampus, a brain region crucial for memory formation, spatial navigation, and executive functions, is especially sensitive to Pb-induced neurotoxicity. Pb readily crosses the blood-brain barrier by mimicking calcium ions and accumulates in brain tissue, where it disrupts synaptic signaling, impairs neurogenesis, and promotes oxidative stress (Popa *et al*., 2023; Song, 2023). These disturbances are often reflected in reduced hippocampal volume, synaptic degeneration, and impaired long-term potentiation—hallmarks of neurodegeneration. In fact, studies have reported Pb-associated cognitive deficits across various domains, including language development, memory retention, problem-solving skills, and visuospatial abilities (Dórea, 2021). Infants and young children exposed to Pb demonstrate measurable deficits in both verbal and performance IQ, as well as in academic performance and executive function (Marshall *et al*., 2021). Real-world consequences of Pb neurotoxicity are tragically evident in cases like the Zamfara Pb poisoning outbreak in Nigeria (Schwartz *et al*., 2021;

Fabolude *et al*., 2025). Since 2010, severe environmental Pb contamination from artisanal gold mining has led to the deaths of over 400 children, with thousands more suffering chronic exposure and requiring medical intervention (de Bakker *et al*., 2021; Schwartz *et al*., 2021; Fabolude *et al*., 2025). The scale of the crisis has underscored the urgent need for sustainable and effective neuroprotective strategies, particularly in underserved and high-risk populations (Al-Worafi, 2023).

A significant concern lies in the long-term persistence of Pb in biological tissues. Pb has a half-life of approximately two years in the brain and up to 30 years in skeletal tissue, creating a prolonged internal source of exposure even after environmental contact has ceased (Collin *et al*., 2022). This chronic bioaccumulation complicates treatment efforts and heightens the need for early preventive and therapeutic interventions. Given the limitations of current medical treatments and the persistent burden of Pb exposure, there is increasing interest in phytotherapeutic agents for neuroprotection.

1.3 AIM OF THE STUDY

The general aim of the study was to investigate the activity of aqueous *R. officinalis* leaf extract on lead acetate-induced hippocampal toxicity in adult Wistar rats.

1.4 SPECIFIC OBJECTIVES

The specific objectives of the study were to:

i. Compare the brain and body weight changes in rats treated with or without lead acetate.
ii. Determine the neurobehavioural activities (Novel object recognition, Y-maze, and Elevated-plus maze tests) in rats treated with or without lead acetate.
iii. Evaluate the antioxidant enzymes (Catalase, Superoxide dismutase, and Glutathione peroxidase) activity and Glutathione concentration in rats treated with or without lead acetate.

iv. Evaluate the Lipid peroxidation (Malondialdehyde concentration) in the hippocampus of rats treated with or without lead acetate.

v. Quantify the acetylcholinesterase activity in the hippocampus of rats treated with or without lead acetate.

vi. Quantify the concentration of Pb in the hippocampus of rats treated with or without lead acetate.

vii. Evaluate nitric oxide levels in the hippocampus of rats treated with or without lead acetate.

viii. Examine the histology of the hippocampus in rats treated with or without lead acetate.

ix. Determine the apoptotic activity in the hippocampus of rats treated with or without lead acetate.

1.5 JUSTIFICATION OF THE STUDY

While oxidative stress is a common pathway through which many neurotoxic agents exert their harmful effects, numerous studies have shown that antioxidants play a critical role in counteracting this damage (Akbari *et al*., 2022). Although the human body produces its own antioxidants, supplementing with external sources can enhance the body’s overall antioxidant defense system and support the activity of endogenous compounds (Engwa *et al*., 2022). Among these external sources, medicinal plants have gained considerable attention due to their strong antioxidant properties (Nwozo *et al*., 2023). Their natural origin, cost-effectiveness, and generally low risk of adverse effects make them especially appealing for therapeutic applications and drug development within the medical and pharmaceutical fields (Singh and Gohil, 2024).

R. officinalis, a medicinal herb widely used in traditional medicine, has attracted scientific attention for its rich composition of bioactive compounds such as flavonoids, phenols, and tannins. These compounds are known to possess strong antioxidant, anti-inflammatory, and anti-apoptotic properties. Several experimental studies have reported *R. officinalis'* effectiveness in improving cognitive function and protecting against neurodegeneration (Hussain *et al*., 2022).

Despite these findings, there is still a notable gap in the literature regarding *R. officinalis'* specific protective effects against Pb-induced damage in the hippocampus. Also, little is known about how *R. officinalis* may influence neurobehavioural outcomes such as memory and learning in animals exposed to Pb (Abdel-Tawwab *et al*., 2024). By bridging this gap in literature, this study can provide novel insights into the therapeutic potential of *R. officinalis*, supporting its possible use as a natural intervention to prevent or ameliorate the effects of Pb-induced hippocampal damage, and inform future strategies for public health and clinical interventions, particularly in regions where Pb exposure remains a serious concern, and access to pharmaceutical neuroprotective agents is limited.

CHAPTER TWO

LITERATURE REVIEW

2.1 THE BRAIN

The brain, a vital and highly complex organ that serves as a central component of the central nervous system, is 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). From an embryological perspective, these three major brain regions originate from the development and differentiation of three primary brain vesicles during early neural development:

A. The Forebrain (Prosencephalon), which consists of;
    i. The Telencephalon: becomes the paired cerebral hemispheres (cerebrum) and the neocortex.
    ii. The Diencephalon: the "in-between" region of the brain forming the thalamus, hypothalamus, and metathalamus (Carstens and Sarnat, 2023).

B. The Midbrain (Mesencephalon), differentiates into the tectum, tegmentum, and cerebral peduncles (Carstens and Sarnat, 2023; Chakravarthi *et al*., 2025).

C. The Hindbrain (Rhombencephalon), includes;
    i. The Metencephalon: gives rise to the pons and cerebellum.
    ii. 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 HIPPOCAMPUS

2.2.1 Gross Anatomy of the Hippocampus

The hippocampus is an infolded area of the cerebral cortex. It is a bilaterally curved structure lying deep within the medial aspect of the temporal lobe of each cerebral hemisphere in the brain (Chauhan *et al*., 2021). The hippocampus, also historically referred to as cornu Ammonis or Ammon's horn, represents a specialized and evolutionarily conserved structure within the medial temporal lobe of the cerebral cortex. During fetal neurodevelopment, this allocortical region undergoes a characteristic infolding process, whereby it migrates medially and inward, ultimately becoming embedded within the floor of the inferior (temporal) horn of the lateral ventricle (Chauhan *et al*., 2021; Catani, 2022; Lang *et al*., 2024).

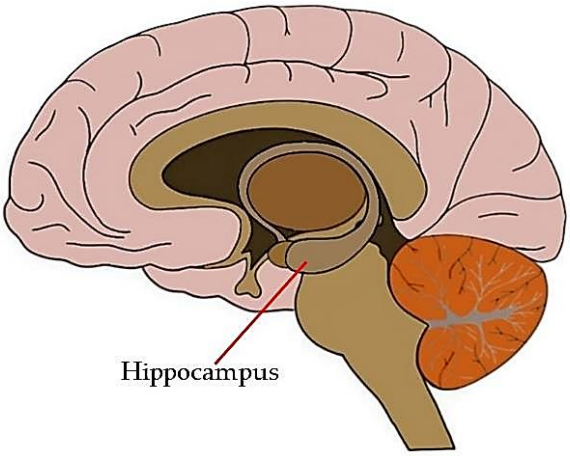

Figure 2.1: The Brain showing the hippocampus (Carstens and Sarnat, 2023)

This developmental folding imparts the hippocampus with its signature curved morphology, visible in various anatomical planes of section (Lang *et al*., 2024). In the adult brain, the hippocampus appears as a prominent longitudinal ridge or elevation in the floor of the temporal horn of the lateral ventricle (Carstens and Sarnat, 2023). Medially, it maintains a continuous anatomical and functional relationship with the subiculum, which in turn merges seamlessly into the parahippocampal gyrus—a critical interface between the hippocampal formation and the neocortex. These interconnections facilitate its central role in memory processing and spatial navigation (Chauhan *et al*., 2021; Estela-Pro and Burwell, 2022).

The term "hippocampus" is derived from the Greek words hippos (horse) and kampos (sea monster), reflecting its seahorse-like appearance in coronal sections of the brain. This description was given by Giulio Cesare Arantius (1477-1552) who gave the hippocampus its name, noting the similarity to a seahorse (Estela-Pro and Burwell, 2022). When observed in frontal (coronal) planes, the hippocampus presents a curved, C-shaped configuration, evocative of a ram's horn—a resemblance that inspired the classical anatomical designation cornu Ammonis (Estela-Pro and Burwell, 2022; Chakravarthi *et al*., 2025). This name is an allusion to the ancient Egyptian deity Amun (or Ammon), traditionally depicted with spiraling ram’s horns, underscoring both the visual and symbolic associations drawn by early neuroanatomists (Bhushan *et al*., 2022).

Figure 2.2: The “Seahorse-shaped” hippocampus (Estela-Pro and Burwell, 2022)

The anterior (rostral) portion of the hippocampus is notably enlarged and displays a series of undulating grooves and ridges on its surface. Owing to its superficial similarity to the shape of an animal’s paw, this anterior segment is often referred to as the Pes hippocampi (Latin for "foot of the hippocampus") (Zhao and Palomero-Gallagher, 2025). As one traces the structure posteriorly, the hippocampus gradually tapers, following a dorsomedial course, and ultimately terminates beneath the splenium of the corpus callosum, adjacent to the crus of the fornix. This posterior continuation links the hippocampus to other components of the limbic system, contributing to its broader integrative functions (Catani, 2022; Zhao and Palomero-Gallagher, 2025).

In the ventricular aspect of the hippocampus lies the alveus, a slender, laminar tract of white matter composed predominantly of the myelinated axons from pyramidal neurons lining the hippocampal formation. Immediately subjacent to the ependymal layer that lines the ventricular cavity, these fibers coalesce along the medial hippocampal margin to give rise to the fimbria hippocampi (Zhao and Palomero-Gallagher, 2025). As the fimbria courses posteriorly, it arches over the dentate gyrus and, upon reaching the splenial region of the corpus callosum, it transitions seamlessly into the fornix, the principal efferent pathway of the hippocampal complex. This continuous conduit—alveus → fimbria → fornix—thus constitutes the major output system by which hippocampal pyramidal cells relay processed information to thalamic, septal, and cortical targets (Estela-Pro and Burwell, 2022; Lang *et al.*, 2024).

2.2.2 Organization of pyramidal cells in the hippocampus

When viewed in coronal section, the Cornu ammonis of the hippocampus is subdivided into four zones: CA1, CA2, CA3, and CA4 (CA - Cornu ammonis) (Kominami *et al.*, 2023). The CA1 field—also referred to as Sommer's sector—comprises pyramidal neurons situated immediately adjacent to the subicular complex (Subiculum) (Chauhan *et al.*, 2021; Kominami *et al.*, 2023). In contrast, the CA2 and CA3 subfields lie more superficially within the hippocampal formation, occupying positions closer to the ventricular surface. A distinctive feature of the CA3 is that its pyramidal cells give rise to recurrent axon collaterals, known as Schaffer collaterals, which project predominantly back to neurons in the CA1 field. The CA4 field contains cells within the hilus of the dentate gyrus, which makes this field considered a part of the dentate gyrus (Chauhan *et al.*, 2021; Sheintuch *et al.*, 2023).

The internal hippocampus consists of archicortex. Within the hippocampal archicortex, the neuronal population is overwhelmingly composed of pyramidal cells, each distinguished by extensive dendritic arbors for input reception and a single axonal projection for output transmission (Sheintuch *et al.*, 2023; Zhao and Palomero-Gallagher, 2025). Notably, the apical dendrites of these neurons ascend away from the ventricular surface toward the dentate gyrus—thereby sampling perforant-path inputs in strata radiatum and lacunosum-moleculare—while their basal dendrites extend inward into stratum oriens adjacent to the inferior horn of the lateral ventricle (Olopade *et al.*, 2021). Once synaptic integration occurs at the somatic and proximal dendritic compartments, the sole axon of each pyramidal neuron courses in the alvear fiber layer—a thin lamina of myelinated hippocampal efferents lining the ventricular margin—before consolidating its fibers into the fimbria as it sweeps posteriorly over the dentate gyrus (Sheintuch *et al.*, 2023). Upon reaching the splenial region of the corpus callosum, these axonal bundles bifurcate: one branch re-enters the entorhinal cortex to complete the perforant pathway feedback loop, while the other continues as the fornix, thus constituting the principal hippocampal efferent tract (van Staalduinen and Zeineh, 2022; Olopade *et al.*, 2021). Through this alveus–fimbria–fornix continuum, hippocampal output is distributed broadly—to the entorhinal and prefrontal cortices, septal nuclei, mammillary bodies, and anterior thalamic nuclei—thereby underpinning the structure's pivotal role in memory consolidation, spatial navigation, and limbic regulation (Zhao and Palomero-Gallagher, 2025; González-Arnay *et al.*, 2024).

The hippocampal archicortex is stratified into four discrete layers, each defined by unique cellular compositions and connectivity profiles: (i) Stratum lacunosum-moleculare layer, (ii) Stratum radiatum layer, (iii) Stratum pyramidale layer, (iv) Stratum oriens layer (Genon *et al.*, 2021)

Stratum lacunosum-moleculare, also called the lacunar-molecular layer, is the deepest layer adjacent to the hippocampal fissure, and is densely populated by inhibitory interneurons. These GABAergic cells form a complex network of dendritic and axonal arbors that gate incoming perforant-path inputs from the entorhinal cortex, thereby shaping the excitatory drive onto CA1 pyramidal neurons (Genon *et al*., 2021; Gonzalez-Ferrer *et al*., 2024). Immediately superficial to this lies stratum radiatum, which is traversed by the apical dendrites of both CA1 and CA3 pyramidal cells as well as the dendritic processes of local stellate neurons. It is also the pathway for Schaffer collateral fibers originating from CA3, which establish en passant synapses on the radiatum dendrites of CA1 pyramidal neurons, mediating a crucial excitatory link within the hippocampal trisynaptic circuit (Estela-Pro and Burwell, 2022; Kominami *et al*., 2023; Zhao and Palomero-Gallagher, 2025).

At the core of the archicortex is stratum pyramidale, the thickest and most prominent layer, composed of densely packed pyramidal neuron somata organized into fields CA1–CA4. This lamina not only serves as the principal excitatory output zone of the hippocampus but also seamlessly merges with layer V of the adjacent neocortex, reflecting its dual role in intrinsic hippocampal processing and broader cortico-hippocampal communication (Estela-Pro and Burwell, 2022; Lang *et al*., 2024). Finally, stratum oriens forms the most superficial layer just beneath the alveus. It contains basket-cell interneurons and oriens-lacunosum moleculare (O-LM) interneurons whose projections target the somatic and distal dendritic compartments of pyramidal cells, respectively. This layer also harbors the basal dendrites of pyramidal neurons and shares many cytoarchitectural features with neocortical layer VI, underscoring its integrative role in modulating hippocampal output (Estela-Pro and Burwell, 2022).

2.2.3 Hippocampal Formation

Spanning the full caudal-to-rostral length of the medial temporal lobe, the hippocampal formation constitutes a pivotal component of the limbic system, whose integrity is essential for the encoding and consolidation of memory (White *et al.*, 2024; Hosseini *et al.*, 2025). Macroscopically, the hippocampal formation presents as a distinctive C-shaped bulge on the floor of the inferior horn of the lateral ventricle, reflecting its curved trajectory along the base of the temporal lobe (van Staalduinen and Zeineh, 2022; Insausti *et al.*, 2023). Functionally, the hippocampal formation integrates multimodal neocortical inputs via the entorhinal cortex, processes information through its intrinsic trisynaptic circuitry, and funnels processed output through the alveus–fimbria–fornix pathway to widespread cortical and subcortical targets, thereby underpinning spatial navigation, contextual learning, and episodic memory. Its vulnerability to stress, ischemia, and neurodegenerative pathology underscores the hippocampal formation's centrality in both healthy cognition and dementia syndromes (Nyberg *et al.*, 2022; Insausti *et al.*, 2023; Hosseini *et al.*, 2025). Embedded deep within the temporal cortex, the hippocampal formation comprises the hippocampus proper (Cornu Ammonis), the dentate gyrus, and the subicular cortex, with the presubiculum, parasubiculum, and entorhinal cortex often considered accessory components of the hippocampal formation (Nyberg *et al.*, 2022; van Staalduinen and Zeineh, 2022; Insausti *et al.*, 2023; Hosseini *et al.*, 2025).

2.2.3.1 Dentate Gyrus

The dentate gyrus is a narrow, convoluted band of allocortex nestled between the superior margin of the parahippocampal gyrus and the subjacent fimbria hippocampi, forming a distinctive C-shaped "tooth-like" ribbon, hence its Latin name, gyrus dentatus, when viewed in coronal section (Scharfman, 2025). This serrated appearance is accentuated by numerous penetrating blood vessels that traverse its ventricular surface, creating the alternating gyri and sulci that give the region its characteristic morphology. Unlike the pyramidal-cell–dominated hippocampus proper, the dentate gyrus is populated primarily by granule cells, small excitatory neurons whose densely packed somata constitute the principal cell layer of this structure (Chauhan *et al*., 2021; Ben-Simon *et al*., 2022). The axons of these granule neurons—known as mossy fibers—project through the polymorphic layer into the stratum lucidum of CA3, where they form powerful, large-bouton synapses on the proximal dendrites of CA3 pyramidal cells, thus providing the main excitatory drive from the dentate gyrus to hippocampal field CA3. This allocortical ribbon is organized into three stratified laminae. Superficially lies the molecular layer, a sparsely cellular zone composed chiefly of granule cell dendritic tufts and perforant-path terminals originating from layer II of the entorhinal cortex (Sinha *et al*., 2022). Beneath this, the granule cell layer contains the tightly packed granule neuron somata that receive afferent inputs and initiate hippocampal processing. Deepest is the polymorphic (hilus) layer, populated by a heterogeneous array of interneurons—including basket cells and mossy cells—and by the initial trajectories of mossy fibers, which contribute to both recurrent excitatory and inhibitory microcircuits (Chauhan *et al*., 2021). Functionally, the dentate gyrus plays a critical role in pattern separation, transforming and sparsifying cortical inputs to minimize interference between similar memory representations and thus supporting the formation of distinct episodic memories (Scharfman, 2025). Additionally, the subgranular zone at the interface of the granule cell layer and hilus remains one of the few adult neurogenic niches in the mammalian brain; newly generated granule cells integrate into existing circuits, contributing to hippocampal plasticity and potentially influencing learning, mood regulation, and resilience to stress throughout life (Sinha *et al*., 2022).

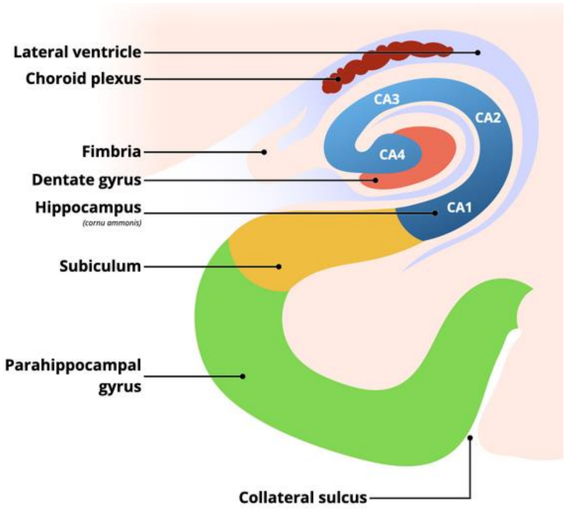

Figure 2.3: Hippocampal Formation and Surrounding Regions (Chauhan *et al*., 2021)

2.2.3.2 Subicular Cortex

The subiculum, or subicular cortex, occupies the transitional zone immediately distal to the CA1 subfield and medial to the entorhinal cortex, forming the most caudal component of the hippocampal formation along the inferior horn of the lateral ventricle (Witter and Amaral, 2021). In contrast to CA1—whose pyramidal layer is typically around ten cells thick—the subicular pyramidal cell layer often exceeds 30 somata in depth, reflecting both an increased neuron count and a potential division into superficial and deep sublaminae that may correspond to distinct projection neuron classes (Lin *et al*., 2021). Axons from subicular pyramidal neurons converge in the adjacent alveus and fimbria before entering the fornix, where they segregate into parallel pathways: one stream targets the medial mammillary nuclei of the hypothalamus via monosynaptic projections, while another innervates the anterior thalamic nuclei (Zhu *et al*., 2023). Through these parallel diencephalic projections, the subiculum serves as a major relay within the Papez circuit, transmitting integrated hippocampal output to thalamic and hypothalamic structures that underpin episodic memory consolidation and spatial navigation (Lin *et al*., 2021; Witter and Amaral, 2021).

2.2.4 Hippocampal Connections

Sensory information from polymodal association cortices enters the hippocampus via two perforant pathways originating in layer II of the lateral and medial entorhinal cortex (Ohara *et al*., 2023). The lateral pathway terminates in the outer two-thirds of the dentate gyrus molecular layer, while the medial pathway feeds the middle molecular layer and sends collaterals to CA3 and CA1 (Vandrey *et al*., 2022). A reciprocal septohippocampal loop—via cholinergic and GABAergic projections from the medial septum and precommissural fornix—paces theta oscillations (Rolls and Treves, 2024). In addition, top-down glutamatergic inputs from medial prefrontal and anterior cingulate cortex (through nucleus reuniens) and modulatory signals from the supramammillary nucleus synchronize rhythms and amplify signals in CA2 and the dentate gyrus (Danieli *et al*., 2023). Ascending monoamines from the locus coeruleus, raphe nuclei, and ventral tegmental area innervate all layers, adjusting arousal, mood, and reward-based consolidation (Rolls *et al*., 2022).

Efferents from CA1 and subiculum form the alveus, which becomes the fimbria and then the fornix (Turner *et al*., 2022). The pre commissural fornix targets the lateral septum, while the postcommissural division innervates mammillary bodies and anterior thalamic nuclei, completing the Papez circuit (Kamali *et al*., 2023). Parallel amygdalar outputs travel via the stria terminalis and ventral amygdalofugal pathway to hypothalamic and brainstem centers. Subicular collaterals project back to entorhinal, cingulate, and prefrontal cortices, and the hippocampal commissure connects both hippocampi (Ohara *et al*., 2023). Together, these pathways distribute processed mnemonic and emotional information across limbic, diencephalic, septal, and cortical networks (Turner *et al*., 2022; Rolls and Treves, 2024).

2.2.4.1 Fornix

The fornix is a C-shaped white-matter bundle forming the main efferent tract of the hippocampus and carrying commissural fibers between both hippocampi (Choi *et al*., 2021).

Originating from CA3 and subicular pyramidal neurons, fibers converge as the alveus, continue as the fimbria, and at the splenium of the corpus callosum become the fornix body (Chauhan *et al.*, 2021). The tract divides into crura, a bilaminar body, and paired columns. In the columns, precommissural fibers project to septal nuclei and basal forebrain, whereas postcommissural fibers target mammillary bodies and anterior thalamic nuclei, linking hippocampal output to diencephalic memory hubs (Ribas and Wen, 2023). Recent diffusion-MRI genetics confirm its central role in spatial, episodic memory, and executive functions, with heritable microstructural variations associated with cognitive performance. Clinically, fornix deep-brain stimulation has yielded memory improvements in Alzheimer's disease cohorts (Li *et al.*, 2022).

2.2.4.2 Papez Circuit

First described by Papez (1937), this loop connects hippocampus → fornix → mammillary bodies → anterior thalamic nuclei → cingulate gyrus, then back via entorhinal cortex to hippocampus, underpinning emotion and episodic memory (Piper *et al.*, 2022). Modern fMRI and effective-connectivity studies in mild cognitive impairment reveal weakened hippocampo-thalamic and cingulate connections, correlating with memory decline and highlighting the circuit's dynamic integration of mnemonic and affective processes (Talwar *et al.*, 2021).

2.2.5 Blood Supply and Drainage of The Hippocampus

The hippocampus receives its arterial blood supply mainly from branches of the posterior cerebral artery (PCA) and the anterior choroidal artery (AChA) (Xu *et al.*, 2021). These sources form a complex vascular network comprising superficial hippocampal arteries that penetrate to form deeper intrahippocampal branches responsible for perfusing key hippocampal subregions such as CA1–CA4, the dentate gyrus, and subiculum (Rao *et al.*, 2022). The PCA, particularly its P2 segment, gives rise to the anterior and posterior hippocampal arteries, along with contributions from the posterior parahippocampal and parieto-occipital arteries, ensuring coverage of the entorhinal cortex and parahippocampal gyrus—regions crucial for memory processing and commonly affected in early Alzheimer’s disease (Agarwal and Carare, 2021; Xu *et al*., 2021; Sumadevi, 2024).

The anterior choroidal artery, a slender branch of the internal carotid artery, provides essential supply to medial temporal lobe structures including the amygdala, hippocampal head, and uncus (Lucifero *et al*., 2021). It also supplies the choroid plexus, optic tract, and posterior limb of the internal capsule, indicating its relevance in both memory and sensorimotor function (Akter *et al*., 2024). Its perforating branches reach the anterior hippocampus through the uncal sulcus, establishing vital collateral pathways (Chauhan *et al*., 2021; Agarwal and Carare, 2021). The intrahippocampal arteries can be classified into four main groups: large/small ventral and large/small dorsal branches, each targeting specific hippocampal layers. For example, large ventral arteries irrigate the stratum lacunosum, stratum pyramidale, and molecular layer of the dentate gyrus, while large dorsal arteries target the granule cell layer and CA3/CA4 subfields. These arteries form a highly anastomotic longitudinal network along the hippocampal sulcus, enhancing vascular resilience in the event of localized ischemia (Thorne *et al*., 2022; Semyachkina-Glushkovskaya *et al*., 2023).

Venous drainage occurs via a dual system of intrahippocampal (deep) and superficial hippocampal veins (Agarwal and Carare, 2021). Deep drainage is provided by two main types: the sulcal intrahippocampal veins, originating from the CA1–CA2 sectors and draining the molecular layers, and the subependymal veins, located near the ventricular surface and responsible for deeper structures including the subiculum. These veins connect to two major superficial venous arcades—one along the fimbriodentate sulcus, the other along the hippocampal sulcus (Inoue *et al*., 2023; Okar *et al*., 2024).

The arcades merge at both poles of the hippocampus: anteriorly draining into the inferior ventricular vein, and posteriorly into the medial atrial vein (Imada and Chen, 2022). Both converge into the basal vein of Rosenthal, which plays a pivotal role in clearing venous blood from the medial temporal lobe (Ota, 2024). This elaborate drainage system supports metabolic homeostasis and protects against venous congestion or ischemic injury, especially in pathologies such as mesial temporal sclerosis, temporal lobe epilepsy, and neurodegeneration (Thorne *et al.*, 2022; Semyachkina-Glushkovskaya *et al.*, 2023; Okar *et al.*, 2024). An intricate understanding of this vascular architecture is essential not only for neurosurgical planning and vascular imaging but also for interpreting hippocampal vulnerability in various disease states. Variability in arterial branching and venous configuration also contributes to individual differences in hippocampal perfusion and neurodegenerative risk (Semyachkina-Glushkovskaya *et al.*, 2023).

2.2.6 Functions of The Hippocampus

Being an integral part of the limbic system, hippocampus plays a vital role in regulating learning, memory encoding, memory consolidation, and spatial navigation (Adedayo *et al.*, 2023).

2.2.6.1 Hippocampus and Memory

The hippocampus—an essential hub within the limbic system—mediates a spectrum of cognitive operations by organizing its principal subfields (CA1–CA3) into a trilaminar loop that underlies long-term memory processing (Slotnick, 2022). Incoming information traverses distinct “polysynaptic” and “direct” pathways: in the polysynaptic or trisynaptic circuit, perforant-path axons from the entorhinal cortex innervate granule cells of the dentate gyrus, whose mossy-fiber projections excite CA3 pyramidal neurons; these, in turn, relay signals via Schaffer collaterals to CA1 pyramidal cells, before hippocampal output returns to neocortical regions such as the inferior temporal and prefrontal cortices (Ramaglia *et al.*, 2021). Parallel to this, a temporoammonic “direct” route conveys layer-III entorhinal inputs straight to CA1, supporting rapid encoding of episodic events and fine spatial contexts (Ohara *et al.*, 2023). Synaptic plasticity in these circuits—most notably long-term potentiation (LTP) at CA3→CA1 synapses—is widely recognized as a cellular substrate for learning and memory, with NMDA-receptor–dependent LTP correlating with improved spatial navigation and memory performance in vivo (Vandrey *et al*., 2022; Donato *et al*., 2021). Moreover, CA3 itself acts as an autoassociative network that enables pattern completion during recall, integrating partial cues into coherent memory traces. Over time, coordinated oscillatory activity (e.g., theta rhythms and sharp-wave ripples) orchestrates hippocampo-cortical dialogue to redistribute and stabilize mnemonic representations across distributed networks (Ramaglia *et al*., 2021; Slotnick, 2022). Thus, through its intricately arranged subfields, dual information channels, and robust plasticity mechanisms, the hippocampus transforms fleeting experiences into enduring, contextually rich memories (Donato *et al*., 2021).

2.2.6.2 Hippocampus and Learning

The hippocampus contributes critically to associative learning paradigms such as eyeblink conditioning by engaging its pyramidal cell populations to encode both the timing and strength of learned responses (O’Keefe and Krupic, 2021). In classical delay eyeblink conditioning—where the conditioned stimulus (CS) and unconditioned stimulus (US) overlap—hippocampal pyramidal neurons adapt their firing patterns to anticipate the onset and magnitude of the US, effectively constructing an internal time–amplitude template of the conditioned blink response (Buss *et al*., 2021). Pharmacological manipulations of hippocampal excitability, for example with calcium-channel blockers like nimodipine, can accelerate acquisition by enhancing pyramidal neuron activity, whereas targeted lesions or optogenetic suppression of hippocampal interneurons markedly slow or block learning, underscoring the modulatory role of hippocampal circuits in shaping learning rates (Crossley *et al*., 2024). Trace eyeblink conditioning—where a gap separates the conditioned stimulus (CS) and unconditioned stimulus (US)—requires an intact hippocampus to bridge that interval, and hippocampal inactivation during the gap prevents learning (Buss *et al*., 2021). After training, CA1 pyramidal cells display increased excitability and enduring LTP at both mossy-fiber (DG→CA3) and Schaffer-collateral (CA3→CA1) synapses, stabilizing the blink memory over days to weeks (Maity *et al*., 2022). Coordinated theta and sharp-wave ripple oscillations further synchronize hippocampal subfields during learning and offline replay, reinforcing the engram and promoting its eventual transfer to neocortex. Together, these synaptic and network changes make the hippocampus essential for temporal associative learning (Vandrey *et al*., 2022; Buss *et al*., 2021; Crossley *et al*., 2024).

2.2.6.3 Hippocampus and Spatial Navigation

The hippocampus serves as the neural substrate for constructing an internal “cognitive map” that encodes the spatial relationships among environmental landmarks and objects, enabling flexible navigation and goal-directed behaviour (Baumann and Mattingley, 2021). Central to this function are place cells—specialized pyramidal neurons that fire when an animal occupies a specific location, or place field, and remain largely silent elsewhere, thereby forming a sparse code that tiles the environment (Mao, 2023). Beyond mere positional tuning, place-cell activity also reflects the animal’s heading direction, intended destination, and task demands, integrating spatial, contextual, and behavioural information into a cohesive representation (Chen *et al*., 2024). The stability and precision of these spatial maps rely on synaptic plasticity—particularly long-term potentiation at Schaffer-collateral synapses—and are further organized by theta and gamma oscillations, which segment neural activity into temporal windows that facilitate interregional communication and synaptic strengthening (Etter *et al*., 2023). Through this richly structured network of place fields, oscillatory coordination, and plasticity mechanisms, the hippocampus not only supports allocentric navigation but also provides the spatial scaffold upon which episodic memory and future planning are built (Rolls, 2023).

2.2.6.4 Hippocampus and Behaviour

The hippocampus supports flexible, goal-directed behaviour by forming and reconstructing relational memory representations that bind disparate elements of experience into coherent schemas, enabling the dynamic recombination of information when novel solutions are required (Burman, 2023). Damage to the hippocampus consistently impairs this cognitive flexibility and gives rise to maladaptive behaviours, demonstrating its indispensable role in evaluating and updating action–outcome associations (Uddin, 2021). Early lesion studies revealed that hippocampal damage provokes hyperlocomotion in animals exploring new environments, implicating the structure in suppressing unnecessary or excessive behavioural activation (Chamberlain and Ahmari, 2021). Moreover, hippocampal dysfunction compromises an animal's ability to inhibit previously learned responses during extinction and reversal learning, underscoring its centrality in response-inhibition processes (Burman, 2023; Chamberlain and Ahmari, 2021). As an evaluative hub, the hippocampus coordinates behavioural inhibition, obsessive-like environmental scanning, and the formation of spatial and relational maps, integrating contextual and emotional information to guide adaptive actions (Uddin, 2021). GABAergic inhibition within hippocampal circuits further contributes to controlling intrusive or unwanted thoughts, illustrating its broader role in inhibitory control beyond motor responses (Anderson and Floresco, 2022). Disinhibition of ventral hippocampal neurons disrupts contextual and elemental fear conditioning, highlighting its involvement in modulating fear-related behaviour. However, acute and chronic stress elevate circulating corticosterone, which in turn attenuates hippocampal neuronal firing rates and impairs synaptic plasticity, thereby undermining its evaluative functions and leading to stress-related cognitive and behavioural deficits (Kalisch *et al*., 2024). This sensitivity to glucocorticoids exemplifies the dynamic interplay between endocrine stress responses and hippocampal-dependent control of behaviour (Davies *et al*., 2022).

2.2.7 Hippocampal Dysfunction

The hippocampus, a bilateral structure located within the medial temporal lobes, plays a pivotal role in various cognitive and emotional processes, including memory consolidation, spatial navigation, and emotional regulation (Rao *et al*., 2022). Given its integral functions, dysfunctions within the hippocampus have been implicated in a range of neuropsychiatric and neurodegenerative disorders (Rao *et al*., 2022; Gupta *et al*., 2023).

2.2.7.1 Alzheimer’s Disease

Alzheimer's disease (AD) is characterized by progressive neurodegeneration, with the hippocampus being one of the earliest and most severely affected regions (Rao *et al*., 2022). Neuropathological studies have consistently demonstrated significant neuronal loss and gliosis within the hippocampal formation, contributing to the hallmark hippocampal atrophy observed in AD patients (Salta *et al*., 2023). The degeneration encompasses multiple hippocampal subfields, including the stratum radiatum, stratum lacunosum, stratum moleculare, subiculum, and stratum pyramidale. These layers are integral to synaptic integration and signal transmission, and their deterioration disrupts the hippocampus's ability to process and relay information effectively (Babcock *et al*., 2021; Rao *et al*., 2022). A pivotal aspect of AD pathology involves the accumulation of hyperphosphorylated tau proteins, leading to the formation of neurofibrillary tangles (Rawat *et al*., 2022). This pathological process initiates in the entorhinal cortex, particularly affecting layer II neurons, and subsequently spreads to the hippocampus and other cortical regions, including the temporal, frontal, and parietal lobes (Jayaraman *et al*., 2021; Rao *et al*., 2022).

The propagation of tau pathology follows a predictable pattern, correlating with the progression of clinical symptoms. In parallel, the aberrant processing of amyloid precursor protein (APP) results in the accumulation of amyloid-beta (Aβ) peptides, which aggregate to form extracellular plaques (Jayaraman *et al*., 2021; Salta *et al*., 2023). These plaques are predominantly located in regions of heightened neuronal activity, such as the hippocampus, and are implicated in synaptic dysfunction and neuronal death. Notably, Aβ accumulation has been shown to disrupt synaptic transmission and plasticity, further exacerbating cognitive deficits (Zhang *et al*., 2022). Furthermore, AD is associated with alterations in several neurotransmitter systems. Dysregulation of glutamatergic, serotonergic, and noradrenergic pathways has been documented, contributing to the complex symptomatology of the disease (Zhang *et al*., 2023). For instance, glutamatergic excitotoxicity, driven by excessive activation of NMDA receptors, leads to neuronal injury and death. Similarly, deficits in serotonergic and noradrenergic signaling have been linked to mood disturbances and cognitive impairments observed in AD patients (Salta *et al*., 2023; Okar *et al*., 2024).

2.2.7.2 Depression

Decades of neuroimaging studies have converged on the finding that patients with major depressive disorder (MDD) exhibit significant reductions in hippocampal volume—typically ranging from 4% to 10% bilaterally—relative to healthy controls, with more pronounced atrophy observed in those with early onset, longer duration, and recurrent episodes of depression (Shah *et al*., 2021; Tartt *et al*., 2022). This vulnerability of the hippocampus has been attributed in part to chronic glucocorticoid neurotoxicity: sustained elevations in cortisol levels, resulting from hypothalamic–pituitary–adrenal (HPA) axis dysregulation in MDD, promote dendritic retraction, neuronal loss, and impaired neurogenesis in hippocampal subfields (Tartt *et al*., 2022). Concurrently, stress-induced downregulation of brain-derived neurotrophic factor (BDNF) deprives hippocampal neurons of critical support for survival and synaptic plasticity, with lower BDNF expression correlating with smaller hippocampal size and poorer clinical outcomes (Xiao *et al*., 2021; Tartt *et al*., 2022).

Animal models of chronic unpredictable stress and corticosteroid administration similarly demonstrate deficits in long-term potentiation at CA3–CA1 synapses, reductions in dendritic spine density, and shifts toward long-term depression that mirror synaptic alterations seen in depressed patients (Xiao *et al*., 2021). Beyond neurons, glial cells critically modulate hippocampal health: astrocyte loss undermines metabolic and glutamate-clearance functions, microglial activation drives maladaptive synaptic pruning and inflammation, and oligodendrocyte deficits impair myelination and circuit conduction—all of which contribute to hippocampal atrophy and the cognitive and affective symptoms of MDD (Shah *et al*., 2021). Together, these intertwined mechanisms—glucocorticoid toxicity, neurotrophic factor depletion, synaptic plasticity disruption, and glial dysfunction—underscore the hippocampus as both a barometer of depressive pathology and a potential target for therapeutic intervention aimed at restoring its structural and functional integrity (Shah *et al*., 2021; Xiao *et al*., 2021; Tartt *et al*., 2022).

2.2.8 Etiology of Hippocampal Disorders

Hippocampal disorders arise from a diverse range of etiologies and can present as isolated conditions or in conjunction with broader neurological, psychiatric, or systemic diseases (Abed, 2023). The hippocampus, being critical for memory consolidation, spatial navigation, and emotional regulation, is vulnerable to a variety of pathological processes. The main causes of hippocampal disorders include:

2.2.8.1 Structural Abnormalities and Genetic Factors

Developmental and acquired lesions in the hippocampus can dramatically alter its architecture and disrupt essential circuits. In some individuals, congenital malformations—like incomplete hippocampal inversion or neuronal migration defects—set the stage for lifelong vulnerability to seizures and memory issues (Wegrzyn *et al*., 2022; Weerasinghe-Mudiyanselage *et al*., 2022). Birth-related insults such as perinatal hypoxia or hydrocephalus also injure hippocampal neurons at a critical time, leading to hippocampal sclerosis and later epilepsy. Beyond development, traumatic brain injury—especially blows to the temporal lobes—can shear delicate hippocampal pathways, and focal strokes in the posterior cerebral artery territory cause acute hippocampal infarcts with sudden amnesia. Finally, neoplasms and demyelinating plaques (e.g., in multiple sclerosis) that encroach on the medial temporal lobe can physically displace or destroy hippocampal tissue (Wegrzyn *et al*., 2022).

Genes set the baseline resilience of hippocampal circuits. Mutations in ion-channel genes such as SCN1A are well known to cause Dravet syndrome, where hippocampal hyperexcitability drives severe childhood epilepsy (Rao *et al*., 2022). In neurodevelopmental disorders like Rett syndrome (MECP2 mutations) and schizophrenia (DISC1 variants), subtle hippocampal network disruptions underlie cognitive and psychiatric symptoms. On the degenerative side, familial Alzheimer's disease often features early hippocampal neurodegeneration and rare familial frontotemporal dementias similarly involve hippocampal atrophy (Berger *et al*., 2020).

2.2.8.2 Autoimmune Disorders and Infections

Sometimes the body's own defense system misfires against the hippocampus. In autoimmune limbic encephalitis, antibodies—most famously anti-NMDA or anti-LGI1—attack hippocampal neurons, causing subacute memory loss, confusion, and seizures. Patients often present over days to weeks, and brain MRI shows swelling in the medial temporal lobes (Lang *et al*., 2024). When linked to an occult tumor (a paraneoplastic process), treating the cancer can dramatically improve hippocampal inflammation and rescue memory function (Fries *et al*., 2020; Jiang *et al*., 2023). The hippocampus is peculiarly vulnerable to certain infections, especially herpes simplex virus type 1 (HSV-1). HSV-1 has a predilection for the dentate gyrus granule cells—key players in forming new memories—and causes necrotizing encephalitis that leaves permanent scars in the hippocampus. Survivors often develop refractory epilepsy and hippocampal sclerosis months to years after the initial infection. Other pathogens—such as human herpesvirus 6, cytomegalovirus, or even some bacteria—can also invade the limbic system, but none strike the hippocampus as relentlessly as HSV-1 (Wouk *et al.*, 2021; Piekut *et al.*, 2022).

2.2.8.3 Vascular Lesions

Any interruption of hippocampal blood flow can be disastrous. A small stroke in the hippocampal branches of the posterior cerebral artery produces sudden anterograde amnesia—patients can't form new memories for hours to days, a syndrome known as transient global amnesia. Chronic small-vessel disease and hypoperfusion, common in older adults with hypertension, lead to gradual hippocampal shrinkage and contribute to vascular cognitive impairment (Perosa *et al.*, 2020; Johnson, 2023).

2.2.8.4 Idiopathic Causes and Paraneoplastic Syndromes

In some people, hippocampal dysfunction arises without a clear culprit. Mesial temporal sclerosis in "idiopathic" epilepsy, for instance, is diagnosed when no tumor, infection, or genetic mutation is found, yet the hippocampus shows neuron loss and gliosis on imaging or pathology (Chen *et al.*, 2022). These cases remind us that our understanding remains incomplete—subtle insults or gene–environment interactions may lie just beneath current detection limits (Prem *et al.*, 2020; Bove *et al.*, 2024). When an immune response to a remote tumor misdirects against the hippocampus, patients can develop paraneoplastic limbic encephalitis. Small-cell lung cancer, breast cancer, and testicular tumors are classic culprits. Memory rapidly declines and seizures emerge, but successful treatment of the underlying malignancy often calms the autoimmune storm and halts further hippocampal damage (Madhavan *et al.*, 2020; Dalmau and Rosenfeld, 2020).

2.2.8.5 Toxic and Metabolic Factors

Toxins and metabolic imbalances can stealthily erode hippocampal integrity (Davidson and Stevenson, 2024). Chronic alcohol misuse produces thiamine deficiency (Wernicke–Korsakoff syndrome), leading to profound hippocampal shrinkage and confabulation (Eva *et al*., 2023). Hypoglycemia, hepatic encephalopathy, and severe electrolyte disturbances (e.g., hyponatremia) also trigger excitotoxic cascades that selectively injure hippocampal pyramidal cells. Heavy metals like lead accumulate in hippocampal neurons, inducing oxidative stress and synaptic loss (Bakulski *et al*., 2020; Liu *et al*., 2021).

2.3 LEAD

Lead (Pb) is a dense, bluish-gray heavy metal with an atomic weight of 207.2 u 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 organolead compounds. Lead is chemically stable and non-degradable, making it a persistent environmental contaminant (Ali *et al*., 2024). 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).

Exposure occurs through inhalation or ingestion, with occupational risks in high-exposure industries and environmental risks from polluted air and contaminated food or dust. In the body, lead distributes among blood, soft tissues, and bones—where it can persist for decades. While blood lead has a half-life of about 30 days, skeletal stores act as long-term reservoirs. Biologically, lead disrupts multiple systems (Collin *et al*., 2022). It impairs heme synthesis, kidney function, and especially the nervous system—posing serious risks to children due to their developing brains. Chronic exposure is linked to cognitive impairment, anemia, hypertension, and reproductive toxicity. Due to its persistence and widespread effects, lead remains a major concern in environmental and neurotoxicology research (Schneider, 2023).

2.3.1 Human Exposure and Metabolism

Lead enters the body through three principal pathways: oral ingestion of contaminated food, water, or soil; inhalation of airborne particulates; and, for organolead compounds, dermal absorption (Collin *et al*., 2022). Most human lead exposure occurs through ingestion, as lead residues accumulated in soil, dust, water, and food enter the gastrointestinal tract. A landmark dietary survey by the European Food Safety Authority (EFSA) in 2012 identified that grains and grain products alone accounted for 16.1 % of dietary lead intake, followed by milk and dairy at 10.4 %, non-alcoholic beverages at 10.2 %, and vegetables (fresh and processed) at 8.4 % of total intake. Occupational settings remain hotspots for lead uptake. People working in battery recycling, metal smelting, glass and pigment manufacture, or paint removal face inhalation of lead fumes and dermal contact with lead-laden dust if controls are lax (Tobias *et al*., 2019). International labor reports highlight that such work can raise blood lead levels far above those seen in the general population. Even coal combustion, used in power plants or industrial boilers, releases trace lead into flue gases, which settles onto crops and soils downwind, adding another subtle but pervasive source of contamination (Odubo and Kosoe, 2024). Ingested lead is taken up predominantly in the small intestine, with the duodenum serving as a particularly active site of absorption via both DMT1-mediated and DMT1-independent pathways (Patwa *et al*., 2022). Factors such as particle solubility, age, nutritional status, and the presence of competing dietary minerals (e.g., calcium, iron) modulate gastrointestinal uptake, with children and pregnant women absorbing up to 40–70 % of an ingested dose compared to 5–15 % in well-nourished adults (Tokarczyk and Koch, 2025). In the blood, less than 1 % of absorbed lead remains dissolved in plasma; about 99 % binds to red blood cells and is ferried to organs such as the brain, liver, kidneys, and even the aorta. Meanwhile, bone serves as a long-term sink, holding on to over 90 % of the body's lead burden and releasing it slowly back into circulation over decades (Collin *et al*., 2022).

Changes like pregnancy or osteoporosis can mobilize that skeletal lead reservoir, causing renewed internal exposure long after external contact has ceased. Unlike many xenobiotics, lead does not undergo enzymatic biotransformation; rather, it exerts toxicity by displacing essential metals (calcium, iron, zinc) at enzyme active sites and membrane transporters (Aslam *et al*., 2021). By binding sulfhydryl groups on proteins and lipids, lead impairs antioxidant defenses, perturbs mitochondrial function, and promotes oxidative stress (Witkowska *et al*., 2021). Disruption of calcium-dependent signaling impairs neurotransmitter release in neurons, inhibits heme synthesis in erythroblasts, and alters glomerular filtration in the kidney, underlining its multi-system toxicity (Penticoff and Fortin, 2023). Lead is eliminated slowly, primarily via urinary excretion (approximately 70–80 % of total output), with the remainder lost in feces, sweat, hair, and nails (Collin *et al*., 2022). Because this detoxification process is slow, and because most lead hides out in bone, even a one-time high exposure can cast a long shadow over a person's health.

2.3.2 Mechanism of Lead-Induced Neurotoxicity

Lead's neurotoxicity stems from its ability to cross the blood–brain barrier (via calcium transporters), accumulate in neurons and glia, and interrupt synapse formation and plasticity—effects that underlie cognitive deficits and behavioural disorders in children (Ramírez Ortega *et al*., 2021). Lead can also bind directly to DNA and disrupt replication and repair enzymes, inducing strand breaks and mutagenesis that contribute to its long-term carcinogenic and developmental toxicity Frontiers. Together, these interconnected pathways form a complex toxicological web, highlighting why lead exposure—even at low levels—remains a critical focus for environmental health research and public policy (Cuomo *et al*., 2022).

2.3.2.1 Oxidative Stress

Lead's principal mode of harm is the induction of oxidative stress, which arises when reactive oxygen species (ROS) generation overwhelms the body's antioxidant defenses. Two intertwined pathways drive this imbalance: enhanced ROS production—such as superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals—and the depletion or inactivation of endogenous antioxidants like glutathione and key enzymes (Huchzermeyer *et al.*, 2022). At the molecular level, lead's propensity to form covalent bonds with sulfhydryl (–SH) groups makes antioxidant enzymes especially vulnerable (Jomova *et al.*, 2025). By binding to cysteine residues in enzymes such as glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT), lead effectively inactivates their catalytic sites, undermining the primary lines of defense against ROS (Sule *et al.*, 2022).

In parallel, lead inhibits δ-aminolevulinic acid dehydratase (δ-ALAD), a zinc-dependent enzyme in the heme synthesis pathway; δ-ALAD inhibition not only disrupts hemoglobin production but also leads to accumulation of δ-aminolevulinic acid, itself a pro-oxidant that amplifies ROS formation. Moreover, lead can displace essential metal cofactors—particularly zinc—from their native binding sites on antioxidant proteins, further compromising enzyme stability and activity (Witkowska *et al.*, 2021). This displacement impairs the conversion of superoxide radicals to hydrogen peroxide (via SOD) and the subsequent reduction of hydrogen peroxide to water (via CAT and GPx), creating a vicious cycle of ROS accumulation and lipid, protein, and DNA damage (Jomova *et al.*, 2025). Over time, chronic oxidative stress from lead exposure contributes to cell dysfunction and death, with especially severe consequences in red blood cells, neuronal tissues, and renal cells—organs highly sensitive to redox imbalance (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. Once in the cytoplasm, cytochrome c binds to apoptotic protease activating factor-1 (Apaf-1) and recruit's procaspase-9 to form the apoptosome, which in turn cleaves and activates executioner caspases such as caspase-3. 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 signaling. 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 defense 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 up-regulates 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 (Qu and Zheng, 2024; Jomova *et al*., 2025).

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 signaling 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 (Pb^{2+}) 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. Once inside the synaptic terminal, Pb^{2+} binds 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. 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, TrkB receptor signaling 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 Pb^{2+} 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 Pb^{2+} 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. Consequently, the "Trojan horse" strategy by which lead hijacks calcium signaling 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 Hippocampus

Numerous studies have converged on the vulnerability of the hippocampus to lead neurotoxicity, consistently revealing profound histopathological alterations across various animal models. Augustine *et al.* (2021) demonstrated that a 21-day regimen of 120 mg/kg lead provoked oxidative-stress–mediated damage in rat hippocampi, manifesting as severe disruption of the pyramidal cell layer and marked atrophy of those neurons. Similarly, Antar *et al*. (2024) found that exposing rats to 0.2 % lead in drinking water for thirty days not only diminished pyramidal cell counts in the CA1, CA3, and dentate hilus regions but also reduced granule-cell populations in the dentate gyrus—changes they attributed chiefly to apoptosis.

Enogieru and Egbon (2022) observed vacuolation and pyknotic nuclei in CA1 following a 28-day, 100 mg/kg lead acetate exposure, implicating oxidative stress as the driving mechanism, and Sharma *et al*. (2022) reported demyelination alongside hippocampal neuron atrophy in rats administered 0.2 % and 0.4 % lead in water for thirty days, again linking the effects to apoptotic pathways. Olatomide *et al*. (2019) extended these findings by documenting pyramidal-cell degeneration, cytoplasmic vacuolation, and nuclear pyknosis in CA3 after 21 days of 60 mg/kg and 90 mg/kg lead treatment, with oxidative stress and necrosis cited as underlying mechanisms. An eight-week, 50 mg/kg exposure led Paulis *et al*. (2018) to note laminar disorganization, vacuolated CA1 and CA3 fields, and shrunken, acidophilic neurons with pyknotic nuclei, effects they likewise linked to oxidative injury. Using an inhalational model, Rahman and Guillemin (2021) exposed mice to 121.7 µg PbO/m³ for six weeks and observed degenerated, shrunken pyramidal neurons in CA1, attributing these changes to lead's substitution for calcium and resultant mitochondrial impairment. Shaban *et al*. (2021) worked with 4 % lead in drinking water over seventeen days and yielded vacuolated pyramidal layers and neuronal loss in rats, while Thangarajan *et al*. (2023) reported degeneration, vacuolation, neuronal depletion, and disorganized cell layering in mouse hippocampi after fourteen days of 10 mg/kg dosing, implicating oxidative stress. Gudadhe *et al*. (2024) described decreased pyramidal-cell numbers and pyknotic degeneration after two months on 0.5 % lead water, attributing these to calcium-ion displacement, and Liu *et al*. (2021) observed hippocampal-layer disruption and cell degeneration after 28 days of 50 mg/kg lead, with apoptosis identified as the key mechanism.

More recently, Annaç *et al*. (2022) reported CA1–CA3 pyramidal-cell degeneration following 30 days of 50 mg/kg exposure, linking these changes to oxidative stress and reductions in cholinesterase activities (CaE and AChE). Long *et al*. (2022) conducted a twelve-week study of 200 mg/L lead and revealed disrupted neuronal lamination, astrocyte loss, and morphological damage in CA1 and CA3, tied to oxidative-stress markers (↓SOD, CAT, GSH; ↑MDA), while Alves Oliveira *et al*. (2020) likewise attributed CA1–CA3 neuronal loss following 55 days of 50 mg/kg lead to oxidative pathways. Shaban *et al*. (2021) documented extreme neuronal depletion, vacuolation, and pyknosis after eight days of 40 mg/kg dosing, again implicating oxidative-stress–mediated enzyme changes (↓SOD, GPx, GST; ↑MDA). Su *et al*. (2025) described ultrastructural destruction of the endoplasmic reticulum, Golgi apparatus, and mitochondria in CA1 following 90 days of 0.2 % exposure. Enogieru and Williams (2024) linked 28 days of 100 mg/kg lead to CA1 vacuolation and nuclear pyknosis via oxidative stress (↓SOD, CAT, GPx; ↑MDA, NO). Finally, Li *et al*. (2016) demonstrated that 21 days of 0.2 % and 0.5 % lead drinking water in mice induced hippocampal inflammation and axonal-regeneration inhibition in CA1, mediated by upregulation of repulsive guidance molecules RGMa and RGMb. The hippocampus is highly susceptible to lead-induced neurotoxicity, as demonstrated by numerous animal studies. Across various dosages and durations, lead exposure consistently results in histopathological damage such as neuronal degeneration, vacuolation, loss of pyramidal and granular cells, and disruption of hippocampal architecture, particularly in the CA1, CA3, and dentate gyrus regions (Chang, 2023). The most frequently implicated mechanisms include oxidative stress, apoptosis, mitochondrial damage, and calcium ion substitution. Some studies also highlight inflammation, inhibition of neurogenesis, and altered expression of neurotrophic factors like BDNF (Nicoletti *et al*., 2022). These findings underscore the multifactorial and severe impact of lead on hippocampal structure and function.

2.3.4 Current Treatment Options for Lead-Induced Hippocampal Dysfunction

Treatment options for hippocampal disorders encompass pharmacological, surgical, neuromodulatory, and emerging regenerative strategies aimed at restoring excitatory–inhibitory balance, halting disease progression, and preserving cognitive function (Gao and Li, 2023). In mesial temporal lobe epilepsy (MTLE) with hippocampal sclerosis, first-line management employs antiseizure medications—such as carbamazepine, levetiracetam, and lamotrigine—to suppress neuronal hyperexcitability and achieve seizure control in approximately two-thirds of patients (Łukawski and Czuczwar, 2021).

In Alzheimer’s disease (AD)—where hippocampal neurodegeneration underlies progressive memory impairment—symptomatic pharmacotherapy remains the mainstay (Akhtar *et al.*, 2024). Cholinesterase inhibitors (donepezil, rivastigmine, galantamine) modestly improve cognition in mild–moderate AD, while memantine, an NMDA-receptor antagonist, stabilizes function in moderate–severe stages; combination therapy offers incremental benefits in slowing clinical decline (Xu *et al.*, 2021). Recently approved anti-amyloid monoclonal antibodies (aducanumab, lecanemab, donanemab) aim to remove cerebral amyloid plaques and have shown reduced brain atrophy and slowed cognitive deterioration in early AD, though long-term efficacy and safety profiles are still under evaluation. Emerging regenerative approaches, such as interneuron precursor transplantation into the hippocampus, are in first-in-human trials to restore inhibitory circuitry and prevent recurrent seizures in drug-resistant epilepsy (Southwell, 2024).

Treatment of lead (Pb) toxicity focuses on elimination of exposure, accelerated excretion, and mitigation of oxidative damage (Collin *et al.*, 2022). Chelation therapy with oral succimer (DMSA; 30 mg/kg/day for five days, then 20 mg/kg/day for 14 days) reduces blood lead levels (BLLs) by 60–77 percent in moderate pediatric poisoning, while intravenous calcium disodium EDTA ($CaNa_2EDTA$), often combined with dimercaprol (BAL), is reserved for BLLs > 70 µg/dL or encephalopathy. Nutritional interventions—adequate calcium, iron, and zinc intake—compete with Pb for gastrointestinal absorption, lowering uptake by up to 30 percent and improving neurodevelopmental outcomes in exposed children (Ventre *et al.*, 2022). Antioxidants scavenge Pb-induced reactive oxygen species, preserving mitochondrial function and reducing neuronal apoptosis; alpha-lipoic acid offers combined chelating and antioxidative benefits in preclinical models (Khan *et al.*, 2022).

2.4 *Rosmarinus officinalis*

Rosmarinus officinalis, popularly known as rosemary, is a powerful herb belonging to the mint family (Lamiaceae). It is a fragrant, bushy, evergreen sun-loving perennial shrub native to the dry and rocky areas of the Mediterranean region, known for its ability to survive with only the moisture from sea air, and cultivated around the world as a medicinal, ornamental, culinary, and essential oil-bearing plant (de Macedo *et al*., 2020; Allegra *et al*., 2020). It is a perennial shrub, and usually grows to about 2–6.5 feet (0.6–2 meters) in height, with dark green glossy leaves which are numerous, opposite, sessile, and linear, measuring about 1.4 inches (3.5 cm) long and 0.08–0.16 inches (2–4 mm) wide, having leathery and curled margins (Pappachan *et al*., 2023). The species can regenerate by both seeds and cuttings, it is heat and drought tolerant, thrives in areas with dry, poor, rocky, and sandy soil, and relatively pest resistant (González-Minero *et al*., 2020). The leaves of *R. officinalis* have a pungent, slightly bitter taste, and, dried or fresh, are commonly used to season and flavor foods (Pappachan *et al*., 2023).

Figure 2.4: *Rosmarinus officinalis* leaves (Melero-Bravo *et al*., 2022)

Spain is the main producer of rosemary in the world for commercial purposes, followed by France, Italy, and Tunisia. Some regions of Asia, the United States, Mexico, and South Africa also grow it. As it is a very small crop, there are no official reliable measurements of its quantity of production (Melero-Bravo *et al*., 2022).

2.4.1 Different Names of *Rosmarinus Officinalis*

The genus name *"Rosmarinus"* comes from the Latin words *ros* (dew) and *marinus* (sea) which means 'dew of the sea', in reference to the ability of this plant to thrive well in coastal areas (sea cliffs) and exposure to ocean mists (Hammer and Junghanns, 2020). Common names for *R. officinalis* relate the species to various myths and folklore, as the plant has been known and used in many cultures since antiquity (Melero-Bravo *et al*., 2022; Banjaw *et al*., 2024). In Portugal, for example, the plant was called *'alecrim'* a word derived from the Scandinavian *'ellegren'*, meaning *'elfin-plant'*, as folklore associated the plant with elves, and in Sicilian tales it was said that baby fairies slept in the rosemary flowers (Hammer and Junghanns, 2020).

In Spain a common name for the species, *'romero'*, is interpreted as *'pilgrim's flower'*, in reference to a traditional tale that the Virgin Mary rested under a rosemary bush during the flight to Egypt (Mehta *et al*., 2023). In China, the plant is called, '*mí dié xiāng'* which means "the winding fragrant herb", painting the picture of an herb that layers and trails in a drifting way, carrying a pleasant scent. In French and German languages, it is called *'rosmarin',* in Italian, *'rosmarino'* (Mehta *et al*., 2023). Locally, in Nigeria, there is no widely attested indigenous term for *R. officinalis*; the English name "rosemary" is simply borrowed, reflecting its relatively recent introduction as a cultivated herb. However, in the Yoruba language, it is referred to as '*ẹwe elewe'* meaning *"leaf-leaf",* a general term used in herbal contexts, perhaps echoing the plants' repeated medicinal uses (Lawal *et al*., 2022; Adepoju *et al*., 2024).

2.4.2 Taxonomy

Taxonomic tree of *Rosmarinus officinalis*

Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Dicotyledonae
Order	Lamiales
Family	Lamiaceae
Genus	*Rosmarinus*
Species	*Rosmarinus officinalis*

2.4.3 Conventional Uses of *Rosmarinus officinalis*

The plant, *R. officinalis* has been cultivated for thousands of years in the Mediterranean region, where it was valued for its culinary, medicinal and symbolic properties (Hendel *et al*., 2024). The Egyptians used rosemary in burial rituals, and traces have been found in tombs dating to 3000 BCE. In ancient Greece and Rome, it was associated with purification, often burned as incense, and thought to banish diseases and evil spirits (Pontillo *et al*., 2021; Hendel *et al*., 2024). Medicinally, rosemary has been used as a digestive aid, stimulant, and a popular constituent of tonics. The oil is used in perfumes, soaps, mouthwashes, and antiseptic solutions. Research suggests that its scent may enhance memory and concentration (Hendel *et al*., 2024).

One of Europe's earliest alcohol-based perfumes, "Hungary water", was made from rosemary and used by Queen Elizabeth of Hungary in the 14th century. French emperor, Napoleon Bonaparte reportedly used dozens of citrus-heavy scents enriched with rosemary and lavender because the aroma evoked fond memories of his time ruling Europe (Meziane *et al*., 2025). In cooking, rosemary is used as a seasoning and spice, used to flavor various dishes, as well as in baking, and herbal teas. Anthropologists and archeologists have found evidence that *R. officinalis* herb has been used as cosmetics in ancient Egypt, Mesopotamia, China, and India. Its antiseptic nature stimulates metabolic processes in the skin's dermal layer and is very beneficial in cases of acne, dermatitis and eczema (Jahanian *et al*., 2024). *R. officinalis* is used as a circulatory stimulant, particularly for those with low blood pressure. It has also been reportedly used to treat respiratory disorders like bronchitis and asthma, possibly due to its anti-inflammatory properties. Rosemary oil has been shown to reduce stress and anxiety.

2.4.4 Phytochemical Composition of *Rosmarinus officinalis*

Rosemary belongs to the class of aromatic and therapeutic plants, which are distinguished by their high essential oil production. These plants are rich in terpenes, phenolic compounds, terpenoids, and other phytochemicals, which are bioactive substances with specific Anti-bacterial and Anti-oxidant effects. The most common phenolic compounds in rosemary are flavonoids (naringenin, eriodyctiol, hesperidin, vicenin-2, luteolin-7-glucuronide, apigenin, and luteolin); diterpenes (carnosic acid, carnosol, and carnosobetulinic acid); triterpenes (ursolic acid, oleanolic acid, and β-Amyrin & α-Amyrin); phenolic acids (rosmarinic acid, caffeic acid, chlorogenic acid); essential oils (1,8-Cineole, also known as eucalyptol, camphor, α-Pinene, camphene, limonene, and β-Caryophyllene), vitamins (Ascorbic acid, Folate, Niacin, Riboflavin, Thiamin, Vitamins A, B12, B6, D and E) (Jafarı-sales and Pashazadeh, 2020; Ouknin *et al*., 2021; Jeevalatha *et al*., 2022; Kamli *et al*., 2022).

2.4.5 Pharmacological Activities of *Rosmarinus officinalis*

With its culinary and medicinal uses dating back centuries, *R. officinalis* has become a staple herb for flavor enhancement and natural health remedies. Rich in bioactive compounds, it boasts an array of pharmacological benefits, including antibacterial, antidiabetic, anti-inflammatory, antitumor, and antioxidant properties.

2.4.5.1 Antioxidant activity

The antioxidant function of *R. officinalis* is elucidated through various investigations. The antioxidant capability of rosemary has been researched significantly compared to its biological function. The antioxidant function of rosemary stems from the phytochemicals found in the plant's natural oils and extracts. The combined effects of different phytoconstituents of rosemary lead to increased antioxidant activity.

Ebrahimi *et al*. (2020) and Kamli *et al*. (2022) investigated the antioxidant properties of *R. officinalis*, attributing its activity to a combination of phytochemicals such as carnosol, carnosic acid, and rosmarinic acid. These compounds, primarily found in the plant's essential oils and extracts, contribute to its notable free radical scavenging activity. Rosemary's antioxidant function results from the synergistic interaction of its bioactive constituents, which enhance its biological activity.

Alavi *et al*. (2021) and Topal and Gulcin (2022) demonstrated that rosemary extracts significantly inhibit free radicals and reduce lipid peroxidation induced by oxidative stress. Similarly, Pisoschi *et al*. (2021) reported that rosemary enhances the production of endogenous antioxidant enzymes, which act as chain-breaking antioxidants and reactive oxygen species (ROS) scavengers. Gulcin and Alwasel (2022) further suggested that these enzymes also function as metal ion chelators, reducing oxidative stress.

Gutiérrez-del-Río *et al*. (2021) observed that carnosic acid and carnosol efficiently scavenge peroxyl radicals in membrane lipids, outperforming synthetic antioxidants such as BHA, BHT, and propyl gallate. Additional support comes from Gulcin and Alwasel (2022), who emphasized rosemary's metal-chelating effects, particularly of Fe^{2+}, thus limiting ROS formation. Alavi *et al*. (2021) linked oxidative stress to the development of chronic diseases, noting rosemary's preventive role via its antioxidative properties.

Kola *et al*. (2024) highlighted the contribution of caffeic acid derivatives in rosemary extracts, which chelate metal ions and stabilize peroxides. Bouammali *et al*. (2023) affirmed that rosemary's antioxidant potential underpins many of its therapeutic benefits. Topal and Gulcin (2022) found that rosmanol exhibited an antioxidant effect four times stronger than synthetic compounds.

The inhibition of lipid peroxidation by rosemary extracts has been quantified, with Iorio *et al*. (2022) and Habtemeriam (2023) reporting that carnosic acid and carnosol inhibit lipid peroxidation by 88–100% and 38–89%, respectively, under oxidative stress. Irakli *et al*. (2023) emphasized that the combined effect of phenolic diterpenes enhances rosemary's antioxidant efficiency.

Cores *et al*. (2023) and Dong *et al*. (2024) associated the plant's antioxidant effect with the presence of isoprenoid quinones. Kaur *et al*. (2023) noted that rosemary extracts are widely used in the food industry to prevent lipid oxidation and extend shelf life, showcasing its commercial potential. Wei *et al*. (2021) and Sun and Shahrajabian (2023) explained that the antioxidant mechanism involves the conversion of phenolic hydroxyl groups in carnosic acid and carnosol into quinone derivatives.

Further studies by Kancheva *et al*. (2021), Aribisala and Sabiu (2022) and Alfieri *et al*. (2022) confirmed that compounds with catechol structures exert radical-scavenging effects, reducing intracellular oxidative stress. Molaei *et al*. (2021) revealed that rosemary facilitates the elimination of xenobiotics by activating phase II detoxifying enzymes through the Nrf2 pathway. Satoh *et al*. (2022) described how electrophilic quinone derivatives from rosemary alkylate cysteine residues of Keap1, promoting Nrf2 translocation and the expression of antioxidant genes.

McCord *et al*. (2023) found that carnosol significantly upregulates Nrf2-regulated genes, including those encoding antioxidant enzymes. Similarly, Aziz *et al*. (2022) reported that both carnosic acid and carnosol protect HT22 neuronal cells against oxidative glutamate toxicity by inducing heme oxygenase-1, NADPH quinone oxidoreductase, and γ-glutamyl cysteine ligase. Wan *et al*. (2024) further demonstrated that carnosic acid alleviates LPS-induced liver damage by enhancing antioxidant enzyme levels in serum and liver tissue.

In addition, Yeddes *et al*. (2022) showed that pre-treatment of RAW264.7 macrophages with carnosic acid significantly reduced ROS and nitric oxide production, while Mirza *et al*. (2023) observed that carnosol increased the activity of quinone reductase and glutathione S-transferase *in vivo*.

2.4.5.2 Antimicrobial

The antimicrobial activity of *R. officinalis* has been well-documented across numerous studies. Both rosemary extract and its essential oil have shown significant antibacterial and antiviral potential. The primary active compounds contributing to these effects include phenolic acids such as carnosic acid and rosmarinic acid, and volatile constituents of the essential oil such as 1,8-cineol, camphor, and α-pinene.

The antimicrobial activity of rosemary is typically assessed using minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) assays. Valková *et al*. (2021) reported the inhibition of microbial growth by rosemary essential oils, determined through MIC values. Essential oils rich in carvacrol (67.0%) and γ-terpinene (15.3%) exhibited significant activity against Gram-negative strains, including *E. coli*, with MIC values ranging from 0.025 mL/mL to 0.78 mL/mL, as determined via the broth microdilution method by Baginska *et al*. (2023).

Al-jaafreh (2024) further confirmed the potent inhibitory effect of rosemary oil on *E. coli* ATCC 25922, with a minimal inhibitory concentration of 6.4 mg/L. Valková *et al*. (2021) also demonstrated the antimicrobial potential of rosemary essential oil against *B. cereus* and *S.*

aureus. Additional findings supported its activity against *Clostridium perfringens*, *Aeromonas hydrophila*, and *Salmonella choleraesuis*.

Rosemary extracts have demonstrated the ability to inhibit the growth of a broad range of microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Saccharomyces cerevisiae*. Ekambaram *et al*. (2024) found that rosemary extract exhibited inhibitory effects on both *S. aureus* and methicillin-resistant *S. aureus* (MRSA), with minimum inhibitory concentrations (MIC) of 0.8 mg/mL and 10.0 mg/mL, respectively. The antimicrobial effect of rosmarinic acid was suggested to be associated with interference in the expression of major virulence factors on the bacterial surface, particularly microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) in *S. aureus* and MRSA.

The antibacterial effects of *R. officinalis* have been widely investigated, particularly in applications involving red meat products such as beef meatballs, cooked beef, and pork sausages. Various studies confirmed the efficacy of rosemary essential oil against a range of bacterial strains, including *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens*, *Aeromonas hydrophila*, and *Salmonella choleraesuis* (Rashidaie *et al*., 2019; Valková *et al*., 2021 and Sulieman *et al*., 2023).

The incorporation of rosemary extract into meat products was shown to inhibit the growth of *Brochothrix thermosphacta* and *Enterobacteriaceae* (Kaur *et al*., 2023) suggesting potential for food preservation. Farhadi *et al*. (2023) compared the effects of carnosic acid and rosmarinic acid, concluding that carnosic acid exhibited stronger antibacterial efficacy against pathogenic bacteria.

Further investigation by Dai and Liu (2021) explored the antibacterial mechanism of six rosemary-derived monomers against *Salmonella*. Among them, 1,8-cineol demonstrated the strongest inhibitory activity, primarily through disruption of bacterial cell membrane integrity and leakage of intracellular contents.

In addition to its antibacterial properties, *R. officinalis* exhibits promising antiviral potential. Rosmarinic acid has been reported to inhibit the growth of viruses such as HIV, hepatitis B virus (HBV), and enterovirus 71 (EV71). It was shown that rosmarinic acid reacts with nitrite ions (NO_2^-) to form 6-nitro and 6,6-dinitro rosmarinic acid derivatives, which significantly enhance antiviral activity. These nitrated derivatives were effective in inhibiting HIV-1 integrase, suppressing replication in lymphocyte MT-4 cells.

Ranga *et al*. (2023) demonstrated that rosmarinic acid could target the epsilon (ε) sequence in the pregenomic RNA of HBV, inhibiting the binding between this sequence and the viral polymerase, thereby interfering with a critical step in HBV replication. Similarly, Lin *et al*. (2021) found that rosmarinic acid protected cells from pathological changes induced by EV71, with the strongest antiviral effect observed during the early phase of infection. These findings suggest its therapeutic potential as an early-stage antiviral agent against EV71.

Additionally, rosemary extracts have been found to suppress influenza virus replication and ameliorate virus-induced pneumonia (Lin *et al*., 2021), further expanding its pharmacological relevance in antiviral therapy.

2.4.5.3 Antitumor

Kakouri *et al*. (2022) and Semreen *et al*. (2024) investigated the antiproliferative effects of *R. officinalis* polyphenols—rosmarinic acid, carnosic acid, and carnosol—on various human cancer cell lines. Using in vitro cell viability and nitric oxide assays, they demonstrated that treatment with these compounds elevates intracellular nitric oxide levels, triggering intrinsic apoptotic pathways. Carnosic acid emerged as the principal regulator of this apoptosis induction, underscoring its central role in rosemary's anticancer activity.

Kallimanis *et al*. (2022) evaluated the anti-tumor efficacy of a methanolic rosemary leaf extract in a two-stage mouse skin carcinogenesis model. Topical application of rosemary 5 minutes before each benzo[a]pyrene (B[a]P) initiation or DMBA exposure reduced covalent binding of these carcinogens to epidermal DNA by up to 64% and decreased tumor incidence per mouse from 7.1 to as low as 2.5. Additionally, rosemary pre-treatment significantly inhibited tumor-promoting markers—including TPA-induced ornithine decarboxylase activity, inflammation, and hyperplasia—thereby blocking both initiation and promotion phases of skin tumorigenesis.

Dandoti (2021) defined evasion of apoptosis and activation of invasion and metastasis as core hallmarks of cancer, highlighting how malignant cells resist programmed cell death and acquire metastatic potential. Rosemary extract has been shown to counter these hallmarks by restoring apoptotic sensitivity in resistant cancer cells and inhibiting early tumor-promotion events in vivo, positioning it as a promising antitumorigenic agent.

Allegra *et al*. (2020) reported that rosemary extract not only blocks carcinogen–DNA interactions in murine models but also exerts direct cytotoxic effects on colon cancer cell lines (HCT116, SW480). In these cells, treatment upregulated the Nrf2 transcription factor and its downstream effector sestrin-2, promoting oxidative-stress defense and apoptosis. In vivo xenograft studies further confirmed tumor growth inhibition, underscoring the therapeutic potential of rosemary in colorectal cancer.

Kakouri *et al*. (2022) explored the impact of a methanolic rosemary extract on human non-small cell lung cancer A549 cells. Through MTT, clonogenic survival assays, and immunoblot analysis of Akt/mTOR/p70S6K signaling, they demonstrated that 50 μg/mL extract dose-dependently inhibited proliferation, reduced long-term survival, and enhanced PARP cleavage. These effects correlated with marked reductions in total and phosphorylated Akt, mTOR, and p70S6K levels, implicating inhibition of the Akt/mTOR axis in rosemary's anticancer mechanism.

2.4.5.4 Anti-inflammatory

Gonçalves *et al*. (2022) and Habtemariam (2023) evaluated the anti-inflammatory potency of rosemary diterpenes carnosic acid and carnosol in standard in vivo and in vitro assays. Using carrageenan-induced paw edema and cell-based nitric oxide (NO) assays, they demonstrated that these compounds inhibit inflammatory mediators with an efficacy nine times greater than indomethacin, indicating a strong synergistic effect of rosemary's phenolic diterpenes.

R. officinalis essential oil and crude extracts have been shown to impede leukocyte migration to sites of inflammation in murine models. In vivo leukocyte-migration assays revealed significant reductions in white blood cell infiltration and edema formation, correlating with decreased NO and pro-inflammatory gene expression at the lesion site. These findings underscore rosemary's capacity to attenuate the early cellular events of inflammation.

Xu *et al*. (2022) investigated the molecular basis of carnosol's anti-inflammatory action in endothelial and immune cells. Through kinase assays and immunoblotting, they found that carnosol inhibits IKK-β phosphorylation, preventing NF-κB nuclear translocation, and simultaneously upregulates HO-1 expression via Nrf2 activation. This dual action led to marked decreases in prostaglandin E_2, TNF-α, and nitrite production at 5–20 μM concentrations, revealing a coordinated suppression of both inflammatory signaling and oxidative stress pathways.

Grigore *et al*. (2022) focused on the endothelial-vascular interface, assessing carnosic acid's effect on cytokine-induced adhesion molecule expression. Using human umbilical vein endothelial cells (HUVECs) treated with TNF-α, they measured ICAM-1 and VCAM-1 levels by flow cytometry and monocyte-adhesion assays. Carnosic acid significantly reduced both adhesion molecule expression and monocyte binding, suggesting a mechanism by which rosemary mitigates leukocyte extravasation during vascular inflammation.

Singh *et al.* (2023) examined the effects of carnosic acid and carnosol on human polymorphonuclear leukocytes. Employing 5-lipoxygenase activity assays and leukocyte activation models, they showed potent inhibition of leukotriene synthesis and suppression of ROS and degranulation responses. This indicates that rosemary diterpenes can directly blunt pro-inflammatory mediator production in innate immune cells.

Iorio *et al.* (2022) explored the neuroinflammatory modulation by carnosic acid in microglia. In LPS-activated MG6 microglial cultures, treatments with carnosic acid (1–20 μM) led to significant reductions in NO release and iNOS expression (Western blot), as well as decreased IL-1β and IL-6 secretion. These results highlight rosemary's potential to counteract neuroinflammation via downregulation of microglial activation pathways.

2.4.5.5 Anti-obesity

Nair *et al.* (2021) investigated the antiadipogenic effect of carnosic acid, the principal bioactive diterpene in *R. officinalis* extracts, on murine preadipocytes. They aimed to elucidate the molecular mechanism by which carnosic acid inhibits adipocyte differentiation. Using 3T3-L1 cells treated at the onset of differentiation, they measured lipid accumulation (Oil Red O staining), and assessed expression of C/EBPα and PPARγ by qPCR and Western blot. They found that carnosic acid dose-dependently suppressed lipid droplet formation by downregulating both C/EBPα and PPARγ pathways, reducing triglyceride content by over 60% at 10 μM. These results imply that carnosic acid may serve as a novel inhibitor of adipogenesis with potential for sustainable weight-management strategies.

Kapoor *et al.* (2021) evaluated whether a rosemary leaf extract (RE) standardized to 20% carnosic acid could limit weight gain via pancreatic lipase inhibition in vivo and in vitro. They aimed to test the hypothesis that reduced dietary fat absorption underlies rosemary's anti-obesity action. In vitro, they performed pancreatic lipase assays (RE vs. orlistat control), reporting 80% inhibition at 100 μg/mL RE ($p < 0.001$). In a high-fat-diet mouse model, oral administration of RE (50 mg/kg/day for 8 weeks) resulted in 30% less weight gain and a 25% reduction in fat mass compared to controls ($p < 0.01$). These findings indicate that rosemary extract can mitigate diet-induced obesity by impairing intestinal lipid assimilation, positioning RE as a potential natural alternative to lipase inhibitors.

Diab *et al*. (2022) explored carnosic acid's metabolic effects in a genetic model of obesity (ob/ob mice) to assess its broader therapeutic potential. They aimed to determine whether chronic CA treatment could improve glucose homeostasis and hepatic steatosis. ob/ob mice received CA (10 mg/kg/day) for 6 weeks, followed by oral glucose tolerance tests, histological liver analysis, and RT-qPCR for lipid-metabolism genes (Srebp-1c, Fasn). CA-treated mice exhibited a 40% improvement in glucose tolerance, a 35% reduction in hepatic triglyceride accumulation, and significant downregulation of Srebp-1c and Fasn expression ($p < 0.05$). These results suggest carnosic acid not only curbs adipogenesis but also ameliorates obesity-related metabolic derangements, supporting its development as a multifunctional anti-obesity agent.

CHAPTER THREE

MATERIALS AND METHODS

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/2021/172.**

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 MATERIAL

Packets of dried *R. officinalis* leaves were bought at a local market in Benin City, Edo State, Nigeria, with NAFDAC Number A1-9793 and batch number 206211.

3.4 EXTRACTION OF PLANT MATERIAL

Plant extraction was performed as previously described by Enogieru and Iyoha (2023), with minor modifications. A total of 5 kg of dried leaf material was blended into fine powder using a mechanical blender. Aliquots of 10 g of the powdered sample were extracted with distilled water using either three successive macerations (3 × 50 mL, 24 hours each at room temperature) or a single decoction step (1 × 50 mL, 10 minutes at 95°C). Following extraction, the mixtures were filtered using Whatman No. 1 filter paper, and the resulting aqueous extracts were freeze dried. The dried extracts were stored in the dark at 4°C until further use. Prior to experimentation, the freeze dried extracts were reconstituted in distilled water to the desired working concentration.

3.5 PHYTOCHEMICAL SCREENING

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

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 *R. officinalis* leaves was carried out in the Pharmacognosy Department, University of Benin. Flavonoids, tannins, phenols, saponins, steroids, alkaloids, and glycosides were screened for their presence in the plant material.

3.5.1.1 Test for Phenols

2 mL of 5% neutral $FeCl_3$ solution were added to 1 mL of aqueous extract, the dark blue colour showed the presence of phenolic compounds (Idu and Igeleke, 2012).

3.5.1.2 Test for Flavonoids

To 1mL of aqueous extract, a few drops of conc. Sulphuric acid (H_2SO_4) was added along the sides of the test tube. The formation of a yellow color indicated the presence of flavonoids (Idu and Igeleke, 2012).

3.5.1.3 Test for Tannins

1 mL of freshly prepared 10% KOH was added to 1 mL of aqueous extract. Dirty white precipitates indicated the presence of tannins (Idu and Igeleke, 2012).

3.5.1.4 Test for Alkaloids (Mayer’s reagent test)

A few quantities of each portion were stirred with 5 mL of 1% aqueous HCl in a water bath and then filtered. Of the filtrate, 1 mL 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 1 mL, Mayer's reagent was added and the appearance of a buffcolored 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. H_2SO_4 was added along the walls of the test tube; a red colour showed the presence of steroids (Idu and Igeleke, 2012).

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 (Idu and Igeleke, 2012).

3.5.1.7 Test for glycosides

Aqueous extract of 2.5 mL was treated with 5 mL of conc. H_2SO_4 and boiled for 15 minutes in a water bath. This mixture was cooled and neutralized with 20% Potassium hydroxide (KOH). Three drops of $FeCl_3$ were added to one-half of the mixture and observed for green to black precipitates (Idu and Igeleke, 2012).

3.5.2 Quantitative Phytochemical Screening

Quantitative analysis provides precise measurements of the concentrations of specific bioactive compounds (Velu *et al*., 2018). Quantitative screening of *R. officinalis leaves* was carried out in the Pharmacognosy Department, University of Benin. Total flavonoids, tannins and phenols content were estimated.

3.5.2.1 Test for Total Phenol Content

1 mg of the plant extract was dissolved in 1 mL of methanol, homogenized in an additional 1 mL of methanol, and centrifuged for 20 minutes. The supernatant was collected. Solutions of Gallic acid, sodium carbonate, and Folin Ciocalteu reagent (FCR) were prepared. A 10% Folin Ciocalteu reagent was made by mixing 10 mL of FCR with 90 mL of water, and a 5% Na_2CO_3 solution was created by dissolving 3 g of Na_2CO_3 in 50 mL of water. In test tubes, 200 µL of

each extract was mixed with 1.5 mL of 10% FCR, kept in the dark for 5 minutes, and then 1.5 mL of 5% Na_2CO_3 was added. The solution was mixed and kept in the dark for 2 hours. Absorbance was measured at 750 nm using a UV-spectrophotometer, and total phenol content, expressed as Gallic acid equivalent, was found to be directly proportional to the absorbance using a microplate reader (Idu and Igeleke, 2012). Total phenol was calculated using the formula:

Total Phenol Content (mg GAE/g) = (Absorbance of the standard / Absorbance of the sample) x Concentration of the standard x Dilution factor

3.5.2.2 Test for Total Flavonoid Content

0.5 mL of plant extract solution was mixed with 2 mL of distilled water, 0.15 mL of 5% aluminum chloride solution and 0.3 mL of 1 M potassium acetate solution. The reaction mixture was incubated at room temperature for 30 minutes. The absorbance of the reaction mixtures at a specific wavelength were measured using a UV-Visible spectrophotometer and a 51 calibration curve was plotted (Idu and Igeleke, 2012). The total flavonoid content was calculated using the formula:

Total Flavonoid Content (mg RE/g) = (Absorbance of the sample / Absorbance of the standard) x Concentration of the standard x Dilution factor

3.5.2.3 Test for Total Tannin Content

0.1 mL plant extract solution was mixed with 0.75 mL of distilled water, 0.1 mL of Folin Ciocalteu reagent and 0.5 mL of 20% sodium carbonate solution, and incubated in the dark at room temperature for 2 hours. The absorbance of the reaction mixtures at a specific wavelength were measured using a UV-Visible spectrophotometer and a calibration curve was plotted (Idu and Igeleke, 2012). The total tannin content was calculated using the formula:

Total Tannin Content (mg GAE/g) = (Absorbance of the standard / Absorbance of the sample) x Concentration of the standard x Dilution factor

3.6 PROCUREMENT AND CARE OF EXPERIMENTAL ANIMALS

For this study, forty-eight (48) adult Wistar rats weighing between 140 g to 160 g 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 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 six (6) groups (A, B, C, D, E, and F) of eight (8) rats each (n=8).

Table 3.1: Summary of Experimental Design

GROUP	**TREATMENT**
Group A	Control
Group B	100 mg/kg body weight of lead acetate only
Group C	100 mg/kg body weight of *R. officinalis* extract + 100 mg/kg body weight of lead acetate
Group D	200 mg/kg body weight of *R. officinalis* extract + 100 mg/kg body weight of lead acetate
Group E	100 mg/kg body weight of *R. officinalis* extract only
Group F	200 mg/kg body weight of *R. officinalis* extract only

Administration lasted for twenty-eight (28) days. All administrations were carried out via oral route using an orogastric tube. Treatment groups were pre-treated with *R. officinalis* extract, 1hr before the administration of lead acetate.

3.8 NEUROBEHAVIOURAL ASSESSMENTS

After administration on the 28th day, neurobehavioural activities were assessed. Neurobehavioural assessment is a clinical study of behaviour that emphasizes the role of brain dysfunction (Zasler *et al*., 2013). It is an essential component of rehabilitation and is used to discern behaviours 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, various neurobehavioural assessment tests were carried out. These include; novel object recognition (NOR) test, Y-maze, and elevated plus maze (EPM).

3.8.1 Novel Object Recognition (NOR) Test

The novel object recognition (NOR) test also known as the object recognition test (ORT) is a standard behavioural assay utilized to investigate many aspects of learning and memory in rodents (Lueptow, 2017). The NOR's key benefit over other rodent memory tests is that it takes advantage of rodents' innate inclination to seek out novelty (Grayson *et al*., 2015). The test was performed as described by Enogieru and Omoruyi (2022). The NORT was performed in a wooden open box apparatus (80 × 60 × 40 cm). The objects to be discriminated was of two different colours (yellow and pink), made up of painted wood, around 10 cm in height and heavy (so it will not be displaced by the animals during test). In addition, these objects had no genuine significance for the rats and were not associated with reinforcement.

Figure 3.1: Novel Object Recognition Apparatus

The day before testing, rats were allowed to explore the apparatus for a 2 min session of habituation. Twenty-four hours later, the first 3 min sample trial test (T1), with two similar objects (termed as sample objects FO1 and FO2) presented at the corners of the box, commenced. Following T1, all the rats were placed back in their home cage and a delay of 60 min was given as 69 inter-trial interval for T2. In the second 5-min choice trial (T2), one of the objects (FO2) presented in T1 was replaced by a new object (NO). To evaluate the effect on long term memory, animals were again exposed to the apparatus and time spent by rats in exploring FO1 and NO was recorded. All the combinations and locations of the objects were used in a balanced manner to reduce potential bias due to preferences for particular locations or objects.

To avoid the presence of olfactory trails, the apparatus and the objects were cleaned thoroughly with 70% ethanol after each trial. Exploration was considered as directing the nose at a distance ≤ 2 cm to the objects and/or touching it with the nose. The times spent by rats in exploring two objects in T1 and T2 was recorded separately. The total time spent in exploring two identical objects in T1 and the time spent in exploring two different objects in T2 was recorded. The discrimination between the familiar and the novel object during T2 was measured by comparing the time spent in exploring the familiar object with that spent in exploring the new object. To remove any kind of biasness in overall levels of exploration, a discrimination index (D) was calculated; $D = N−F/N + F$.

3.8.2 Y-Maze Test

The Y-maze test (YMT) is used to measure the spontaneous alternation performance, which allowed the evaluation of cognitive searching behaviour, as an index for the cognitive dysfunction (Prieur and Jadavji, 2019). Animals were gently placed individually in the Y-maze apparatus, which consist of three identical arms (33 × 11 × 12cm each) in which the arms are symmetrically separated at 120° (Kraeuter *et al.*, 2019). The parameters measured include; number of total arm entries, number of spontaneous alternation and percentage of spontaneous alternation. Specifically, each rat was placed at the end of arm A, and allowed to explore all the three arms (labeled A, B, C) freely for 5 min, taking the following parameters: the number of arm visits and sequence (alternation) of arm visits visually. An alternation was defined as entries in all three arms on consecutive occasions (Prieur and Jadavji, 2019). The percentage of alternation was calculated as total of alternations divided by (total arm entries –2), and then multiplied by 100% as previously described (Kraeuter *et al.*, 2019). After each rat session, the observation chamber was cleaned with 70% ethanol to remove residual odor.

Figure 3.2: Y-Maze Apparatus

3.8.3 Elevated-plus Maze (EPM)

According to Gari and Varshney (2020), the Elevated Plus Maze (EPM) is commonly employed to assess anxiety-like behaviour in laboratory rodents, though it can also be utilized to evaluate cognitive function. The apparatus consists of two open arms (50 × 10 cm) and two closed arms of the same dimensions, with the closed arms enclosed by 30 cm high walls. These arms are arranged in a plus-shaped configuration and connected by a central platform (10 × 10 cm), with the entire maze elevated 60 cm above the ground.

Each rat was placed at the center of the maze facing an open arm and allowed to explore freely for 5 minutes. During this period, transfer latency parameters was recorded. Transfer latency is defined as the time taken for the animal to move from the open arm into a closed arm upon initial placement in the maze, often interpreted as an index of cognitive function or exploratory motivation. An arm entry was recorded when all four paws of the animal (or at minimum, both hind paws) were fully within the arm. To prevent olfactory cues from affecting behaviour, the maze was cleaned with 70% ethanol between trials. (Casteller *et al*., 2006; Naqvi *et al*., 2012).

Figure 3.3: Elevated Plus Maze Apparatus

3.9 EVALUATION OF BODY AND BRAIN WEIGHTS

After the neurobehavioural assessments were completed, the rats were euthanized with low level anesthesia, 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:

Relative brain weight = [absolute brain weight (g) / body weight (g)] x 100

3.10 HIPPOCAMPAL OXIDATIVE STRESS PARAMETERS

The hippocampus was removed from the brain after being harvested, blotted of blood, and weighed right away with an electronic balance calibrated in milligrams and recorded to the nearest two decimal places. The harvested and weighed hippocampi were 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 × 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 Hadwan *et al.* (2024).

A. *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.

B. *Preparation of reagent*

i. 0.01M $KMnO_4$ was prepared by dissolving 0.158 g of $KMnO_4$ in 100 mL of distilled water.

ii. Phosphate buffer (pH 7.4); 0.426 of $NaHPO_4$ NaH_2PO_4 was weighed and dissolved in 100 mL of distilled water.

iii. 6M H_2SO_4: 32.3 mL of conc. H_2SO_4 was added to 66.7 mL of distilled water.

iv. 30Mm H_2O_2 solution was prepared by measuring 0.34 mL of 30% of H_2O_2 in 1001 mL of phosphate buffer.

C. *Procedure*

To a known volume of supernatant (0.5 mL), 5.0 mL of H_2O_2 was added. This was then mixed by inversion and allowed to stand for 30 minutes. The reaction was stopped by adding 6M H_2SO_2.

The absorbance was taken at 480nm within 30-60 seconds against distilled water.

D. *Calculation*

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

Where, OD = absorbance

L = light path = 1cm

V_1 = total volume of the reaction sample

M = molar extinction co-efficient of H_2O_2 (40/M/cm).

3.10.2 Estimation of Glutathione (GSH) Concentration

This was determined using the method described by Nuhu *et al.* (2021).

A. Reagents

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

B. Procedure

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

C. Calculation

$$Concentration\ of\ GSH = [Atest\ x\ Conc.\ of\ Standard] / A_{stand}$$

$$\%\ Glutathione\ Reduced = [(A_0\text{-}A_1)\ x\ 100] / A_0$$

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 Zhao *et al.* (2021).

A. 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 catalyzing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD which is determined at 420 nm.

B. Preparation of reagents

Carbonate buffer (0.05 M) pH 10.2: this was prepared by dissolving 0.2014 g of Na_2CO_3. 0.2604 g of $NaHCO_3$ and 0.0372 g of EDTA in 100 mL of distilled water.

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

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

C. Procedure

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

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

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 Ahmed *et al*. (2021).

A. Principle

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

B. Preparation of reagent

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

C. Procedure

To an aliquot of supernatant (0.2 mL), 2.5 mL of phosphate buffer, 2.5 mL of H_2O_2, 1.5 mL of distilled water and 2.5 ml of pyrogallol was added. The reaction was allowed to stand for 30 minutes at room temperature. A deep brown color was formed which was read at 420nm.

D. Calculation

Activity = [(OD/Min) x (VtDf)] / [E x Vs x 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

Malondialdehyde was determined using the thiobarbituric acid assay (Fauziah *et al.,* 2018).

A. Principle

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

B. Preparation of reagent

Stock TCA-TBA-HCL was prepared by mixing 15 g 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.

C. Procedure

A volume of supernatant (1.0 mL) was added to 2.0 mL 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 1000 g for 10 minutes. The absorbance was determined at 535nm against a blank.

The concentration of MDA was determined using the formula:

MDA (unit/mg protein) = (A x Vt x 1000) / (M x V x 1 x Y)

A = absorbance of sample test at 535nm

Vt = total volume of the reaction = 3mL

M = molar extinction co-efficient of product = 1.56 × 105m-1cm-1

l = light path = 1cm

V = volume of tissue extract used = 1mL

Y = mg of tissue in the volume of sample used

3.11 DETERMINATION OF NITRIC OXIDE (NO) LEVEL

Nitric oxide, measured as nitrite, was determined using Griess reagent, according to the method of Vargas-Maya *et al*. (2021). Briefly, 100 mL of sample were incubated with 100 mL of Griess reagent (Sigma) at room temperature for 20 min. Nitrite level was determined by measuring the absorbance at 550 nm using a spectrophotometer.

3.12 ESTIMATION OF LEAD CONTENT

Lead concentration was assayed for using AAS according to methods described by Omotuyi *et al*. (2018). Fresh tissue samples were thoroughly digested in 3.0 mL solution containing 15 mL 5 nitric acid (HNO3, 69%) and 25 mL of perchloric acid (HClO4, 58%) at 100°C for 48 hours. The digestate was filtered and diluted to 100 mL using distilled/deionized water. The metal concentrations were determined by atomic absorption spectrophotometer (AAS BUCK Scientific Model 211 VGP) at 283.2 nm wavelength.

3.13 HISTOLOGY OF THE HIPPOCAMPUS

Briefly, the excised hippocampus 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 Bancroft and Layton (2018) 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 molds.

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 5 µm 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.

3.14 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 Bancroft and Layton (2018). 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 hematoxylin for 15-20 minutes
iv. Excess hematoxylin 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).

3.15 PHOTOMICROGRAPHY

The sections of the hippocampus 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 color 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.

3.16 ASSESSMENT OF APOPTOSIS

For hippocampus homogenates, commercially available kits were utilized to evaluate cleaved caspase 3 (Sigma-Aldrich, St Louis, MO, USA) expression according to the manufacturer's protocol. Samples were lysed, centrifuged, and supernatants collected. Protein concentrations were normalized. Kit reagents and standards were prepared, and the reaction was assembled in a 96-well plate with samples, controls, and substrate. Plates were incubated in the dark, and absorbance or fluorescence was measured accordingly.

3.17 STATISTICAL ANALYSIS

The obtained data were analyzed, and graph production was carried out, using GraphPad Prism software, version 9.0 (GraphPad Software, San Diego, CA, USA). All values were analyzed 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 ± 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 qualitative phytochemical constituents in *R. officinalis.* Results showed the presence of alkaloids, tannins, phenols, saponins, flavonoids, and glycosides, while steroids were absent.

Table 4.2 shows the quantitative estimation of phytochemical constituents in *R. officinalis.* Results showed that phenols had the highest concentration, while tannins had the least concentration.

Table 4.1: Qualitative screening of phytochemical constituents of Aqueous *R. officinalis* Leaf Extract

PHYTOCHEMICALS	**RESULT**
Alkaloids	+
Tannins	+
Phenols	+
Saponins	+
Flavonoids	+
Steroids	-
Glycosides	+

+: Present

-: Absent

Table 4.2: Quantitative estimation of phytochemical constituents in Aqueous *R. officinalis* Leaf Extract

PHYTOCHEMICALS	**CONCENTRATION (µg/g)**
Total Phenolic Content	25602 ± 96.34
Total Flavonoids Content	119552 ± 54.01
Total Tannins Content	19556 ± 45.34

4.2 ACUTE TOXICITY STUDY

Table 4.3 shows the acute toxicity study observations after 72 hours. Results showed that there was no observable morbidity, mortality or diarrhea across the experimental groups following oral administration of *R. officinalis* at doses from 10 mg/kg to 5000 mg/kg body weight.

Table 4.3: Acute toxicity study and observations after 72 hours

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

n=3/group

4.3 EFFECT OF TREATMENT ON BRAIN AND BODY WEIGHT

Table 4.4 shows the body and organ weights across the experimental groups. There was a significant decrease ($p<0.05$) in the final body weight of rats exposed to Pb only when compared to control. There was no significant difference ($p>0.05$) in the final body weight of Pb-exposed rats pre-treated with *R. officinalis* when compared to Pb only. There was also no significant difference ($p>0.05$) in the *R. officinalis* only treated groups when compared to control.

Results showed a significant decrease ($p<0.05$) in weight change in Pb-exposed rats when compared to control. However, a significant increase ($p<0.05$) was observed in Pb-exposed rats pre-treated with *R. officinalis* when compared to Pb only. Also, no significant difference ($p>0.05$) was observed in the *R. officinalis* only treated groups when compared to control.

For whole brain weight, Pb-exposed rats showed significant decrease ($p<0.05$) when compared to control. However, there was no significant difference ($p>0.05$) in the Pb-exposed rats pretreated with *R. officinalis* when compared with Pb only. There was also no significant difference ($p>0.05$) was observed in the *R. officinalis* only treated groups when compared to control.

No significant differences ($p>0.05$) were observed in relative brain weight across the experimental groups.

Table 4:4 Weights across experimental groups.

Groups	**Initial Body Weight (g)**	**Final Body Weight (g)**	**Weight Change (g)**	**Whole Brain Weight (g)**	**Relative Brain Weight (%)**
Control	157.0 ± 5.431	177.0 ± 4.378	20.00 ± 1.080	1.80 ± 0.041	1.018 ± 0.036
Pb	149.0 ± 8.276	154.0 ± 8.495 $^{\#}$	5.00 ± 2.858 $^{\#}$	1.50 ± 0.070 $^{\#}$	0.975 ± 0.016
RO1 + Pb	150.8 ± 5.513	167.3 ± 5.089	18.00 ± 1.225 *	1.68 ± 0.048	1.000 ± 0.007
RO2 + Pb	154.5 ± 2.872	173.3 ± 2.869	18.75 ± 1.377 *	1.70 ± 0.041	0.983 ± 0.009
RO1	149.5 ± 10.18	172.8 ± 10.04	23.25 ± 1.652	1.75 ± 0.065	1.015 ± 0.028
RO2	152.0 ± 9.416	175.0 ± 5.431	23.00 ± 4.262	1.75 ± 0.029	1.003 ± 0.020

$^{\#}$ and * represent $p < 0.05$ following comparison to the control and Pb-only group respectively.

4.4 EFFECT OF TREATMENT ON NEUROBEHAVIOURAL ACTIVITY

4.4.1 Novel Object Recognition (NOR)

Figure 4.1 and 4.2 shows the mean exploration times (Trial test) of familiar object 1 (FO1) and familiar object 2 (FO2) respectively. There were no significant differences (*p*>0.05) in the mean exploration times across experimental groups.

Figure 4.3 and 4.4 shows the mean exploration times (real test) of familiar object 1 (FO1) and novel object (NO) respectively. There were no significant differences (*p*>0.05) in the mean exploration times across experimental groups in both FO1 and NO.

Figure 4.5 and 4.6 shows the total exploration times during trial (T1) and real test (T2) across experimental groups respectively. There was a significant decrease (*p*<0.05) in the total exploration time of trial (T1) in rats treated with Pb only when compared to control. No significant differences (*p*>0.05) were observed in the Pb-exposed rats pre-treated with *R. officinalis* as well as *R. officinalis*-only treated groups. Also, there were no significant differences (*p*>0.05) in the total exploration time across experimental groups during real test (T2).

Figure 4.7 shows the discrimination index across experimental groups. There was a significant decrease (*p*<0.05) in the discrimination index in rats treated with Pb only when compared to control. However, there was a significant increase (*p*<0.05) in Pb-exposed rats pretreated with *R. officinalis* when compared to Pb only group. Also, there was no significant difference (*p*>0.05) in the *R. officinalis* only groups when compared to control.

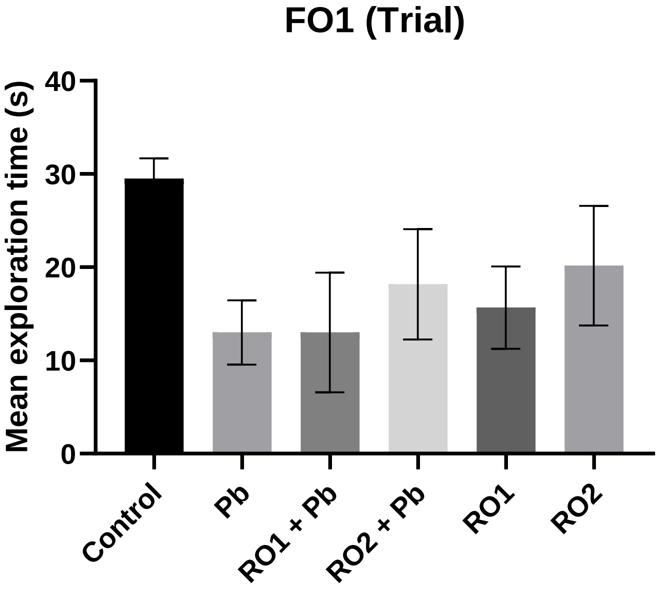

Figure 4.1: Mean exploration times of familiar object 1 (FO1) across experimental groups.

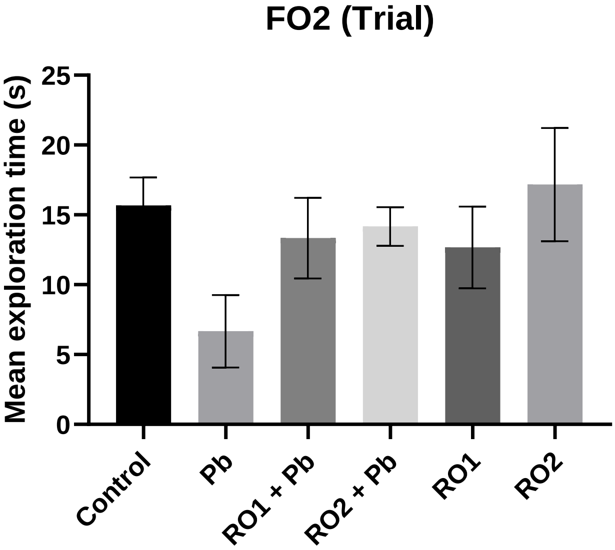

Figure 4.2: Mean exploration times of familiar object 2 (FO2) across experimental groups.

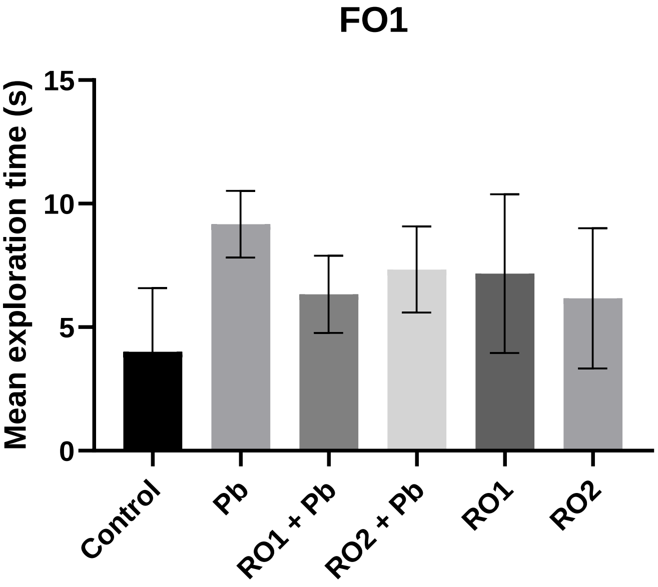

Figure 4.3: Mean exploration times (real test) of familiar object 1 (FO1) across experimental groups.

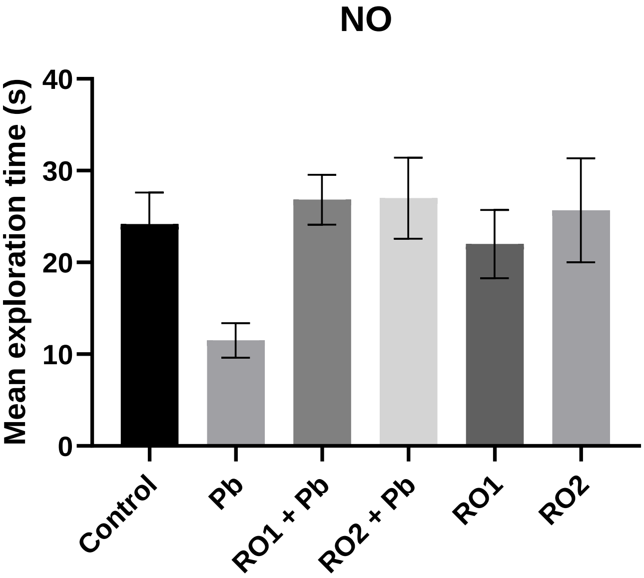

Figure 4.4: Mean exploration times of novel object (NO) across experimental groups.

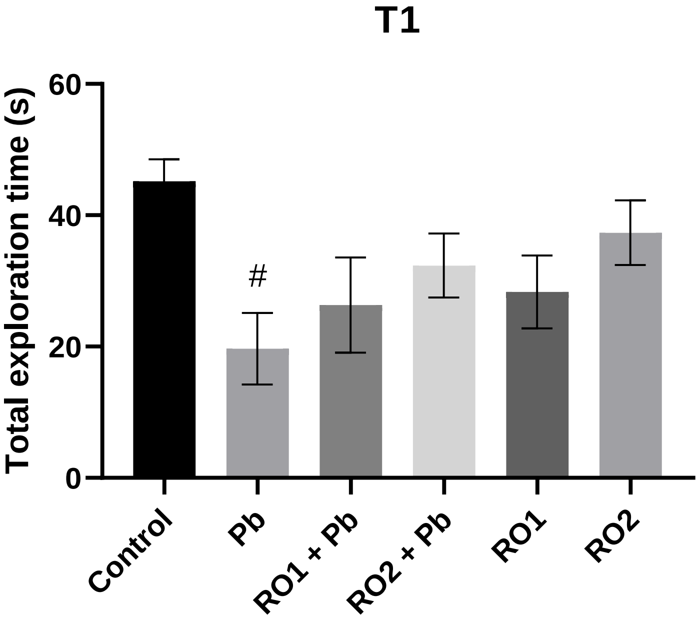

Figure 4.5: Total exploration times (T1) across experimental groups.

# $p<0.05$ compared with control group.

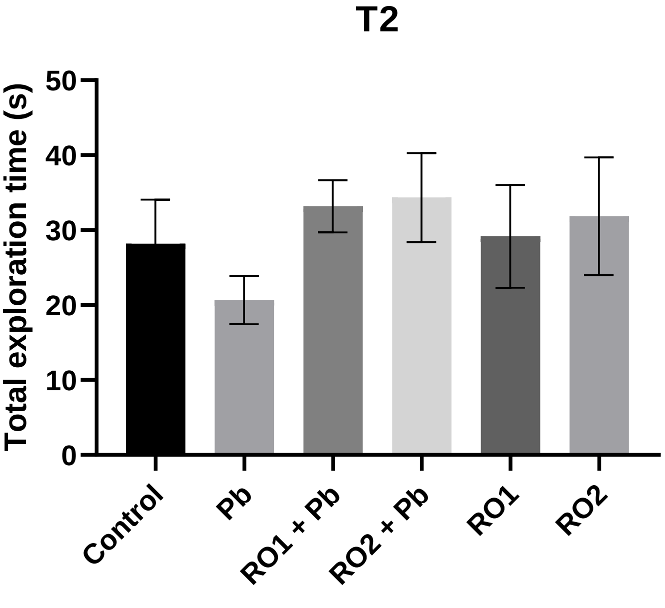

Figure 4.6: Total exploration times (T2) across experimental groups.

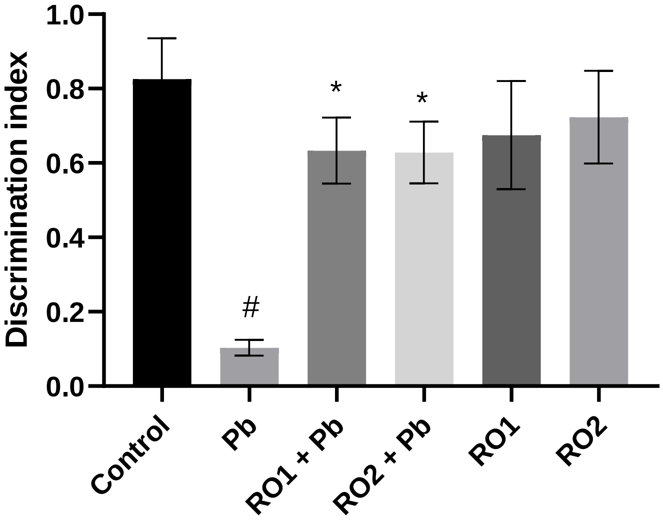

Figure 4.7: Discrimination index across experimental groups.

# *p*<0.05 compared with control group; **p*<0.05 compared with Pb only group.

4.4.2 Y-Maze

Figure 4.8 shows the number of total arm entries across the experimental groups. Rats treated with Pb only showed a significant decrease ($p<0.05$) in the number of total arm entries when compared to control. However, there was a significant increase ($p<0.05$) in the number of total arm entries of Pb-exposed rats pretreated with 200 mg/kg *R. officinalis* when compared to Pb only group. There were no significant differences ($p>0.05$) in the *R. officinalis* only treated groups compared to control.

Figure 4.9 shows the number of total alternations across the experimental groups. There were no significant differences in the number of total alternations in rats treated with Pb only compared to control. However, there was a significant increase ($p<0.05$) in the number of total alternations in Pb-exposed rats pretreated with 200 mg/kg *R. officinalis,* when compared to Pb only group. There were no significant differences ($p>0.05$) in the *R. officinalis* only treated groups compared to control.

Figure 4.10 shows the spontaneous alternation across the experimental groups. There was a significant decrease ($p<0.05$) in spontaneous alternation of rats treated with Pb only when compared to control. Conversely, there was a significant increase ($p<0.05$) in spontaneous alternation of Pb-exposed rats pretreated with *R. officinalis* when compared to Pb only group. There were no significant differences ($p>0.05$) in the *R. officinalis* only treated groups compared to control.

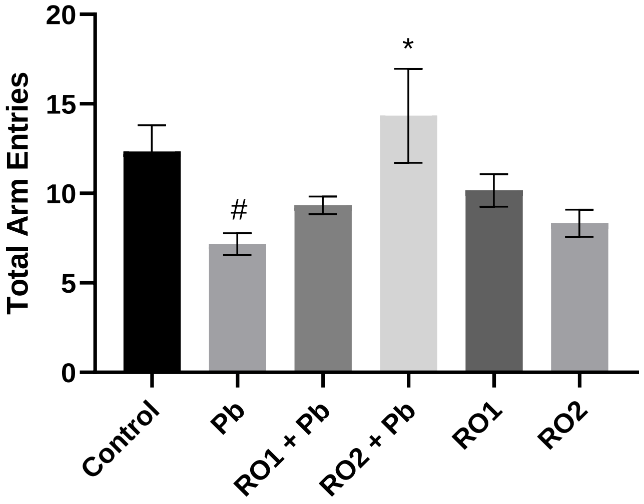

Figure 4.8: Number of total arm entries across experimental groups.

# *p*<0.05 compared with control group; **p*<0.05 compared with Pb only group.

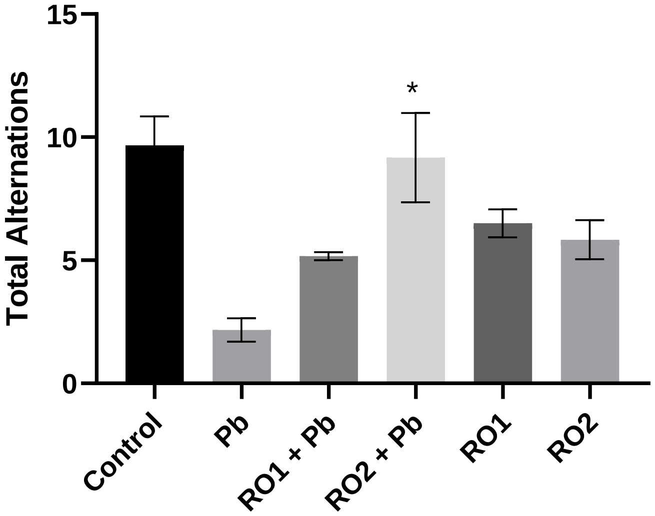

Figure 4.9: Number of total alternations across experimental groups.

**p*<0.05 compared with Pb only group.

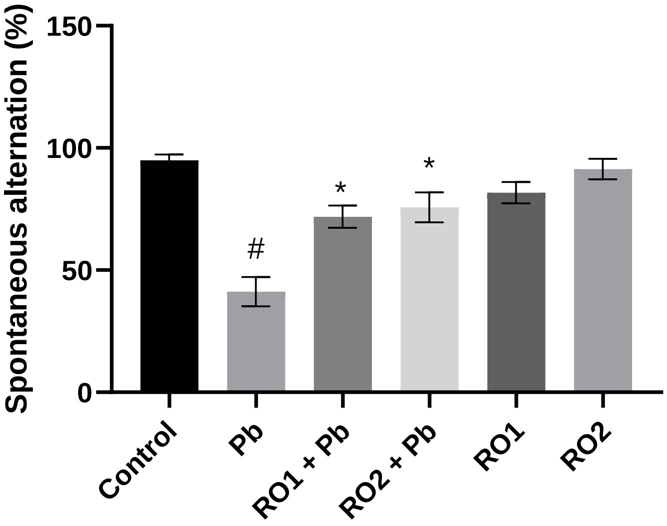

Figure 4.10: Spontaneous alternation across experimental groups.

# *p*<0.05 compared with control group; **p*<0.05 compared with Pb only group.

4.4.3 Elevated Plus Maze (EPM)

Figure 4.11 shows the transfer latency across the experimental groups. A significant increase ($p<0.05$) in the transfer latency was observed in rats treated with Pb only when compared to control. However, there was a significant decrease ($p<0.05$) in the transfer latency of Pb-exposed rats pretreated with *R. officinalis* when compared with Pb only group. There were no significant differences ($p>0.05$) in the *R. officinalis* only treated groups compared to control.

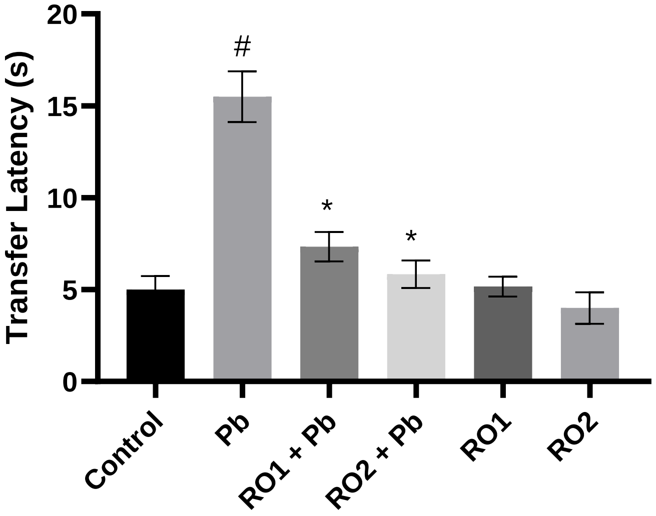

Figure 4.11: Transfer latency across experimental groups.

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

4.5 EFFECT OF TREATMENT ON ANTIOXIDANT ENZYMES ACTIVITY

Figure 4.12 shows hippocampal Superoxide dismutase (SOD) activity across the experimental groups. Rats treated with Pb only showed a significant decrease ($p<0.05$) in SOD activity when compared to control. However, there was a significant increase ($p<0.05$) in SOD activity in Pb-exposed rats pretreated with *R. officinalis,* when compared to Pb only group. There were no significant differences ($p>0.05$) in the *R. officinalis* only treated groups compared to control.

Figure 4.13 shows hippocampal catalase (CAT) activity across experimental groups. There was a significant decrease ($p<0.05$) in the CAT activity in rats treated with Pb only when compared to control. Conversely, there were a significant increase ($p<0.05$) in CAT activity in Pb-exposed rats pretreated with 200 mg/kg *R. officinalis,* when compared to Pb only group. There were no significant differences ($p>0.05$) in the *R. officinalis* only treated groups compared to control.

Figure 4.14 shows hippocampal glutathione peroxidase (GPx) activity across experimental groups. There was a significant decrease ($p<0.05$) in GPx activity in rats treated with Pb only when compared to control. However, there were significant increase ($p<0.05$) in GPx activity in Pb-exposed rats pretreated with *R. officinalis*, when compared to Pb only group. There were no significant differences ($p>0.05$) in the *R. officinalis* only treated groups compared to control.

Figure 4.15 shows glutathione (GSH) concentration in the hippocampus across experimental groups. Rats treated with Pb 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 *R. officinalis.* There were no significant differences ($p>0.05$) in the *R. officinalis* only treated groups compared to control.

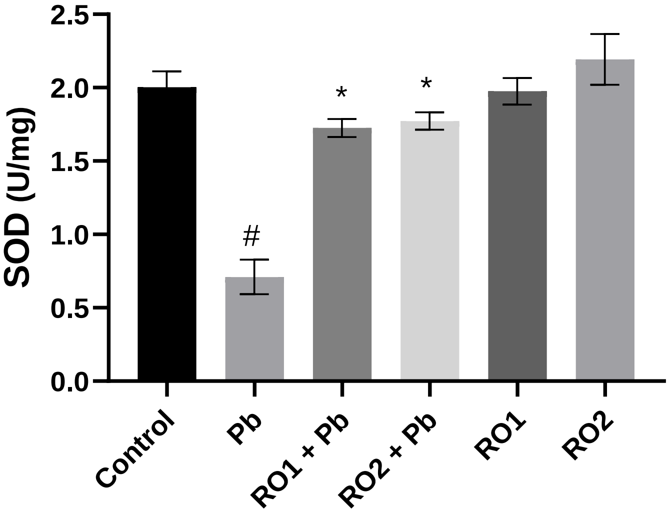

Figure 4.12: SOD activity in the hippocampus of rats across experimental groups. #$p<0.05$ compared with control group; *$p<0.05$ compared with Pb only group.

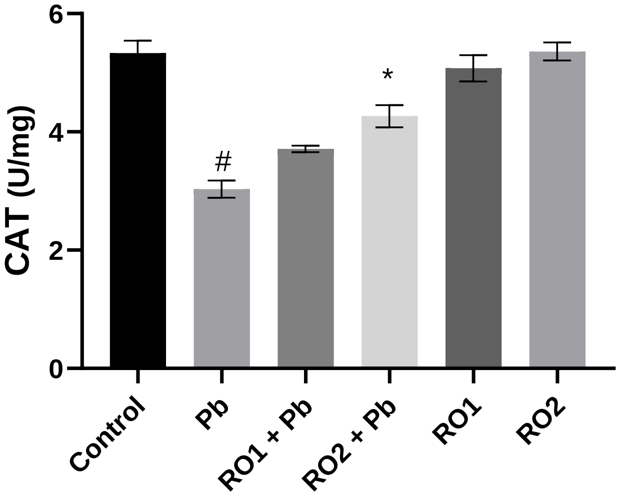

Figure 4.13: CAT activity in the hippocampus of rats across experimental groups. #$p<0.05$ compared with control group; *$p<0.05$ compared with Pb only group.

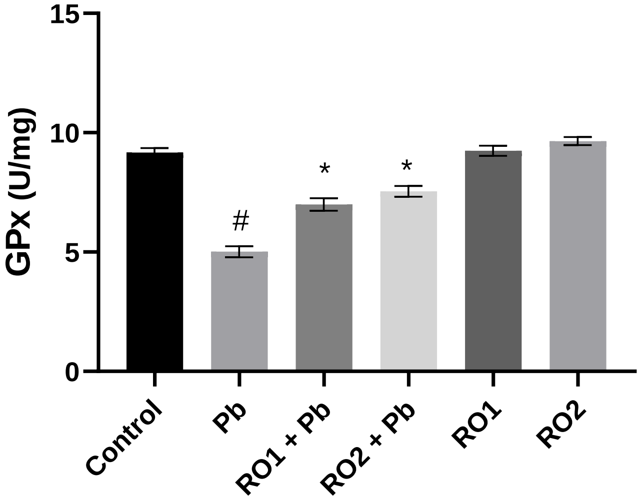

Figure 4.14: GPx activity in the hippocampus of rats across experimental groups. #$p<0.05$ compared with control group; *$p<0.05$ compared with Pb only group.

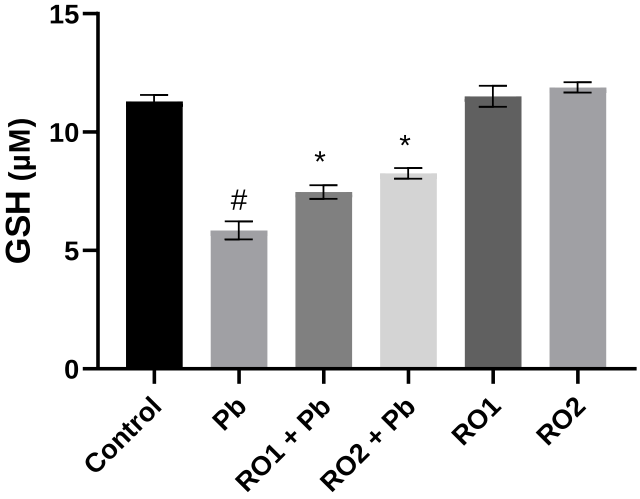

Figure 4.15: GSH concentration in the hippocampus of rats across experimental groups. #$p<0.05$ compared with control group; *$p<0.05$ compared with Pb only group.

4.6 EFFECT OF TREATMENT ON LIPID PEROXIDATION

Figure 4.16 shows hippocampal Malondialdehyde (MDA) concentration across the experimental groups. There was a significant increase ($p<0.05$) in MDA concentration in rats treated with Pb only when compared to control. However, there was a significant decrease ($p<0.05$) in the Pb-exposed rats pretreated with *R. officinalis* when compared to Pb only group. There were no significant differences ($p>0.05$) in the *R. officinalis* only treated groups compared to control.

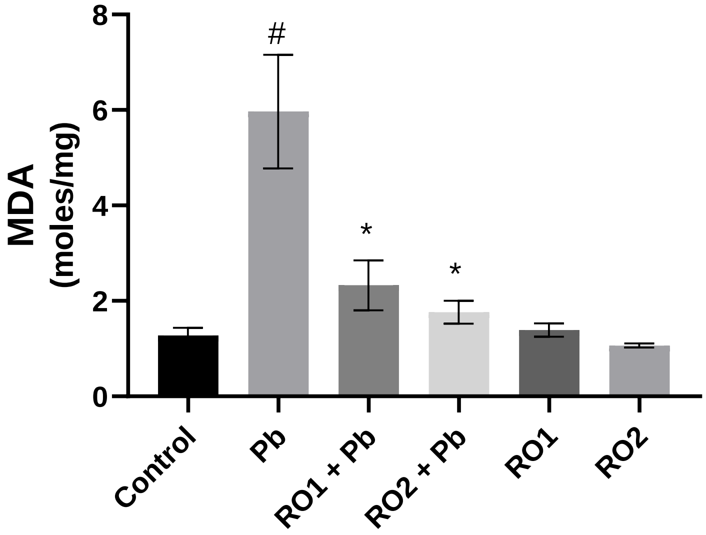

Figure 4.16: MDA concentration in the hippocampus of rats across experimental groups. #$p<0.05$ compared with control group; *$p<0.05$ compared with Pb only group.

4.7 EFFECT OF TREATMENT ON AChE CONCENTRATION

Figure 4.17 shows Acetylcholinesterase (AChE) concentration in the hippocampus across the experimental groups. There was a significant increase ($p<0.05$) in AChE concentration in the hippocampus of rats treated with Pb only when compared to control. However, there was a significant decrease ($p<0.05$) in AChE concentration in the hippocampus of Pb-exposed rats pretreated with *R. officinalis*, when compared to Pb only group. Also, no significant difference ($p>0.05$) was observed in AChE concentration in the hippocampus of rats treated with *R. officinalis* only when compared to control.

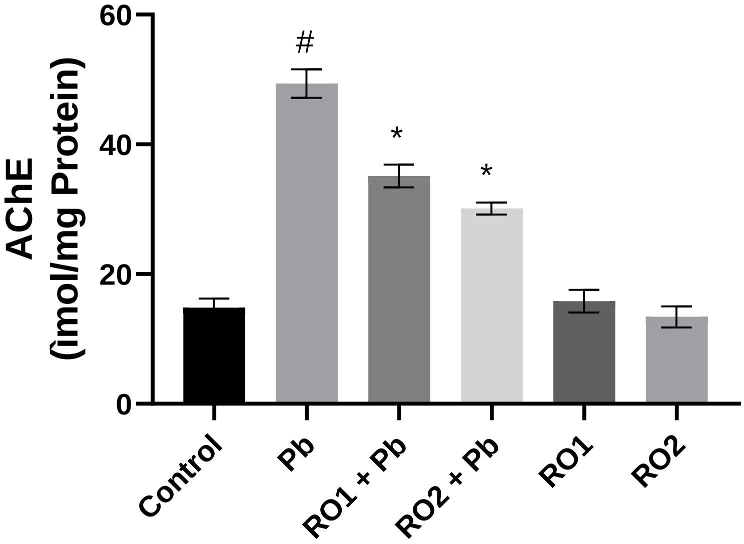

Figure 4.17: AChE concentration in the hippocampus of rats across experimental groups. #$p<0.05$ compared with control group; *$p<0.05$ compared with Pb only group.

4.8 EFFECT OF TREATMENT ON LEAD (Pb) ACCUMULATION

Figure 4.18 shows Pb concentration in the hippocampus across experimental groups. There was a significant increase ($p<0.05$) in Pb concentration in the hippocampus of rats treated with Pb only when compared to control. However, there was a significant decrease ($p<0.05$) in Pb-exposed rats pre-treated with *R. officinalis*, when compared to Pb only exposed rats. There were no significant differences ($p>0.05$) observed in Pb accumulation in the hippocampus of rats treated with *R. officinalis* only when compared to control.

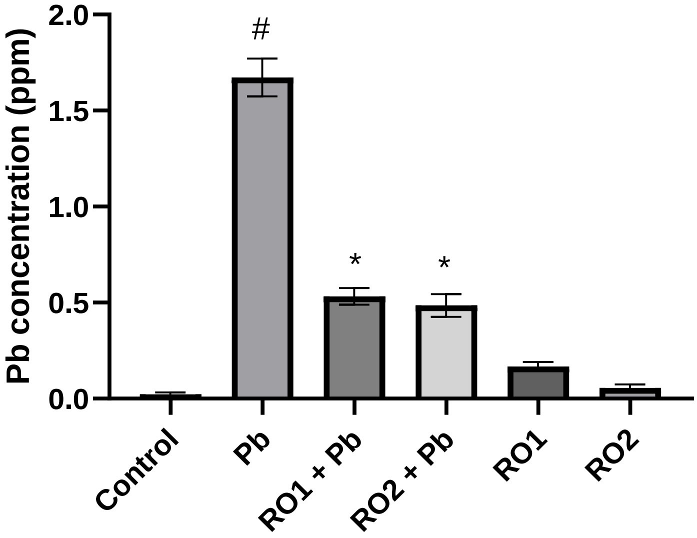

Figure 4.18: Pb concentration in the hippocampus of rats across experimental groups. #$p<0.05$ compared with control group; *$p<0.05$ compared with Pb only group.

4.9 EFFECT OF TREATMENT ON NITRIC OXIDE (NO) CONCENTRATION

Figure 4.19 shows the concentration of nitric oxide (NO) in the hippocampus across experimental groups. There was a significant increase ($p<0.05$) in NO concentration in the hippocampus of rats treated with Pb only when compared to control. However, there was a significant decrease ($p<0.05$) in NO concentration in the hippocampus Pb-exposed rats pre-treated with *R. officinalis* when compared to Pb only exposed rats. There were no significant differences ($p>0.05$) observed in NO accumulation in the hippocampus of rats treated with *R. officinalis* only when compared to control.

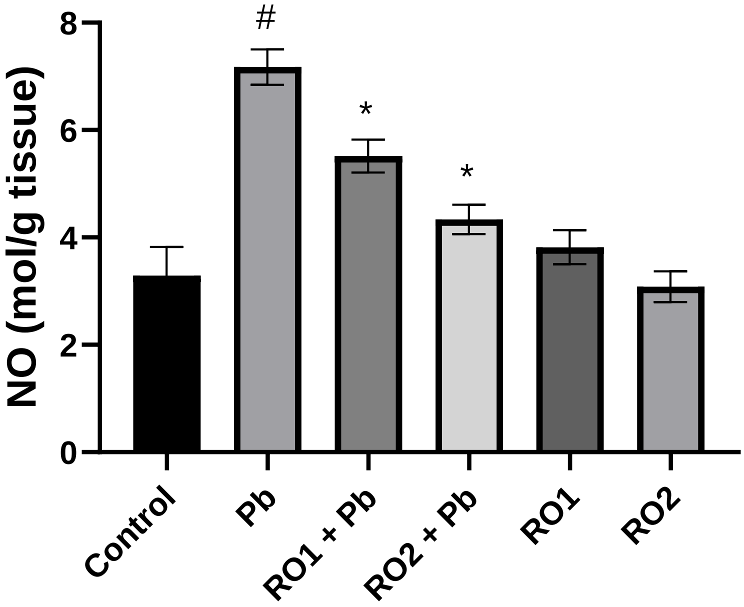

Figure 4.19: NO concentration in the hippocampus of rats across experimental groups. #$p<0.05$ compared with control group; *$p<0.05$ compared with Pb only group.

4.10 EFFECT OF TREATMENT ON THE HISTOLOGY OF THE HIPPOCAMPUS

Plate 4.1-4.6 show the histology of the hippocampus (CA1) of rats across experimental groups. Plate 4.1 shows the histology of rats in control group with normal structure of pyramidal cells. Plate 4.2 shows the hippocampal histology of Pb only treated rats showing atrophy and vacuolated pyramidal cells and astrocytes. The hippocampus of Pb-exposed rats pretreated with *R. officinalis* (Plate 4.3 and 4.4) showed relatively normal histological structures. Plates 4.5 and 4.6 shows the hippocampal histology of rats treated with *R. officinalis* only, showing relatively normal hippocampal features.

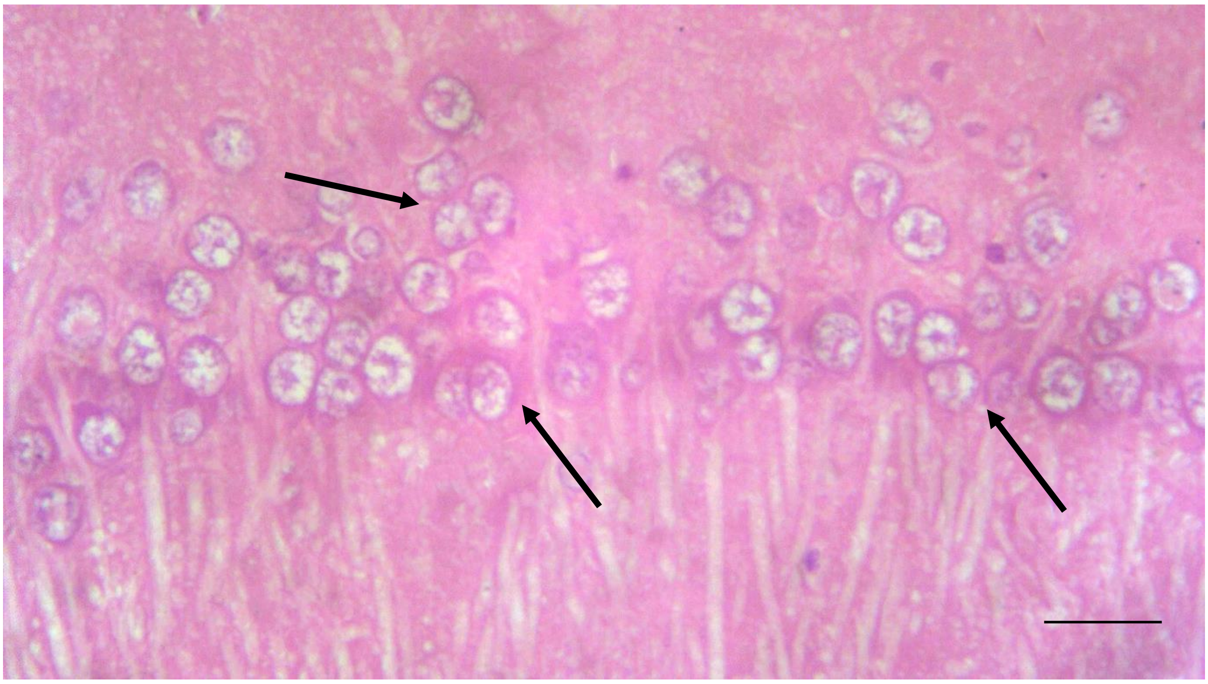

Plate 4.1: Representative histology of the hippocampus CA1 in Control group revealing normal structure of pyramidal cells (arrows). (H&E; Scale bar: 25µm).

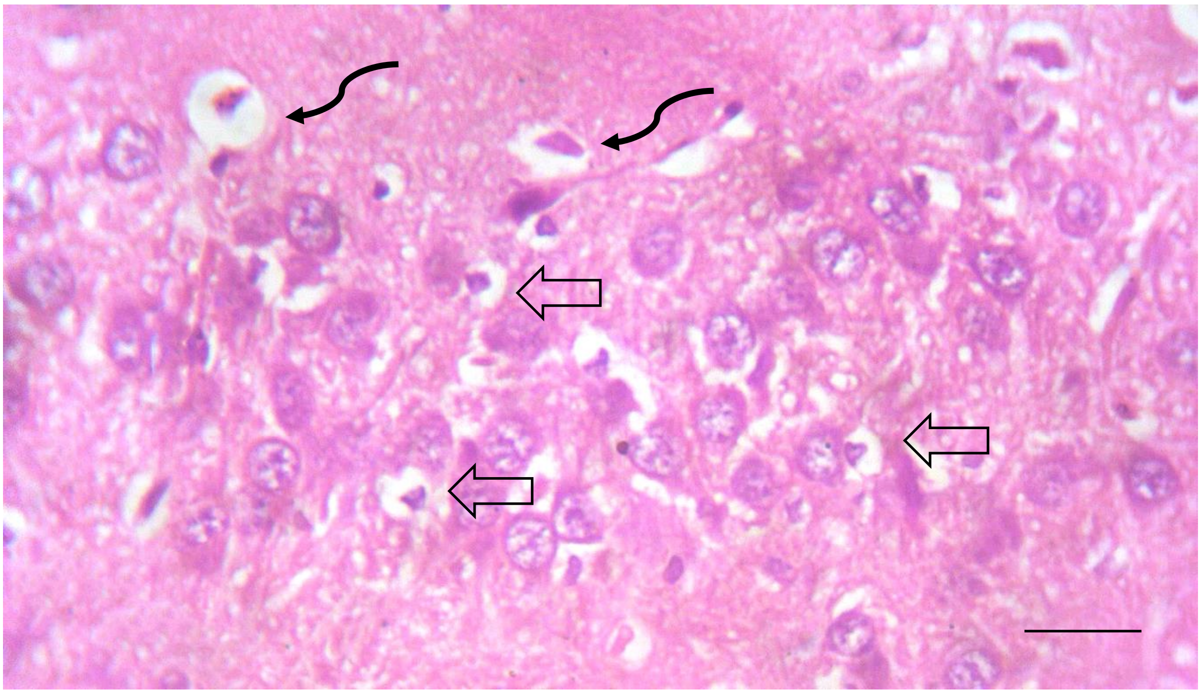

Plate 4.2: Representative histology of the hippocampus CA1 in Pb-treated rats showing atrophy and vacuolated pyramidal cells (transparent double arrows) and astrocytes (curved arrows. (H&E; Scale bar: 25µm).

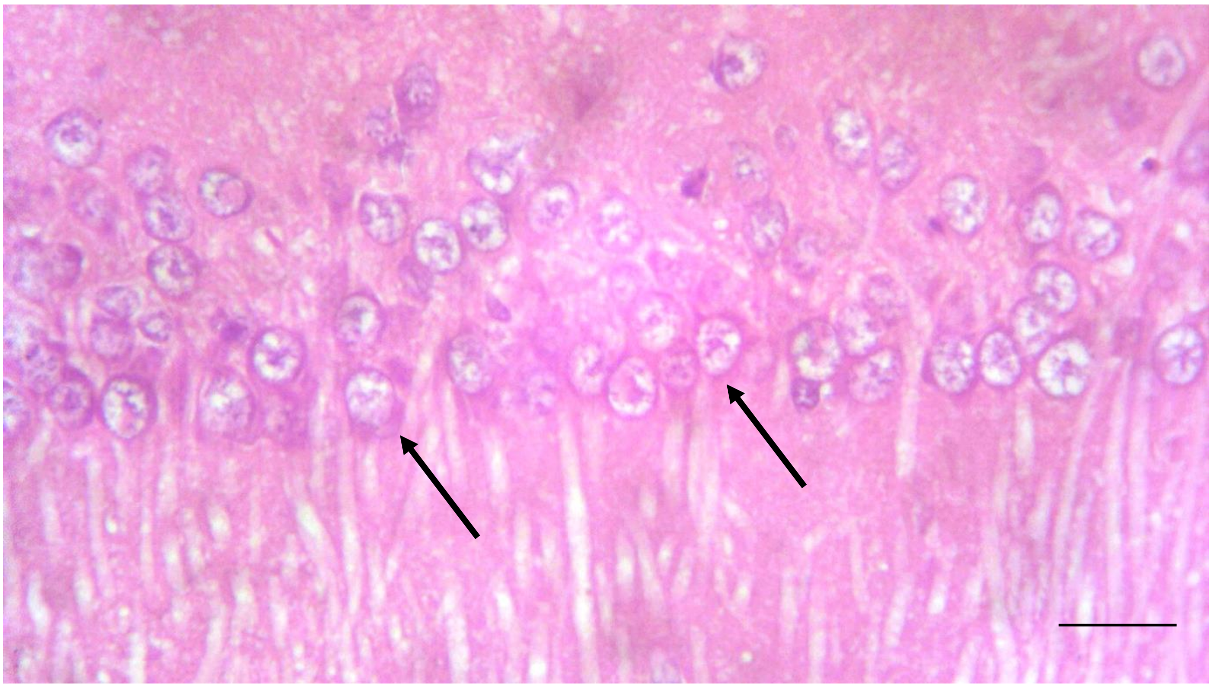

Plate 4.3: Representative histology of the hippocampus CA1 in Pb-treated rats pre-treated with 100 mg/kg *R. officinalis* showing relatively normal histological structure of pyramidal cells (arrows). (H&E; Scale bar: 25µm).

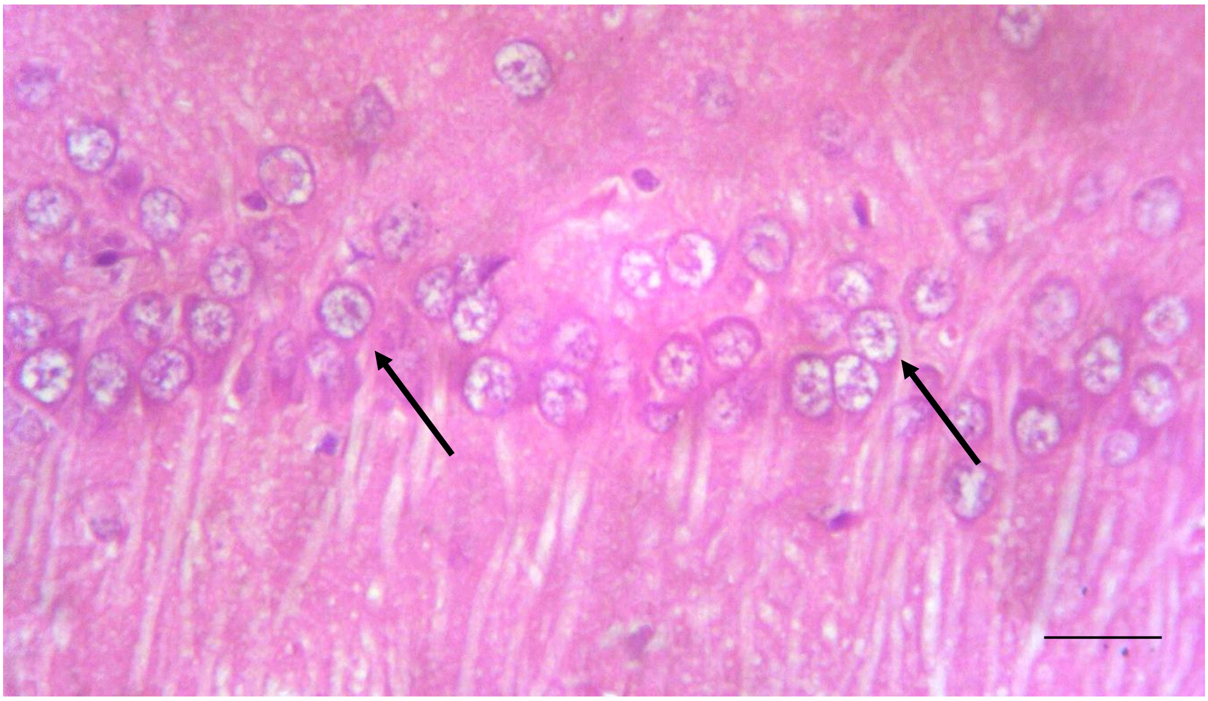

Plate 4.4: Representative histology of the hippocampus CA1 in Pb-treated rats pre-treated with 200 mg/kg *R. officinalis* showing relatively normal histological structure of pyramidal cells (arrows). (H&E; Scale bar: 25µm).

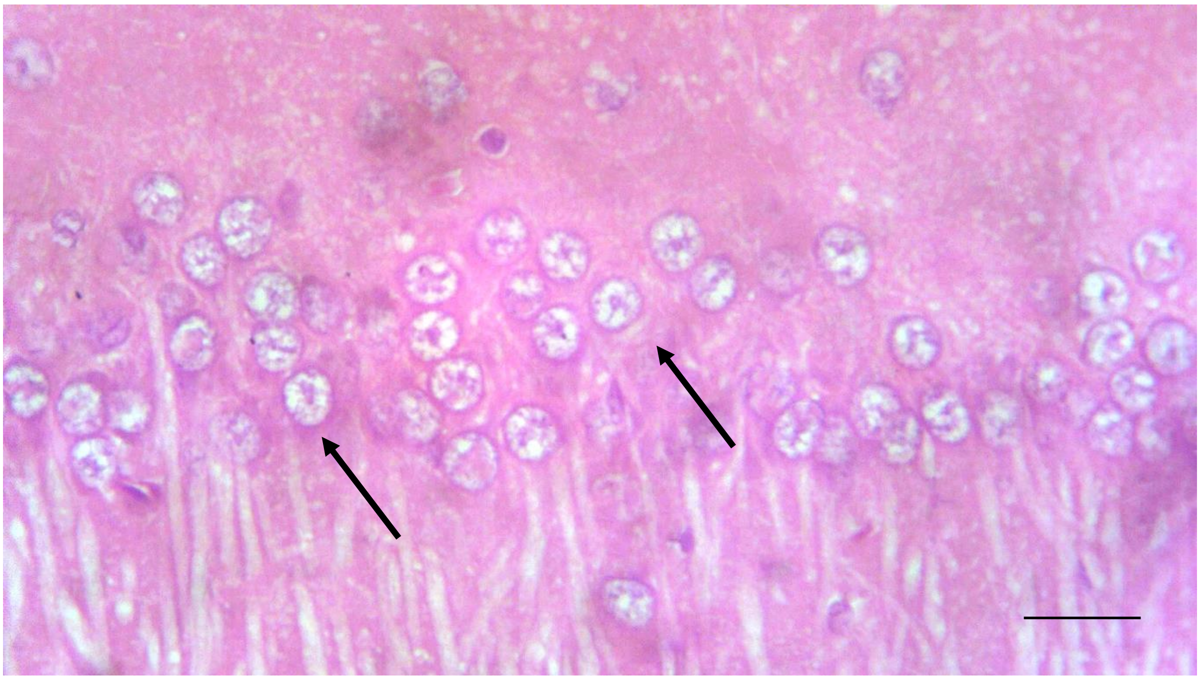

Plate 4.5: Representative histology of the hippocampus CA1 in rats treated with 100 mg/kg *R. officinalis* only showing relatively normal histological structure of pyramidal cells (arrows). (H&E; Scale bar: 25µm).

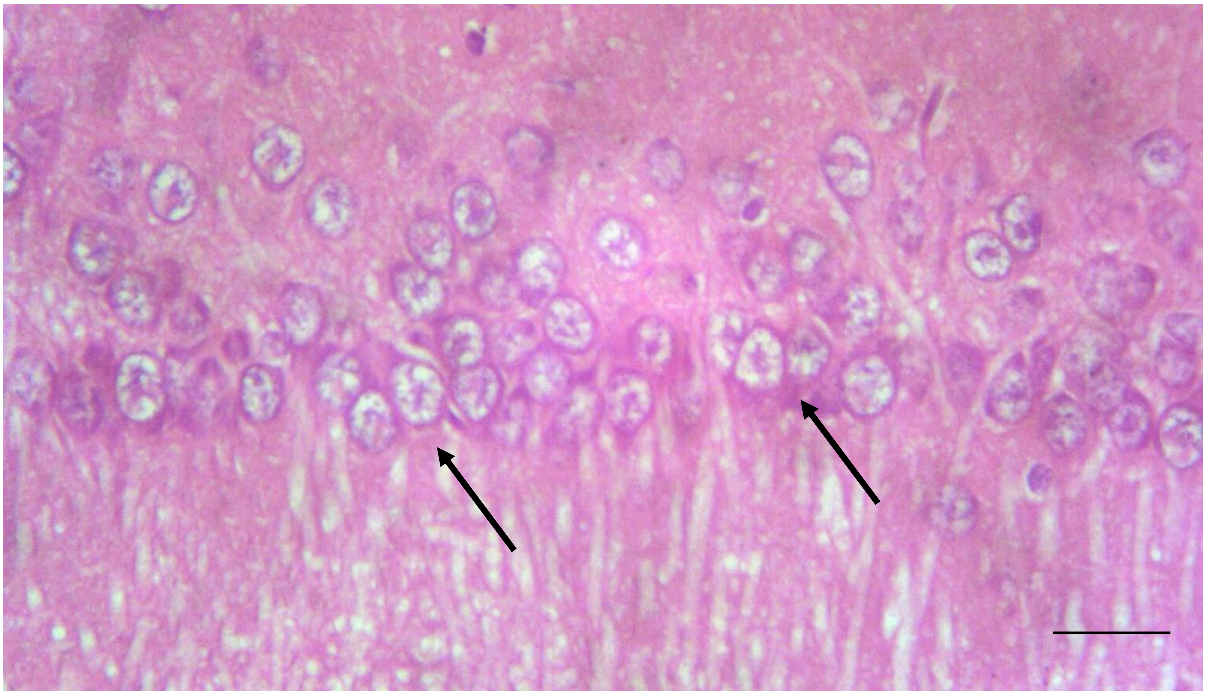

Plate 4.6: Representative histology of the hippocampus CA1 in rats treated with 200 mg/kg *R. officinalis* only showing relatively normal histological structure of pyramidal cells (arrows). (H&E; Scale bar: 25µm).

4.11 EFFECT OF TREATMENT ON APOPTOSIS

Figure 4.20 shows the caspase-3 expression in the hippocampus of rats across experimental groups. There was a significant increase (p<0.05) of caspase-3 in the hippocampus of Pb exposed rats when compared to control. However, there was a significant decrease (p<0.05) of caspase-3 in Pb-exposed rats pre-treated with *R. officinalis* when compared to Pb-exposed rats. There were no significant differences (p>0.05) observed in the *R. officinalis* only treated groups when compared to control.

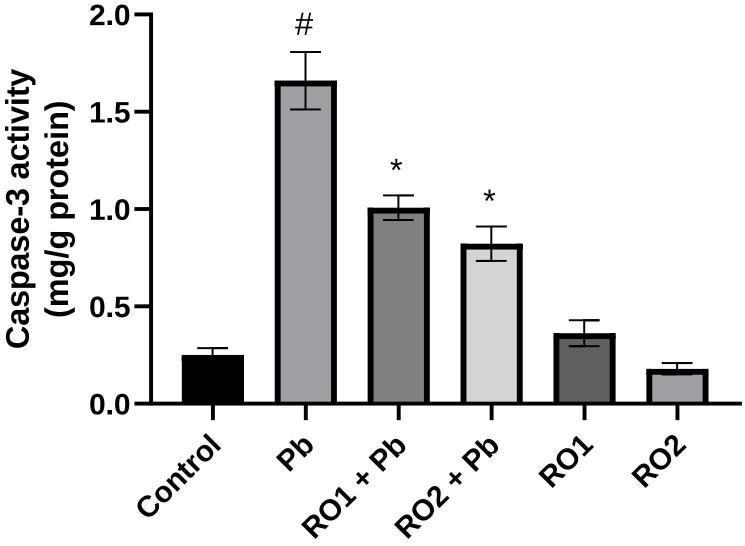

Figure 4.20: Caspase-3 expression in the hippocampus of rats across experimental groups. #p<0.05 compared with control group; *p<0.05 compared with Pb only group.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 *R. officinalis* is a Rich Source of Phytochemicals

Phytochemical screening constitutes a fundamental analytical approach for the systematic identification and characterization of bioactive constituents in medicinal plants (Yu *et al*., 2021; Halder and Jha, 2023). This process is pivotal in elucidating the chemical basis of their pharmacological activities, thereby providing a robust scientific foundation for their therapeutic applications and facilitating the development of evidence-based phytotherapeutics (Yu *et al*., 2021). *R. officinalis* is renowned for its nutritional richness and abundance of antioxidant bioactive compounds, which are believed to contribute significantly to its therapeutic properties. The phytochemical screening of *R. officinalis* revealed the presence of several key secondary metabolites, including alkaloids, tannins, phenols, saponins, flavonoids, and glycosides. Quantitative analysis indicated that flavonoids were the most abundant among these compounds, underscoring their potentially significant role in the biological activities of the plant. Flavonoids are widely recognized for their neuroprotective effects, largely due to their ability to modulate cellular signaling pathways and counteract neurotoxic insults (Calderaro *et al*., 2022; Bellavite, 2023).

Phenolic compounds were also found in substantial amounts, further emphasizing their importance in the plant's neuroprotective profile. Phenols are crucial antioxidants that help neutralize reactive oxygen species (ROS) and inhibit neuroinflammatory signaling pathways. Their ability to activate genes related to neuronal survival and resilience provides a biochemical defense against both environmental and metabolic stressors affecting brain health (Fakhri *et al*., 2022; Houldsworth, 2024). Tannins complement this neuroactive profile by exerting antioxidant effects, neutralizing free radicals, and stabilizing neural membranes. These actions help maintain neuronal integrity and reduce lipid peroxidation, which is implicated in the progression of neurodegenerative disorders (Angelova *et al*., 2021; Olufunmilayo *et al*., 2023). Although steroids were absent in the extract, the presence of alkaloids, saponins, and glycosides adds to the comprehensive neuroprotective potential of *R. officinalis*. Alkaloids contribute by modulating neurotransmitter activity and safeguarding neurons from excitotoxicity (Nunes *et al*., 2020; Ali *et al*., 2023). Saponins are believed to enhance neural communication and promote tissue repair through their interaction with various cellular receptors (Tan *et al*., 2022). Glycosides, meanwhile, provide anti-inflammatory and antioxidant effects essential for preserving neuronal function and preventing degeneration (Ebrahimi *et al*., 2023; Zhao *et al*., 2023).

5.2 ***R. officinalis*** **Attenuates Pb-Induced Weight Loss**

Changes in body weight are important markers of general health and physiological condition (Wang and Wu, 2022). Noticeable fluctuations often signal systemic imbalances and can be early signs of underlying health problems (Wang and Wu, 2022). Exposure to certain drugs and toxic substances, such as lead (Pb), has been shown to influence body weight in animals (Collin *et al*., 2022; Enogieru and Momodu, 2022). These weight alterations, particularly reductions in body mass, can serve as valuable indicators of the harmful effects of such compounds (Montoro-Huguet *et al*., 2021). Pb toxicity interferes with normal growth and developmental processes, often resulting in developmental delays (Ortega *et al*., 2021). In more severe instances, it can lead to stunted growth (Ortega *et al*., 2021). Consequently, Pb exposure poses a significant health risk due to its potential to impair both body and organ weight, as well as overall developmental outcomes.

Findings from this study showed a significant decrease in the body and brain weights of Pb-exposed rats when compared to control. The observed reduction in body and brain weights may be linked to various factors previously associated with heavy metal exposure. Notably, Pb ingestion has been reported to induce anorexia—a condition marked by reduced food intake in experimental animals (Wang *et al*., 2022). In addition, Pb exposure has been shown to decrease muscle mass and trigger cachexia, a syndrome characterized by a sustained elevation in basal metabolic rate without a corresponding increase in caloric or protein intake (Rausch *et al*., 2021). These mechanisms potentially contribute to the significant weight loss observed in Pb-exposed rats. However, Pb-exposed rats pretreated with *R. officinalis* showed a significant increase in body weight, indicating that *R. officinalis* potentially mitigates Pb-induced weight loss. This improvement in body weight can potentially be attributed to *R. officinalis'* known nutritional and therapeutic properties. *R. officinalis* contains bioactive compounds such as saponins and alkaloids, which have been reported to enhance appetite and improve nutrient absorption (Meziane *et al*., 2025). Additionally, its antioxidant properties may potentially support metabolic efficiency and protect against muscle degradation, contributing to improved overall body condition (Lommelli *et al*., 2025).

5.3 *R. officinalis* Mitigates Pb-Induced Neurobehavioural Changes

Previous studies emphasize that behavioural assessments are essential for monitoring the neurotoxic effects of environmental exposures, as they provide functional insights into the impact of neurotoxins on the central nervous system. (Starnes *et al*., 2022). According to Panina *et al*. (2023), neurobehavioural evaluation is regarded as the main method for the methodical monitoring of neurotoxic exposure, which may result in alterations in mood, emotional response, social behaviour, and cognitive and motor abilities. Research indicates that exposure to neurotoxins, specifically Pb, affects working memory, processing speed, visuospatial skills, and motor function on neurobehavioural tests (Ortega *et al*., 2021).

The Novel Object Recognition (NOR) test serves as a robust behavioural paradigm for evaluating non-spatial working memory and recognition memory in rodents, relying heavily on the functional interplay between the hippocampus and the prefrontal cortex (Lissner *et al*., 2021; Barbosa and Castelo-Branco, 2022). Recognition memory entails identifying previously encountered stimuli, typically using the familiarity of objects as cues (Atkinson *et al*., 2024). Studies have shown that Pb exposure inhibit the formation of episodic memory which is a function of the hippocampus (Zou *et al*., 2024). In this study, a significant decrease in total exploration time was observed in Pb-exposed rats. This reduction suggests a Pb-induced decline in exploratory motivation or a potential deficit in attentional engagement, consistent with prior findings that link Pb neurotoxicity with hypoactivity and diminished environmental interaction (Shvachiy *et al*., 2022). Also, discrimination index (DI) is a measure of recognition memory. It is calculated as the difference in time spent exploring the novel and familiar objects, divided by the total exploration time. A higher DI indicates better memory performance, reflecting a preference for the novel object. A significant decrease in discrimination index, a key indicator of recognition memory, was observed in Pb exposed rats, consistent with earlier reports of Pb-induced deficits in recognition memory (Alves Oliveira *et al*., 2020; Rashno *et al*., 2022). This decline in discrimination index and exploratory behaviour may be attributed to Pb-induced temporary or permanent long-term memory impairment (Shvachiy *et al*., 2022). However, pretreatment with *R. officinalis* effectively mitigated Pb-induced recognition memory deficits as evidenced by significantly increased discrimination index in the experimental rats.

The Y-maze test, a well-established method for assessing short-term spatial memory and spontaneous alternation behaviour, provides insights into hippocampal-dependent cognitive functions (d'Isa *et al*., 2021; Callahan *et al*., 2021). Results from this study showed that Pb-only treated rats had a significant reduction in total arm entries, which may reflect reduced exploratory activity or motor suppression, both of which are characteristic of Pb neurotoxicity (Callahan *et al*., 2021). Additionally, spontaneous alternation, a direct index of spatial working memory, was significantly decreased in the Pb-only group, indicating cognitive impairment. This is consistent with previous findings of Wang *et al*. (2024) and Zygan-Filipiak *et al*. (2025) who also reported decreased spontaneous alternation in Pb-exposed rats. Conversely, Pb-exposed rats pretreated with *R. officinalis* demonstrated a significant increase in total arm entries, total alternations and spontaneous alternations indicating an attenuation of Pb-induced deficit and suggesting restored exploratory behaviour as well as enhancement of working memory performance.

The Elevated Plus Maze (EPM) test was employed to assess hippocampal-dependent learning via transfer latency. Transfer latency, the time taken by an animal to move from the open to the closed arms, reflects cognitive performance and anxiety-related decision making (d'Isa *et al*., 2021; Bijlsma *et al*., 2024). Rodents, when introduced to an unfamiliar open space, tend to modify their behaviour based on perceived risk levels (d'Isa *et al*., 2021). Typically, their first instinct upon being placed on the open arm of the EPM is to seek refuge in the closed arms, which are perceived as more secure. An increased transfer latency is indicative of compromised cognitive processing linked to hippocampal function (Dues *et al*., 2023). A significant increase in transfer latency was observed in Pb-only treated rats compared to control, suggesting impaired cognitive processing and increased anxiety, both of which are linked to hippocampal dysfunction which has been reported to play a role in stress sensitivity and resiliency in connection to mood and anxiety (Larosa and Wong, 2022; Enogieru and Williams, 2024). Pb exposure has been shown to cause neurodegeneration in the hippocampus and disrupt synaptic transmission in its neurons, ultimately leading to impaired hippocampal function (Larosa and Wong, 2022). However, Pb-exposed rats pretreated with *R. officinalis* demonstrated a significant reduction in transfer latency compared to Pb-only rats, indicative of improved cognitive function and possibly reduced anxiety.

Collectively, these findings highlight the neurotoxic impact of Pb on hippocampal-dependent behaviours, particularly in domains of recognition memory, spatial working memory, and decision-making latency. More importantly, they demonstrate the potential of *R. officinalis* to attenuate these deficits, thus affirming *R. officinalis* as a promising candidate for mitigating Pb-induced cognitive and behavioural impairments.

5.4 *R. officinalis* Inhibits Pb-Induced Oxidative Stress

Oxidative stress is widely recognized as a central mechanism through which heavy metals exert toxic effects on the nervous system (Pyatha *et al*., 2022). Metals such as Pb contribute to this process by enhancing the formation of ROS, which are chemically unstable molecules that can initiate cellular damage (Nowicka, 2022). This condition arises when there is a disruption in the equilibrium between oxidative agents and the body's antioxidant systems—essentially, when ROS production surpasses the cell's ability to neutralize them or repair the resulting damage (Nowicka, 2022; Liu *et al*., 2022; Pyatha *et al*., 2022). Such an imbalance can stem from increased ROS generation, weakened antioxidant defenses, or both (Liu *et al*., 2022). ROS are particularly damaging due to their unpaired electrons, which drive them to interact destructively with proteins, lipids, and DNA (Juan *et al*., 2021). Pb has the capacity to cross the blood–brain barrier (Li *et al*., 2021; Collin *et al*., 2022), where it disrupts enzyme systems essential for brain homeostasis. The brain is especially prone to oxidative injury due to its high oxygen demand and lipid-rich structure, which necessitates strong antioxidant protection to maintain cellular integrity (Collin *et al*., 2022).

To combat oxidative stress, the body relies on antioxidants—molecules capable of safely donating electrons to ROS, thereby stabilizing them and reducing their reactivity (Shaw *et al*., 2022). Among the most critical of these defenses are the enzymes superoxide dismutase (SOD), catalase (CAT), and the tripeptide glutathione (GSH), all of which help prevent or repair oxidative damage (Rao *et al*., 2025). These enzymes are especially vulnerable to lead toxicity because their function depends on trace elements necessary for structural and catalytic activity (Jomova *et al*., 2022). SOD serves as the first line of defense by converting highly reactive superoxide radicals into hydrogen peroxide and oxygen, thus minimizing cellular damage (Saxena *et al*., 2022). This function is vital in the brain, where oxidative activity is high and damage from ROS can impair neurological function (Bhatt *et al*., 2021; Houldsworth, 2024). Following this, CAT acts to neutralize hydrogen peroxide—a compound that, if left unchecked, can exacerbate oxidative stress—by breaking it down into water and oxygen (Anwar *et al*., 2024). CAT also plays a protective role by preserving the activity of SOD, which can be compromised in the presence of accumulated hydrogen peroxide (Jomova, 2024). Without proper CAT function, elevated hydrogen peroxide levels may harm brain tissue, especially in areas like the hippocampus (Anwar *et al*., 2024; Jomova, 2024). GSH complements these defenses by directly binding to and neutralizing free radicals, serving as a crucial protector of cellular components (Lana *et al*., 2024). On the other hand, malondialdehyde (MDA) is a lipid peroxidation by-product often used as a biomarker for oxidative stress. Formed during the breakdown of polyunsaturated fatty acids, MDA reflects the extent of lipid membrane damage caused by ROS (Zengin, 2021; Zhang *et al*., 2025). Measuring MDA levels provides a reliable estimate of oxidative injury in tissues (Zengin, 2021; Zhang *et al*., 2025).

Findings from this study showed significant decrease in antioxidant enzymes (SOD, CAT and GPx) activity, GSH concentration and a corresponding increase in MDA concentration in Pb-exposed rats. This aligns with earlier reports that the neurotoxic effects of Pb are largely driven by oxidative stress mechanisms (Ortega *et al*., 2021; Pyatha *et al*., 2022). Previous studies have shown that Pb exposure leads to oxidative damage in brain tissue by suppressing the activity of key antioxidant enzymes and elevating lipid peroxidation levels in experimental animals (Li *et al*., 2023; Zhang *et al*., 2025). However, pretreatment with *R. officinalis* attenuated the effects of Pb by improving antioxidant enzymes (SOD, CAT and GPx) activity, GSH concentration and inhibiting MDA levels, thus demonstrating the antioxidant potential of *R. officinalis*.

5.5 *R. officinalis* Inhibits AChE, NO, and Pb Accumulation in the Hippocampus

Acetylcholinesterase (AChE) is a critical enzyme in the central nervous system responsible for terminating synaptic transmission by hydrolyzing the neurotransmitter acetylcholine into acetate and choline (Pawar *et al*., 2023). This breakdown is essential for regulating cholinergic signaling and maintaining proper neuronal communication, particularly in the hippocampus, a brain region vital for learning and memory (Liu *et al*., 2023). Pb exposure has been shown to disrupt cholinergic neurotransmission by increasing AChE activity in the hippocampus (Ortega *et al*., 2021). Elevated AChE activity accelerate the breakdown of acetylcholine at synaptic junctions, leading to reduced cholinergic signaling. This decrease in acetylcholine availability impairs synaptic plasticity and contributes to cognitive deficits (Huang *et al*., 2022). Increased AChE activity is thus considered a biomarker of neurotoxicity, and its upregulation by Pb is one of the key mechanisms underlying Pb-induced learning and memory impairments. By enhancing AChE activity, Pb disrupts neurotransmitter balance and contributes to broader neurochemical disturbances that compromise hippocampal integrity and cognitive performance (Ortega *et al*., 2021). Findings from this study showed significant increase in AChE activity in the hippocampus of Pb-exposed rats. This increase is indicative of disruption to cholinergic neurotransmission leading to neuronal stress in the hippocampus. However, pre-treatment with *R. officinalis* significantly decreased AChE activity in Pb-exposed rats. Thus, highlighting its ability to restore cholinergic balance and its anti-cholinesterase effects.

Evidence suggests that Pb accumulation in the hippocampus leads to neurochemical alterations and structural damage, contributing to deficits in spatial learning and memory (Pyatha *et al*., 2022). As a result, therapeutic strategies such as the use of chelating agents have been explored to mitigate Pb-induced neurotoxicity (Leal *et al*., 2023). Chelators are compounds capable of binding metal ions through multiple coordination sites, forming stable complexes that facilitate the excretion of the metal from the body via urine or feces (Jomova *et al*., 2022; Li *et al*., 2023). This approach offers a potential means of reducing Pb burden in neural tissue and preserving hippocampal function. Findings from this study revealed a marked elevation in hippocampal Pb levels in rats exposed to Pb. However, pre-treatment with *R. officinalis* significantly decreased Pb accumulation in the hippocampus, indicating its potential as an effective metal chelating agent. The chelating activity of *R. officinalis* is likely due to its rich content of bioactive molecules such as flavonoids and polyphenols. These compounds can bind heavy metal ions through multiple coordination sites, thereby reducing their solubility and systemic absorption. By forming stable complexes with Pb, these phytochemicals may hinder its ability to cross the blood–brain barrier and limit its deposition in brain regions such as the hippocampus (Liu *et al*., 2022; Mishra *et al*., 2022).

Nitric oxide (NO) is a versatile signaling molecule that functions as a neurotransmitter and plays essential roles in both normal and pathological processes within the central nervous system, particularly in the hippocampus (Andrabi *et al*., 2023; Yang *et al*., 2025). In neurons, NO is synthesized primarily by neuronal nitric oxide synthase (nNOS). While NO contributes to neuroprotection under physiological conditions, excessive NO production has been linked to neuronal injury (Iova *et al*., 2023). In pathological states, elevated NO can react with superoxide radicals to form highly reactive nitrogen and oxygen species, which contribute to oxidative and nitrosative stress (Kiran *et al*., 2023; Jomova *et al*., 2023). Additionally, NO plays a dual role in inflammation, acting as both a pro-inflammatory and anti-inflammatory mediator depending on its concentration, cellular source, and context (Kölliker-Frers *et al*., 2021). It is produced by inducible nitric oxide synthase (iNOS) in activated glial cells and immune cells during neuroinflammation, contributing to the regulation of cytokine production, leukocyte recruitment, and blood-brain barrier permeability (Liy *et al*., 2021; Kölliker-Frers *et al*., 2021). Dysregulated NO signaling can exacerbate inflammatory damage in neurological disorders. The findings from this study showed a significant increase in NO levels in the hippocampus of Pb-exposed rats. However, following pre-treatment with *R. officinalis*, there was a significant decrease in NO levels, indicating the ability of *R. officinalis* to scavenge these toxic reactive nitrogen species and mitigate excessive NO production.

5.6 *R. officinalis* Attenuates Pb-Induced Hippocampal Histological Alterations

The hippocampus is especially vulnerable to the toxic effects of Pb exposure due to its complex neural circuits and sensitive cellular structures (Gudadhe *et al*., 2024). This brain region plays a critical role in learning, memory, and cognitive function, all of which depend on the health and integrity of its neurons (Abed, 2023). Research has shown that hippocampal pyramidal cells are particularly susceptible to damage from neurotoxins such as Pb, which can impair synaptic transmission and lead to cognitive deficits (Ortega *et al*., 2021; Enogieru and Iyoha, 2023; Gudadhe *et al*., 2024).

In the present study, histological examination of the hippocampus in rats exposed to Pb revealed marked cellular alterations, including atrophy, vacuolated pyramidal cells, and reactive astrocytes. These findings suggest that Pb exposure induces significant neurotoxic damage, particularly affecting the pyramidal neurons, which are crucial for cognitive processes such as learning and memory (Ramírez Ortega *et al*., 2021; Gudadhe *et al*., 2024). The presence of vacuolations indicates disruptions in cellular integrity and possible impairment in synaptic connectivity, which could underlie functional deficits associated with Pb toxicity (Carmona *et al*., 2021). Conversely, rats pre-treated with *R. officinalis* demonstrated relatively preserved hippocampal histology with pyramidal cells appearing normal with minimal signs of cellular damage. This protective effect aligns with previous studies reporting that antioxidants derived from natural sources can mitigate heavy metal-induced neurodegenerative changes (Enogieru and Momodu, 2022; Enogieru and Egbon, 2022). The neuroprotective action of *R. officinalis* may be attributed to its rich content of phenolic compounds and flavonoids, which have demonstrated efficacy in reducing oxidative stress and inflammation, thereby preserving neuronal architecture (Kölliker-Frers *et al*., 2021; Bellavite, 2023; Tavan *et al*., 2024).

5.7 *R. officinalis* Downregulates Caspase-3 expression in the Hippocampus

Apoptosis, or programmed cell death, is a vital biological process that enables cells to undergo controlled self-destruction (Kalkan, 2022; Hajibabaie *et al*., 2023). This mechanism plays a key role in maintaining cellular homeostasis and is essential for development, immune regulation, and tissue remodeling (Meizlish *et al*., 2021). In neurodegenerative diseases, apoptosis is a major contributor to neuronal loss (Erekat, 2022). Conditions such as Alzheimer’s disease, Parkinson’s disease, and amyotrophic lateral sclerosis (ALS) are characterized by the gradual loss of specific neurons, often involving apoptotic pathways (Pathak *et al*., 2021). The mechanisms through which apoptosis drives neurodegeneration are multifaceted. They include caspase activation, protein misfolding such as alpha-synuclein aggregation, and calcium imbalance (Gupta *et al*., 2021; Saramowicz *et al*., 2024). Caspase-3, a cysteine-aspartic protease, serves as a key executor of apoptosis (Asadi *et al*., 2022; Srivastava and Saxena, 2023). Its activation can occur via two principal pathways: the extrinsic pathway initiated by death ligand binding and caspase-8 activation (Orning and Lien, 2021; Pang and Vince, 2023), and the intrinsic pathway triggered by internal stressors like DNA damage or oxidative stress, which cause mitochondrial release of cytochrome c (Sharma *et al*., 2023; Sadiq, 2023). Both pathways lead to caspase-3 activation, which then cleaves numerous cellular substrates to execute apoptosis (Eskandari and Eaves, 2022; Asadi *et al*., 2022; Srivastava and Saxena, 2023). The expression of caspase-3 is significantly elevated in the hippocampal neurons of patients with Alzheimer's disease, suggesting a direct link between apoptotic mechanisms and hippocampal neurodegeneration (Španić *et al*., 2022; Wójcik *et al*., 2024).

In the present study, rats exposed to Pb exhibited a significant increase in hippocampal caspase-3 expression compared to controls, indicating that Pb induces apoptotic neuronal death. These findings align with previous research linking Pb toxicity to caspase-3-mediated neurodegeneration (Jayaraman *et al*., 2021; Ortega *et al*., 2021; Sharma *et al*., 2021; Asadi *et al*., 2022; Balusu and De Strooper, 2024). Notably, pre-treatment with *R. officinalis* resulted in a significant reduction of caspase-3 levels in Pb-exposed rats, suggesting that *R. officinalis* possesses anti-apoptotic properties.

5.8 CONCLUSION

Findings from this study indicate that *R. officinalis* attenuates Pb-induced hippocampal toxicity primarily through its antioxidant, metal chelating, anti-cholinesterase and anti-apoptotic effects. Therefore, *R. officinalis* may serve as a promising candidate for developing novel therapeutic agents aimed at managing Pb neurotoxicity and its associated cognitive impairments.

5.9 RECOMMENDATIONS

Further investigations on *R. officinalis* focusing on the isolation of specific bioactive compounds responsible for its neuroprotective effects against heavy metal-induced hippocampal toxicity are necessary. Additional mechanistic studies using diverse experimental models of neurotoxicity are warranted to better understand its protective pathways. Such investigations will pave the way for advancing *R. officinalis* as a promising candidate for clinical evaluation in humans. Ultimately, this could support its development as a therapeutic neuroprotective agent targeting hippocampal dysfunction and related cognitive impairments.

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