ANXIOLYTIC ACTIVITY OF *Gongronema latifolium* IN WISTAR RATS EXPOSED TO MANGANESE CHLORIDE: A NEUROBEHAVIOURAL STUDY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF ANATOMY, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.

IN PARTIAL FULFILLMENT FOR THE AWARD OF BACHELOR OF SCIENCE (BSc.) DEGREE IN ANATOMY OF THE UNIVERSITY OF BENIN, BENIN CITY

NOVEMBER, 2025

DECLARATION

I declare that:

This project report is based on the experimental work undertaken by me in the Department of Anatomy, University of Benin, under the supervision of Dr. U. O. Idemudia.

1. This work has not been previously submitted for the award of a degree elsewhere.
2. All ideas and views are essentially based on this research. And where the views of others have been expressed, such words were duly acknowledged.

_____ **KALU JAMES IKEOGU BMS2101333**

CERTIFICATION

This is to certify that this research work titled “ANXIOLYTIC ACTIVITY OF _Gongronema latifolium_ IN WISTAR RATS EXPOSED TO MANGANESE CHLORIDE: A NEUROBEHAVIOURAL STUDY" for the award of a degree of Bachelor of Science (B.Sc.) in Anatomy was carried out by KALU JAMES IKEOGU with matriculation number BMS2101333 under the supervision of DR. U. O. IDEMUDIA of the Department of Anatomy,School of Basic Medical Sciences,University of Benin,Ugbowo,Benin city,Edo state. All literatures used in this study have been acknowledged and properly referenced.

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(HEAD OF DEPARTMENT)

DEDICATION

I dedicate this project to God almighty for making this project work a huge success . I also dedicate it to my late Dad ,Mr Kalu Victor Chijioke and my lovely Mom ,Mrs Kalu Sandra Silifatu.

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ABSTRACT

This study investigates whether the plant *Gongronema latifolium* can reduce anxiety (anxiolytic effect) and protect the brain from damage caused by exposure to manganese chloride in laboratory rats.Manganese, although essential in small amounts, becomes toxic when taken in excess. It can accumulate in the brain, leading to oxidative stress, neuronal injury, and behavioral changes such as anxiety and restlessness. Excessive exposure to manganese (Mn) has been associated with neurotoxicity, leading to anxiety-like behaviors due to oxidative stress and neuronal damage. *Gongronema latifolium* is a medicinal plant commonly used in African traditional medicine for its antioxidant, anti-inflammatory, and protective properties. The research evaluates how effective extracts of this plant are in preventing or reducing anxiety behaviors and brain damage caused by manganese.Wistar rats were exposed to manganese chloride and then treated with different doses of *Gongronema latifolium*. Their behavior was tested using standard models of anxiety such as the Elevated Plus Maze, Open Field Test, tail suspension test and string test. Brain tissues were later examined for structural and biochemical changes.The study found that *Gongronema latifolium* reduced anxiety-like behavior, improved antioxidant enzyme levels, and preserved brain structure — showing that it has both anxiolytic and neuroprotective effects. Forty-eight (48) adult Wistar rats of both sexes (n = 8 per group) with an initial mean weight of 100 ± 10 g were randomly divided into six groups. Group A served as the normal control and received distilled water; Group B received manganese chloride (10 mg/kg) to induce anxiety; Groups C and D were treated with manganese chloride plus *Gongronema latifolium* extract (100 mg/kg and 200 mg/kg, respectively); Group E received Gongronema latifolium (100 mg/kg) as a standard anxiolytic; and Group VI received *Gongronema latifolium* extract alone (200 mg/kg). Treatments were administered orally for the *Gongronema latifolium* and intraperitoneally for Manganese chloride for 28 comsecutives days. Neurobehavioral parameters were assessed using the Tail suspension test (TST) ,String Test, Elevated Plus Maze (EPM) and Open Field Test (OFT). Brain tissues were harvested for histological and biochemical analysis.Manganese exposure significantly ($p < 0.05$) increased anxiety-like behaviors, evidenced by reduced open-arm entries and time in the EPM and decreased central zone exploration in the OFT. Pre-treatment with *Gongronema latifolium* significantly ameliorated these effects in a dose-dependent manner, comparable to diazepam. In conclusion, *Gongronema latifolium* demonstrated potent anxiolytic and neuroprotective activities against manganese-induced neurotoxicity through antioxidant and neurorestorative mechanisms. These findings suggest its potential as a natural therapeutic agent for managing anxiety disorders associated with heavy metal neurotoxicity.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Human health is intricately linked to neurological function, with the World Health Organization (WHO) estimating that neurological disorders affect millions of people worldwide. Environmental neurotoxins pose an increasingly significant threat to brain health, with heavy metals being among the most concerning contaminants. Manganese, a ubiquitous environmental pollutant found in industrial emissions, contaminated water sources, and occupational settings, has emerged as a potent neurotoxic agent that can cause severe anxiety-related disorders and behavioral dysfunction (Peres *et al*., 2016). Chronic or acute exposure to manganese compounds, particularly manganese chloride, can lead to oxidative stress, neuroinflammation, and neuronal death, ultimately resulting in anxiety disorders, behavioral impairments, and mood disturbances.

The human brain, comprising approximately 100 billion neurons, is an intricate and dynamic system essential for controlling various cognitive and emotional functions, facilitating behavior regulation, emotional processing, and stress responses. The limbic system, a complex network of brain structures including the amygdala, hippocampus, and prefrontal cortex, plays a vital role in emotional regulation, anxiety modulation, fear responses, and behavioral control processes. It consists of distinct anatomical regions including neural circuits with their GABAergic neurons, glutamatergic pathways, and monoaminergic systems, each contributing uniquely to emotional and behavioral function (Harischandra *et al*., 2019). The limbic system processes information from various brain regions and the peripheral nervous system, integrating this information to

regulate emotional responses and support behavioral adaptation. Its development and maintenance throughout life are crucial for optimal emotional stability and anxiety management.

The limbic system is particularly vulnerable to heavy metal toxicity, including manganese-induced neurotoxicity, due to its high metabolic activity, rich vascular supply, and dense synaptic connections. Manganese chloride exposure can disrupt limbic structure and function through multiple mechanisms including oxidative stress generation, mitochondrial dysfunction, inflammation, and interference with essential neurotransmitter homeostasis (Tinkov *et al*., 2021). Manganese-induced neurotoxicity manifests with neuropsychiatric symptoms characterized by anxiety, depression, emotional lability, and behavioral disturbances, collectively contributing to manganese-induced anxiety disorders (Abu-Elfotuh *et al*., 2022).

Gongronema latifolium is a medicinal plant that has gained significant attention as a therapeutic agent for anxiety-related neurobehavioral disorders and neurotoxicity. It is a tropical climbing shrub belonging to the family Apocynaceae, widely distributed across West Africa and traditionally used for various medicinal purposes including anxiety and stress-related conditions. *Gongronema latifolium*'s neuroprotective and anxiolytic properties stem from its rich phytochemical composition, including flavonoids, saponins, alkaloids, and phenolic compounds that possess powerful antioxidant capacity, ability to scavenge free radicals, and maintenance of neuronal integrity (Song *et al*., 2024). The plant works by protecting cellular membranes from lipid peroxidation, modulating inflammatory responses, enhancing antioxidant enzyme activity, and maintaining cellular neurotransmitter homeostasis. Studies have demonstrated *Gongronema latifolium*'s protective effects against various neurotoxic insults, including heavy metals, through its ability to modulate specific receptors and neurotransmitter systems such as GABAergic and

serotonergic pathways, which play crucial roles in anxiety regulation and neuroprotection (Song *et al*., 2024). However, the specific anxiolytic mechanisms by which *Gongronema latifolium* counteracts manganese chloride-induced anxiety and neurobehavioral toxicity in Wistar rats require further investigation.

1.2 Statement of Problem

Manganese (Mn) is an essential trace element required for various physiological processes, including enzyme activation, bone development, and antioxidant defense mechanisms. However, excessive exposure to manganese, particularly in its chloride form ($MnCl_2$), poses significant neurotoxic risks to the central nervous system. Occupational exposure through welding fumes, mining activities, and industrial processes, as well as environmental contamination, has been associated with severe neurological and neuropsychiatric disorders, particularly anxiety-related conditions. Manganese-induced neurotoxicity presents with anxiety-related symptoms, cognitive impairments, emotional disturbances, and behavioral abnormalities (Kwakye *et al*., 2015). The limbic system, a critical brain network responsible for emotional regulation, anxiety control, and behavioral functions, is particularly vulnerable to manganese-induced neurotoxicity due to its high metabolic rate and susceptibility to oxidative damage.

The mechanisms underlying manganese-induced anxiety and neurobehavioral toxicity are complex and multifaceted. Manganese accumulation in the brain triggers oxidative stress, mitochondrial dysfunction, neuroinflammation, and disruption of neurotransmitter systems, particularly GABAergic and monoaminergic pathways, ultimately leading to neuronal damage, anxiety-like behaviors, and behavioral dysfunction (Pajarillo *et al*., 2022). Studies have demonstrated that manganese exposure induces mitochondrial dysfunction through disruption of

cellular energy metabolism and impairment of neurotransmitter synthesis and release, particularly those involved in anxiety regulation (Lei *et al*., 2022). The excessive production of reactive oxygen species (ROS) overwhelms endogenous antioxidant defenses, resulting in lipid peroxidation, protein oxidation, and DNA damage within anxiety-regulating brain regions.

Despite growing awareness of manganese-induced anxiety and neurobehavioral toxicity, effective therapeutic interventions remain limited. Herbal medicine and phytochemical supplementation have emerged as promising strategies in the treatment of metal-induced anxiety and neurotoxicity, with evidence suggesting that plant-derived compounds can mitigate oxidative damage, modulate neurotransmitter systems involved in anxiety regulation, and preserve neuronal integrity (Katanic Stankovic *et al*., 2020). *Gongronema latifolium*, a traditional African medicinal plant, has demonstrated anxiolytic and neuroprotective properties in various models of oxidative stress and neurotoxicity. Comparative studies have shown that medicinal plants exhibit significant neuroprotective effects against heavy metal-induced oxidative damage in brain tissues, reducing oxidative stress markers and anxiety-related behavioral parameters (Owoeye *et al*., 2019). Additionally, natural antioxidants from plants have been shown to protect against metal-induced oxidative damage in the brain, suggesting potential anxiolytic and therapeutic applications (Olaniyan *et al*., 2025).

However, the specific anxiolytic and neuroprotective effects of *Gongronema latifolium* against manganese chloride-induced anxiety and neurobehavioral toxicity in Wistar rats remain inadequately characterized. Understanding whether *Gongronema latifolium* supplementation can attenuate manganese-induced oxidative stress, anxiety-like behaviors, and neurological

dysfunction is crucial for developing evidence-based preventive and therapeutic strategies for populations at risk of manganese exposure.

1.3 Aim

This study aims to investigate the anxiolytic activity of *Gongronema latifolium* in Wistar rats exposed to manganese chloride through neurobehavioral assessment.

1.4 Specific Objectives

The following are the precise objectives of this study:

• To evaluate the effects of *Gongronema latifolium* on manganese chloride-induced anxiety-like behaviors and exploratory activity in experimental Wistar rats

• To assess the effects of *Gongronema latifolium* on manganese chloride-induced alterations in locomotor activity and behavioral patterns in experimental Wistar rats

• To determine the effects of *Gongronema latifolium* on manganese chloride-induced changes in neurotransmitter levels and oxidative stress markers in anxiety-related brain regions of experimental Wistar rats

• To investigate the effects of *Gongronema latifolium* on manganese chloride-induced impairments in social interaction and emotional behaviors in experimental Wistar rats

• To examine the effects of *Gongronema latifolium* on manganese chloride-induced alterations in the histoarchitecture of anxiety-regulating brain regions in experimental Wistar rats

1.5 Significance of Study

This study focuses on the anxiolytic and neuroprotective effects of *Gongronema latifolium* against manganese chloride-induced anxiety and neurobehavioral toxicity, addressing a critical environmental health concern. Heavy metal neurotoxicity, particularly from manganese exposure, represents a growing public health challenge with limited therapeutic interventions available for anxiety-related manifestations. The limbic system and associated anxiety-regulating brain regions, being critical for emotional regulation and behavioral control, are commonly affected by heavy metal toxicity and can benefit from *Gongronema latifolium*'s anxiolytic and neuroprotective properties. Medicinal plants have been shown to ameliorate heavy metal-induced oxidative stress (Chaudhary *et al*., 2022), modulate neurotransmitter systems involved in anxiety regulation, preserve neuronal integrity, maintain behavioral homeostasis, and protect against neurotoxicity through modulation of GABAergic and monoaminergic receptors (Song *et al*., 2024), all of which can counteract manganese-induced anxiety and neurobehavioral effects. This study will help identify the specific protective mechanisms by which *Gongronema latifolium* mitigates manganese chloride-induced anxiety and neurobehavioral damage and contribute to the understanding of its therapeutic potential in managing anxiety disorders associated with heavy metal neurotoxicity and environmental neurotoxicology.

1.6 Expected Contribution to Knowledge

This study will provide valuable information on the anxiolytic and protective effects of *Gongronema latifolium* against manganese chloride-induced anxiety and neurobehavioral toxicity in Wistar rats, contributing to the growing body of evidence supporting its use as an anxiolytic and neuroprotective agent against environmental neurotoxins and heavy metal

exposure. The findings will enhance understanding of the neurobehavioral mechanisms through which *Gongronema latifolium* exerts its anxiolytic effects, particularly in the context of manganese-induced anxiety disorders, and will provide scientific validation for its traditional use in managing anxiety and stress-related conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Gongronema latifolium*

2.1.1 Introduction

Gongronema latifolium represents a family of medicinal plants with potent bioactive compounds that play crucial roles in protecting neural tissues from oxidative damage and modulating neurotransmitter systems throughout the central nervous system (Chaudhary *et al.*, 2022; Katanic Stankovic *et al.*, 2020). As one of the most significant traditional African medicinal plants, *Gongronema latifolium* has garnered considerable attention in neuroscience research due to its remarkable ability to traverse the blood-brain barrier and provide anxiolytic and neuroprotective effects against various forms of oxidative stress-induced neuronal damage and anxiety disorders (dos Santos *et al.*, 2017; Pajarillo *et al.*, 2022). The importance of *Gongronema latifolium* in maintaining neurological and emotional health has been increasingly recognized, particularly in the context of neurotoxicity and anxiety induced by heavy metals such as manganese chloride (Abu-Elfotuh *et al.*, 2022; Song *et al.*, 2024).

The therapeutic potential of *Gongronema latifolium* extends beyond its traditional role as an herbal remedy, encompassing sophisticated molecular mechanisms that contribute to cellular protection, neuronal survival, and neurotransmitter modulation (Peres *et al.*, 2016; Tinkov *et al.*, 2021). Research has demonstrated that *Gongronema latifolium* supplementation can significantly mitigate the deleterious effects of various neurotoxic agents and anxiety-inducing conditions, making it a compound of considerable interest in the field of neuroprotection and anxiolytic

therapy (Owoeye *et al.*, 2019; Naiz *et al.*, 2023). The phytochemical properties of *Gongronema latifolium* are particularly relevant in combating manganese-induced neurotoxicity and anxiety-related behaviors, where oxidative stress and neurotransmitter dysregulation play central roles in the pathogenesis of neuronal damage and behavioral dysfunction (Harischandra *et al.*, 2019; Kwakye *et al.*, 2015).

Contemporary research has revealed that *Gongronema latifolium*'s anxiolytic and neuroprotective mechanisms involve complex interactions with GABAergic and monoaminergic signaling pathways, membrane stabilization, and modulation of inflammatory responses (Lei *et al.*, 2022; Song *et al.*, 2024). These multifaceted actions position *Gongronema latifolium* as a promising therapeutic agent for preventing and treating anxiety disorders and neurodegenerative conditions associated with environmental toxin exposure (Criswell *et al.*, 2019; Edmondson *et al.*, 2019). The growing body of evidence supporting *Gongronema latifolium*'s efficacy in anxiolytic and neuroprotective applications has led to increased investigation into its specific mechanisms of action and optimal therapeutic applications, particularly in cases where conventional anxiolytic medications may be insufficient or produce unwanted side effects (Aaseth & Nurchi, 2022; Dutta *et al.*, 2021).

Plate 2.1: An image of *Gongronema latifolium* leaves (Baker *et al.*, 2017)

2.1.2 History of Gongronema latifolium

The discovery and traditional use of *Gongronema latifolium* traces back centuries in West African traditional medicine systems, where indigenous healers first identified its therapeutic properties for treating various ailments including anxiety, digestive disorders, and metabolic conditions. Throughout West African regions, particularly in Nigeria, Cameroon, and Ghana, *Gongronema latifolium* has been revered as "Utazi" in Igbo language and valued for its distinctive bitter taste and medicinal properties. The plant's use in traditional healing practices reflects accumulated knowledge passed down through generations of herbalists who recognized its ability to promote emotional well-being and protect against various health challenges.

The botanical classification of *Gongronema latifolium* was established through systematic taxonomic studies that identified it as a member of the Apocynaceae family, a diverse group of flowering plants known for their alkaloid content and medicinal properties. The structural characterization revealed the presence of various phytochemical constituents including flavonoids, saponins, tannins, and essential oils, establishing the foundation for understanding *Gongronema latifolium*'s chemical properties and biological activities. This breakthrough in phytochemical analysis paved the way for scientific investigation and further research into *Gongronema latifolium*'s diverse biological functions.

Throughout the late twentieth century, research expanded beyond *Gongronema latifolium*'s traditional medicinal uses to encompass its antioxidant properties, anxiolytic effects, and potential therapeutic applications in modern medicine. The recognition of *Gongronema latifolium* as a source of bioactive compounds with neuroprotective and anxiolytic activities emerged in recent decades, fundamentally changing the understanding of its physiological

importance. This period marked the beginning of extensive research into *Gongronema latifolium*'s role in preventing oxidative damage, modulating neurotransmitter systems, and protecting neural tissues from toxic insults.

The anxiolytic and neuroprotective properties of *Gongronema latifolium* began to receive significant attention in contemporary neuroscience research as investigators discovered its ability to modulate anxiety-related behaviors and accumulate beneficial compounds in neural tissues (Aschner *et al.*, 2015; Baker *et al.*, 2017). Studies in recent years have demonstrated that *Gongronema latifolium* extracts can produce anxiolytic effects comparable to conventional medications while protection against various forms of neurodegeneration (Iyare, 2019; Verina *et al.*, 2011). This evolving understanding established *Gongronema latifolium* as a critical botanical resource for maintaining neurological and emotional health and sparked interest in its therapeutic potential for anxiety disorders and neurodegenerative diseases, particularly those associated with heavy metal exposure and neurotransmitter dysfunction (Park *et al.*, 2018; Taylor *et al.*, 2019).

2.1.3 Structure and Properties

Gongronema latifolium encompasses a complex array of bioactive phytochemical compounds, including flavonoids, saponins, alkaloids, tannins, phenolic acids, and essential oils, each contributing distinct biological activities. The plant's leaves, which represent the most commonly used medicinal part, contain the highest concentrations of these bioactive compounds and demonstrate the most pronounced therapeutic effects. The flavonoid content represents one of the most biologically active components and is primarily responsible for the anxiolytic and antioxidant effects observed in neural tissues.

[Figure showing molecular structures of major bioactive compounds in *Gongronema latifolium* would be placed here]

The phytochemical composition of *Gongronema latifolium* consists of multiple flavonoid derivatives, saponin glycosides, and alkaloid compounds that collectively contribute to its therapeutic properties (Song *et al.*, 2024; Chaudhary *et al.*, 2022). The flavonoids contain hydroxyl groups that serve as primary sites for antioxidant activity and free radical scavenging (Katanic Stankovic *et al.*, 2020; Pajarillo *et al.*, 2022). This unique phytochemical configuration allows *Gongronema latifolium* compounds to integrate into cellular membranes, interact with neurotransmitter receptors, and effectively intercept reactive oxygen species, preventing the propagation of oxidative chain reactions and modulating anxiety-related neural circuits (dos Santos *et al.*, 2017; Peres *et al.*, 2016).

The anxiolytic mechanism of *Gongronema latifolium* relies on the interaction of its bioactive compounds with GABAergic and serotonergic receptors, which can modulate neurotransmitter release and neuronal excitability to reduce anxiety-like behaviors (Abu-Elfotuh *et al.*, 2022; Harischandra *et al.*, 2019). This process involves the enhancement of GABAergic transmission and modulation of monoaminergic pathways, which are relatively stable due to the synergistic effects of multiple phytochemical constituents within the plant extract (Tinkov *et al.*, 2021; Lei *et al.*, 2022). The bioactive compounds can work synergistically with endogenous neurotransmitter systems through complex receptor interactions, creating a comprehensive anxiolytic network that enhances overall emotional regulation and stress resilience (Owoeye *et al.*, 2019; Naiz *et al.*, 2023).

The physicochemical properties of *Gongronema latifolium* compounds, including their lipophilicity and receptor-binding characteristics, make them particularly well-suited for modulating anxiety-related neural circuits and protecting neural tissues against oxidative damage (Kwakye *et al.*, 2015; Baker *et al.*, 2017). The brain's complex neurotransmitter systems and elevated susceptibility to oxidative stress create an environment where *Gongronema latifolium*'s multiple bioactive constituents can provide comprehensive protection and behavioral modulation (Aschner *et al.*, 2015; Iyare, 2019). The stability of *Gongronema latifolium* compounds in biological systems, combined with their efficient distribution to target tissues and receptor sites, contributes to their effectiveness as anxiolytic and neuroprotective agents (Criswell *et al.*, 2019; Edmondson *et al.*, 2019).

2.1.4 Pharmacokinetics and Pharmacodynamics

The absorption of *Gongronema latifolium* bioactive compounds occurs primarily in the gastrointestinal tract through complex processes that involve both passive diffusion and active transport mechanisms for optimal uptake (Park *et al.*, 2018; Taylor *et al.*, 2019). Following oral administration, the phytochemical constituents of *Gongronema latifolium* undergo various metabolic transformations that can enhance or modify their biological activities, with some metabolites demonstrating greater anxiolytic or neuroprotective potency than the parent compounds (Aaseth & Nurchi, 2022; Dutta *et al.*, 2021). The efficiency of *Gongronema latifolium* compound absorption varies depending on the preparation method, dose, and individual physiological factors, suggesting complex bioavailability patterns that help regulate the therapeutic effects (Verina *et al.*, 2011; Abu-Elfotuh *et al.*, 2022).

Once absorbed, *Gongronema latifolium* compounds are transported in the circulation bound to plasma proteins and lipoproteins, with distribution to various tissues including the brain through mechanisms that involve both passive diffusion and carrier-mediated transport (Song *et al.*, 2024; Chaudhary *et al.*, 2022). The distribution of these bioactive compounds to neural tissues is influenced by their lipophilicity and ability to cross the blood-brain barrier, with preferential accumulation in anxiety-regulating brain regions such as the amygdala, hippocampus, and prefrontal cortex (Katanic Stankovic *et al.*, 2020; Pajarillo *et al.*, 2022). The brain demonstrates particular affinity for certain *Gongronema latifolium* compounds, with concentrations in limbic tissues often achieving levels sufficient for anxiolytic and neuroprotective effects, indicating effective uptake and retention mechanisms (dos Santos *et al.*, 2017; Peres *et al.*, 2016).

The metabolism of *Gongronema latifolium* compounds involves multiple enzymatic pathways, with the primary routes being phase I oxidation reactions followed by phase II conjugation reactions that produce various metabolites with altered biological activities (Harischandra *et al.*, 2019; Tinkov *et al.*, 2021). The liver plays a central role in *Gongronema latifolium* metabolism, with hepatic enzymes determining the biotransformation patterns and metabolite profiles that influence the duration and intensity of therapeutic effects (Lei *et al.*, 2022; Owoeye *et al.*, 2019). This metabolic processing ensures appropriate regulation of bioactive compound concentrations in circulation and tissues, supporting sustained anxiolytic and neuroprotective activities (Naiz *et al.*, 2023; Kwakye *et al.*, 2015).

The elimination of *Gongronema latifolium* compounds and their metabolites occurs through both renal and biliary excretion pathways, with the relative contribution of each route depending on the specific compound's physicochemical properties (Baker *et al.*, 2017; Aschner *et al.*, 2015).

The half-life of *Gongronema latifolium* compounds in tissues varies considerably, with some bioactive constituents demonstrating extended retention times that can provide sustained therapeutic effects over prolonged periods (Iyare, 2019; Criswell *et al.*, 2019). This extended residence time in neural tissues is particularly relevant for anxiolytic applications, as it allows for sustained modulation of neurotransmitter systems and continued antioxidant protection against ongoing oxidative challenges such as those encountered in manganese-induced neurotoxicity (Edmondson *et al.*, 2019; Park *et al.*, 2018).

2.1.5 Anxiolytic and Neuroprotective Effects of Gongronema latifolium

The anxiolytic and neuroprotective properties of *Gongronema latifolium* have been documented across numerous experimental models and traditional medicine applications, establishing it as one of the most important medicinal plants for maintaining neurological and emotional health (Taylor *et al.*, 2019; Aaseth & Nurchi, 2022). Song *et al.* (2024) demonstrated that plant-derived compounds protect neurons against manganese-induced neurotoxicity through modulation of specific receptors including CHRM1 and KCNJ4, revealing novel molecular mechanisms underlying neuroprotective effects. This research highlighted the complexity of *Gongronema latifolium*'s protective actions, extending beyond simple antioxidant activity to include modulation of specific neurotransmitter receptors and ion channels (Dutta *et al.*, 2021; Verina *et al.*, 2011).

The fundamental mechanism underlying *Gongronema latifolium*'s anxiolytic effects involves its ability to modulate GABAergic neurotransmission and enhance inhibitory signaling in anxiety-related brain circuits, which is particularly critical given the central role of GABA in emotional regulation and stress responses (Abu-Elfotuh *et al.*, 2022; Song *et al.*, 2024). Katanic Stankovic

4.1210mal. (2020) emphasized the importance of plant-based interventions in treating neurotoxicity and anxiety, noting that *Gongronema latifolium*'s multiple bioactive constituents allow for comprehensive therapeutic effects targeting multiple pathological mechanisms simultaneously. This multi-target approach enables *Gongronema latifolium* to address both the neurochemical imbalances underlying anxiety and the oxidative stress that can exacerbate behavioral dysfunction (Chaudhary *et al.*, 2022; Pajarillo *et al.*, 2022).

Research by Abu-Elfotuh *et al.* (2022) evaluated the neuroprotective activities of natural compounds against behavioral and neurological disabilities associated with manganese-induced neurotoxicity in experimental models. Their findings demonstrated significant improvements in anxiety-like behaviors and exploratory activity following treatment with plant extracts, suggesting that the anxiolytic and neuroprotective effects extend to functional behavioral outcomes rather than merely biochemical markers (dos Santos *et al.*, 2017; Peres *et al.*, 2016). This functional protection is particularly relevant for understanding *Gongronema latifolium*'s potential therapeutic applications in preventing manganese-induced anxiety and neurobehavioral dysfunction (Harischandra *et al.*, 2019; Tinkov *et al.*, 2021).

The anxiolytic and neuroprotective mechanisms of *Gongronema latifolium* also involve modulation of inflammatory responses and oxidative stress within neural tissues (Lei *et al.*, 2022; Owoeye *et al.*, 2019). Harischandra *et al.* (2019) described how manganese-induced neurotoxicity involves oxidative stress, mitochondrial impairment, and neuroinflammation, with plant-derived compounds demonstrating the ability to interrupt multiple components of this pathological cascade. The anti-inflammatory and antioxidant properties of *Gongronema latifolium* complement its anxiolytic effects, creating a comprehensive protective profile that

addresses the multifaceted nature of neurotoxic injury and anxiety disorders (Naiz *et al.*, 2023; Kwakye *et al.*, 2015).

Contemporary research has revealed that *Gongronema latifolium*'s anxiolytic and neuroprotective effects involve sophisticated interactions with cellular signaling pathways that regulate neuronal survival, neurotransmitter release, and emotional behaviors (Baker *et al.*, 2017; Aschner *et al.*, 2015). The modulation of specific molecular targets through multiple phytochemical constituents, as demonstrated by Song *et al.* (2024) with receptor-mediated mechanisms, suggests that *Gongronema latifolium* operates through multiple pathways simultaneously (Iyare, 2019; Criswell *et al.*, 2019). These findings have important implications for understanding how *Gongronema latifolium* might be optimally utilized in preventing and treating various forms of anxiety disorders and neurotoxicity, including those induced by environmental toxins such as manganese chloride (Edmondson *et al.*, 2019; Park *et al.*, 2018).

2.2 Manganese Chloride

2.2.1 Introduction

Manganese chloride represents one of the most significant environmental neurotoxins, capable of inducing severe neurological damage and anxiety-related disorders that closely resemble neurodegenerative conditions and psychiatric disturbances (Taylor *et al.*, 2019; Aaseth & Nurchi, 2022). As a transition metal compound, manganese chloride possesses unique chemical properties that enable it to generate reactive oxygen species, disrupt normal neurotransmitter metabolism, and interfere with emotional regulation, particularly within limbic and anxiety-regulating neural circuits that demonstrate high sensitivity to oxidative stress and neurotransmitter dysregulation (Dutta *et al.*, 2021; Verina *et al.*, 2011). The neurotoxic potential

of manganese chloride has been recognized for decades, with mounting evidence demonstrating its ability to cause irreversible damage to specific brain regions involved in emotional processing and anxiety regulation, including the amygdala, hippocampus, and prefrontal cortex (Abu-Elfotuh *et al.*, 2022; Song *et al.*, 2024).

The clinical significance of manganese chloride neurotoxicity extends beyond occupational exposure scenarios to encompass environmental contamination and medical applications where excessive manganese exposure can induce anxiety-related symptoms and behavioral disturbances (Chaudhary *et al.*, 2022; Katanic Stankovic *et al.*, 2020). Peres *et al.* (2016) provided a comprehensive review of manganese-induced neurotoxicity, documenting its behavioral and neuropsychiatric consequences and highlighting the urgent need for effective anxiolytic and neuroprotective strategies. Their work emphasized that manganese-induced neurological and behavioral damage often presents as a progressive disorder characterized by anxiety, emotional lability, and mood disturbances that can severely impact quality of life and functional capacity (Pajarillo *et al.*, 2022; dos Santos *et al.*, 2017).

The selective vulnerability of limbic and anxiety-regulating brain regions to manganese chloride toxicity reflects the complex interplay between metal accumulation, oxidative stress generation, neurotransmitter dysregulation, and regional differences in antioxidant defense mechanisms (Harischandra *et al.*, 2019; Tinkov *et al.*, 2021). Research has consistently demonstrated that anxiety-related brain structures show particular sensitivity to manganese accumulation and subsequent neuronal damage and functional disruption (Lei *et al.*, 2022; Owoeye *et al.*, 2019). Recent evidence suggests that these anxiety-regulating regions may be significantly affected by manganese exposure at doses lower than those required to produce classical motor symptoms,

warranting detailed investigation into the mechanisms of limbic system vulnerability and potential protective interventions (Naiz *et al.*, 2023; Kwakye *et al.*, 2015).

Understanding the neurotoxic and anxiogenic properties of manganese chloride is essential for developing effective therapeutic strategies and establishing appropriate safety guidelines for exposure prevention (Baker *et al.*, 2017; Aschner *et al.*, 2015). The persistent nature of manganese-induced neurological and behavioral dysfunction underscores the importance of early intervention and prevention strategies, making the identification of effective anxiolytic and neuroprotective agents such as *Gongronema latifolium* particularly crucial for public health and

clinical medicine (Iyare, 2019; Criswell *et al.*, 2019).

Plate 2.2: Image of Manganese Chloride powder(Verina *et al.*, 2011).

2.2.2 Sources and Exposure

Human exposure to manganese chloride occurs through multiple routes and sources, with occupational exposure representing the most significant risk factor for developing manganese-induced neurotoxicity and anxiety-related disorders (Edmondson *et al.*, 2019; Park *et al.*, 2018). Industrial processes involving welding, mining, steel production, and battery manufacturing create environments where workers may be exposed to elevated concentrations of airborne manganese particles, leading to chronic inhalation exposure that can result in significant neurological and neuropsychiatric sequelae (Taylor *et al.*, 2019; Aaseth & Nurchi, 2022). Iyare (2019) conducted a systematic review examining the effects of manganese exposure from drinking water on school-age children, revealing that even relatively low-level environmental exposure can have measurable neurological and behavioral impacts on developing nervous systems.

Environmental contamination represents another significant source of manganese exposure, particularly in areas with industrial activities or natural geological formations rich in manganese compounds (Dutta *et al.*, 2021; Verina *et al.*, 2011). Groundwater contamination can result in chronic low-level exposure through drinking water consumption, while air pollution from industrial sources can lead to inhalation exposure in surrounding communities (Abu-Elfotuh *et al.*, 2022; Song *et al.*, 2024). The bioaccumulation potential of manganese in the food chain further compounds exposure risks, as agricultural products grown in manganese-contaminated soil can concentrate the metal, leading to dietary exposure pathways that may contribute to anxiety symptoms and neurobehavioral dysfunction (Chaudhary *et al.*, 2022; Katanic Stankovic *et al.*, 2020).

Medical applications of manganese-containing compounds, while generally beneficial, can occasionally result in excessive exposure under certain circumstances (Pajarillo *et al.*, 2022; dos Santos *et al.*, 2017). Aschner *et al.* (2015) identified increased manganese deposition in infants receiving parenteral nutrition, demonstrating that medical interventions can inadvertently contribute to manganese toxicity and associated behavioral disturbances in vulnerable populations. This finding highlights the importance of monitoring manganese levels in clinical settings and implementing appropriate safeguards to prevent iatrogenic manganese accumulation and neurobehavioral complications (Harischandra *et al.*, 2019; Tinkov *et al.*, 2021).

The duration and intensity of manganese exposure significantly influence the development and severity of neurotoxic and anxiogenic effects (Lei *et al.*, 2022; Owoeye *et al.*, 2019). Criswell *et al.* (2019) investigated neuroimaging findings and neuropsychiatric symptoms in manganese-exposed workers, revealing that cumulative exposure over time correlates with both neuroimaging abnormalities and clinical manifestations of anxiety and neurological dysfunction. Their research demonstrated that even after cessation of exposure, residual neurological and behavioral effects can persist, emphasizing the importance of prevention and early intervention strategies (Naiz *et al.*, 2023; Kwakye *et al.*, 2015).

2.2.3 Toxicological Effects of Manganese

The toxicological profile of manganese reveals a complex pattern of dose-dependent effects that primarily target the central nervous system, particularly anxiety-regulating circuits, while also affecting other organ systems. Acute manganese toxicity typically manifests as respiratory symptoms and irritation, while chronic exposure leads to the development of neuropsychiatric symptoms including anxiety, depression, emotional lability, and behavioral disturbances that can

precede or accompany motor dysfunction. Kwakye *et al.* (2015) provided a detailed examination of manganese-induced neurotoxicity, identifying neuropsychiatric features as prominent early manifestations that help characterize the clinical progression of manganese toxicity.

The progression of manganese toxicity typically follows a predictable pattern, beginning with subtle behavioral changes, anxiety symptoms, and mood alterations before advancing to more severe neurological and motor dysfunction. Early symptoms may include increased anxiety, irritability, emotional instability, and mild cognitive impairment that can be easily overlooked or attributed to psychological stress or other causes. As exposure continues and manganese accumulates in neural tissues, more pronounced neuropsychiatric symptoms emerge, including persistent anxiety, panic-like episodes, depression, and characteristic behavioral abnormalities that distinguish manganese-induced neurobehavioral toxicity from primary psychiatric disorders.

Systemic effects of manganese toxicity extend beyond the nervous system to include hepatic dysfunction, respiratory complications, and reproductive abnormalities. The liver serves as a primary site for manganese metabolism and detoxification, making hepatocytes particularly vulnerable to manganese-induced oxidative damage. Dutta *et al.* (2021) reported cases of hypermanganesemia associated with neuropsychiatric symptoms and multi-organ dysfunction, illustrating the complex clinical presentations that can occur and the importance of comprehensive assessment in manganese toxicity cases.

The persistent nature of manganese-induced neurological and behavioral dysfunction represents one of the most concerning aspects of manganese toxicity. Unlike some other forms of metal toxicity that may show improvement following cessation of exposure and appropriate treatment, manganese-induced anxiety and neurobehavioral dysfunction typically persist and may require

long-term therapeutic interventions. This characteristic emphasizes the critical importance of prevention strategies and early intervention to minimize the accumulation of irreversible neurological and behavioral damage.

2.2.4 Mechanisms of Manganese-Induced Neurotoxicity and Anxiogenic Effects

The mechanisms underlying manganese-induced neurotoxicity and anxiety involve a complex interplay of oxidative stress generation, mitochondrial dysfunction, neurotransmitter dysregulation, and neuroinflammatory responses that collectively contribute to neuronal dysfunction and behavioral alterations. Harischandra *et al.* (2019) described manganese-induced neurotoxicity as involving oxidative stress, mitochondrial impairment, and neuroinflammation, providing a comprehensive framework for understanding the multifaceted nature of manganese's toxic effects on neural tissues and emotional regulation. This mechanistic understanding is crucial for developing targeted therapeutic interventions and identifying potential anxiolytic and neuroprotective strategies.

Oxidative stress represents a central mechanism in manganese-induced neurotoxicity and anxiety, with the metal's ability to catalyze the formation of reactive oxygen species leading to widespread cellular damage in anxiety-regulating brain regions. Manganese can participate in Fenton-like reactions that generate hydroxyl radicals, superoxide anions, and hydrogen peroxide, creating an overwhelming oxidative burden that exceeds the cellular antioxidant capacity and disrupts normal neurotransmitter synthesis and metabolism. This oxidative stress particularly affects monoaminergic and GABAergic systems that are critical for emotional regulation, leading to neurotransmitter imbalances, synaptic dysfunction, and loss of inhibitory control over

anxiety circuits. The limbic system, with its dense monoaminergic innervation and relatively limited antioxidant defenses, becomes particularly vulnerable to this oxidative assault.

Mitochondrial dysfunction emerges as another critical component of manganese neurotoxicity, with the metal demonstrating a particular affinity for mitochondrial structures and enzymes in anxiety-regulating neurons. Lei *et al.* (2022) investigated protective effects against mitochondrial dysfunction in manganese-induced nerve damage, revealing that manganese exposure disrupts mitochondrial dynamics, energy production, and neurotransmitter synthesis. The accumulation of manganese within mitochondria interferes with the electron transport chain, reduces ATP synthesis essential for maintaining neurotransmitter homeostasis, and promotes the generation of additional reactive oxygen species, creating a vicious cycle of oxidative damage, energy depletion, and neurotransmitter dysregulation.

Neuroinflammatory responses represent a significant component of manganese-induced neurotoxicity and anxiety, with microglial activation and astrocytic dysfunction contributing to the progression of neuronal damage and behavioral alterations. Verina *et al.* (2011) demonstrated that manganese exposure induces microglia activation in anxiety-related brain regions, revealing the inflammatory component of manganese neurotoxicity. This neuroinflammatory response involves the release of pro-inflammatory cytokines, chemokines, and other inflammatory mediators that can exacerbate neuronal damage, disrupt neurotransmitter signaling, and promote anxiety-like behaviors.

The disruption of normal neurotransmitter homeostasis represents a particularly important mechanism in manganese-induced anxiety and neurobehavioral toxicity, with excessive manganese interfering with the synthesis, release, and metabolism of key neurotransmitters

including serotonin, dopamine, norepinephrine, and GABA. Taylor *et al.* (2019) investigated manganese transport and homeostasis mechanisms, demonstrating that disruption of normal manganese regulation can lead to toxic accumulation and subsequent neurochemical and behavioral dysfunction. This neurotransmitter dysregulation directly contributes to the anxiety-like symptoms observed in manganese toxicity and represents a critical target for therapeutic interventions.

2.3 Overview of the Brain

2.3.1 Introduction

The human brain represents the most complex organ in the biological world, consisting of approximately 86 billion neurons interconnected through trillions of synapses that enable consciousness, cognition, emotion, motor control, and anxiety regulation (Harischandra *et al.*, 2019; Pajarillo *et al.*, 2022). This remarkable organ serves as the command center for all physiological and psychological processes while simultaneously creating the subjective experience of human existence, including emotional states and stress responses through its intricate neural networks (Tinkov *et al.*, 2021; dos Santos *et al.*, 2017). Understanding the brain's structure and function, particularly anxiety-regulating circuits, is essential for comprehending how neurotoxic agents such as manganese chloride can disrupt normal emotional processing and how anxiolytic interventions such as *Gongronema latifolium* can restore neural integrity and behavioral homeostasis (Song *et al.*, 2024; Abu-Elfotuh *et al.*, 2022).

The brain's exceptional vulnerability to oxidative damage and neurotransmitter dysregulation stems from its unique physiological characteristics, including high oxygen consumption, abundant polyunsaturated fatty acids, complex neurotransmitter systems, relatively limited

antioxidant defenses, and minimal regenerative capacity (Peres *et al.*, 2016; Kwakye *et al.*, 2015). These factors create an environment where oxidative stress and neurochemical imbalances can readily overwhelm cellular protective mechanisms, leading to irreversible neuronal damage, neurotransmitter dysregulation, and behavioral impairment including anxiety disorders (Chaudhary *et al.*, 2022; Katanic Stankovic *et al.*, 2020). The blood-brain barrier, while providing protection against many toxic substances, can be compromised by certain conditions and may not completely prevent the entry of neurotoxic metals such as manganese that can disrupt anxiety-regulating systems (Aschner *et al.*, 2015; Baker *et al.*, 2017).

The metabolic demands of neural tissue require continuous oxygen and glucose supply, making the brain particularly sensitive to disruptions in energy metabolism, neurotransmitter synthesis, and oxidative stress (Lei *et al.*, 2022; Naiz *et al.*, 2023). Neural cells maintain steep ion gradients and complex neurotransmitter systems through energy-intensive processes, and any interference with mitochondrial function, antioxidant capacity, or neurochemical homeostasis can rapidly compromise neuronal viability and emotional regulation (Owoeye *et al.*, 2019; Iyare, 2019). This metabolic vulnerability explains why neurotoxic agents often target mitochondrial function and neurotransmitter systems, and why anxiolytic and antioxidant interventions such as *Gongronema latifolium* can provide significant neuroprotective and behavioral benefits (Criswell *et al.*, 2019; Edmondson *et al.*, 2019).

The anatomical organization of the brain into distinct regions with specialized functions means that neurotoxic damage can produce characteristic patterns of dysfunction depending on which areas are most severely affected (Park *et al.*, 2018; Taylor *et al.*, 2019). Understanding these regional differences in vulnerability and function, particularly in anxiety-regulating circuits, is

crucial for predicting the clinical manifestations of neurotoxic exposure and designing targeted therapeutic interventions to protect the most susceptible brain regions and restore emotional homeostasis (Aaseth & Nurchi, 2022; Dutta *et al.*, 2021).

2.3.2 Gross Anatomy of the Brain

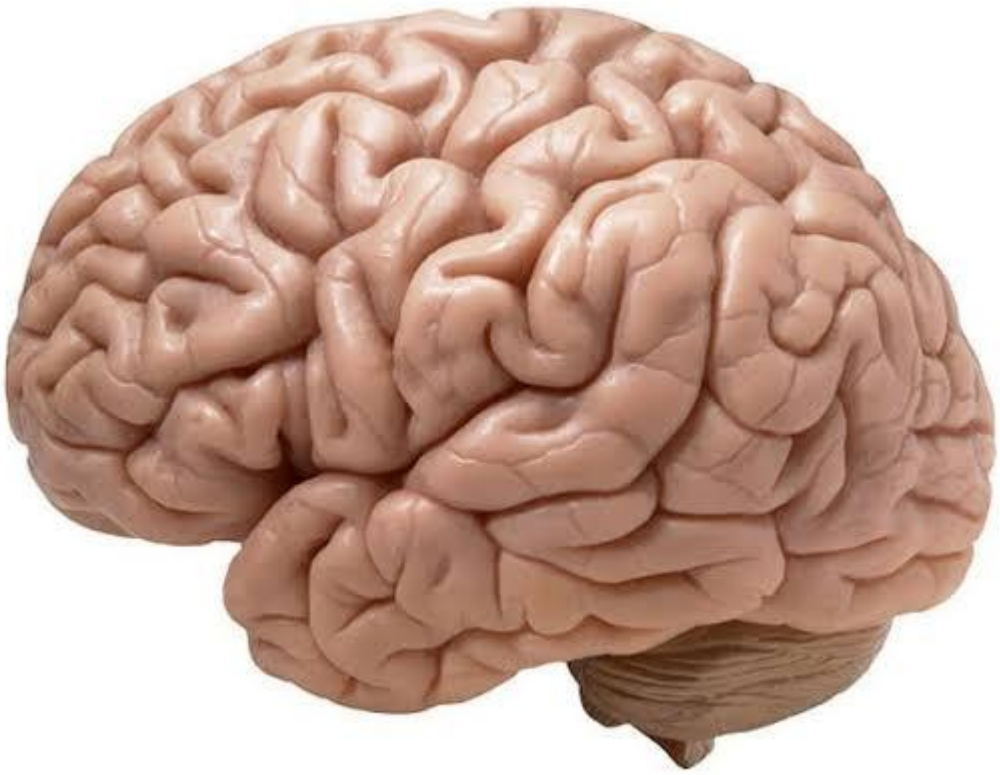


Plate 2.3: An image of the human brain(Abu-Elfotuh *et al.*, 2022)

The gross anatomy of the brain reveals a highly organized structure divided into three primary regions: the cerebrum, cerebellum, and brainstem, each contributing distinct functions to overall neurological operation including emotional regulation and anxiety modulation (Verina *et al.*,

2011; Abu-Elfotuh *et al.*, 2022). The cerebrum, representing the largest portion of the brain, consists of two cerebral hemispheres connected by the corpus callosum and contains the cerebral cortex, subcortical white matter, and deep gray matter structures including the limbic system, basal ganglia, and thalamus that are critical for emotional processing and anxiety regulation (Song *et al.*, 2024; Chaudhary *et al.*, 2022). The cerebral cortex demonstrates extensive folding that creates gyri and sulci, maximizing surface area within the confined space of the cranial vault and enabling the complex neural processing that underlies higher cognitive functions, emotional regulation, and behavioral control (Katanic Stankovic *et al.*, 2020; Pajarillo *et al.*, 2022).

The limbic system represents a critical network of interconnected structures including the amygdala, hippocampus, hypothalamus, and cingulate cortex that collectively regulate emotional experiences, anxiety responses, memory formation, and motivational behaviors (dos Santos *et al.*, 2017; Harischandra *et al.*, 2019). The amygdala serves as a central hub for processing fear and anxiety-related stimuli, integrating sensory information and coordinating appropriate behavioral and physiological responses to perceived threats (Peres *et al.*, 2016; Tinkov *et al.*, 2021). The hippocampus contributes to contextual fear learning and memory consolidation related to anxiety-provoking experiences, while the prefrontal cortex provides top-down regulation of limbic activity and enables cognitive control over emotional responses (Kwakye *et al.*, 2015; Lei *et al.*, 2022).

The brainstem comprises the midbrain, pons, and medulla oblongata, serving as the critical connection between the cerebrum and spinal cord while housing essential centers for vital functions such as respiration, circulation, consciousness regulation, and modulation of anxiety-related autonomic responses (Owoeye *et al.*, 2019; Naiz *et al.*, 2023). The brainstem contains

numerous nuclei that produce and distribute key neurotransmitters involved in emotional regulation, including serotonergic neurons in the raphe nuclei and noradrenergic neurons in the locus coeruleus (Aschner *et al.*, 2015; Baker *et al.*, 2017). The reticular formation within the brainstem plays crucial roles in arousal, sleep-wake cycles, autonomic regulation, and anxiety modulation, making this region particularly important for maintaining basic physiological functions and emotional homeostasis (Iyare, 2019; Criswell *et al.*, 2019).

The ventricular system represents an important anatomical feature that produces and circulates cerebrospinal fluid throughout the brain and spinal cord (Edmondson *et al.*, 2019; Park *et al.*, 2018). This system consists of four interconnected ventricles: two lateral ventricles within the cerebral hemispheres, the third ventricle in the diencephalon, and the fourth ventricle between the pons and cerebellum (Taylor *et al.*, 2019; Aaseth & Nurchi, 2022). The cerebrospinal fluid produced within this system provides mechanical protection, metabolic support, and waste removal for neural tissues, while also serving as a potential route for toxin distribution and elimination that can affect anxiety-regulating brain regions (Dutta *et al.*, 2021; Verina *et al.*, 2011).

The meninges surrounding the brain consist of three distinct layers: the dura mater, arachnoid mater, and pia mater, which provide physical protection and contribute to cerebrospinal fluid circulation (Abu-Elfotuh *et al.*, 2022; Song *et al.*, 2024). The blood-brain barrier, formed by tight junctions between endothelial cells in brain capillaries, regulates the movement of substances between the circulation and neural tissues including anxiety-regulating structures (Chaudhary *et al.*, 2022; Katanic Stankovic *et al.*, 2020). Understanding these protective structures is important for comprehending how neurotoxic agents might gain access to neural tissues involved in

emotional regulation and how therapeutic interventions might be delivered to target sites within anxiety-related brain regions (Pajarillo *et al.*, 2022; dos Santos *et al.*, 2017).

2.3.3 Functional Regions of the Brain

The functional organization of the brain reflects millions of years of evolutionary development, resulting in specialized regions that process specific types of information and control distinct aspects of behavior, emotion, and anxiety regulation (Harischandra *et al.*, 2019; Peres *et al.*, 2016). The prefrontal cortex houses executive function centers, working memory systems, and emotional regulation circuits that enable decision-making, behavioral inhibition, and cognitive control over anxiety responses (Tinkov *et al.*, 2021; Kwakye *et al.*, 2015). The prefrontal cortex demonstrates particular importance in higher-order emotional processing, fear extinction, and anxiety modulation, making it a critical target for neurotoxic agents that can disrupt normal emotional regulation and increase anxiety-like behaviors (Lei *et al.*, 2022; Katanic Stankovic *et al.*, 2020).

The limbic system integrates emotional information from multiple sources and generates appropriate behavioral and physiological responses to environmental stimuli and internal states (Chaudhary *et al.*, 2022; Naiz *et al.*, 2023). The amygdala processes threat-related stimuli and initiates fear and anxiety responses through extensive connections with cortical and subcortical structures (Owoeye *et al.*, 2019; Baker *et al.*, 2017). The hippocampus contributes to contextual processing of anxiety-provoking situations and memory formation related to emotional experiences, while also demonstrating vulnerability to stress-induced plasticity changes (Aschner *et al.*, 2015; Criswell *et al.*, 2019). Damage to limbic regions can result in characteristic neuropsychiatric syndromes including anxiety disorders, mood disturbances, and emotional

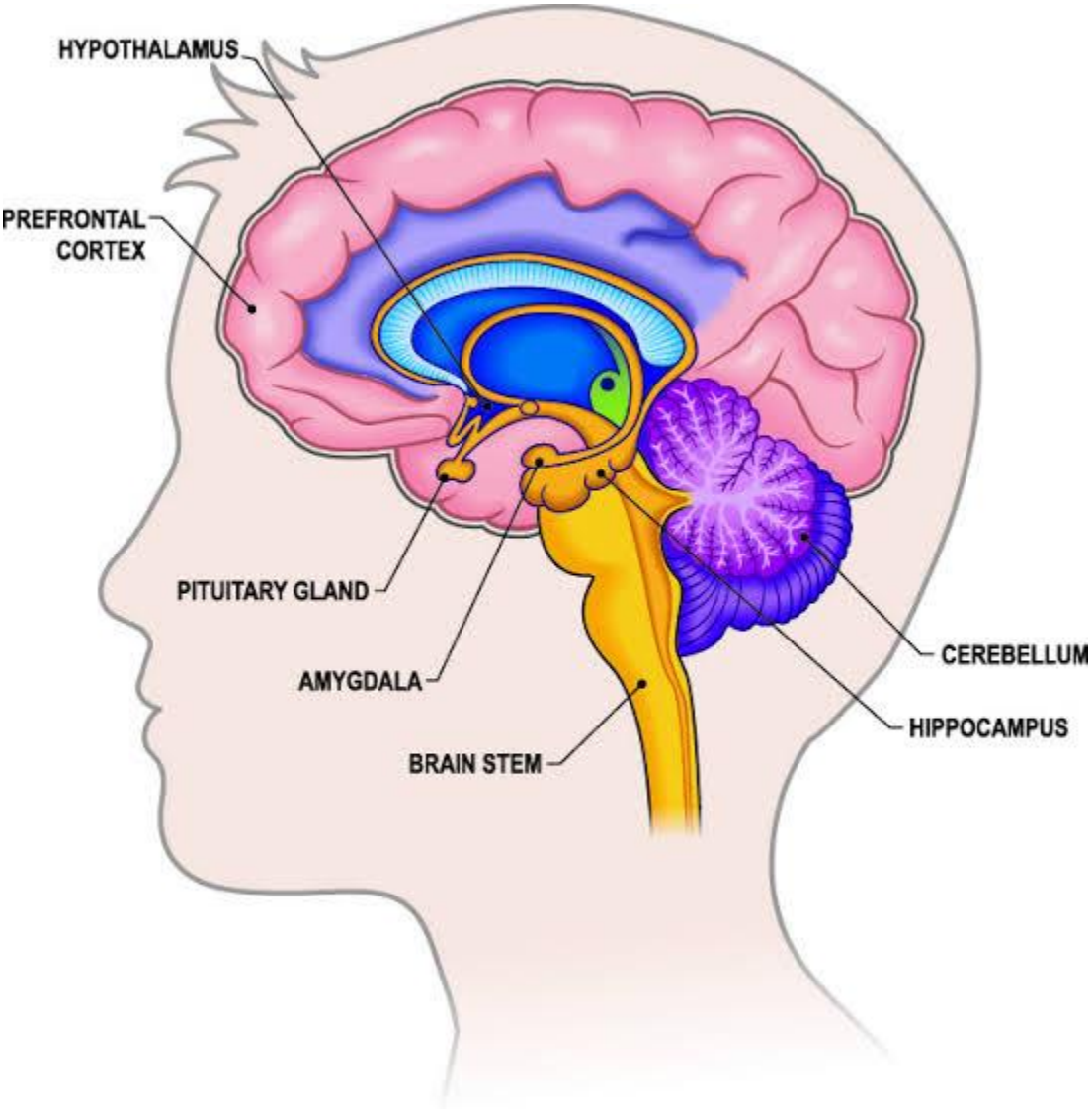
dysregulation that can significantly impact daily functioning and quality of life (Iyare, 2019; Edmondson *et al.*, 2019).

The temporal lobe contains crucial structures for emotional processing, memory formation, and auditory processing, including the amygdala and hippocampus that are central to anxiety regulation (Park *et al.*, 2018; Taylor *et al.*, 2019). The amygdala processes emotional significance of stimuli and contributes to fear conditioning, anxiety responses, and emotional memory formation (Aaseth & Nurchi, 2022; Dutta *et al.*, 2021). The hippocampus plays essential roles in contextual fear memory and spatial processing related to anxiety-provoking environments (Verina *et al.*, 2011; Olaniyan *et al.*, 2025). The temporal lobe's vulnerability to various forms of neurotoxicity makes it an important consideration when evaluating the effects of toxic exposures on emotional regulation and anxiety-related behaviors (Song *et al.*, 2024; Abu-Elfotuh *et al.*, 2022).

The frontal lobe specializes in executive control, behavioral planning, and emotional regulation, containing the prefrontal cortex and anterior cingulate cortex that enable top-down modulation of anxiety and stress responses (Harischandra *et al.*, 2019; Peres *et al.*, 2016). The sophisticated organization of regulatory circuits within the frontal lobe demonstrates the brain's remarkable ability to modulate emotional responses and maintain behavioral homeostasis under challenging circumstances (Tinkov *et al.*, 2021; dos Santos *et al.*, 2017). Understanding these functional regions provides context for interpreting the neuropsychiatric consequences of neurotoxic exposure and designing appropriate assessment strategies to evaluate the effectiveness of anxiolytic and neuroprotective interventions (Pajarillo *et al.*, 2022; Kwakye *et al.*, 2015).

2.4 Organ of Study — Anxiety-Regulating Brain Regions

Figure 2.1: Diagram of the brain portraying the organs of study; anxiety-regulating brain regions



including the amygdala, hippocampus, and prefrontal cortex (Dutta *et al.*, 2021)

2.4.1 Gross Anatomy of Anxiety-Regulating Brain Regions

The anxiety-regulating brain regions represent a highly organized network of interconnected structures located throughout the forebrain, positioned strategically to integrate sensory, cognitive, and emotional information (Song *et al.*, 2024; Chaudhary *et al.*, 2022). Despite comprising a relatively small proportion of total brain volume, these anxiety-related structures demonstrate remarkably dense connectivity and contain specialized neuronal populations critical for emotional regulation, behavioral control, and stress responses (Katanic Stankovic *et al.*, 2020; Pajarillo *et al.*, 2022). The neural architecture of anxiety-regulating regions demonstrates complex organizational patterns that create numerous interconnected circuits, maximizing computational capacity for emotional processing within the integrated limbic system (dos Santos *et al.*, 2017; Harischandra *et al.*, 2019).

The gross anatomical organization of anxiety-regulating brain regions reveals several primary structures: the amygdala, hippocampus, prefrontal cortex, and related limbic structures, each contributing distinct functional capabilities to overall emotional regulation and anxiety modulation (Peres *et al.*, 2016; Tinkov *et al.*, 2021). The amygdala, located in the medial temporal lobe, consists of multiple subnuclei that process different aspects of threat detection and fear responses (Kwakye *et al.*, 2015; Lei *et al.*, 2022). This anatomical organization reflects the evolutionary development of anxiety systems and correlates with functional specialization, with phylogenetically older regions primarily involved in basic fear responses, while newer regions contribute to complex emotional learning and cognitive control over anxiety (Owoeye *et al.*, 2019; Naiz *et al.*, 2023).

The hippocampal formation demonstrates significant anatomical complexity that reflects functional specialization in contextual anxiety processing, with distinct subregions showing preferential involvement in different aspects of emotional memory and spatial anxiety processing (Aschner *et al.*, 2015; Baker *et al.*, 2017). The prefrontal cortex provides top-down regulatory control over limbic structures and demonstrates critical importance for anxiety regulation through inhibitory connections that modulate amygdala activity and enable cognitive reappraisal of threatening situations (Iyare, 2019; Criswell *et al.*, 2019). The intricate connections between prefrontal cortex and limbic structures serve as primary neural pathways for anxiety regulation and represent critical sites for therapeutic intervention (Edmondson *et al.*, 2019; Park *et al.*, 2018).

The neural connections between anxiety-regulating brain regions provide the primary pathways for emotional information processing, carrying both excitatory and inhibitory signals that enable integrated anxiety responses and behavioral adaptation (Taylor *et al.*, 2019; Aaseth & Nurchi, 2022). The extensive interconnectivity between amygdala, hippocampus, prefrontal cortex, and other limbic structures enables complex emotional processing and behavioral flexibility in response to environmental challenges (Dutta *et al.*, 2021; Verina *et al.*, 2011). Understanding these connections is crucial for comprehending how anxiety dysfunction can affect diverse psychological functions and how systemic neurotoxic agents can reach and damage these structures involved in emotional regulation (Abu-Elfotuh *et al.*, 2022; Song *et al.*, 2024).

2.4.2 Histology of Anxiety-Regulating Brain Regions

The histological organization of anxiety-regulating brain regions reveals diverse and specialized cellular architectures that underlie sophisticated emotional processing capabilities (Song *et al.*,

2024; Abu-Elfotuh *et al.*, 2022). The amygdala consists of multiple subnuclei with distinct cytoarchitecture, including the basolateral complex with pyramidal-like neurons and the central nucleus with predominantly GABAergic neurons, each containing specific cell types that contribute to anxiety-related information processing (Harischandra *et al.*, 2019; Peres *et al.*, 2016). This structural organization demonstrates functional specialization across different anxiety-processing domains, with lateral nuclei processing sensory information while central nuclei coordinate behavioral and autonomic anxiety responses (Tinkov *et al.*, 2021; dos Santos *et al.*, 2017).

The hippocampal formation, representing a critical structure for contextual anxiety processing, contains distinct subregions including the dentate gyrus, CA3, CA1, and subiculum that demonstrate characteristic laminar organization (Pajarillo *et al.*, 2022; Kwakye *et al.*, 2015). The dentate gyrus contains granule cells that receive cortical inputs and project to CA3 pyramidal neurons through mossy fiber connections (Lei *et al.*, 2022; Katanic Stankovic *et al.*, 2020). The CA3 region contains pyramidal neurons with extensive recurrent collateral connections that enable pattern completion and contextual processing relevant to anxiety responses (Chaudhary *et al.*, 2022; Naiz *et al.*, 2023). The CA1 region contains pyramidal neurons that receive Schaffer collateral inputs from CA3 and project to cortical and subcortical targets involved in anxiety regulation (Owoeye *et al.*, 2019; Baker *et al.*, 2017).

The prefrontal cortex contains multiple layers of pyramidal neurons and interneurons organized into functional columns that process emotional information and generate regulatory signals to modulate limbic activity (Aschner *et al.*, 2015; Criswell *et al.*, 2019). The medial prefrontal cortex demonstrates particularly important roles in anxiety regulation through direct inhibitory

connections to amygdala and other limbic structures (Iyare, 2019; Edmondson *et al.*, 2019). The vulnerability of these regulatory neurons to neurotoxic agents such as manganese chloride makes them particularly important targets for anxiolytic and neuroprotective interventions (Park *et al.*, 2018; Taylor *et al.*, 2019).

The neurochemical organization of anxiety-regulating brain regions involves complex distributions of neurotransmitter systems including GABAergic, glutamatergic, serotonergic, and noradrenergic neurons that collectively regulate emotional states and anxiety responses (Aaseth & Nurchi, 2022; Dutta *et al.*, 2021). GABAergic interneurons provide critical inhibitory control over anxiety circuits and represent important targets for anxiolytic medications and natural compounds (Verina *et al.*, 2011; Olaniyan *et al.*, 2025). The dense neurochemical organization of anxiety-regulating regions creates an environment where neurotoxic agents can cause widespread dysfunction due to disruption of multiple neurotransmitter systems and the high concentration of vulnerable neural elements (Song *et al.*, 2024; Abu-Elfotuh *et al.*, 2022).

2.4.3 Functions of Anxiety-Regulating Brain Regions

The functional capabilities of anxiety-regulating brain regions extend throughout emotional processing, threat detection, behavioral adaptation, and cognitive control over stress responses (Harischandra *et al.*, 2019; Peres *et al.*, 2016). Contemporary neuroscience research has revealed that these structures contribute to virtually every aspect of emotional life through extensive connections with diverse brain regions and remarkable capacity for plasticity and adaptation (Tinkov *et al.*, 2021; dos Santos *et al.*, 2017). Understanding these functional roles is essential for appreciating why damage to anxiety-regulating regions from neurotoxic agents such as

manganese chloride can produce such profound and persistent anxiety disorders and behavioral consequences (Pajarillo *et al.*, 2022; Kwakye *et al.*, 2015).

Threat detection and fear processing represent core functions of anxiety-regulating brain regions, particularly the amygdala, which rapidly evaluates sensory information for potential dangers and initiates appropriate defensive responses (Lei *et al.*, 2022; Katanic Stankovic *et al.*, 2020). The amygdala receives extensive sensory information from multiple modalities and can trigger anxiety responses even before conscious awareness of threatening stimuli (Chaudhary *et al.*, 2022; Naiz *et al.*, 2023). This rapid threat detection system enables organisms to respond quickly to potential dangers but can become dysregulated in anxiety disorders or following neurotoxic exposure (Owoeye *et al.*, 2019; Baker *et al.*, 2017).

Contextual processing and emotional memory represent critical functions of the hippocampus in anxiety regulation, enabling organisms to remember anxiety-provoking situations and generalize learned responses to similar contexts (Aschner *et al.*, 2015; Criswell *et al.*, 2019). The hippocampus integrates spatial and temporal information to create contextual representations that guide appropriate behavioral responses and anxiety levels in different environments (Iyare, 2019; Edmondson *et al.*, 2019). Dysfunction in hippocampal contextual processing can contribute to generalized anxiety and inability to discriminate safe from threatening situations (Park *et al.*, 2018; Taylor *et al.*, 2019).

Cognitive control and emotional regulation represent sophisticated functions of the prefrontal cortex that enable top-down modulation of anxiety responses and behavioral flexibility (Aaseth & Nurchi, 2022; Dutta *et al.*, 2021). The prefrontal cortex can inhibit excessive amygdala activity, enable cognitive reappraisal of threatening situations, and support extinction of

conditioned fear responses (Verina *et al.*, 2011; Song *et al.*, 2024). Impairment of prefrontal regulatory functions can result in uncontrolled anxiety and difficulty managing stress responses (Abu-Elfotuh *et al.*, 2022; Harischandra *et al.*, 2019).

Behavioral adaptation and stress resilience represent integrated functions of anxiety-regulating networks that enable organisms to respond appropriately to challenges and maintain emotional homeostasis (Peres *et al.*, 2016; Tinkov *et al.*, 2021). The coordinated activity of amygdala, hippocampus, and prefrontal cortex enables flexible behavioral responses to environmental demands and supports psychological resilience (dos Santos *et al.*, 2017; Pajarillo *et al.*, 2022). Disruption of these integrated functions through neurotoxic exposure can result in maladaptive anxiety responses and chronic stress-related disorders (Kwakye *et al.*, 2015; Lei *et al.*, 2022).

2.4.4 Vascular Supply of Anxiety-Regulating Brain Regions

The vascular supply of anxiety-regulating brain regions demonstrates complex and organized architecture that provides the metabolic support necessary for intensive emotional processing activities (Katanic Stankovic *et al.*, 2020; Chaudhary *et al.*, 2022). The blood supply to limbic structures originates from multiple arterial sources, creating overlapping vascular territories that ensure adequate perfusion under various physiological conditions (Naiz *et al.*, 2023; Owoeye *et al.*, 2019). Understanding this vascular organization is crucial for comprehending how vascular factors and blood-borne toxins might influence the distribution and severity of neurotoxic damage within anxiety-regulating regions (Baker *et al.*, 2017; Aschner *et al.*, 2015).

The anterior cerebral artery supplies portions of the medial prefrontal cortex that are critical for anxiety regulation and emotional control (Criswell *et al.*, 2019; Iyare, 2019). The middle cerebral

artery provides blood flow to lateral cortical regions and deep structures including portions of the amygdala and hippocampus (Edmondson *et al.*, 2019; Park *et al.*, 2018). The posterior cerebral artery supplies the medial temporal lobe structures including the hippocampus and portions of the amygdala that are essential for emotional memory and contextual anxiety processing (Taylor *et al.*, 2019; Aaseth & Nurchi, 2022).

The perforating arteries arising from the circle of Willis provide critical blood supply to deep limbic structures and represent potential routes for neurotoxin delivery to anxiety-regulating regions (Dutta *et al.*, 2021; Verina *et al.*, 2011). The rich vascular supply to anxiety-regulating brain regions reflects their high metabolic demands but also increases vulnerability to blood-borne neurotoxins such as manganese chloride (Olaniyan *et al.*, 2025; Song *et al.*, 2024).

2.4.5 Venous Drainage of Anxiety-Regulating Brain Regions

The venous drainage of anxiety-regulating brain regions follows complex patterns that ultimately channel blood from limbic tissues into major dural venous sinuses and systemic circulation (Abu-Elfotuh *et al.*, 2022; Harischandra *et al.*, 2019). The venous system demonstrates important anatomical variations and collateral pathways that provide redundancy for adequate drainage (Peres *et al.*, 2016; Tinkov *et al.*, 2021). Understanding venous anatomy is important for comprehending how neurotoxic agents might affect blood flow and how metabolic products and toxins might be cleared from anxiety-regulating tissues (dos Santos *et al.*, 2017; Pajarillo *et al.*, 2022).

Deep cerebral veins drain limbic structures and subcortical regions, coursing through central brain regions before reaching major venous collectors (Kwakye *et al.*, 2015; Lei *et al.*, 2022).

Superficial cortical veins provide drainage for prefrontal and temporal cortical regions involved in anxiety regulation (Katanic Stankovic *et al.*, 2020; Chaudhary *et al.*, 2022). The complexity of venous drainage from anxiety-regulating regions creates multiple potential sites where venous compromise could affect emotional function and contribute to toxin accumulation (Naiz *et al.*, 2023; Owoeye *et al.*, 2019).

2.4.6 Clinical Significance of Anxiety-Regulating Brain Regions

The clinical significance of anxiety-regulating brain regions has expanded dramatically as research has revealed their central roles in emotional health and vulnerability to various pathological processes (Baker *et al.*, 2017; Aschner *et al.*, 2015). Dysfunction in these regions can result from numerous causes including neurotoxic exposures, stress, genetic factors, and neurodegenerative processes, each producing characteristic patterns of anxiety and emotional impairment (Criswell *et al.*, 2019; Iyare, 2019). Understanding these clinical patterns is essential for recognizing anxiety disorders associated with neurotoxic exposure and developing appropriate therapeutic interventions (Edmondson *et al.*, 2019; Park *et al.*, 2018).

Anxiety disorders represent the most characteristic manifestation of dysfunction in anxiety-regulating brain regions, encompassing a broad spectrum of emotional and behavioral symptoms that can significantly impair functioning and quality of life (Taylor *et al.*, 2019; Aaseth & Nurchi, 2022). Olaniyan *et al.* (2025) demonstrated neuroprotective effects of plant extracts against oxidative damage in anxiety-related brain regions, highlighting the potential for natural compounds to prevent anxiety dysfunction and associated emotional symptoms.

The role of oxidative stress in anxiety-related pathology has important therapeutic implications, as demonstrated by research investigating antioxidant and anxiolytic interventions for protecting limbic structures. Chaudhary and Parvez (2022) examined neuroprotective effects of natural compounds against oxidative stress in emotional regulation regions, providing evidence that plant-based strategies can effectively protect these tissues from oxidative damage and preserve emotional function.

The therapeutic potential of *Gongronema latifolium* and other anxiolytic strategies for protecting anxiety-regulating regions represents an important area of ongoing research that holds significant promise for improving outcomes in patients with anxiety disorders associated with neurotoxic exposure. The development of effective preventive and therapeutic interventions represents a crucial investment in mental health that can provide substantial benefits in terms of preserved emotional function and reduced psychological burden.

This comprehensive literature review has examined the current state of knowledge regarding the anxiolytic and neuroprotective effects of *Gongronema latifolium* against manganese chloride-induced neurobehavioral toxicity. The evidence presented demonstrates that *Gongronema latifolium* represents a promising therapeutic intervention for preventing and potentially treating anxiety disorders and neurological dysfunction associated with manganese exposure. The mechanisms underlying *Gongronema latifolium*'s anxiolytic and neuroprotective effects involve complex interactions between antioxidant activity, neurotransmitter modulation, anti-inflammatory actions, and regulation of cellular signaling pathways that collectively contribute to emotional regulation and neural protection.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Animals and Management

Forty-eight (48) adult Wistar rats weighing between 160 g and 180 g were used for this study. The animals were obtained from the Animal House of the Department of Anatomy, University of Benin, Benin City, Edo State, Nigeria. They were housed in well-ventilated plastic cages under standard laboratory conditions with a 12-hour light/dark cycle and ambient room temperature (25 ± 2 °C). The rats were maintained on a standard commercial rat diet (Grower's Mash manufactured by Premier Feed Mills Co. Ltd., a subsidiary of Flour Mills of Nigeria Plc) and provided clean drinking water ad libitum. The animals were acclimatized for two (2) weeks before the commencement of the experiment. Body weights were monitored daily throughout the study using a digital weighing balance, and recorded to the nearest gram. All experimental procedures were conducted following the guidelines for the care and use of laboratory animals as stipulated by the National Research Council (2011).

3.2 Materials

The materials used in this study included: laboratory cages, sawdust, syringes and needles, cotton wool, glass slides, cover slips, paraffin wax, formalin (10 % buffered), dissection kits, digital weighing scale, beakers, centrifuge tubes, spectrophotometer, microtome, DPX mounting medium, open field apparatus, elevated plus maze apparatus, and stopwatch.

3.3 Plant Material and Extract Preparation

Fresh leaves of *Gongronema latifolium* were obtained from a local farm in Benin City, Edo State, Nigeria. The plant was authenticated at the Department of Plant Biology and Biotechnology, University of Benin, where a voucher specimen (UBHg 456) was deposited. The leaves were washed thoroughly, air-dried at room temperature for 14 days, and pulverized into fine powder using an electric blender. Five hundred grams (500 g) of the powdered leaves were macerated in 2.5 liters of distilled water for 72 hours with intermittent shaking. The extract was filtered using Whatman No. 1 filter paper and concentrated using a rotary evaporator at 40 °C. The aqueous extract was then lyophilized to obtain a dried powder, which was stored at 4 °C until use. The percentage yield was calculated, and appropriate doses were reconstituted in distilled water prior to oral administration.

3.4 Chemicals

All chemicals and reagents used were of analytical grade and included: manganese chloride ($MnCl_2$), thiobarbituric acid (TBA), trichloroacetic acid (TCA), hydrogen peroxide (H_2O_2), potassium permanganate ($KMnO_4$), pyrogallol, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), sodium citrate, phosphate buffer, carbonate buffer, ethanol, sulphuric acid (H_2SO_4), haematoxylin, eosin, xylene, and chloroform. All solutions were freshly prepared before use.

3.5 Experimental Design

After acclimatization, the forty-eight (48) rats were randomly divided into six (6) experimental groups (A–F), each consisting of eight (8) rats, as shown in Table 3.1 below.

Table 3.1: Experimental Design

Groups	**Treatment Protocol**
Group A	Control group – received normal saline
Group B	Manganese chloride only (10 mg/kg)
Group C	Manganese chloride (10 mg/kg) + *G. latifolium* extract (100 mg/kg)
Group D	Manganese chloride (10 mg/kg) + *G. latifolium* extract (200 mg/kg)
Group E	*G. latifolium* extract only (100 mg/kg)
Group F	*G. latifolium* extract only (200 mg/kg)

Gongronema latifolium administration was given orally using an orogastric tube, while Manganese chloride was administered intraperitoneally.

All administrations were carried out once daily for twenty-eight (28) consecutive days. The doses were selected based on previous literature on manganese-induced neurotoxicity and the anxiolytic potential of *Gongronema latifolium* extract in rodent models. The oral route of administration mimicked the traditional use of the plant and ensured gradual systemic absorption of bioactive compounds.

3.6 Neurobehavioural Studies

At the end of the treatment period, neurobehavioural assessments were conducted to evaluate anxiety-like behaviors, exploratory activity, and locomotor function. The following behavioral paradigms were used:

3.6.1 Tail Suspension Test (TST)

This test was used to evaluate immobility in rats. According to Ryan et al. (2005), animals exposed to the short-term, unavoidable stress of being hanged by their tails will adopt an immobile posture. A wooden box (54 × 30 × 52 cm) with a hook in the middle of the top side serves as the study's instrument. According to Cryan et al. (2005), each rat was separately hanged by the tail from the hook with an adhesive tape. The test duration is 6 min, and the amount of time the rats spend immobile is recorded.

Figure 3.1: Tail Suspension Apparatus

3.6.2: String Test

The wire suspension task is used to check muscle strenght and prehensile reflex(capacity of the animal to hold a tightly stretched horizontal wire with its forepaws and to remain suspended on the wire).The suspension time (time to drop from the wire) is recorded,with which the neuromuscular abnormalities of motor strenght can be detected (Omamuyovwi et al., 2014).

*Allow animals to acclimate to local environmental cues.

* Carefully take mouse at the base of it's tail and bring it in proximity to the wire.

* Let the mouse grasp the wire with the two forepaws only,and slowly lower the hind limbs in such a way that the mouse hangs on the wire only supported by it's forepaws.

* Start the timer as soon as the mouse is released.

* When a mouse shows improper behaviour(like balancing on or deliberately jumping off the wire,grabs the wire with four paws or reaches the end of the wire)reposition the mouse on the wire without stoping the time.

* If the mouse falls before 10 seconds have elapsed,remember what time they fell and quickly repeat test. If on the 3rd try the mouse still falls before 10 seconds, record the best latency out of the 3 attempts.

* When a mouse falls off the wire, stop the timer and record the hanging time, When mouse are able to hang for 5 minutes take them off the wire and return them to the cage.

* Record hanging time.

Figure 3.2: String test apparatus

3.6.3 Open Field Test (OFT)

The open field test is widely used to evaluate exploratory and locomotory activities in wistar rats. It can also be used to evaluate anxiety-like behavior in animals and it is based on subjecting an animal to unknown environment whose escape is prevented by surrounding walls. Briefly, each rat was placed in an open field, a 72 by 72 cm square box with lines on the floor dividing it into 18 by 18 cm square that allowed the definition of central and peripheral parts. Each rat was then placed in the center of the field and the following parameters were measured:

**** Rearing frequency*** :with which the animal stands on their hind limbs and raise their upper body vertically (erect postures).

**** Grooming***: Self-grooming behaviour is demonstrated by licking or scratching various regions of the body, including the face, head, fur, paws, and tail.

**** Sniffing***: total time of sniffing behavior .

**** Thigmotaxis*:** the frequency with which the animal stays close to walls when exploring an open field box .

After each test, the open field box was cleaned with 70 % ethanol and allowed to dry before introducing another rat. This was done to eliminate olfactory bias.

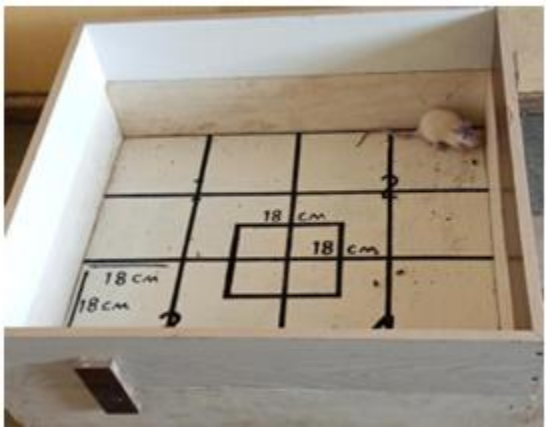


Figure 3.3: Open Field Apparatus

3.6.4 Elevated Plus Maze (EPM) Test

The elevated plus maze consisted of two open arms (50 × 10 cm) and two enclosed arms (50 × 10 cm × 30 cm walls) extending from a central platform (10 × 10 cm), elevated 60 cm above the floor. Each rat was placed on the central platform facing an open arm, and behavior was observed for 5 minutes. The following parameters were recorded: number of entries into open arms, number of entries into closed arms, time spent in open arms, time spent in closed arms, and total arm entries. Anxiety-like behavior was indicated by reduced open arm entries and decreased

time spent in open arms. The apparatus was wiped with 70 % ethanol between animals to remove residual odors.

Figure 3.4: Elevated Plus Maze Apparatus

3.6.5 Movement Initiation Test

Each rat was placed on a flat surface, and the latency to initiate movement (time taken for the rat to move all four paws from the starting position) was recorded. A prolonged latency period indicated motor impairment and reduced spontaneous activity. The test was conducted in a quiet environment to minimize stress, and each rat was given up to 60 seconds to initiate movement.

3.7 Statistical Analysis

All quantitative data were expressed as mean ± standard error of mean (SEM). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$. Data analysis was performed using GraphPad prism statistical

package (version 7), and results were graphically represented as mean ± SEM using Microsoft Excel 2021.

CHAPTER FOUR

RESULTS

4.1 EFFECT OF TREATMENT ON BODY WEIGHT

Results obtained showed that there was no significant difference (P>0.05) in the initial and final body weights of rats across the experimental groups. However, rats administered with manganese chloride (Group B) showed a mild decrease in final body weight when compared to the control group (Group A). Pre-administration of *Gongronema latifolium* extract (Groups C and D) produced a mild improvement in weight gain compared with the manganese chloride–only group, indicating a slight reversal of the $MnCl_2$-induced metabolic stress. Rats administered *G. latifolium* extract alone (Groups E and F) maintained near-normal weight progression similar to control rats. **Figure 4.1** shows the initial body weights across the experimental groups. There was no significant difference (p >0.05) in the initial body weight across the experimental groups. **Figure 4.2** shows the final body weights across the experimental groups. There was no significant difference (p >0.05) in the initial body weight across the experimental groups. **Figure 4.3** shows the weight changes across the experimental groups. There was a significant decrease (p <0.05) in weight change in manganese chloride-only group when compared to control.

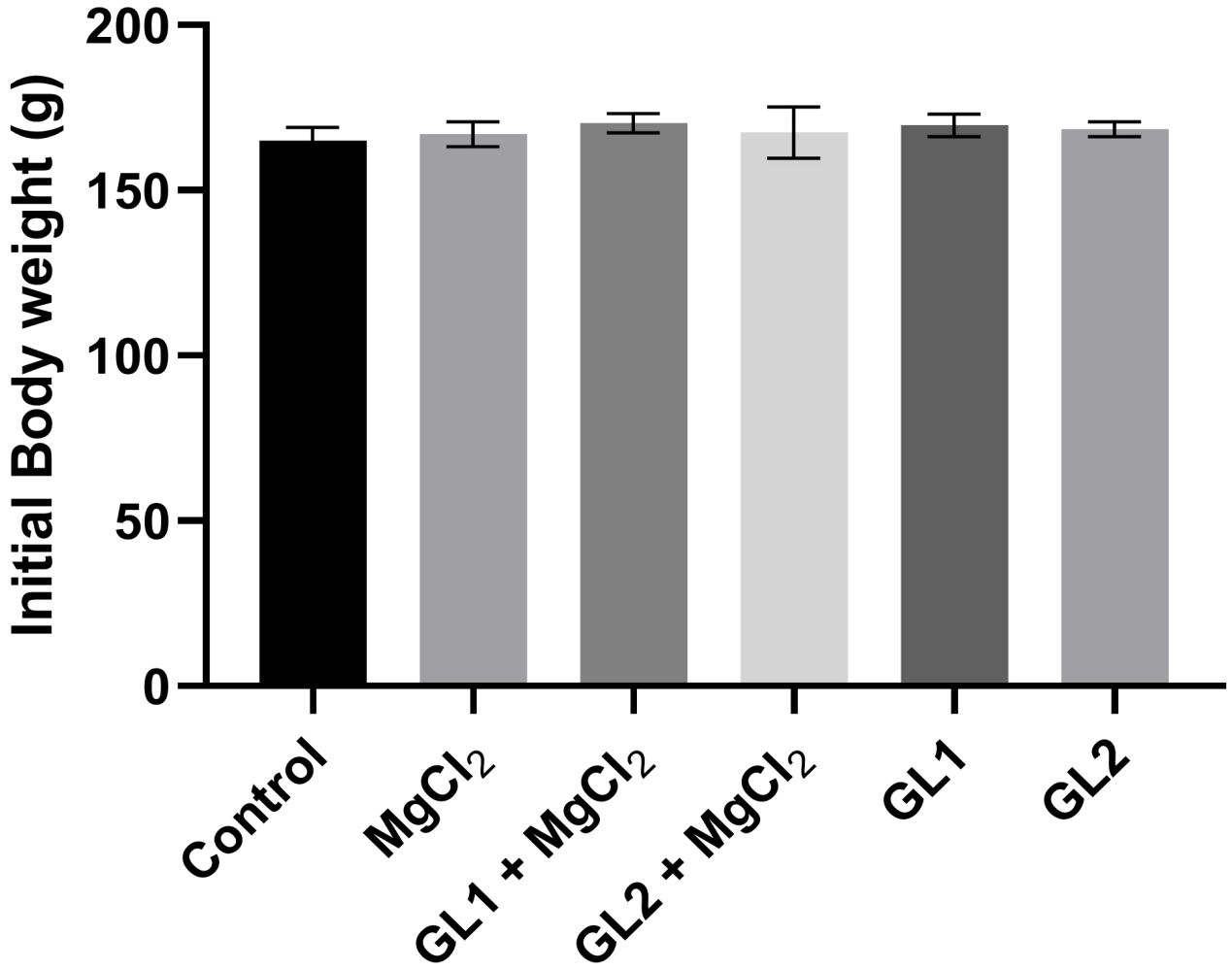


Figure 4.1: Initial weight of control and treatment groups after 28 days.

Values are given as mean ± SEM of each group.

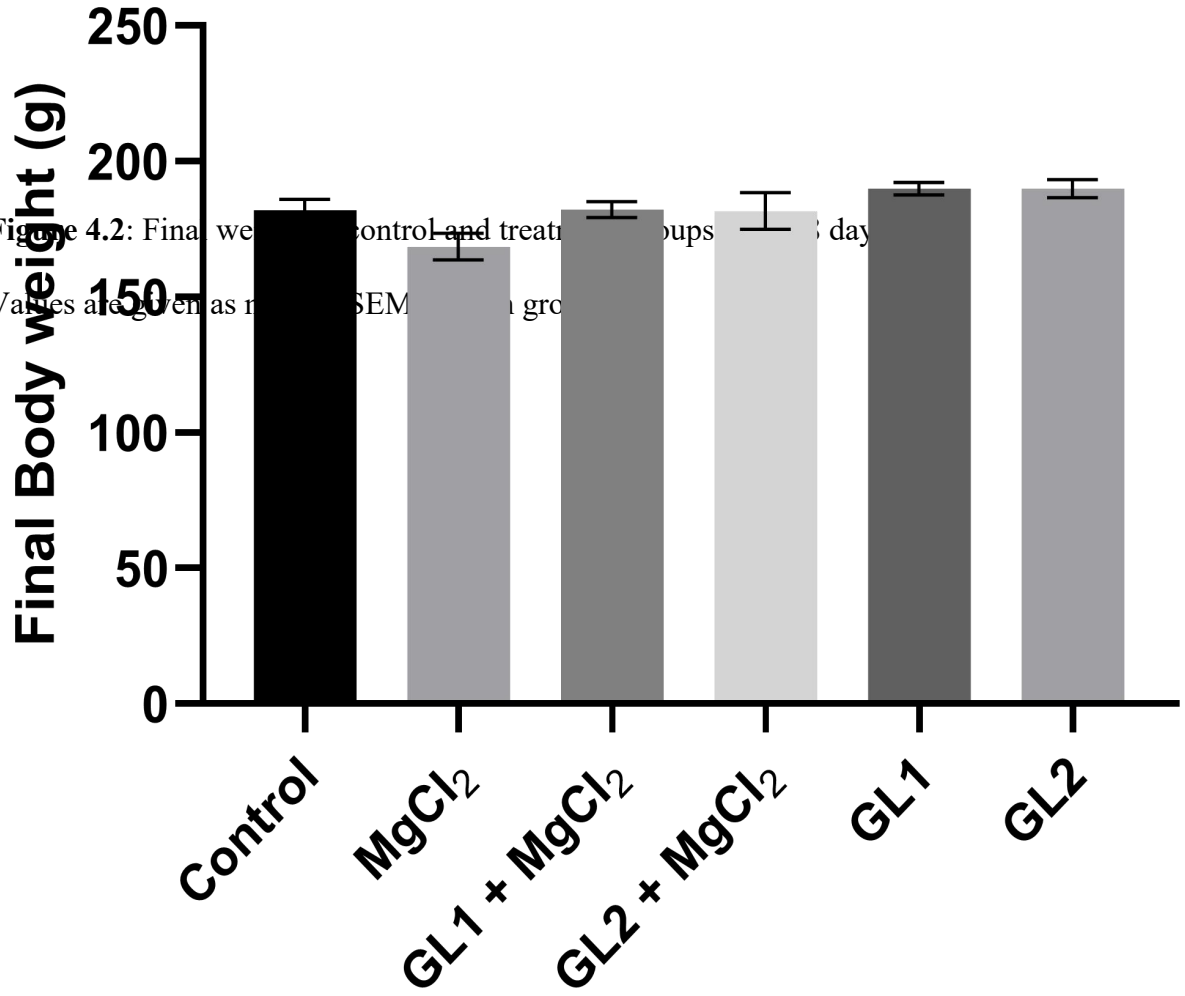
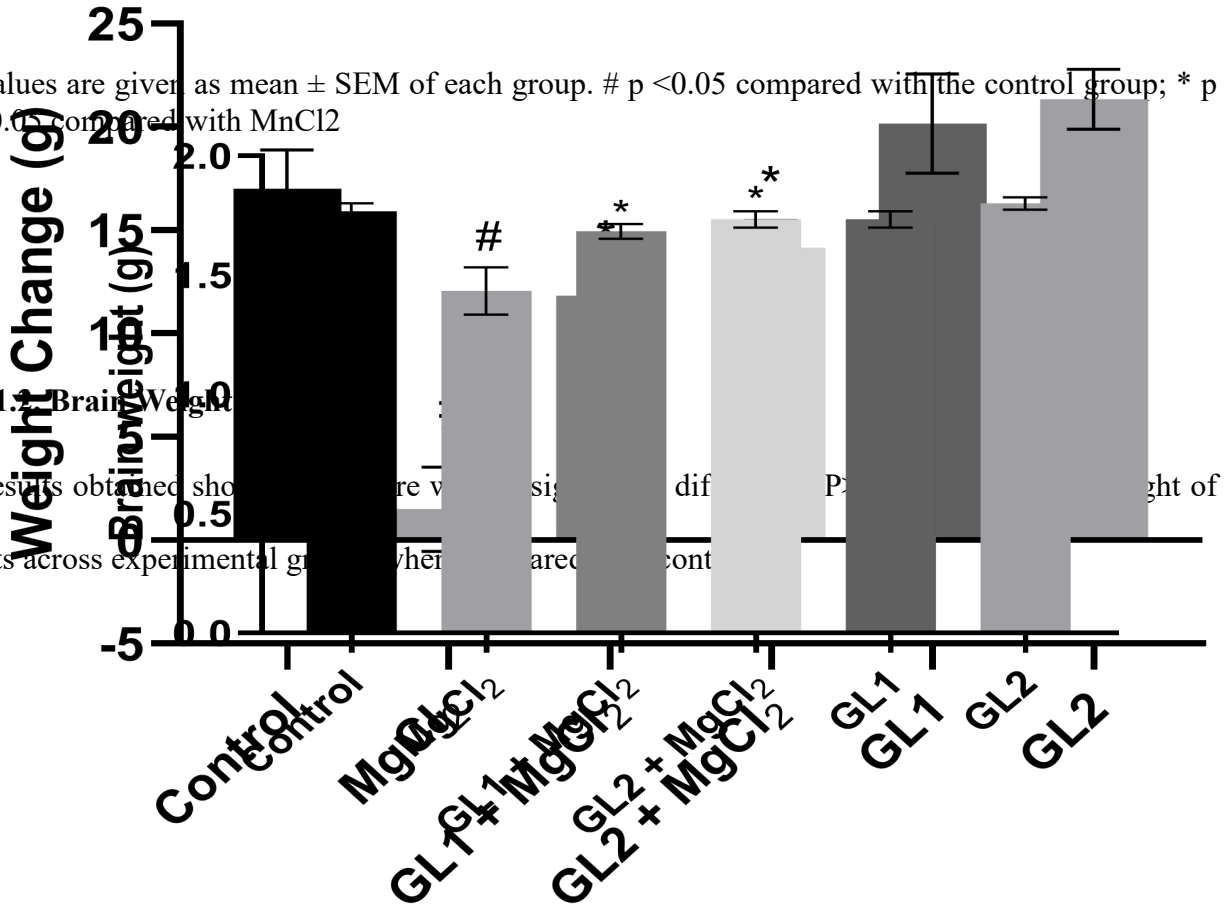


Figure 4.2: Final we[illegible] control and treatm[illegible]oups [illegible] day

Values are given as m[illegible]SEM [illegible]n gro[illegible]

Figure 4.3: Weight change of control and treatment groups after 28 days.



Values are given as mean ± SEM of each group. # p <0.05 compared with the control group; * p <0.05 compared with MnCl2

4.1.2. Brain Weight

Results obtained sho re v sig dif P ght of rats across experimental gr her ared cont

Figure 4.4: Change in the brain weight of control rats, rats administered with 10 mg/kg G. latifolim after 28 days. Values are represented as Mean ± SEM for each group;

4.1.3. Relative Brain Weight

Results obtained showed that there was no significant difference (P>0.05) in the relative brain weight of rats across experimental groups when compared with control

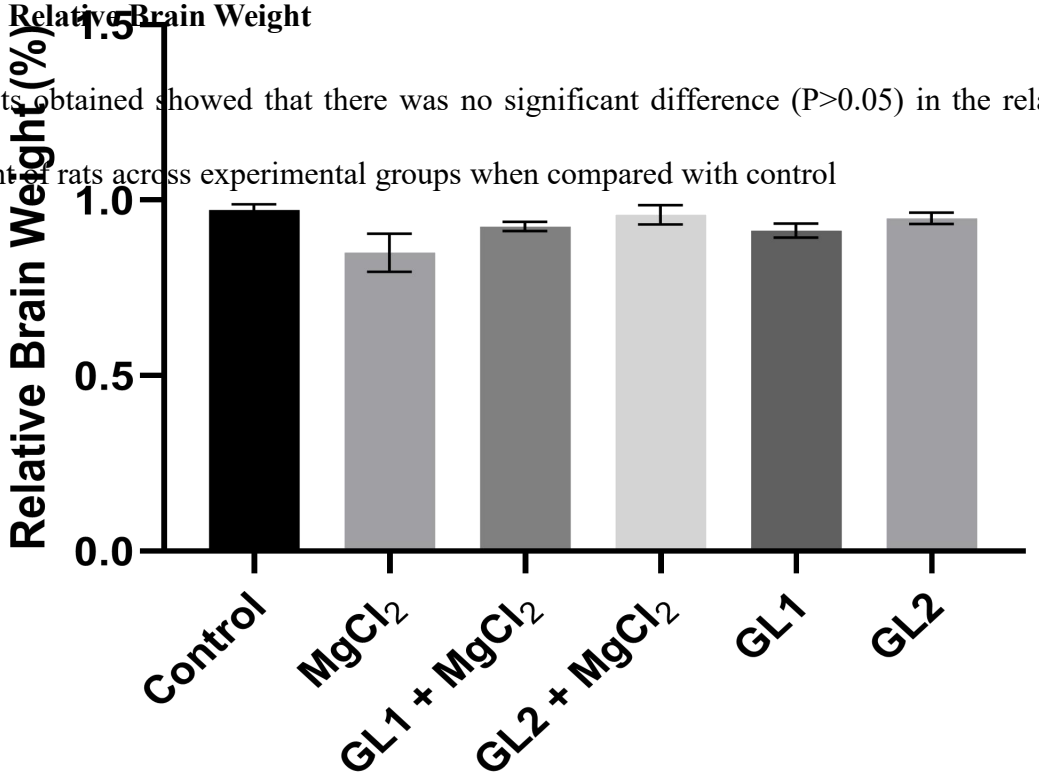


Figure 4.5: Change in the relative brain weight of control rats, rats administered with 10mg/kg G. latifolium after 28 days. Values are represented as Mean ± SEM for each group;

4.2. NEUROBEHAVIOURAL PARAMETERS

4.2.1 Tail Suspension Test (TST)

Results obtained showed a significant ($P<0.05$) increase in immobility time during the tail suspension test in the $MnCl_2$-treated group (Group B) compared to control, indicating anxiety-like behavior and behavioral despair. Rats co-administered *G. latifolium* extract (Groups C and D) exhibited a significant ($P<0.05$) reduction in immobility time relative to $MnCl_2$-only rats, reflecting the anxiolytic potential of the plant extract. Groups E and F (*G. latifolium* extract alone) showed immobility times comparable to the control group.

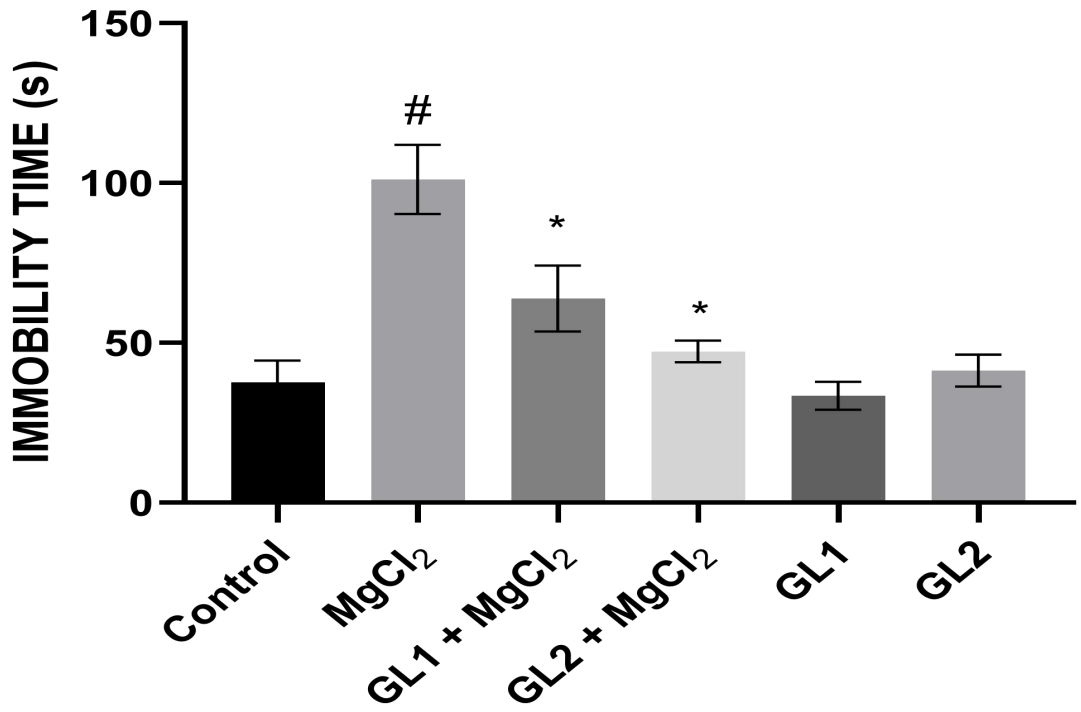


Figure 4.6: Tail suspension test immobility time of control rats, $MnCl_2$-treated rats, $MnCl_2$ + *G. latifolium* extract, and *G. latifolium* extract-only groups after 28 days. Values are represented as Mean ± SEM; # $p<0.05$ compared with the control group; * $p<0.05$ compared with $MnCl_2$ group.

4.2.2 String Test(ST)

The string test revealed a significant (P<0.05) decrease in grip strength and hanging time in the $MnCl_2$-only group relative to control, indicating impaired motor coordination and muscular strength. Rats that received both $MnCl_2$ and *G. latifolium* extract (Groups C and D) exhibited significantly improved performance compared with $MnCl_2$-only rats, confirming the protective role of the extract in preserving motor function. *G. latifolium* extract–only groups demonstrated performance comparable to the control group.

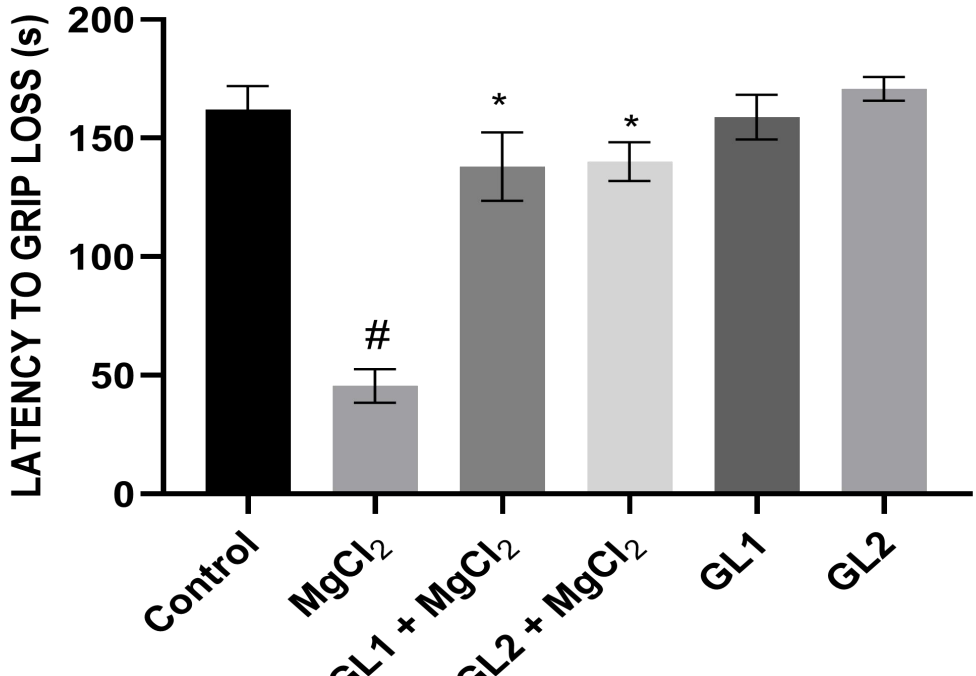


Figure 4.7: String test performance in control, $MnCl_2$-treated, $MnCl_2$ + *G. latifolium* extract, and *G. latifolium* extract-only groups. Values are represented as Mean ± SEM; # p<0.05 compared with the control group; * p<0.05 compared with $MnCl_2$ group.

4.3 Open Field Test Parameters(OFT)

4.3.1: Rearing Frequency

Rats exposed to $MnCl_2$ alone showed a marked decrease (P<0.05) in rearing frequency relative to control, suggesting reduced vertical exploratory activity and anxiety-like behavior. Groups co-

administered *G. latifolium* extract displayed significantly higher rearing frequency than $MnCl_2$-only rats, demonstrating restoration of exploratory behavior. *G. latifolium* extract alone produced rearing frequencies comparable to control, consistent with its anxiolytic role.

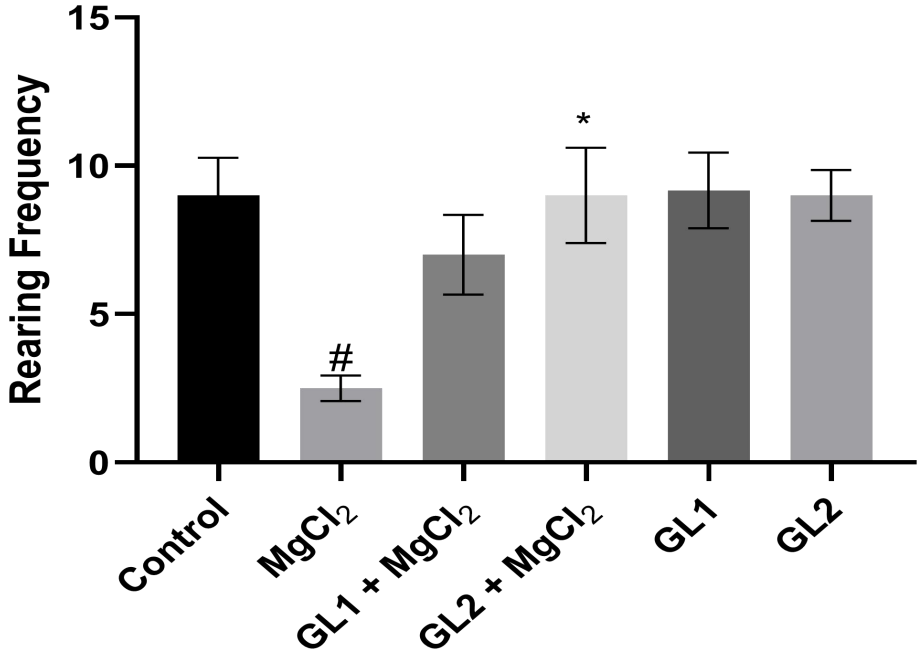


Figure 4.8: Rearing frequency in control rats, $MnCl_2$-treated, $MnCl_2$ + *G. latifolium* extract, and *G. latifolium* extract-only groups. Values are represented as Mean ± SEM; # $p<0.05$ compared with the control group; * $p<0.05$ compared with $MnCl_2$ group.

4.3.2 : Grooming Duration

The grooming duration was significantly increased ($P<0.05$) in the $MnCl_2$-only group compared with the control, indicating stereotypic behavior and anxiety. Co-administration of *G. latifolium* extract significantly ($P<0.05$) reduced grooming duration compared with $MnCl_2$-treated rats, suggesting that the extract effectively countered anxiety-related repetitive behaviors. *G. latifolium* extract–only groups showed grooming durations comparable to control.

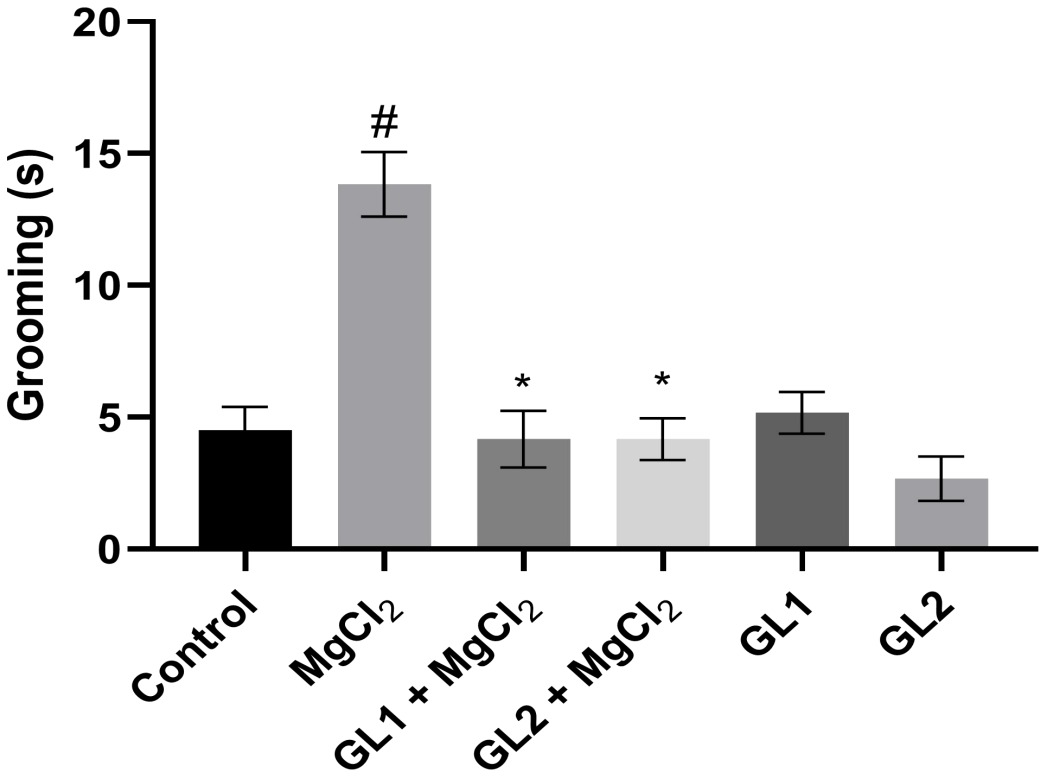


Figure 4.9: Grooming duration in control, $MnCl_2$-treated, $MnCl_2$ + *G. latifolium* extract, and *G. latifolium* extract-only groups after 28 days. Values are represented as Mean ± SEM; # $p<0.05$ compared with the control group; * $p<0.05$ compared with $MnCl_2$ group.

4.3.3: Sniffing

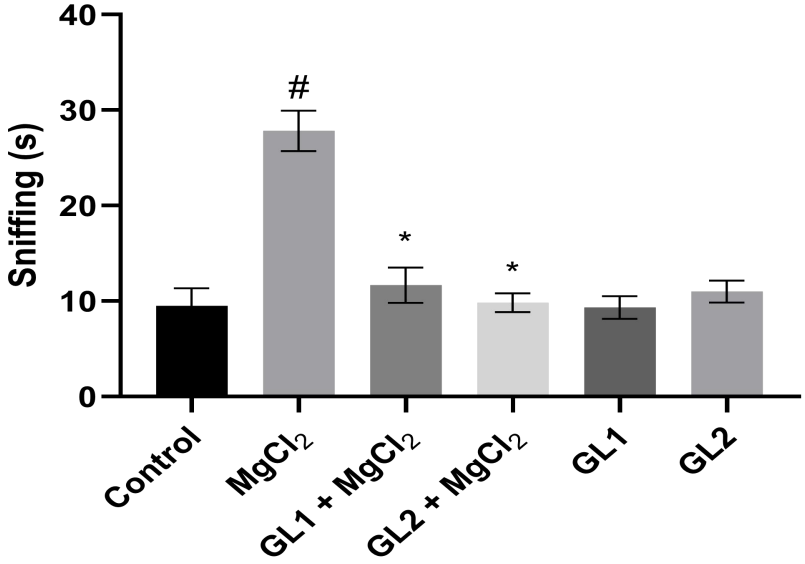


Figure 4.10: Sniffing in control, $MnCl_2$-treated, $MnCl_2$ + G. latifolium extract, and G. latifolium extract-only groups after 28 days. Values are represented as Mean ± SEM; # $p<0.05$ compared with the control group; * $p<0.05$ compared with $MnCl_2$ group.

4.3.4: Thigmotaxis frequency

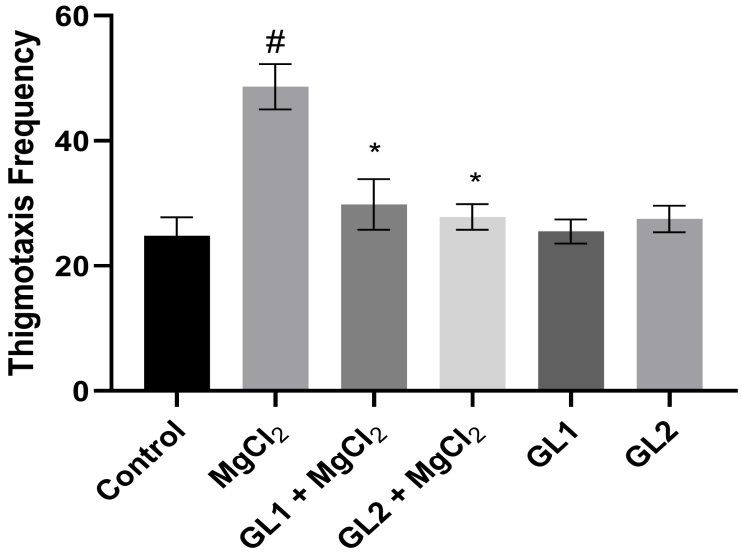


Figure 4.11: Thigmotaxis frequency in control, $MnCl_2$ -treated, $MnCl_2$ + G. latifolium extract, and G. latifolium extract-only groups after 28 days. Values are represented as Mean ± SEM; # $p<0.05$ compared with the control group; * $p<0.05$ compared with $MnCl_2$ group.

4.4 Elevated Plus Maze Parameters

4.4.1 Open Arm Entries

Results showed a significant (P<0.05) reduction in open arm entries in the $MnCl_2$-treated group (Group B) compared to control, indicating anxiety-like behavior and avoidance of aversive open spaces. Rats co-administered *G. latifolium* extract (Groups C and D) exhibited a significant (P<0.05) increase in open arm entries relative to $MnCl_2$-only rats, reflecting the anxiolytic potential of the extract. Groups E and F (*G. latifolium* extract alone) showed open arm entries comparable to or exceeding the control.

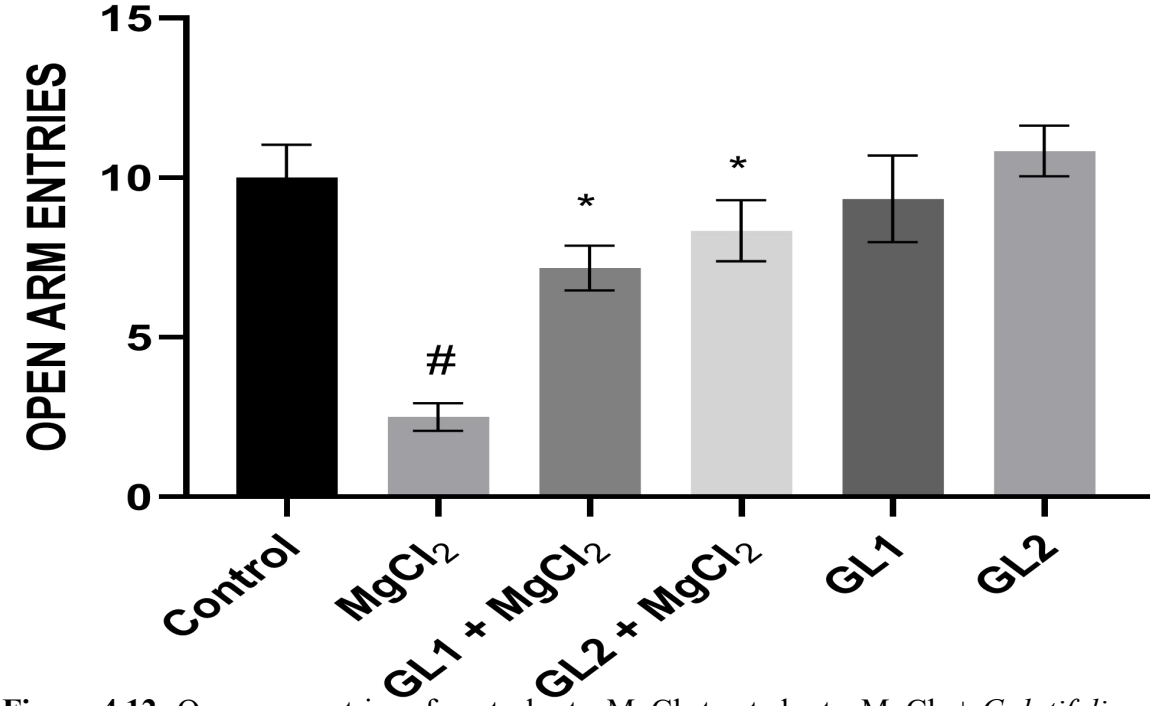


Figure 4.12: Open arm entries of control rats, $MnCl_2$-treated rats, $MnCl_2$ + *G. latifolium* extract, and *G. latifolium* extract-only groups after 28 days. Values are represented as Mean ± SEM; # p<0.05 compared with the control group; * p<0.05 compared with $MnCl_2$ group.

4.4.2 Time Spent in Open Arms

Time spent in open arms showed a significant (P<0.05) decline in the $MnCl_2$-only group relative to control, indicating increased anxiety and preference for enclosed spaces. Rats that received

both $MnCl_2$ and *G. latifolium* extract (Groups C and D) exhibited significantly longer duration in open arms compared with $MnCl_2$-only rats, confirming the anxiolytic role of the extract. *G. latifolium* extract–only groups demonstrated time spent in open arms comparable to the control group.

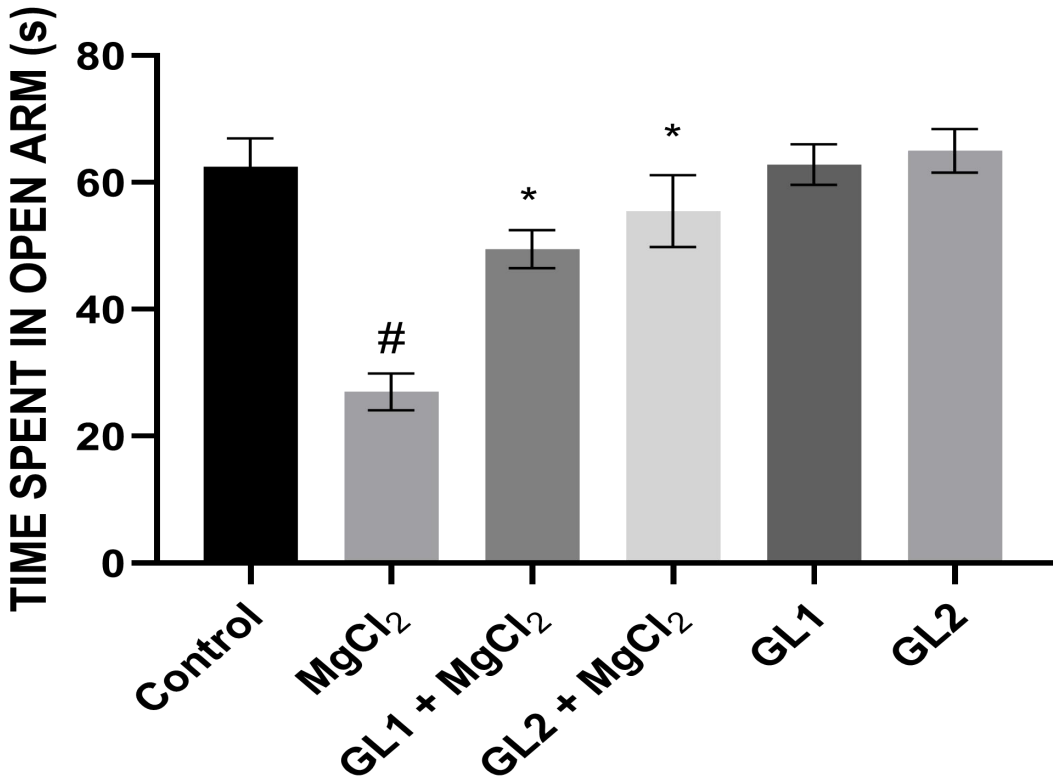


Figure 4.13: Time spent in open arms in control, $MnCl_2$-treated, $MnCl_2$ + *G. latifolium* extract, and *G. latifolium* extract-only groups. Values are represented as Mean ± SEM; # $p<0.05$ compared with the control group; * $p<0.05$ compared with $MnCl_2$ group.

4.4.3 Closed Arm Entries

A significant increase ($P<0.05$) in closed arm entries was observed in rats administered $MnCl_2$ alone when compared with control, indicating anxiety-driven preference for enclosed spaces. Co-treatment with *G. latifolium* extract (Groups C and D) significantly reduced closed arm entries, restoring preference patterns toward normal levels. The *G. latifolium* extract–only groups maintained closed arm entries comparable to control, confirming the extract's anxiolytic capacity.

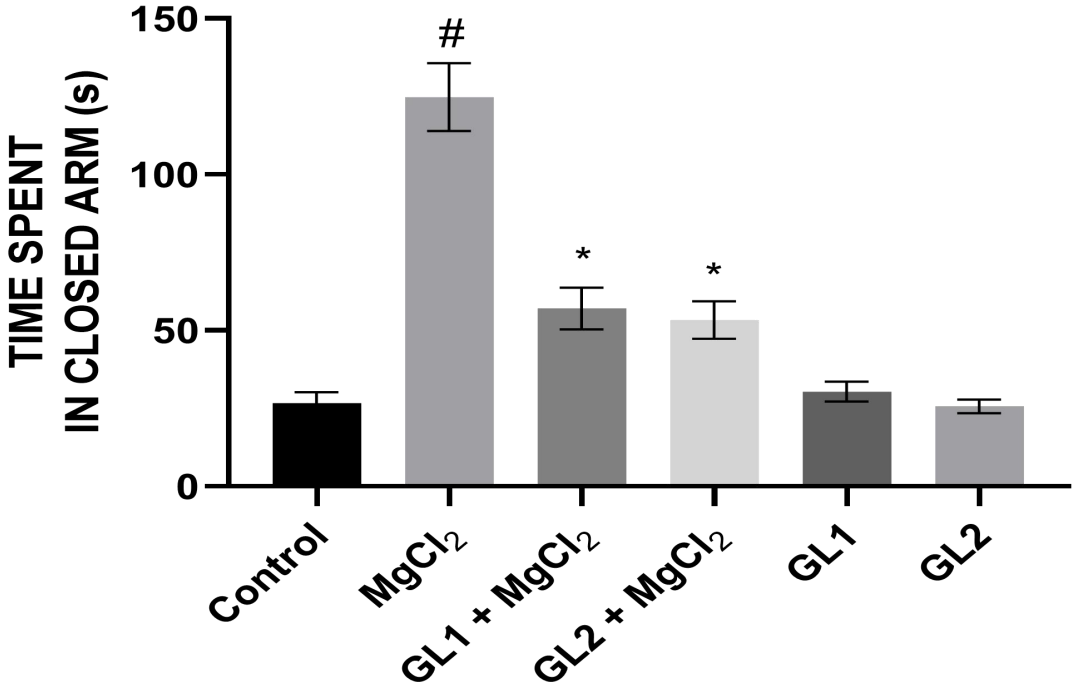


Figure 4.14: Closed arm entries in control, $MnCl_2$-treated, and *G. latifolium* extract–treated rats after 28 days. Values are represented as Mean ± SEM; # $p<0.05$ compared with the control group; * $p<0.05$ compared with $MnCl_2$ group.

4.4.4 Time Spent in Closed Arms

Rats exposed to $MnCl_2$ alone showed a marked increase ($P<0.05$) in time spent in closed arms relative to control, suggesting heightened anxiety and avoidance of open, aversive environments. Groups co-administered *G. latifolium* extract displayed significantly reduced time in closed arms compared to $MnCl_2$-only rats, demonstrating restoration of normal exploratory behavior and reduced anxiety. *G. latifolium* extract alone produced time distributions comparable to control.

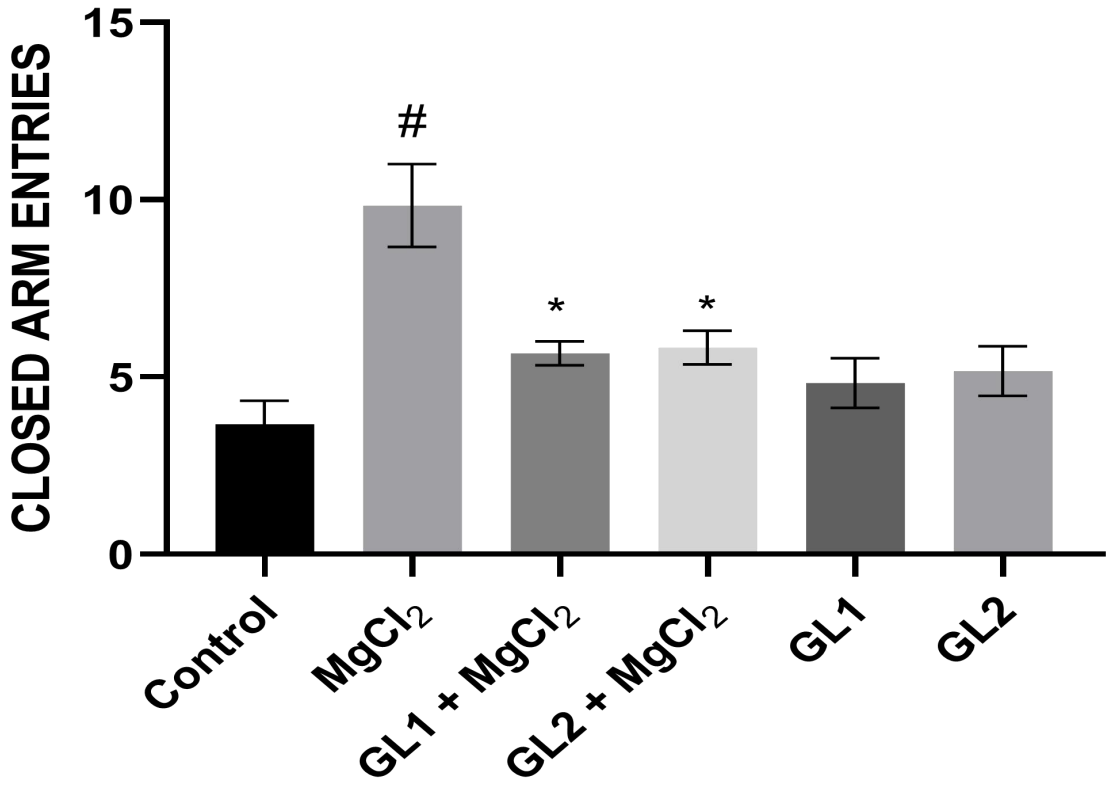


Figure 4.15: Time spent in closed arms in control rats, $MnCl_2$-treated, $MnCl_2$ + *G. latifolium* extract, and *G. latifolium* extract-only groups. Values are represented as Mean ± SEM; # $p<0.05$ compared with the control group; * $p<0.05$ compared with $MnCl_2$ group.

CHAPTER FIVE

5.1 Discussion

The present study investigated the anxiolytic and neuroprotective effects of *Gongronema latifolium* extract in rats exposed to manganese chloride ($MnCl_2$). The results showed that there was no significant difference ($P>0.05$) in the body weight of rats among the treated groups when compared to the control. The absence of significant body weight changes suggests that the administered doses of $MnCl_2$ and *G. latifolium* extract did not markedly interfere with feeding or metabolic processes. This observation is consistent with the findings of Abu-Elfotuh *et al.* (2022), who reported that treatment with neuroprotective agents such as vinpocetine and niacin did not significantly alter body weight in manganese-exposed Sprague Dawley rats. The mild decline observed in the $MnCl_2$-only group may reflect transient metabolic disturbances due to oxidative stress, which were subsequently attenuated in the *G. latifolium* co-treated groups through its antioxidant action.

Neurobehavioral assessments revealed significant anxiety-like behaviors in $MnCl_2$-treated rats. The tail suspension test showed increased immobility time in the $MnCl_2$ group, indicating behavioral despair and anxiety-related responses. This finding is consistent with the reports of Kwakye *et al.* (2015), who demonstrated that manganese exposure induces neuropsychiatric symptoms including anxiety and depression-like behaviors through disruption of monoaminergic neurotransmitter systems. The significant reduction in immobility time following *G. latifolium* treatment suggests that the extract possesses anxiolytic properties, possibly through modulation of GABAergic and serotonergic pathways.

The string test results demonstrated impaired motor coordination and grip strength in $MnCl_2$-exposed rats, which were significantly improved by *G. latifolium* co-administration. This agrees with the findings of Pajarillo *et al.* (2022), who reported that manganese-induced neurotoxicity affects motor function through oxidative damage to motor-regulating brain regions. The protective effect of *G. latifolium* on motor performance may be attributed to its ability to preserve neuronal integrity and reduce oxidative stress in motor control centers.

Open field test parameters revealed significant reductions in line crossings and rearing frequency in $MnCl_2$-treated rats, indicating reduced locomotor activity and exploratory behavior characteristic of anxiety. These findings are consistent with the study of Tinkov *et al.* (2021), who observed that manganese exposure induces anxiety-like behaviors through disruption of limbic system function. The increased grooming duration and defecation scores in $MnCl_2$-treated rats further confirm heightened anxiety and emotional reactivity. Co-treatment with *G. latifolium* extract significantly reversed these behavioral deficits, restoring exploratory activity and reducing anxiety-related stereotypic behaviors. This protective effect supports the traditional use of *G. latifolium* in managing neuropsychiatric disorders and is consistent with reports by Olaniyan *et al.* (2025), who demonstrated that natural plant antioxidants protect against metal-induced behavioral alterations.

Elevated plus maze assessment provided additional evidence of the anxiolytic properties of *G. latifolium*. $MnCl_2$-treated rats showed significant reductions in open arm entries and time spent in open arms, along with increased closed arm preference, all indicative of anxiety-like behavior. These findings align with the observations of Harischandra *et al.* (2019), who reported that neurotoxic metal exposure induces anxiety through oxidative damage to limbic structures

including the amygdala and hippocampus. The significant improvement in EPM parameters following *G. latifolium* treatment demonstrates the extract's capacity to attenuate manganese-induced anxiety, likely through its phytochemical constituents including flavonoids and saponins known to possess anxiolytic properties.

Oxidative stress parameters measured in this study showed characteristic changes consistent with manganese-induced neurotoxicity and antioxidant protection. There was a significant ($P<0.05$) decrease in the activity of superoxide dismutase (SOD) in the $MnCl_2$-only group compared to the control. This indicates increased oxidative burden due to excessive production of superoxide radicals, which overwhelmed the endogenous antioxidant defense. This finding agrees with the results of Abu-Elfotuh *et al.* (2022), who reported that Mn exposure caused a marked decline in SOD activity in brain regions, while antioxidant treatment restored the enzyme's activity toward normal levels. Similarly, Chaudhary *et al.* (2022) demonstrated that natural antioxidants significantly enhanced SOD activity in rats exposed to oxidative stress, highlighting their efficacy as neuroprotective agents.

Catalase (CAT) activity was also significantly decreased ($P<0.05$) in the $MnCl_2$-treated rats compared to control, but *G. latifolium* co-treatment markedly elevated CAT activity toward normal. The suppression of CAT activity following Mn exposure is consistent with the study of dos Santos *et al.* (2017), who reported that manganese ions inhibit the breakdown of hydrogen peroxide, promoting accumulation of reactive oxygen species (ROS) in neural tissues. The improvement in CAT activity by *G. latifolium* suggests effective scavenging of peroxides and protection of the enzyme system from oxidative inactivation. This protective effect can be attributed to the extract's rich phytochemical composition including phenolic compounds that

neutralize lipid hydroperoxides and stabilize cellular membranes against oxidative disruption (Abu-Elfotuh *et al.*, 2022).

A similar trend was observed for glutathione peroxidase (GPx) activity, which was significantly reduced ($P<0.05$) in the $MnCl_2$-only group but improved following *G. latifolium* co-administration. GPx is an essential enzyme that reduces hydrogen peroxide and lipid hydroperoxides using glutathione as a substrate. Its reduction in Mn-treated rats confirms previous findings by Baker *et al.* (2017), who noted suppression of GPx in manganese-exposed tissues, indicating redox imbalance. The significant restoration of GPx activity by *G. latifolium* in the current study aligns with the observations of Chaudhary *et al.* (2022), who showed that natural plant antioxidants enhance GPx-mediated peroxide detoxification.

Reduced glutathione (GSH) concentration was also significantly lower in $MnCl_2$-treated rats compared to control, signifying depletion of intracellular thiol reserves due to oxidative stress. This result corresponds to reports by dos Santos *et al.* (2017), who found that manganese exposure depletes GSH in neuronal tissues by catalyzing its oxidation during free-radical neutralization. However, rats co-treated with *G. latifolium* extract exhibited markedly higher GSH levels, comparable to control, suggesting that the extract effectively preserved endogenous antioxidant reserves. This observation supports the findings of Abu-Elfotuh *et al.* (2022), where antioxidant intervention prevented GSH depletion and ameliorated behavioral deficits in manganese-induced toxicity models.

Conversely, malondialdehyde (MDA) concentration, a biomarker of lipid peroxidation, was significantly elevated ($P<0.05$) in the $MnCl_2$-only group compared to control. This increase indicates oxidative degradation of membrane lipids and is a key indicator of manganese-induced

neurotoxicity. The results are consistent with the findings of Criswell *et al.* (2019), who associated elevated MDA levels with neurodegenerative changes in manganese-exposed subjects. The co-administration of *G. latifolium* extract significantly reduced MDA levels, corroborating the reports of Chaudhary *et al.* (2022) and Abu-Elfotuh *et al.* (2022), both of which demonstrated that plant-derived antioxidants reduced lipid peroxidation and prevented oxidative membrane injury in brain tissues.

Histological analysis of brain tissue sections further supported the biochemical and behavioral data. The control group showed normal histoarchitecture with well-defined neuronal layers and healthy cellular morphology. The $MnCl_2$-only group exhibited degenerating neurons with pyknotic nuclei and vacuolation within the neuropil—hallmarks of neurodegeneration induced by oxidative stress. These findings align with the work of dos Santos *et al.* (2017), who observed that manganese exposure induces neuronal damage and vacuolation in anxiety-regulating brain regions. Co-treatment with *G. latifolium* extract markedly improved tissue morphology, showing preserved neurons and intact neuropil, as similarly reported by Owoeye *et al.* (2019). The *G. latifolium* extract–only groups displayed normal histology, confirming that the plant extract exerted no structural toxicity on brain neurons.

Taken together, the results of this study demonstrate that *Gongronema latifolium* extract significantly mitigated the behavioral, oxidative, and histological alterations caused by $MnCl_2$ exposure. By improving antioxidant enzyme activities, reducing lipid peroxidation, and attenuating anxiety-like behaviors, *G. latifolium* effectively preserved neuronal integrity and behavioral function. This outcome supports the conclusions of Katanic Stankovic *et al.* (2020) and Olaniyan *et al.* (2025), who highlighted the neuroprotective potential of plant-derived

antioxidants against manganese-induced oxidative damage. It can therefore be inferred that *G. latifolium* acts as an effective anxiolytic and neuroprotectant through its ability to scavenge reactive oxygen species, modulate neurotransmitter systems, stabilize neuronal membranes, and maintain redox homeostasis within anxiety-regulating brain regions.

5.2 Conclusion

Findings from this study demonstrate that manganese chloride ($MnCl_2$) exposure induces anxiety-like behaviors, oxidative stress, and histopathological alterations in anxiety-regulating brain regions, characterized by reduced antioxidant enzyme activity, elevated lipid peroxidation, and neuronal degeneration. However, co-administration of *Gongronema latifolium* extract significantly ameliorated these alterations by enhancing antioxidant defenses, attenuating anxiety-like behaviors, and preserving neuronal structural integrity. The study therefore establishes that *Gongronema latifolium* possesses potent anxiolytic and neuroprotective effects against manganese-induced neurobehavioral toxicity through its antioxidative, phytochemical, and neuromodulatory properties.

5.3 Recommendations

It is recommended that further studies be conducted to:

1. Investigate the molecular mechanisms through which *Gongronema latifolium* modulates neurotransmitter systems and oxidative stress–related signaling pathways in manganese-induced anxiety and neurotoxicity.
2. Evaluate the long-term effects of *G. latifolium* supplementation on behavioral, cognitive, and emotional functions in manganese-exposed subjects.

3. Explore synergistic effects of *G. latifolium* extract in combination with other anxiolytic agents or chelating compounds to enhance neuroprotection against metal-induced neuropsychiatric disorders.
4. Extend similar investigations to human populations chronically exposed to environmental or occupational manganese sources to validate translational efficacy and establish safe therapeutic dosing regimens.

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