

**THE NEUROPROTECTIVE EFFECTS OF AQUEOUS LEAF EXTRACT OF
Gongronema latifolium AGAINST MANGANESE CHLORIDE-INDUCED
CEREBELLAR TOXICITY IN ADULT WISTAR RATS**

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BENIN CITY**

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**PRESENTED TO THE DEPARTMENT OF ANATOMY, SCHOOL OF BASIC
MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES UNIVERSITY OF
BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) IN ANATOMY.**

CERTIFICATION

This is to certify that this research work titled “ **THE NEUROPROTECTIVE EFFECTS OF AQUEOUS LEAF EXTRACT OF *Gongronema latifolium* AGAINST MANGANESE CHLORIDE-INDUCED CEREBELLAR TOXICITY IN ADULT WISTAR RATS** ” for the award of a degree of Bachelor of Science (B.Sc.) in Anatomy was carried out by **OVIOSUN FULNESS OLOHIJE** under the supervision of **DR. U. O. IDEMUDIA**. All literatures used in this study have been acknowledged and properly referenced.

DR. U. O. IDEMUDIA
(PROJECT SUPERVISOR)

DATE

DR. A.B ENOGIERU
(HEAD OF DEPARTMENT)

DATE

EXTERNAL EXAMINER

DATE

DEDICATION

This project is dedicated first, to God almighty for his grace to get this far in my academic pursuit and for the gift of life. I would also love to dedicate this project to my wonderful parents and siblings for their constant encouragement, love and support.

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My deepest gratitude goes to God almighty, for his divine help, grace and tender mercies during the course of this program. My sincere gratitude also goes out to my parents (Mr. and Mrs. Oviosun) for their financial support, love, prayers and encouragement. I would also love to appreciate my siblings for their unwavering love and support all through the course of my study. My appreciation also goes to my supervisor, Dr. U. O. Idemudia for his guidance, advice and immense support during the course of this research .

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TABLE OF CONTENTS

DEDICATION.....	Error! Bookmark not defined.
ACKNOWLEDGEMENTS.....	Error! Bookmark not defined.
LIST OF FIGURES.....	9
LIST OF TABLES.....	Error! Bookmark not defined.
LIST OF PLATES.....	12
ABSTRACT.....	Error! Bookmark not defined.
CHAPTER ONE.....	Error! Bookmark not defined.
INTRODUCTION.....	Error! Bookmark not defined.
1.2 Aim.....	Error! Bookmark not defined.
1.3 Specific Objectives.....	Error! Bookmark not defined.
1.4 Justification of Study.....	Error! Bookmark not defined.
1.5 Expected Contribution to Knowledge.....	17
CHAPTER TWO.....	Error! Bookmark not defined.
2.1 Manganese chloride.....	Error! Bookmark not defined.
2.1.1 Introduction.....	Error! Bookmark not defined.
2.1.2 Sources and exposure pathway.....	Error! Bookmark not defined.
2.1.3 Mechanism of Toxicity.....	Error! Bookmark not defined.
2.1.4 Neurological and Cerebellar effect.....	Error! Bookmark not defined.
2.2 Overview of the Brain.....	Error! Bookmark not defined.
2.2.1 Introduction.....	Error! Bookmark not defined.
2.3 Organ of Study— Cerebellum.....	Error! Bookmark not defined.
2.3.1 Gross anatomy of the cerebellum.....	Error! Bookmark not defined.
2.3.2 Histology of the cerebellum.....	Error! Bookmark not defined.
2.3.3 Functional region of the cerebellum.....	28
2.3.4 Development of the cerebellum.....	Error! Bookmark not defined.
2.3.5 Function of the cerebellum.....	Error! Bookmark not defined.
2.3.6 Arterial supply of the cerebellum.....	30
2.3.7 Venous drainage of the cerebellum.....	31

2.3.8 Clinical significance of the cerebellum.....	Error! Bookmark not defined.
2.4 Gongronema latifolium.....	Error! Bookmark not defined.
2.4.1 Scientific classification.....	Error! Bookmark not defined.
2.4.2 Phytochemical composition.....	Error! Bookmark not defined.
2.4.3 Traditional and Pharmacological Uses.....	Error! Bookmark not defined.
2.4.4 Mechanism of Neuroprotective action.....	Error! Bookmark not defined.
CHAPTER THREE.....	Error! Bookmark not defined.
MATERIALS AND METHODS.....	Error! Bookmark not defined.
3.1 Animals and Management.....	40
3.2 Experimental Design.....	40
3.3 Sacrifice of Animals and Sample Collection.....	44
3.4 Antioxidants and Lipids Peroxidation.....	Error! Bookmark not defined.
3.4.1 Determination of MDA concentration.....	Error! Bookmark not defined.
3.4.2 Determination of Superoxide Dismutase (SOD) activity.....	Error! Bookmark not defined.
3.4.3 Determination of catalase activity.....	Error! Bookmark not defined.
3.4.4 Determination of glutathione peroxidase activity.....	Error! Bookmark not defined.
3.4.5 Determination of concentration of reduced glutathione.....	Error! Bookmark not defined.
3.5 Histological Procedure.....	50
3.5.1 Paraffin tissue processing of Drury and Wallington (1980).....	50
3.5.2 Haematoxylin and Eosin staining method of Drury and Wallington (1980).....	51
3.6 Photomicrography.....	52
3.7 Statistical Analysis.....	Error! Bookmark not defined.
CHAPTER FOUR.....	Error! Bookmark not defined.
4.0: RESULTS.....	Error! Bookmark not defined.
4.1 Change in Body Weight.....	Error! Bookmark not defined.
4.2 Brain Weight.....	Error! Bookmark not defined.
4.3 Relative Brain Weight.....	Error! Bookmark not defined.
4.4 Cerebellar Weight.....	Error! Bookmark not defined.
4.5 Relative Cerebellar Weight.....	Error! Bookmark not defined.
4.6 Superoxide Dismutase.....	Error! Bookmark not defined.

4.7 Catalase.....	Error! Bookmark not defined.
4.8 Glutathione Peroxidase.....	Error! Bookmark not defined.
4.9 Glutathione.....	Error! Bookmark not defined.
4.10 Malondialdehyde.....	Error! Bookmark not defined.
CHAPTER FIVE.....	Error! Bookmark not defined.
5.1 Discussion.....	Error! Bookmark not defined.
5.2 Conclusion.....	Error! Bookmark not defined.
5.4 Recommendations.....	Error! Bookmark not defined.
REFERENCES	Error! Bookmark not defined.

LIST OF FIGURES

Figure 2.1: Manganese Chloride structural formula

Figure 2.2: Structure of the human Brain

Figure 2.3: Gongrenema latifolium (Utazi leaf)

Figure 4.1: comparing Initial body weight within the groups, Values are given as mean \pm SEM.

Figure 4.2: Comparing Final body weight within the groups, Values are given as mean \pm SEM.

Figure 4.3: Comparing the weight change within the groups, Values are given as mean \pm SEM.

Figure 4.4: Comparing the brain weight within the groups, Values are given as mean \pm SEM.

Figure 4.5: Comparing the relative brain weight within the groups, Values are given as mean \pm SEM.

Figure 4.6: Comparing Movement initiation test within the groups, Values are given as mean \pm SEM.

Figure 4.7: Comparing Step test, Values are given as mean \pm SEM.

Figure 4.8: Comparing Ambulation test, Values are given as mean \pm SEM.

Figure 4.9: Comparing Immobility test, Values are given as mean \pm SEM.

Figure 4.10: Comparing central entry test, Values are given as mean \pm SEM.

Figure 4.11: Comparing Line crossing test, Values are given as mean \pm SEM.

Figure 4.12: Comparing the cerebellar superoxide dismutase level within the groups, Values are given as mean \pm SEM.

Figure 4.13: Comparing catalase level within the groups. Values are given as mean \pm SEM.

Figure 4.14: Comparing the glutathione peroxidase level within the group. Values are given as mean \pm SEM

Figure 4.15: Comparing the glutathione level within the groups, Values are given as mean \pm SEM.

Figure 4.16: Comparing the Malondialdehyde level within the groups, Values are given as mean \pm SEM.

LIST OF TABLES

Figure 2.4.1: Scientific Classification

Figure 3.6: Experimental Design

LIST OF PLATES

Plate 4.1: Photomicrograph of cerebellum of control rats group showing normal architecture (H&E; Scale bar: 25 μ m)

Plate 4.2: Photomicrograph of cerebellum of rats administered 10mg/kg of Manganese chloride showing degenerating and irregular architecture (H&E; Scale bar: 25 μ m)

Plate 4.3: Photomicrograph of cerebellum of rats administered 100mg/kg body weight of aqueous leaf extract of *Gongronema latifolium* and 10mg/kg of Manganese chloride showing relatively normal architecture (H&E; Scale bar: 25 μ m)

Plate 4.4: Photomicrograph of cerebellum of rats administered 200mg/kg body weight of aqueous leaf extract of *Gongronema latifolium* and 10mg/kg of Manganese chloride showing relatively normal architecture (H&E; Scale bar: 25 μ m)

Plate 4.5: Photomicrograph of cerebellum of rats administered 100mg/kg body weight of aqueous leaf extract of *Gongronema latifolium* showing relatively normal architecture (H&E; Scale bar: 25 μ m)

Plate 4.6: Photomicrograph of cerebellum of rats administered 200mg/kg body weight of aqueous leaf extract of *Gongronema latifolium* showing relatively normal architecture (H&E; Scale bar: 25 μ m)

ABSTRACT

Excessive exposure to Manganese chloride ($MnCl_2$) has been shown to induce neurotoxicity, particularly within the cerebellum, due to oxidative stress and neuronal degeneration. The cerebellum's high metabolic activity and synaptic density make it especially vulnerable to heavy metal accumulation and oxidative injury. *Gongronema latifolium* (Utazi leaf), a tropical West African herb rich in flavonoids, saponins, and alkaloids, has demonstrated antioxidant and anti-inflammatory properties in experimental studies. This study was aimed at investigating the neuroprotective effects of aqueous leaf extract of *Gongronema latifolium* against Manganese chloride induced cerebellar toxicity in adult wistar rats. Forty-eight (48) adult wistar rats were used in this study. They were randomly divided into six groups (n=8) and treated for 28 days as follows: Group A served as control and received 1mL of distilled water, Group B received 10mg/kg of $MnCl_2$, Group C received 100mg/kg of aqueous leaf extract of *Gongronema latifolium* and 10mg/kg of $MnCl_2$, Group D received 200mg/kg of aqueous leaf extract of *Gongronema latifolium* and 10mg/kg of $MnCl_2$, Group E received 100mg/kg of aqueous leaf extract of *Gongronema latifolium* and rats in Group F received 200mg/kg of aqueous leaf extract of *Gongronema latifolium*. Administration of aqueous leaf extract of *Gongronema latifolium* was done orally, using an orogastric tube while the administration of Manganese chloride was done via intraperitoneal injection for 28 days respectively. At the end of administration, the neurobehavioral activity was evaluated using the open field and Y-maze tests. The rats were sacrificed by cervical dislocation and the organ (cerebellum) was harvested. This organ was further analyzed for antioxidant enzymes activity, lipid peroxidation and histopathological changes. Graphpad prism software was used for all statistical analysis and data was expressed as mean with standard error of mean (SEM). Results obtained showed no significant change ($p > 0.05$) in the initial body weight and final body weight. A significant decrease ($p < 0.05$) was observed in the weight change of rats in group B (10mg/kg b.wt of $MnCl_2$) when compared to control, however a significant increase was observed in the weight change groups of C and D when compared to B. No significant change ($p > 0.05$) was observed in the cerebellar and relative cerebellar weight of rats across experimental groups. A significant decrease ($p < 0.05$) was observed in cerebellar SOD, CAT, GPx AND GSH activity of rats in group B (10mg/kg b.wt. $MnCl_2$) when compared to the control. However, a significant increase ($p < 0.05$) was observed in cerebellar SOD, CAT, GPx and GSH activity of rats in group C and D when compared to group B. A significant increase ($p < 0.05$) was observed in MDA concentration of rats in group B (10mg/kg b.wt. $MnCl_2$) when compared to control. However, a significant decrease was observed in group C and D when compared to B. Histological analysis revealed cerebellar degeneration in rats exposed to $MnCl_2$. However, administration of aqueous leaf extract of *Gongronema latifolium* mitigated the adverse effects induced by manganese chloride. In conclusion, findings from this study shows that *Gongronema latifolium* leaf extract mitigated cerebellar damage caused by $MnCl_2$ exposure.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study.

Although Manganese (Mn) is an essential trace element involved in enzymatic and metabolic functions, it is considered a heavy metal in toxicological terms due to its potential to accumulate and cause harm when present in excess. Human industrial activities have significantly increased environmental manganese levels. It is widely used in steel production, battery manufacturing, welding, and as an additive in fuel. Additionally, mining, smelting, and agricultural runoff from manganese-based fertilizers have contributed to its release into air, soil, and water systems (Crossgrove & Zheng, 2004; Bouchard *et al.*, 2011). Inhalation of manganese-containing dust or consumption of contaminated water can result in its accumulation in the brain, leading to neurotoxic effects such as motor dysfunction and cognitive impairments. These concerns highlight the importance of studying safe, natural agents that may offer protection against manganese-induced neurotoxicity.

Manganese (Mn) is an essential trace element required for normal physiological functions, including enzyme activation, immune response, and antioxidant defense. However, excessive exposure, particularly in the form of $MnCl_2$, has been linked to neurotoxicity. High levels of Mn can accumulate in the brain, especially in the basal ganglia and cerebellum, leading to symptoms similar to Parkinson's disease; a condition known as manganism (chen *et al.* 2015). The neurotoxic effects are mediated through increased oxidative stress, mitochondrial dysfunction, apoptosis, and neuroinflammation (Aschner 2005). The growing incidence of Mn-related toxicity due to industrial pollution, contaminated water sources, and occupational

hazards poses a public health challenge, especially in low- and middle-income countries. There is a pressing need for neuroprotective strategies that are not only effective but also affordable and accessible.

Medicinal plants have been used from time immemorial to treat diseases. These plants are considered rich sources of phytochemicals which can be used in drug development (Ahad *et al.* 2021). World Health Organization estimates that herbal medication is used in basic healthcare in a number of Asian and African countries (WHO, 2019). These plants can synthesize a wide variety of chemical compounds that are important for routine biological functions (Abdel-Aziz, 2016). It is estimated that more than 500,000 plants have medicinal qualities that have not yet been fully understood (Tomilson, 2015).

Gongronema latifolium, commonly known as Utazi leaf in Nigeria, is a tropical plant cherished for its rich nutritional content and diverse health benefits. *Gongronema latifolium* (Utazi) is a climbing shrub with broad, heart-shaped leaves that has a characteristic sharp, bitter and slightly sweet taste, especially when eaten fresh. It belongs to the family of plants known as Asclepiadaceae and it is widespread in tropical rainforests of west African countries, such as Nigeria, Sierra Leone, Ghana, etc. In southern Nigeria, where it is commonly grown, the Igbos call it Utazi, the Yorubas know it as arokeke and the Efiks and Ibibio call it Utazi. Unique for its versatility, utazi is used in the preparation of many cuisines and is also adopted in herbal medicine therapy with all of its part playing roles and aiding different needs.

Utazi is abundant in nutrients as bioactive compounds, making it highly beneficial for health. Fresh leaves contain protein, magnesium, and cinnamic acid, among other compounds. These include anetol, boron compounds, eugenol, stigmasterol, tannin, zinc, and tryptophan. The

presence of these compounds contributes to their medicinal properties. Research indicates that extracts from *Gongronema latifolium* possess antioxidant, anti-inflammatory, and antimicrobial properties, making it a subject of interest for further scientific investigation. The bioactive compounds found in Utazi leaf have shown promising therapeutic potential in the management of oxidative stress, inflammation and infectious diseases.

1.2 Aim

This study is aimed at investigating the protective activity of aqueous *Gongronema latifolium* (Utazi leaf) extract on Manganese chloride cerebellar induced toxicity in adult Wistar rats.

1.3 Specific Objectives

The following are the precise objectives of study:

1. To evaluate the changes in body and brain weight of rats exposed to manganese chloride and treated with aqueous leaf extract of *Gongronema latifolium*.
2. Assess the neurobehavioral activity (Novel object recognition test, Y-maize and open field test) in manganese chloride exposed rats treated with or without the extract.
3. Determine the effect of aqueous leaf extract of *Gongronema latifolium* on oxidative stress markers (Superoxide dismutase, catalase, and Malondialdehyde) in the cerebellum.
4. Examine the histological changes in the cerebellar tissue of rats treated with aqueous leaf extract of *Gongronema latifolium* compared to manganese chloride only.

1.4 Justification of Study

Manganese (Mn) is an essential trace element involved in numerous physiological processes; however excessive exposure, particularly to MnCl₂, has been linked to neurotoxicity and neurodegenerative disorders such as parkinsonism. Due to increased industrial use and environmental contamination, understanding the mechanisms of manganese-induced neurotoxicity has become a public health priority. Current treatment options for metal-induced neurotoxicity are limited and often come with undesirable side effects. As a result, there is growing interest in natural compounds with antioxidant and neuroprotective properties. *Gongronema latifolium* (utazi) is a medicinal plant traditionally used in Nigeria for its anti-inflammatory, antioxidant, and detoxifying effects, but its specific neuroprotective potential against MnCl₂-induced toxicity remains underexplored.

This study aims to provide scientific evidence for the neuroprotective properties of *Gongronema latifolium* and its possible application in preventing or mitigating heavy metal-induced neurodegeneration. The findings may contribute to the development of affordable, plant-based therapeutic agents with fewer side effects, especially in low-resource settings where traditional medicine is widely used. Additionally, this study aligns with the global pursuit of integrating ethnopharmacology with modern neuroscience and may offer insights into safe natural alternatives for neuroprotection.

1.5 Expected Contribution to Knowledge

This study will provide additional information on the neuroprotective effects of aqueous leaf extract of *Gongronema latifolium* on Manganese chloride-induced cerebellar toxicity in Adult wistar rats.

CHAPTER TWO

2.1 Manganese Chloride

2.1.1 Introduction

Manganese (Mn) is an essential trace element necessary for normal physiological and neurological processes, including enzyme activation, metabolism, and antioxidant defense (Aschner 2020). However, excessive manganese accumulation in the brain can disrupt neuronal integrity and lead to neurotoxicity characterized by motor and cognitive impairments (Caito 2021). When combined with other elements, it forms several compounds used for industrial and experimental purposes. Among these is Manganese chloride.

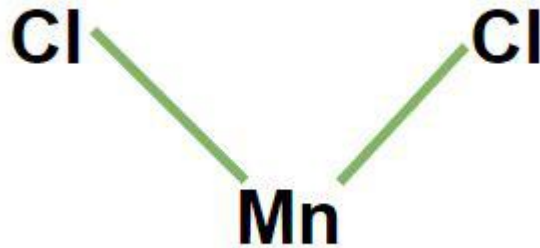


Figure 2.1 Manganese chloride structural formula

MnCl₂ has been shown to have industrial uses such as in battery manufacturing, welding, ceramics and metallurgical operations generate respirable and ingestible manganese dust or fumes that can elevate systemic loading (Eriskson et al., 2004). While inhalation is recognized as

a major route of exposure, ingestion of contaminated food or water also contributes, especially where manganese is elevated in the environment.

Once absorbed, Manganese chloride readily enters systemic circulation and is transported to organs via plasma proteins such as transferrin and albumin. Manganese homeostasis is tightly regulated; however chronic exposure can overwhelm the body's excretory mechanisms, particularly biliary excretion leading to accumulation in the brain and other tissues (Dobson *et al.*, 2003). Among neural structures, the basal ganglia, cerebellum, hippocampus, and cerebral cortex are particularly vulnerable (Guilarte 2013). $MnCl_2$ crosses the blood-brain barrier (BBB) primarily through divalent metal transporter -1 (DMT-1) and transferrin-dependent mechanisms, resulting in selective deposition in dopaminergic and cerebellar neurons (Aschner 2019).

Excessive accumulation of $MnCl_2$ in neural tissue induces oxidative stress, mitochondrial dysfunction, and apoptotic neurodegeneration (Fitsanakis *et al.*, 2006). The compound catalyzes the production of reactive oxygen species (ROS), which in turn disrupt cellular macromolecules, lipid membranes, and DNA. Studies have reported increased levels of malondialdehyde (MDA) a marker of lipid peroxidation and decreased antioxidant enzyme activity (such as catalase, superoxide dismutase, and glutathione peroxidase) following $MnCl_2$ administration in rodents (Ajibade *et al.*, 2011; Zhang *et al.*, 2021).

Furthermore, $MnCl_2$ interferes with dopaminergic transmission by impairing dopamine release and uptake, leading to neurological symptoms resembling Parkinson's disease commonly referred to as manganism (Guilarte, 2013). The toxicity of the brain is not limited to the nervous system.

Chronic exposure can lead to hepatic, renal, and reproductive impairments, as manganese interferes with energy metabolism and enzymatic activity (Martinez-Finley *et al.*, 2013).

Nonetheless, the brain remains the primary target, with $MnCl_2$ -induced oxidative stress and

inflammation forming the major pathways of neurodegeneration. Due to the public health and occupational risks associated with MnCl_2 there is growing research interest in natural antioxidants and neuroprotective agents capable of mitigating its effects.

2.1.2 Sources and exposure pathways

MnCl_2 is one of the most soluble and bioavailable manganese compounds, making it a major environmental and occupational source of manganese exposure. The presence of MnCl_2 in the environment arises primarily from natural processes such as soil erosion, volcanic activity, and weathering of manganese-bearing rocks, as well as anthropogenic activities including industrial operations and agricultural use (Santamaria *et al.*, 20007; O'Neal & Zheng, 2015).

Environmental sources

Industrially, MnCl_2 is used in dry-cell batteries, textile dyeing, fertilizers, welding fluxes, ceramics and catalysts, all of which serve as significant contributors to environmental contamination when waste is not properly managed (Crossgrove 2004). Emissions from ferromanganese and steel industries constitute one of the largest sources of atmospheric manganese, often in particulate form that can settle on soil and water surfaces (Chen *et al.*, 2015).

In agricultural settings, MnCl_2 and other manganese-deficient soils, leading to increased manganese content in crops and runoff into nearby water bodies (Martinez-Finley *et al.*, 2013).

Water pollution remains a major route of environmental dissemination. Manganese from industrial effluents, mining discharge, and leaching of contaminated soil can enter groundwater and surface water systems. Due to its high solubility, MnCl_2 can persist in aqueous environments thereby entering the food chain (Zhen et al, 2011). Studies have reported elevated manganese levels in drinking water sources, especially in areas close to mining sites and metallurgical

industries (Spencer, 2020). Prolonged ingestion of contaminated water can cause neurological and developmental impairments, particularly in children (Rahaman *et al.*, 2017).

Occupational and Dietary Exposure

Occupational exposure represents critical concern, especially among welders, miners and battery workers who are exposed to airborne MnCl_2 dust or fumes. Inhalation of these particles leads to direct absorption through the respiratory tract and translocation to the brain via olfactory pathways, bypassing the blood-brain barrier (Lucchini *et al.*, 2012). Chronic exposure can be linked to neurological symptoms such as motor dysfunction, reduced coordination, and cognitive impairment.

Dietary intake is another important route of exposure. While manganese is naturally present in grains, nuts, and leafy vegetables, contamination from fertilizers, water and food packaging materials containing MnCl_2 can elevate dietary levels (Zoni & Lucchini, 2013). Moreover, cooking with contaminated water or using metal utensils can further increase manganese ingestion. Studies have shown that excessive dietary manganese, especially in its chloride form, can accumulate in the liver and brain over time, leading to oxidative stress and neurotoxicity (Wang *et al.*, 2018).

Pathways of Absorption and Distribution

Manganese chloride can enter the body through inhalation, ingestion, or dermal contact, though inhalation and ingestion are the most significant routes. Once absorbed, MnCl_2 is transported in the bloodstream bound to transferrin, albumin, and citrate (Dobson *et al.*, 2003). It readily crosses the blood-brain barrier and accumulates in the brain regions such as the basal ganglia, hippocampus, and cerebellum, which are responsible for motor coordination and cognitive

function (Aschner 2019). The compound is primarily excreted via the bile, but prolonged or high-dose exposure can overwhelm the excretory system resulting in bioaccumulation and toxicity (Erikson et al.,).

2.1.3 Mechanism of Toxicity

The toxic effects of manganese chloride (MnCl_2) arise primarily from its ability to disrupt essential cellular and biochemical processes in the central nervous system. One of the central mechanisms of MnCl_2 is oxidative stress. Manganese ions catalyze the formation of reactive oxygen species (ROS), leading to lipid peroxidation, protein oxidation, and DNA damage. This oxidative imbalance depletes antioxidants such as glutathione and catalase, impairing mitochondrial respiration and neural viability (Roth, 2009). Mitochondrial dysfunction is a key hallmark of manganese neurotoxicity; Mn^{2+} disrupts oxidative phosphorylation and inhibits enzymes of the electron transport chain, particularly complexes II and III, resulting in ATP depletion and neuronal energy failure (Guilarte, 2013).

Additionally, MnCl_2 interferes with neurotransmitter systems, especially dopamine metabolism. It alters dopamine release and reuptake in the striatum and damages dopaminergic neurons, producing Parkinson-like symptoms collectively called managanism (Bowman *et al.*, 2011; O'Neal & Zheng, 2015). These dopaminergic alterations explain the motor deficits and tremors associated with chronic Mn exposure.

Another mechanism involves neuroinflammation. MnCl_2 activates microglia and astrocytes, leading to the release of pro-inflammatory cytokines such as $\text{TNF-}\alpha$ and IL-6, which further exacerbate oxidative injury and neuronal apoptosis (Filipov et al., 2009; Moreno et al., 2009).

The resulting neuroinflammatory milieu disrupts synaptic signaling and promotes neuronal loss, particularly in the cerebellum and basal ganglia.

Collectively, MnCl₂ exerts its toxic effects through a combination of oxidative stress, mitochondrial impairment, neuroinflammation, and neurotransmitter disruption. These interrelated pathways culminate in neuronal degeneration and behavioral impairments that mirror human neurodegenerative disorders such as Parkinson's disease. Understanding these mechanisms provides a basis for developing antioxidant and anti-inflammatory interventions aimed at mitigating MnCl₂-induced neurotoxicity.

2.1.4 Neurological and cerebellar effects

The central nervous system (CNS) is the principal target of MnCl₂ toxicity. Chronic exposure to MnCl₂ induces profound neurological impairments that closely resemble Parkinsonian symptoms, including tremors, motor incoordination, and cognitive deficits. The neurotoxicity of MnCl₂ arises from its preferential accumulation in brain regions with high metabolic and dopaminergic activity most notably the basal ganglia, cerebellum, and hippocampus (Bowman et al., 2011).

One of the major neurological effects of MnCl₂ exposure is dopaminergic dysfunction.

Manganese disrupts dopamine synthesis, release, and reuptake in the striatum, leading to the degeneration of nigrostriatal neurons (Erikson 2019). This dopaminergic damage results in behavioral manifestations similar to Parkinson's disease, often termed *manganism* (Peres et al., 2016). Unlike idiopathic Parkinson's disease, manganism is characterized by less responsiveness to L-DOPA therapy and distinct neuropathological patterns (Guilarte, 2013). The accumulation of Mn²⁺ in astrocytes and neurons alters mitochondrial respiration and increases oxidative stress, thereby impairing neuronal signaling and synaptic plasticity (Aschner et al., 2009).

In addition to basal ganglia involvement, the cerebellum is highly susceptible to MnCl_2 toxicity due to its rich vascular supply and dense network of Purkinje neurons (Inyang *et al.*, 2023; Moreno *et al.*, 2009). Histological studies in rats have shown that exposure to MnCl_2 leads to degeneration of Purkinje cells, vacuolation in the molecular layer, and loss of normal cortical lamination within the cerebellar cortex (Peres *et al.*, 2016). Such structural damage correlates with marked motor incoordination, decreased locomotor activity, and impaired balance observed in behavioral tests such as the open field and rotarod tests (Oluwole & Adedayo, 2020).

2.2 Overview of the Brain

2.2.1 Introduction

The brain is an extraordinarily intricate and sophisticated organ that is in charge of almost every part of the human experience, including perception, emotion, and thought. It is made up of billions of neurons and glial cells that serve as support structures. These neurons are arranged into an intricate web of circuits and pathways that enable perception, thought, and behaviour in the environment (Sousa *et al.*, 2016). The central nervous system command center is located in the human brain. It transmits data to the muscles after absorbing it from the body's sensory organs. It is made up of billions of glia, which are supporting cells, and more than 100 billion neurons, which are nerve cells (Purves *et al.*, 2018). Neuroglial cells are classified into multiple categories. The CNS receives support from astrocytes, which establish synapses, store glycogen, preserve the blood brain barrier, and provide an anionic homeostatic environment for neuronal communication. The brain is separated into the cerebrum, cerebellum, and brainstem and is located within the skull. The brain's main region, the cerebrum, is in charge of conscious thought, voluntary movement, and sensation. The cerebellum, which sits beneath the cerebrum,

is in charge of balance and coordination. According to Kandel *et al.* (2012), the brainstem regulates vital bodily processes like blood pressure, heart rate, and respiration in addition to serving as a link between the brain and spinal cord. The nervous system, which consists of the spinal cord and a network of nerves that carry information from the brain to various body parts, allows the brain to connect with the rest of the body. Additionally, the brain regulates behavior, emotion, and mental processes like perception, memory and learning. According to Kandel *et al.* (2012), the brain is fundamental to our capacity for thought, emotion, and interaction with the outside world. Brains have centralized physiological control over the body's organs (Carlson *et al.*, 2013). Through the production of patterns in muscle activity and the stimulation of chemical release known as hormones, they exert an influence on the rest of the body (Squire *et al.*, 2012). Quick and well-coordinated reactions to environmental changes are made possible by this centralized control.

2.3 Organ of Study

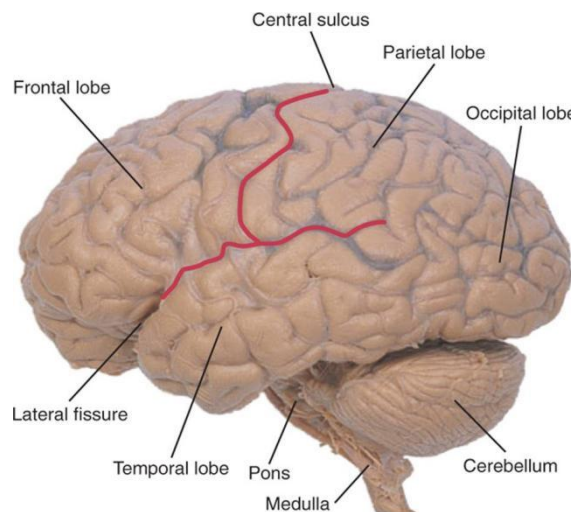


Figure 2.2

Structure of the human brain.

Adapted from Koeppen & Stanton: Berne and Levy Physiology (6th ed.)

2.3.1 Gross anatomy of the cerebellum

The cerebellum often called the “little brain” occupies the posterior cranial fossa beneath the occipital lobes and above the foramen magnum, separated from the cerebrum by the tentorium cerebelli and overlaying the dorsal aspect of the brainstem (Voogd & Marani, 2016). On gross inspection the structure consists of two lateral hemispheres flanking a slender mid-line vermis.

The surface is extensively foliated: numerous narrow folia separated by shallow fissures give the cerebellum its characteristic lobulated external appearance (Glickstein, Strata & Voogd, 2009).

The grey matter cortex of the cerebellum envelops the white matter which consists of four paired deep cerebellar nuclei. They include; dentate, emboliform, globose and fatigial nucleus. They serve as the principal channel for cerebellar efferent output to the thalamus and brainstem (Voogd & Glickstein 1998). The cerebellum is further divided into three transverse lobes : the anterior lobe, the posterior lobe, and the flocculonodular lobe. The anterior lobe is separated from the posterior lobe by the primary fissure, while the the posterior lobe is seperated from the flocculonodular lobe by the posterolateral fissure. (Marzban, 2015).

The cerebellum is attached to the brainstem by three paired peduncles: the superior, middle and inferior cerebellar peduncles. The middle peduncle carries massive pontocerebellar affrerents, the inferior peduncle carries spinocerebellar and vestibulocerebellar inputs, and the superior peduncle conveys cerebellar output and some afferents to the midbrain and thalamus (Nolte, 2025). The average transverse diameter of the cerebellum ranges from 10 to 12 cm, with a superior–inferior height of about 5 cm and an anteroposterior length of 6 cm (Snell, 2019). The cerebellum typically weighs between 130–150 grams, representing roughly 1/8th of the brain’s total weight, which averages about 1350–1400 grams (Standring, 2021; Nolte, 2025). The hue of

the cerebellum is pinkish gray (Waxman, 2020). The cerebellum is a highly ordered structure that is essential for controlling posture, movement, and balance.

2.3.2 Histology of the cerebellum

The cerebellum consists of three histological layers: the molecular layer, the purkinje cell layer, and the granular layer, all arranged over a core of white matter known as the arbor vitae (Standring, 2021). The molecular layer is the outermost layer and relatively cell-sparse region. It contains stellate and basket interneurons interspersed among the dendritic arbors of purkinje cells and the parallel fibers of granule cells.

The purkinje cell layer lies beneath the molecular cell layer, composed of a single row of large flask-shaped purkinje neurons that represent the sole output of the cerebellar cortex. Each purkinje cell extends a vast dendritic tree into the molecular layer, where it gets excitatory input from parallel fibers and climbing fibers. These cells project inhibitory (GABAergic) signals to the deep cerebellar nuclei, supplying the main route by which the cerebellar cortex has influence on the motor control and learning (Llinás, 2011).

The granular layer is the innermost and densest cortical layer which consists of small granule cells, Golgi cells, and glomerular synaptic complexes where mossy fibers, Golgi cell axons, and granule cell dendrites converge (Mugnaini, 2003). This layer usually serves as the first site for excitatory input integration before signals ascend through the purkinje cells to the deep nuclei. These layers work together to maintain a highly ordered microcircuit crucial for timing, adaptation, and sensorimotor prediction (Schmahmann & Pandya, 1997).

2.3.3 Functional region of the cerebellum

- The cerebellum plays a major role in balance and movement coordination (Loffe *et al.*, 2010).
- The cerebellum also plays a role in learning and muscle control (Yang *et al.*, 2018)
- It is also responsible for cognitive functions and memory (Kozi *et al.*, 2014).
- Research has demonstrated that the brain regions involved in emotions regulation are related to the cerebellum (Phillips *et al.*, 2015)
- Studies has also proven that it's involved decision making (Schmahmann *et al.*, 2019).

2.3.4 Development of the cerebellum

The development of the cerebellum is a complex, process that begins early in embryogenesis, arising from the dorsal aspect of the metencephalon within the hindbrain (rhombencephalon) (Larsell & Jansen, 1972). During the fifth gestational week, the rhombic lips appear as bilateral ridges along the metencephalic alar plate and their medial fusion gives rise to the cerebellar plate, the primordium of the cerebellum (Sidman & Rakic, 1982). The midline vermis and two lateral hemispheres are formed by the differentiation of the cerebellar plate which expand and fold to form the cerebellar lobules that characterize the mature cerebellum (Hatten & Heintz, 1995).

During development, the ventricular zone and the rhombic lip produce distinct neuronal populations. The ventricular zone gives rise mainly to GABAergic neurons, including Purkinje cells, while the rhombic lip produces glutamatergic neurons, including granule cells and deep cerebellar nuclei neurons (Hoshino *et al.*, 2005). Granule cells migrate tangentially to form the external germinal layer, which later descends inward to become the internal granular layer,

while Purkinje cells migrate radially to form a monolayer that defines cerebellar cortical organization (Sillitoe & Joyner, 2007).

The growth of the cerebellum is majorly controlled by molecular signals such as FGF8, Wnt1, and Shh, which regulate cell proliferation and patterning within the isthmic organizer region (Martinez et al., 2013). The final maturation of cerebellar circuitry occurs after birth, involving synaptic refinement and myelination of afferent fibers (Wang & Zoghbi, 2001). Disruption of these developmental events results in cerebellar malformations such as hypoplasia or ataxia, reflecting the cerebellum's sensitivity to genetic and environmental perturbations during early neurodevelopment (Millen & Gleeson, 2008).

2.3.5 Function of the cerebellum

The cerebellum serves as a core centre for motor coordination by processing extensive afferent input from spinal, vestibular and pontine (cortical) sources. The Purkinje cells transmit this input to the deep cerebellar nuclei which then influence descending motor and vestibular systems (Gilman, 1985; De Zeeuw, 2015). Due to its anatomic design, the cerebellum is responsible for facilitating fine regulation of timing, force and coordination of voluntary movements by comparing intended motor commands with incoming sensory feedback (Ito, 1972; Martinez, 2014).

One of its primary operational roles is predictive motor control and error-correction: the cerebellum generates internal models of intended movement and uses mismatches between expected and actual sensory outcomes to adjust ongoing motor output, ensuring fluid execution of multi-joint actions (De Zeeuw, 2015; Lisberger, 2020). As such, patients with cerebellar

lesions display ataxia, dysmetria and reduced adaptation of movement — phenomena consistent with the cerebellum's role in calibrating motor programs rather than initiating them (Glickstein, 1992; Gilman, 1985).

In relation to motor learning, the cerebellum contributes to the consolidation and automation of skilled behaviour: different modules within the cerebellum employ distinct encoding schemes (for example rate-coding vs temporal burst-coding) to acquire procedural memories (De Zeeuw *et al.*, 2015). Early studies by Dow and Moruzzi (1958) and subsequently by Marr (1969) and Albus (1971) laid the foundation for the concept that synaptic plasticity in the cerebellar cortex underlies adaptive motor learning (Martinez, 2014; Lisberger, 2020).

Beyond traditional motor functions, emerging evidence indicates that the cerebellum contributes to cognitive, linguistic and affective domains. The description of the cerebellar cognitive-affective syndrome (CCAS) by Schmahmann and Sherman (1998) demonstrated that damage to the posterior cerebellum may lead to impairments in executive functions, visuospatial processing, language and personality change (Schmahmann & Sherman, 1998; Manto & Mariën, 2015). From a network standpoint, the cerebellum is closely connected to cerebral association and limbic regions, supporting this broader role (Prati, 2024; Manto, 2022).

2.3.6 Arterial supply of the cerebellum

Three pairs of cerebellar arteries irrigate the cerebellum.

- a. The superior surface of the cerebellum is supplied by the superior cerebellar artery, a branch of the basilar artery.

- b. A branch of the basilar artery, the anterior inferior cerebellar artery supplies the anterior inferior surface of the cerebellum.
- c. The inferior surface of the cerebellum is irrigated by the posterior inferior cerebellar artery, which is a branch of the vertebral artery (Vishram 2014)

2.3.7 Venous drainage of the cerebellum

The cerebellar venous system, a sophisticated network of veins, drains the cerebellum. The great cerebral vein, posterior inferior cerebellar vein, and superior cerebellar vein make up this system's three primary veins (Ribas *et al.*, 2005). The superior and inferior cerebellar veins, along with their tributaries, empty the cerebellum's venous system into the superior petrosal, transverse, and straight dural venous sinuses (Querol-Pascual *et al.*, 2019).

2.3.8 Clinical significance of the cerebellum

The cerebellum is essential not only for coordinating movement but also for cognitive and emotional processing. Damage or dysfunction can lead to a wide range of motor, cognitive, and neurodegenerative disorders.

1. Gait and posture instability: Injury to the cerebellum disrupts the circuitries that maintain balance and ambulatory stability, leading to unsteady gait, frequent falls, and truncal sway as seen in cerebellar vermis or flocculonodular lobe lesions (Uysal, 2023).
2. Ataxia and movement decomposition: The cerebellum's role in coordinating multi-joint movements means that damage often results in ataxia and the breakdown of smooth motion into segments.

3. Speech and articulatory disorders (ataxic dysarthria): The cerebellum fine-tunes timing and coordination of the speech musculature. Lesions can manifest as slurred, slow or scanning speech, reflecting its non-limb motor contributions (Uysal, 2023).
4. Cognitive- affective dysfunction (Cerebellar Cognitive Affective Syndrome, CCAS): Beyond motor control, the cerebellum influences executive functions, language processing, and emotional regulation. Damage can produce impairments in planning, abstract reasoning, behavioural control and affect (Schmahmann & Sherman, 1998; Schmahmann, 2021).
5. Involvement in neurodegenerative disorders such as Parkinson's disease: Recent evidence highlights cerebellar contributions to the pathophysiology of Parkinson's: altered cerebellar- basal ganglia connectivity and cerebellar tremor- generating circuits are implicated (Wu & Hallett, 2005).
6. Influence on cognition after cerebellar stroke: Focal lesions of the cerebellum can lead to mild but broad cognitive deficits, especially in executive and attention domains, via disruption of cerebro- cerebellar loops (Schutter *et al.*, 2022).
7. Compromise in demyelinating conditions (e.g., Multiple Sclerosis) : Cerebellar involvement in multiple sclerosis leads to worsened coordination, tremor, dysarthria and gait issues; early cerebellar lesions predict poorer prognosis in MS (Wilkins, 2017).
8. Vestibular and ocular- motor disturbances: The cerebellum interacts with vestibular nuclei and ocular motor pathways. Dysfunction can manifest as nystagmus, impaired vestibulo- ocular reflex suppression, dizziness or spatial disorientation (Strupp & Brandt, 2018).

2.4 *Gongronema latifolium* (Utazi leaf)



Figure 2.4 Diagram of Utazi leaf (*Gongronema latifolium*)

Source: [utazi-leaf-millenora.jpg \(720×405\)](#)

2.4.1 Scientific Classification

kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Gentianales
Family	Apocynaceae
Genus	Gongronema
species	latifolium

Figure 2.4.1

2.4.2 Phytochemical composition

The leaves of *Gongronema latifolium* are rich in a variety of secondary metabolites that may underlie their neuroprotective potential. For example, a study of the dried leaf meal found phytochemicals including phytic acid (0.25 %), oxalates (5.26 %), saponins (0.57 %), tannins (3.85 %), glycosides (4.62 %), and alkaloids (6.01 %) among others (Elijah *et al.*, 2024). Another quantitative screening reported that alkaloids, flavonoids, saponins, tannins and reducing compounds were present in significant amounts in ethanol-extracted leaves, with flavonoids and polyphenols particularly abundant (Ebana *et al.*, 2016).

There are other comprehensive reviews that have summarized a broad spectrum of phytoconstituents in this species, listing flavonoids, saponins, alkaloids, steroids, glycosides, phenols and other compounds (Balogun *et al.*, 2016). Furthermore, a comparative analysis found that in *Gongronema latifolium* leaves there were measurable amounts of terpenoids, glycosides, polyphenols, steroids and reducing sugars, with an especially high percentage of alkaloids, saponins and tannins relative to some other medicinal plants (Mgbeje, Umoh & Emmanuel- Ikpeme, 2019).

2.4.3 Traditional and pharmacological uses

Traditional Uses of *Gongronema latifolium*

Gongronema latifolium, commonly called Utazi, has long been used in West African traditional medicine for both dietary and therapeutic purposes. In many Nigerian communities, the leaves are consumed as a bitter vegetable or incorporated into soups, serving as both a food source and a remedy for ailments (Faluyi, 2021). Traditionally, the plant is used to treat digestive disorders

such as constipation, dyspepsia, and nausea, and is also applied in managing malaria, anorexia, and hypertension (Asuquo, Nwachukwu, & Onoyeraye, 2023). In addition, local practices include using the leaves or extracts to aid postpartum recovery and promote general well-being (Faluyi, 2021). The broad traditional applications of *Gongronema latifolium* reflect empirical recognition of its medicinal properties, particularly its effects on digestion, metabolism, and general health.

Pharmacological Uses of *Gongronema latifolium*

Scientific studies have supported many of the traditional claims, demonstrating a variety of pharmacological effects of *Gongronema latifolium*. The plant has been reported to exhibit anti-inflammatory, antioxidant, antimicrobial, antidiabetic, and cytoprotective properties (Morebise 2015). There was also a discovery that the saponin fraction of *Gongronema latifolium* significantly reduced fasting blood glucose levels and improved lipid profiles in diabetic rats, confirming its antidiabetic activity (Igile *et al.* 2017). Similarly, a finding demonstrated that methanolic leaf extract exerted antioxidant effects and reduced markers of oxidative stress in animal models, indicating tissue-protective potential (Edenta, Nwobodo, & Nweje-Anyalowu 2015).

Additionally, antimicrobial activity has been demonstrated against various bacterial strains, confirming its traditional use in treating infections (Igile *et al.*, 2017). Collectively, these pharmacological studies suggest that *Gongronema latifolium* contains bioactive compounds capable of antioxidant, anti-inflammatory, and neuroprotective effects, making it a promising candidate for neuroprotection studies in cerebellar toxicity models.

2.4.4 Mechanism of neuroprotective action

Gongronema latifolium leaf extract is believed to protect the nervous system through several complementary mechanisms. One of the primary ways it acts is by reducing oxidative stress. Experimental studies in animal models have shown that administration of the leaf extract increases the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). At the same time, markers of oxidative damage like malondialdehyde (MDA) and nitric oxide (NO) are significantly lowered (Amangian- Kalawa, Udo, & Akpan, 2020; Ebena, Ekanemesang, Edet, & Omoruyi, 2016). This antioxidant effect helps protect cerebellar neurons from reactive oxygen species generated during toxic insults. In addition, the extract exhibits anti-inflammatory properties. It has been shown to reduce levels of proinflammatory cytokines, including interleukin-2 (IL-2) and interleukin-6 (IL-6), while simultaneously restoring antioxidant enzyme activity in affected tissues (Okoya, Adekunle, & Obiyan, 2020). By controlling inflammation, the extract may prevent secondary neuronal damage and glial activation, which are common features of cerebellar toxicity. Studies indicate that phytochemicals from the leaf inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), enzymes that break down acetylcholine and butyrylcholine, thereby preserving neurotransmission (Smith, Njoku, & Ibe, 2023). This action helps maintain proper neuronal signalling and coordination, which is particularly relevant for cerebellar function. The leaf extract may also protect neurons through metal-chelating and free radical scavenging activities. Some bioactive compounds in *Gongronema latifolium* can bind metal ions and neutralize free radicals, reducing oxidative damage caused by metals such as manganese (Johnson, Adebayo, & Okokon, 2023). This mechanism is crucial in situations where heavy metal exposure contributes to neuronal injury.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Ethical Approval

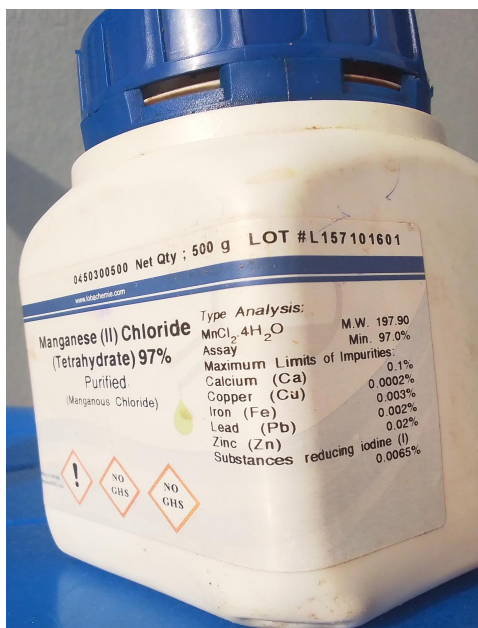
An ethical approval was conducted, which was reviewed and approved by the college Research Ethics Committee of School of Basic Medical Sciences, University of Benin, with this approval number CMS/REC/2025/803

3.2 Reagents and Chemicals

Distilled water

Reagent: Solution of *Gongronema latifolium* leaf extract

Reagent: Solution Manganese chloride



Made in India. CAS No: 13446-34-9

Equipments

Orogastric tubes, cotton wool, plastic cages, Refrigerator, sterile plain containers, plain sample containers, Dissecting set, surgical latex gloves, masking tape, weighing balance, measuring cylinder.

3.3 Plant collection and Authentication

Reagent 1: *Gongronema latifolium* leaf

Fresh *Gongronema latifolium* leaves were gotten from the market, New Benin market, Benin city, Edo state. The plant was authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria, and a voucher specimen was deposited with herbarium number UBH-G325.

3.4 Extract Preparation

Reagent 1: *Gongronema latifolium*

Aqueous extraction of Utazi leaf was prepared using a freeze-drying technique. The first process involves air drying at room temperature, the utazi leaf was then grounded to powder by an electric blender. Five hundred and seventy five grams (575g) of the powdered sample were soaked in 3 litres of distilled water for 24 hours. The mixture was filtered using a cheesecloth to separate the residue from the liquid extract. The resulting filtrate was sent for freeze-drying at Tigras lab, Department of Medical Biochemistry, University of Benin to obtain a dried powdered form of

the utazi leaf extract. The powdered extract was stored in the Department of anatomy for further use.

3.5 Animals and Management

Forty-eight (48) adult wistar rats were used in this study. The rats were bred in the animal house of the department of Anatomy, University of Benin, Benin city, where they were kept in plastic cages under room temperature and were fed daily with Grower's mash (manufactured by Premier feed Mills Co LTD, a subsidiary of Flour Mills of Nigeria Plc) and water. During the course of this experiment they were weighed initially and finally using a digital weighing scale calibrated in gram and recorded to the nearest whole number. Protocols for this experiment were in accordance with the guide for care and use of laboratory animals.

3.6 Experimental Design

In this study, Forty-eight (48) rats were assigned into six (6) groups A, B, C, D, E and F each containing eight (8) rats, after acclimatization for 2 weeks with free access to feed and water. The administration of the extract and toxicant were then carried out for 28 days. Animals were divided into groups receiving (100mg/kg) and (200mg/kg) doses of *Gongronema latifolium* leaf extract orally an hour prior to the administration of manganese chloride. The manganese chloride (toxicant) was administered daily for 28 consecutive days. The extract was given throughout the 28-day toxicant period, to assess its protective effect against cerebellar toxicity. The 1-hour interval between extract and toxicant administration was maintained each day to ensure that the

bioactive compounds were present in the system at the time of toxicant exposure. Animals were monitored daily for behavioral changes, signs of toxicity, and general health..

Table 3.6 Experimental design

GROUPS	DOSAGE
GROUP A	Control group
GROUP B	10mg/kg body weight of Manganese chloride only
GROUP C	100mg/kg body weight of aqueous leaf extract of <i>Gongronema latifolium</i> and 10mg/kg of Manganese chloride
GROUP D	200mg/kg body weight of aqueous leaf extract of <i>Gongronema latifolium</i> and 10mg/kg of Manganese chloride
GROUP E	100mg/kg of body weight of aqueous leaf extract of <i>Gongronema latifolium</i>
GROUP F	200mg/kg of body weight of aqueous leaf extract of <i>Gongronema latifolium</i>

Administration was done orally using an orogastric tube and intraperitoneally using an intraperitoneal injection for the extract and toxicant respectively.

3.7 Neurobehavioral Assessments

Neurobehavioral assessments were carried at the Department of Anatomy, University of Benin to evaluate the effects of *Gongronema latifolium* and manganese chloride on motor coordination, exploratory behavior, and locomotor activity in experimental rats. The tests were carried out 24 hours after the final administration and prior to sacrifice. All behavioral tests were performed in a quiet room under uniform illumination to minimize interference.

3.7.1 Open Field Test (OFT)

The Open Field Test was used to assess exploratory activity and locomotor behavior. Each rat was placed individually at the center of an open field apparatus (made of a square wooden box divided into equal squares). The following parameters were observed and recorded over a period of 5 minutes:

1. **Ambulation:** Number of floor units crossed with all paws, indicating locomotor activity.
2. **Immobility:** Duration of inactivity, reflecting reduced motor drive or anxiety.
3. **Central Square Entry:** Number of entries into the central squares, an index of exploratory drive.
4. **Line Crossing:** Frequency of line crossings, representing general activity level.

The apparatus was cleaned with 70% ethanol between trials to eliminate odor cues. Behavioral data were analyzed using standard protocols as described by Prut and Belzung (2003) and Walsh and Cummins (1976).

3.7.2 Movement and Initiation Test

The Movement and Initiation Test was used to evaluate motor drive and the animal's ability to initiate movement following mild stimulation. Each rat was gently placed in an observation area, and the latency period before spontaneous movement initiation was recorded. Reduced initiation time indicates improved motor function.

3.7.3 Step Test

The Step Test assessed motor coordination and balance. Each rat was held such that its fore paw contacted a flat surface while the hind limbs were lifted slightly. The time taken to initiate a stepping movement was recorded. Prolonged latency in step initiation indicates motor impairment.

3.8 Sacrifice of Animals and Sample Collection

The rats were weighed at the beginning and at the end of the study using a weighing balance. They were sacrificed by cervical dislocation, the skulls were opened, brain organs harvested and blotted free of blood and weighed using digital weighing scale calibrated in grams. The harvested brain organs were fixed in 10% buffered formalin and tissues processed for light microscopic examination.

3.9 Antioxidants and Lipid peroxidation

Reagents

The chemicals and reagents used in this study were of analytical grade. The 5, 5-dithio-bis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH) standard, thiobarbituric acid (TBA), trichloroacetic acid (TCA), were products of Merck (Germany). Ethylene diamine tetra acetic acid (EDTA), epinephrine (adrenaline), hydrogen peroxide (H₂O₂), potassium hydroxide (KOH), sodium carbonate (Na₂CO₃), sodium citrate, sodium chloride (NaCl), sodium dihydrogen phosphate (NaH₂PO₄), sodium hydrogen carbonate (NaHCO₃), sodium hydrogen phosphate (NaHPO₄), sodium hydroxide (NaOH), and sulphuric acid (H₂SO₄) were purchased from British Drug House (BDH) (England). Hydrochloric acid (HCl), potassium permanganate (KMnO₄), and pyrogallol were obtained from May and Bayer (England).

Determination of Concentration of Total Protein

Principle

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

Assay Procedure

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

Calculations

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

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

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

➤ When using a standard

$$\text{Total Protein Concentration} = \frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times \text{Standard Conc.}$$

Determination of MDA Concentration

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

Assay Procedure

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

Calculation

The MDA concentration of each sample was calculated as follows:

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

where,

O.D = Absorbance of sample test at 535 nm

V_t = Total volume of the reaction mixture = 3.6 mL

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

L = Light path = 1.0 cm

V = Volume of sample homogenate used = 0.6 mL

Y = mg of tissue in the sample used

The unit of MDA is moles/mg wet tissue

Determination of Superoxide Dismutase (SOD) Activity

Principle

The activity of SOD was assessed based on the method of Misra and Fridovich (1972). Adrenaline auto-oxidizes rapidly in aqueous solution to adrenochrome whose concentration can be determined spectrophotometrically at 420 nm. The auto-oxidation depends on the presence of superoxide anions (O_2^-). Superoxide dismutase (SOD) inhibits this auto-oxidation by catalyzing the breakdown of superoxide anions. The degree of inhibition is thus a measure of SOD activity. The amount of enzyme producing 50 % inhibition is defined as one unit of the enzyme activity.

Assay Procedure

Sample homogenate (0.2 mL) was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) and allowed to equilibrate. The reaction was initiated by the addition of 0.3 mL of freshly prepared 0.03 mM adrenaline as substrate. The solution was mixed by inversion. The reference tube contained 2.7 mL of carbonate buffer and 0.3 mL of adrenaline, while the blank contained 2.5 mL of carbonate buffer, 0.2 mL of distilled water and 0.3 mL of 0.03 mM adrenaline. The

increase in absorbance at 420 nm due to the formation of adrenochrome was monitored every 30 sec for 120 sec. One unit of SOD activity was taken as the amount of SOD necessary to cause 50 % inhibition of the oxidation of adrenaline to adrenochrome within 120 sec.

Calculation

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

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

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

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

Determination of Catalase Activity

Principle

This is based on the method of Cohen, *et al.*, (1970). This estimation is based on the measurement of the rate of decomposition of hydrogen peroxide (H₂O₂), after the addition of the material containing the enzyme.

Catalase catalyses the reaction: $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$

The quantity of hydrogen peroxide decomposed is directly proportional to the concentration of the enzyme in the sample. The hydrogen peroxide produced in tissues is measured by reacting it with excess potassium permanganate (KMNO₄) and then measuring the residual KMNO₄ spectrophotometrically at 480 nm.

Assay Procedure

Sample homogenate (0.5 mL) was placed in ice – cold test tubes, the blank contained 0.5 mL distilled water. Cold phosphate-buffered H₂O₂ (30 mM, 5 mL) was added to both blank and sample tubes at fixed intervals, and were mixed by inversion. After 3 min, the reaction was stopped by rapid addition of 1 mL of 6 M H₂SO₄. The tubes were mixed thoroughly by inversion after which 7 mL of 0.01 M KMNO₄ was added. Absorbance was read at 480 nm within 3 min.

Calculation

The activity of catalase in each sample is calculated thus:

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

where,

O.D = Absorbance of sample test at 480 nm

V_t = Total volume of the reaction mixture = 13.5 mL

M = Molar extinction coefficient of H₂O₂ = 43.6M⁻¹ cm⁻¹

L = Light path = 1.0 cm

V = Volume of sample homogenate used = 0.5 mL

Y = mg of protein in tissue used

Determination of Glutathione Peroxidase Activity

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

Principle

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

Procedure

To an aliquot of sample (0.2 mL), 5 mL of phosphate-buffered H₂O₂, and 1.5 mL of pyrogallol were added. The reaction mixture was allowed to stand for 30 min at room temperature. A deep colour was formed, which was read at 430 nm.

Calculation

$$\text{Enzyme Activity} = \frac{OD/min \times V_t \times Df}{E \times V_s \times Y}$$

where OD = Absorbance of test

V_t = Total volume of reaction mixture

Df = Dilution factor

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

V_s = Volume of sample

Y = mg of protein used

Determination of Concentration of Reduced Glutathione

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

Reagents

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

Procedure

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

Calculation

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

3.10 Histological Procedure

3.10.1 Paraffin tissue processing of Drury and Wallington (1980).

Following fixation in 10% buffered formalin, the tissues were processed as follows:

- Dehydrate in ascending grades of ethanol: 70 % ethanol, 90 % ethanol and 100 % (absolute) ethanol for one hour each.
- Dehydrated tissues were cleared in three changes of xylene for 60 minutes each.
- Infiltration of tissues was done in three changes of paraffin wax at 60°C for 60 minutes each.
- Paraffin wax was used in embedding the tissue. The paraffin blocked tissues were trimmed and mounted on wooden block for a rotary microtome.

3.10.2 Haematoxylin and Eosin staining method of Drury and Wallington (1980).

Sections were dewaxed in Xylene

Sections were rehydrated in decreasing grades of alcohol (100 %, 90 %, and 70 %) and then transferred to water.

Staining of sections was carried out in Iron Haematoxylin for 10-15 minutes.

Excess stain was removed by washing under tap water.

Differentiated in 1% acid alcohol for 10 seconds.

Blued in running tap water for 5minutes.

Sections were counter-stained in 1% Eosin for 5-10minutes.

Sections were rinsed in water.

Dehydrated in ascending grades of alcohol (50% to absolute alcohol).

Cleared in Xylene and mounted in DPX (Distrene Plasticizer and Xylene).

Result: Nuclei stained blue while Cytoplasm stained pink or red.

3.11 Photomicrography

A Leica DM750 research microscope with attached digital camera was used to examine the sections. Photomicrograph of the tissue sections were taken at different magnifications.

3.12 Statistical Analysis

Data were statistically analyzed using Graphpad Prism and the relevant analytical values were obtained. Statistical significance was determined by means of one-way analysis of variance,

followed by Turkey's multiple comparison post-hoc, and data were presented as Mean \pm Standard Error of Mean (SEM). The post-hoc test was carried out for all groups compared with control. Values of $p < 0.05$ was considered statistically significant. The statistical values obtained was converted into graphical representation in form of bar charts.

CHAPTER FOUR

RESULTS

4.1 Change in Body Weight

Results obtained showed that there was no significant change ($P>0.05$) in the body weight of rats across experimental groups (Initial body weight and Final body weight).

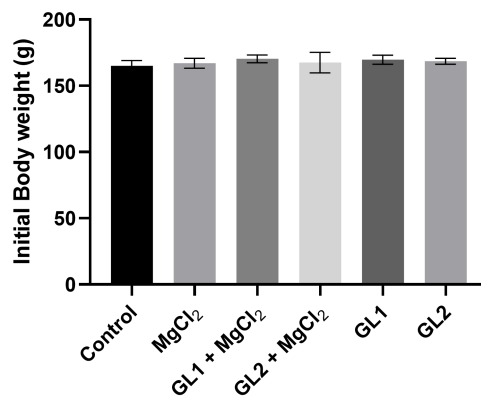


Figure 4.1

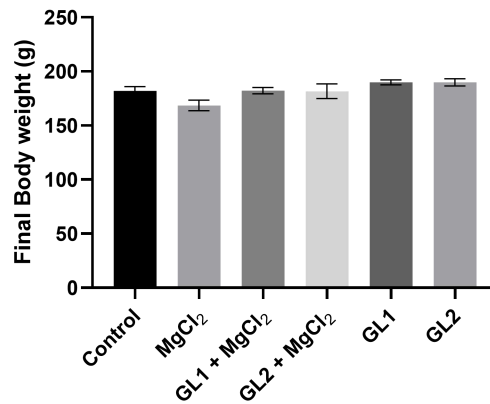


Figure 4.2

Results obtained showed that there was a significant change ($p < 0.05$) in the weight of rats in group B across the experimental group (Figure 4.3)

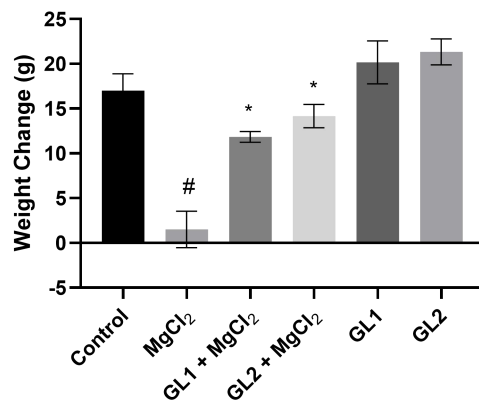


Figure 4.3

4.2 Brain weight

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

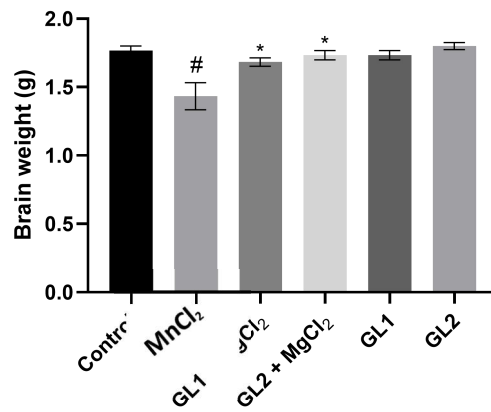


Figure 4.4

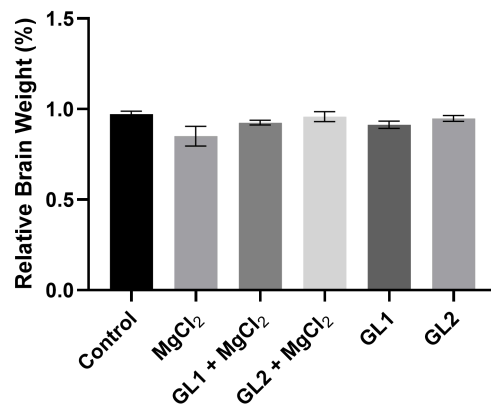


Figure 4.5

4.3 Effect of treatment on Neurobehavioural activity (Movement Initiation and Step Tests)

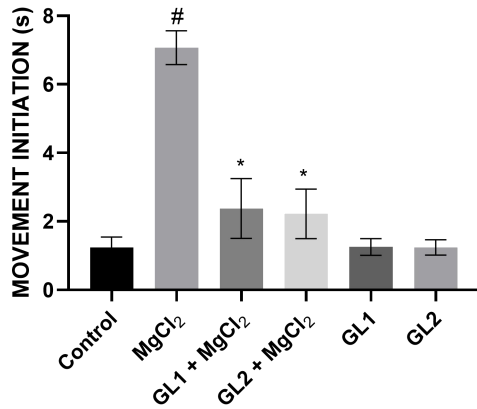


Figure 4.6

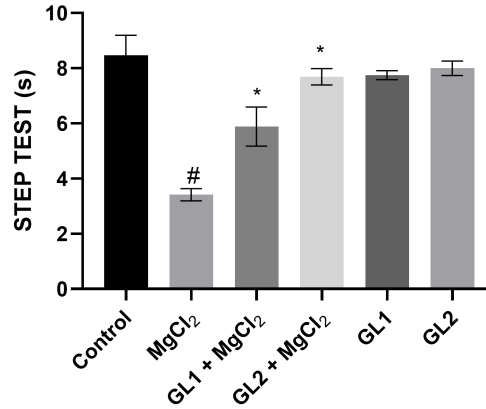


Figure 4.7

Neurobehavioral parameters of control and treatment groups after 28 days.

Values are represented as Mean \pm SEM for each group. [#] $p < 0.05$ compared with the control group, ^{*} $p < 0.05$ compared with the MnCl₂ group.

4.4 Effect of treatment on Neurobehavioural activity (Open Field Test)

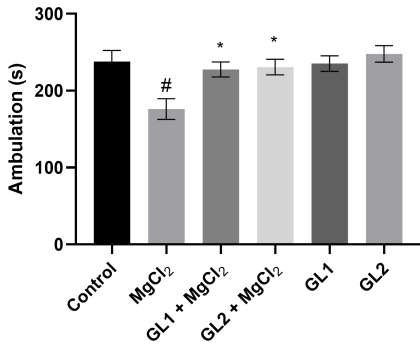


Figure 4.8

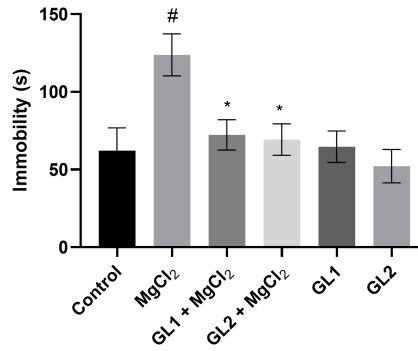


Figure 4.9

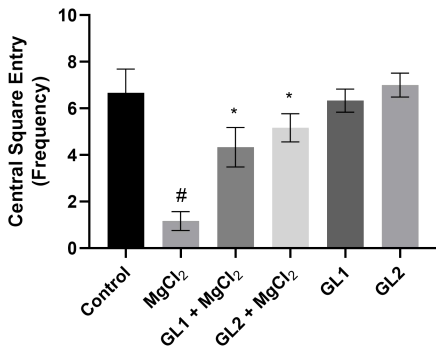


Figure 4.10

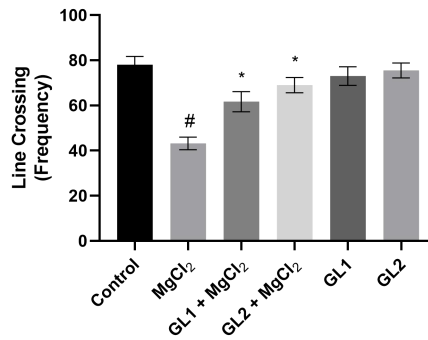


Figure 4.11

Open field parameters of control and treatment groups after 28 days.

Values are represented as Mean \pm SEM for each group. [#] $p < 0.05$ compared with the control group, ^{*} $p < 0.05$ compared with the MnCl₂ group.

4.5 Superoxide Dismutase

Chart showing Superoxide dismutase activity across experimental groups.

Values are represented as Mean \pm SEM for each group. # $p < 0.05$ compared with the control group, * $p < 0.05$ compared with the $MnCl_2$ group.

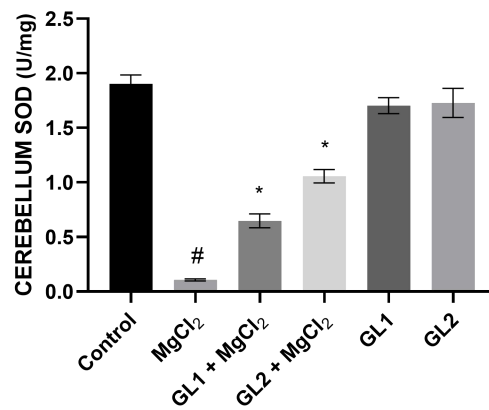


Figure 4.12

4.6 Catalase

Chart showing catalase activity across experimental groups.

Values are represented as Mean \pm SEM for each group. # $p < 0.05$ compared with the control group, * $p < 0.05$ compared with the $MnCl_2$ group.

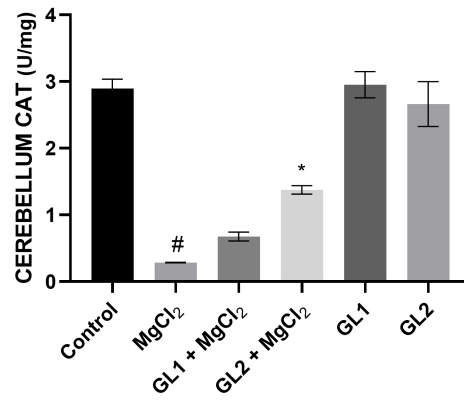


Figure 4.13

4.7 Glutathione peroxidase

Chart showing Glutathione peroxidase activity across experimental groups.

Values are represented as Mean \pm SEM for each group. # $p < 0.05$ compared with the control group, * $p < 0.05$ compared with the $MnCl_2$ group.

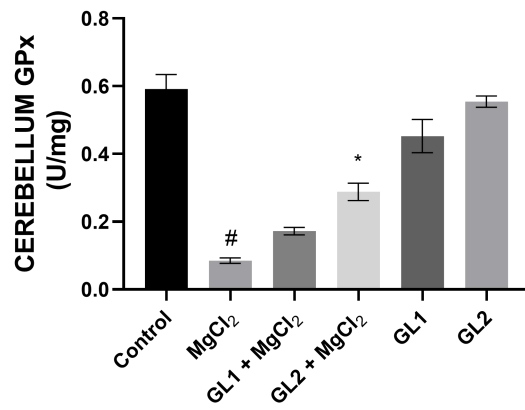


Figure 4.14

4.8 Glutathione

Chart showing Glutathione activity across experimental groups.

Values are represented as Mean \pm SEM for each group. # $p < 0.05$ compared with the control group, * $p < 0.05$ compared with the $MnCl_2$ group.

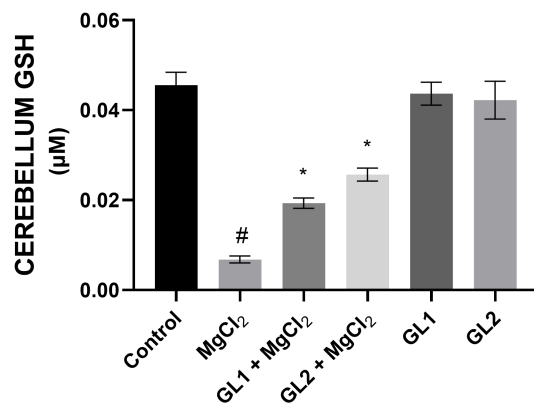


Figure 4.15

4.9 Malondialdehyde

Chart showing Malondialdehyde activity across experimental groups.

Values are represented as Mean \pm SEM for each group. # $p < 0.05$ compared with the control group, * $p < 0.05$ compared with the $MnCl_2$ group.

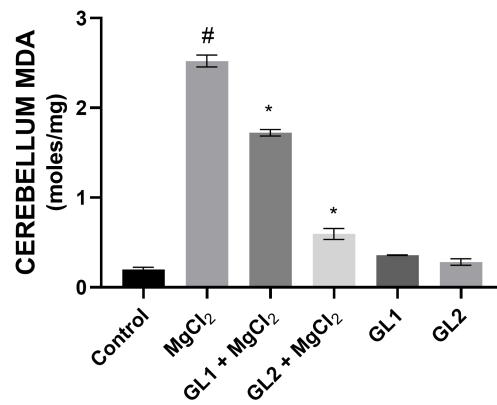


Figure 4.16

4.10 Histology of the cerebellum

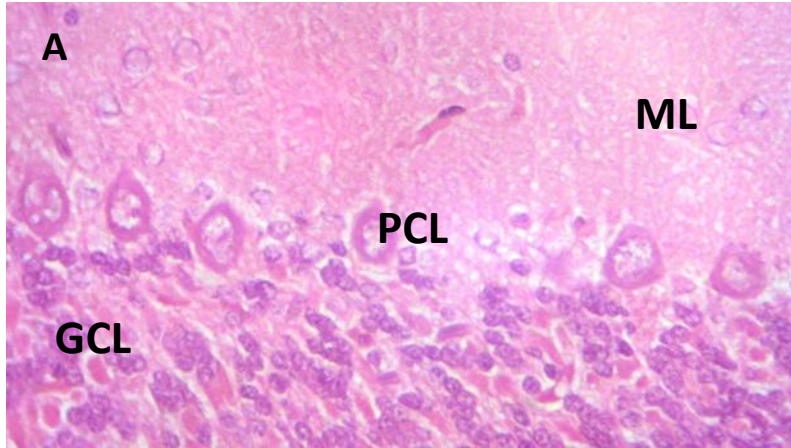


Plate 1: Representative histology of the cerebellum in control rats. (A) Normal histological structure of cerebellum layers – Molecular layer (ML); Purkinje Cell layer (PCL); Granular Cell layer (GCL) (H&E; Scale bar: 25 μ m).

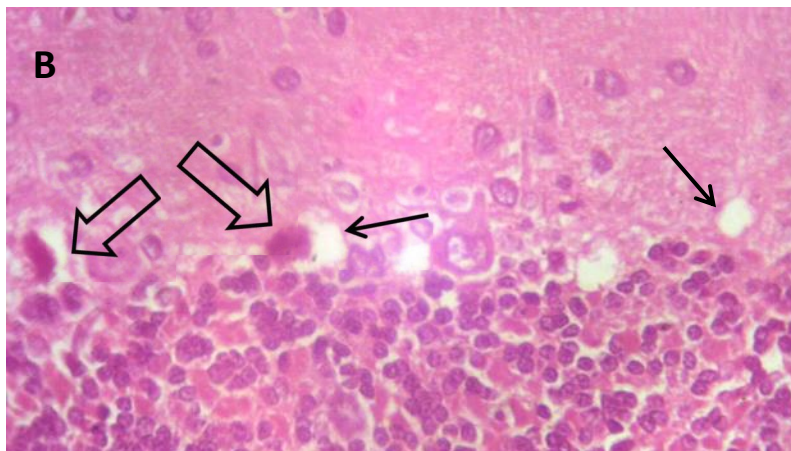


Plate 2: Representative histology of the cerebellum in treatment rats. (B) cerebellum showing degenerating Purkinje cells (big arrows), with nuclei appearing irregular, darkly stained and pyknotic. Also observed are vacuolations in the Purkinje cell layer (arrows). (H&E; Scale bar: 25 μ m).

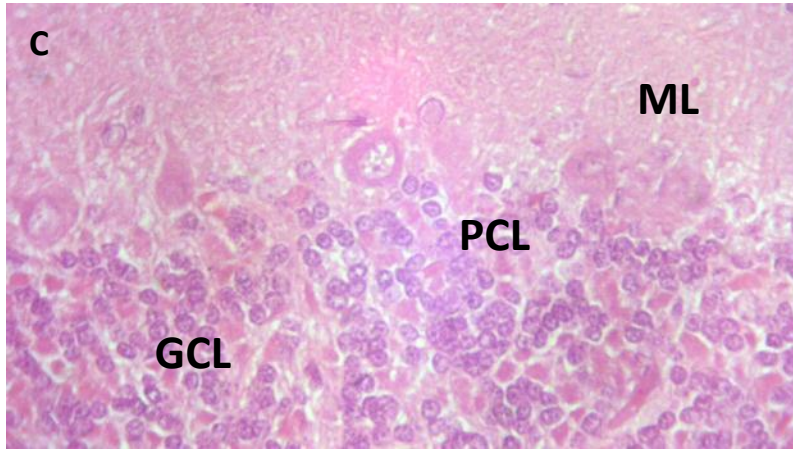


Plate 3: Representative histology of the cerebellum in treatment rats. (C) Relatively Normal histological structure of cerebellum layers observed – Molecular layer (ML); Purkinje Cell layer (PCL); Granular Cell layer (GCL). (H&E; Scale bar: 25 μ m).

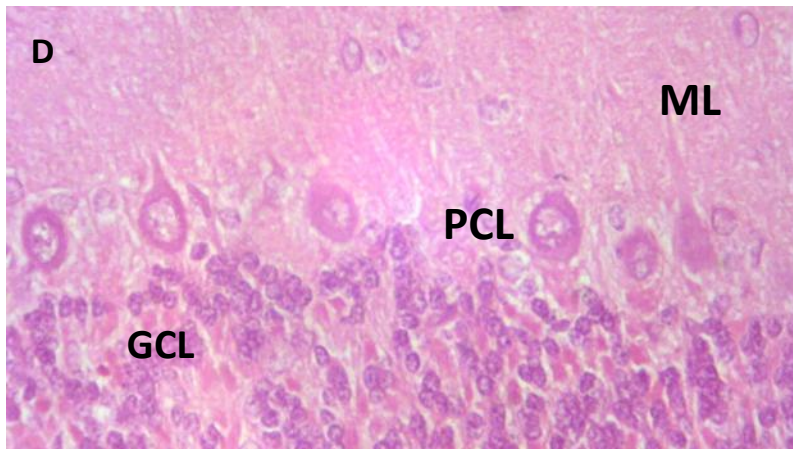


Plate 4: Representative histology of the cerebellum in treatment rats. (D) Relatively Normal histological structure of cerebellum layers observed – Molecular layer (ML); Purkinje Cell layer (PCL); Granular Cell layer (GCL). (H&E; Scale bar: 25 μ m).

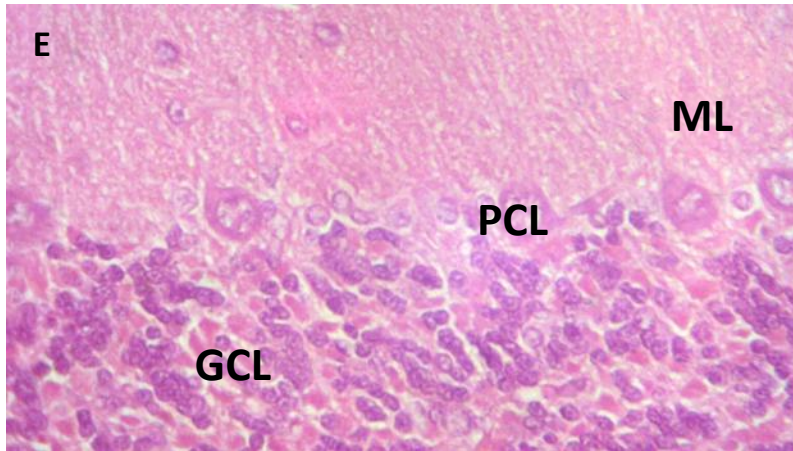


Plate 5: Representative histology of the cerebellum in treatment rats. (E) Relatively Normal histological structure of cerebellum layers observed – Molecular layer (ML); Purkinje Cell layer (PCL); Granular Cell layer (GCL). (H&E; Scale bar: 25 μ m).

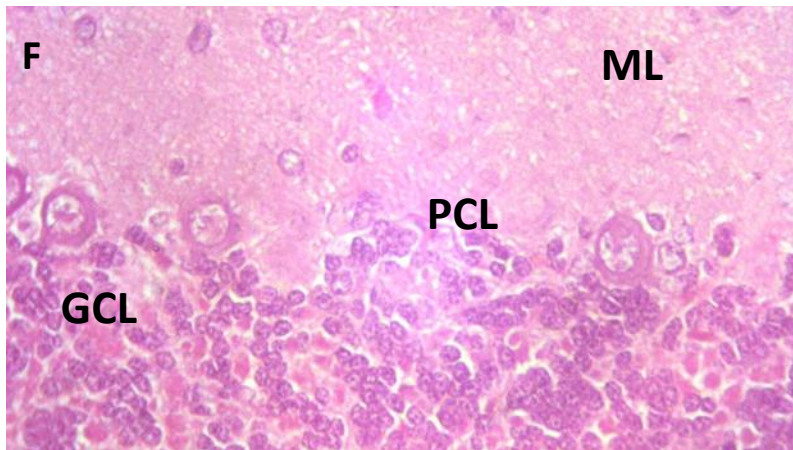


Plate 6: Representative histology of the cerebellum in treatment rats. (F) Relatively Normal histological structure of cerebellum layers observed – Molecular layer (ML); Purkinje Cell layer (PCL); Granular Cell layer (GCL). (H&E; Scale bar: 25 μ m).

CHAPTER FIVE

5.1 Discussion

The result findings showed that there was a significant decrease in the body weight of rats in group B which were administered 10mg/kg of MnCl₂ only compared to the control group. This observation is consistent with previous studies, where daily manganese chloride exposure led to dose-dependent reductions in body weight in rats done by Rahman *et al.*, 2014..Result from the present study showed that there was a significant increase in the body weight of rats treated with 10mg/kg of MnCl₂ followed by 100mg/kg of *Gongronema latifolium* (Group C) and in the rats treated with 10mg/kg of MnCl₂ followed by 200mg/kg of *Gongronema latifolium*(Group D). This suggests that the extract may exert a protective effect, likely through its antioxidant and anti-inflammatory properties, which help maintain metabolic function and overall health (Amangian-Kalawa, Udo, & Akpan 2020; Okoya, Adekunle & Obiyan, 2020). Results obtained showed that there no significant change ($p > 0.05$) was observed in the brain weight of rats across experimental groups. This is in line with the research carried out by Dorman *et al.*, 2000 which mentioned that brain weight change was not significantly altered by manganese exposure. Results obtained showed that there was no significant difference in the relative brain weight of rats among treated group when compared to control. In the movement initiation test, rats administered 10 mg/kg MnCl₂ showed a significant increase in initiation time compared to the control, indicating motor impairment consistent with manganese-induced cerebellar dysfunction (Rodrigues et al., 2015). However, pretreatment with 100 mg/kg and 200 mg/kg *Gongronema latifolium* and 10 mg/kg MnCl₂ respectively, markedly reduced initiation time, suggesting improved motor function. Similarly, *Gongronema latifolium* treatment enhanced step test performance relative to the MnCl₂-only group, reflecting restored coordination and balance.

These results align with Igile *et al.* (2003) and Ojo *et al.* (2019), who reported that *Gongronema latifolium* enhances antioxidant defenses and protects against oxidative-stress-induced cellular damage. In the Open Field Test, present results showed a significant decrease in ambulation, central square entry, and line crossing in rats administered 10 mg/kg MnCl₂ compared to the control, indicating reduced exploratory and locomotor activity (Reichel *et al.*, 2016). Pretreatment with 100 mg/kg and 200 mg/kg *Gongronema latifolium* and 10 mg/kg MnCl₂ respectively significantly increased these parameters compared to the MnCl₂-only group, reflecting improved motor and exploratory behavior. Immobility time increased significantly in the MnCl₂ group but was reduced following *Gongronema latifolium* administration. These findings align with those of Adefegha and Oboh (2012) and Eleyinmi *et al.* (2008), who reported that *Gongronema latifolium* enhances neurobehavioral performance and mitigates oxidative stress-induced neuronal dysfunction. Results obtained showed that there was a significant decrease of SOD level in 10 mg/kg of MnCl₂ group when compared to the control group. This corresponds with previous research carried out by Latronico *et al.*, 2024 that Mn exposure reduces SOD activity in rat brain tissue. The endogenous antioxidant enzymes such as SOD, present a cellular antioxidative defense against free-radical damage (Hassani *et al.* 2015). Inhibition in the activities of antioxidant enzymes like SOD in various rat tissues leads to oxidative stress which can later cause a huge damage to the rat tissues (Pedraza, 2014). Result from the present study showed that there was a significant increase in the SOD level of rats treated with 10mg/kg of MnCl₂ followed by 100mg/kg of *Gongronema latifolium* (Group C) and in the rats treated with 10mg/kg of MnCl₂ followed by 200mg/kg of *Gongronema latifolium*(Group D). This is in line with a research done by Ogunro & Olasehinde, 2024 which showed neuroinflammatory response and Redox-regulation in rats exposed to managanese

chloride and treated with hyperoside from *Gongronema latifolium*. Results obtained showed that there was a significant decrease of CAT level in 10 mg/kg of $MnCl_2$ group when compared to the control group. This corresponds to a study carried out by Zawadzki & Sliwiska-Mosson, 2021. Result from the present study showed that there was a significant increase in the CAT level of rats treated with 10mg/kg of $MnCl_2$ followed by 100mg/kg of *Gongronema latifolium* (Group C) and in the rats treated with 10mg/kg of $MnCl_2$ followed by 200mg/kg of *Gongronema latifolium*(Group D). Treatment with *Gongronema latifolium* has been shown to restore catalase activity in oxidative stress models (Ugochukwu & Cobourne, 2003), supporting its potential neuroprotective role against $MnCl_2$ -induced oxidative damage. Results obtained showed that there was a significant decrease of GPx level in 10 mg/kg of $MnCl_2$ group when compared to the control group which is consistent with reports by Erikson *et al.*, 2004. Result from the present study showed that there was a significant increase in the GPx level of rats treated with 10mg/kg of $MnCl_2$ followed by 100mg/kg of *Gongronema latifolium* (Group C) and in the rats treated with 10mg/kg of $MnCl_2$ followed by 200mg/kg of *Gongronema latifolium*(Group D). This improvement aligns with findings by Ebong *et al.*, 2020. Results obtained showed that there was a significant decrease of GSH level in 10 mg/kg of $MnCl_2$ group when compared to the control group which is consistent with findings by Aschner *et al.* 2007 and Prabhakar *et al*, 2017. Result from the present study showed that there was a significant increase in the GSH level of rats treated with 10mg/kg of $MnCl_2$ followed by 100mg/kg of *Gongronema latifolium* (Group C) and in the rats treated with 10mg/kg of $MnCl_2$ followed by 200mg/kg of *Gongronema latifolium*(Group D). This result agrees with previous reports by Nwachujor *et al.* 2013 which attributed to the increase to the extract's rich polyphenolic and flavonoid constituents that enhance endogenous antioxidant defense mechanisms. Results obtained showed that there was a

significant increase in MDA level in group B when compared to the control which corresponds to findings by Prabhakar et al. 2017 and Sharma *et al* 2018. However group C and D showed a marked decrease in MDA concentration, aligning with reports by Ezejiofor *et al.*, 2016 who attributed this effect to the plant's potent antioxidative and free-radical scavenging phytochemicals.

Result obtained showed normal histological structure of cerebellum layers – Molecular layer (ML); Purkinje Cell layer (PCL); Granular Cell layer (GCL) in group (A) while degenerating Purkinje cells with nuclei appearing irregular, darkly stained and pyknotic was observed as well as vacuolation in the molecular layer in group B. Findings agreed with the research of Weber *et al.*, 2002 which reported that exposure to MnCl₂ alters the histoarchitecture of the cerebellum. However, Group C to F which were treated with *Gongronema latifolium* extract had relatively normal histological architecture. This corresponds with the findings of Ujong *et al.*, 2022, who reported preserved neural structure with *Gongronema latifolium*.

5.2 Conclusion

Aqueous leaf extract of *Gongronema latifolium* was able to mitigate Manganese chloride-induced cerebellar toxicity in adult wistar rats. The extract reduced oxidative damage and preserved cerebellar function, suggesting its potential as a natural antioxidant and neuroprotective agent.

5.3 Recommendations

It is recommended that further studies identify and characterize the active neuroprotective effects of *Gongronema latifolium* and explore its potential as a safe, affordable herbal therapy against oxidative stress and manganese chloride-induced toxicity.

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