EVALUATION OF NEUROBEHAVIOURAL AND BIOCHEMICAL EFFECTS OF THE ETHANOL EXTRACT OF *Afzelia africana* STEM BARK IN ROTENONE-INDUCED PARKISONISM IN RODENTS

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CERTIFICATION

We certify that this work was carried out by JUMOKE VICTORIA AFOLABI-ADUNMO, with matriculation number PHA1908444, in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

I dedicate this work to God Almighty for his love and mercy.

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I am grateful to God for the grace to be able to complete my academic journey and for his protection and love throughout my six years in school. I also want to thank Mama Maria and St. Joseph of Cupertino for all the intercession made on my behalf throughout my time in school.

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ABSTRACT

Parkinson's disease is a neurodegenerative disease that occurs due to progressive loss of dopaminergic neuron resulting in motor dysfunction with symptoms such as bradykinesia, akinesia and non-motor symptoms such as anxiety and cognitive deficits. Experimental models using neurotoxins such as rotenone mimic the features of Parkinson's disease. *Afzelia africana*, a medicinal plant with antioxidant and anti-inflammatory properties has shown potential neuroprotective properties. The purpose of this study is to evaluate the biochemical and behavioural effects of *Afzelia africana* in rotenone-induced Parkinsonism using catalepsy and beam walking.

Phytochemical screening of the stem bark of *Afzelia africana* was conducted to determine the bioactive phytoconstituents present and acute toxicity studies was done to determine the safety profile of the plant. Rats randomly divided into different groups viz: no treatment, vehicle only, rotenone plus vehicle, rotenone plus 250 mg/kg of stem bark extract, and rotenone plus 500 mg/kg of stem bark extract were used for the study. These were treated daily with either the vehicle or two doses of *Afzelia africana.* Rats subject to rotenone treatment received rotenone on days 1,4,7 and 10 of the study. Catalepsy and beam walking were conducted to assess motor performance on days 0, 5 and 10. Animals were sacrificed on the 11^{th} day and organs harvested for biochemical analysis.

The mean lethal dose of *Afzelia africana* was estimated to be greater than 5000 mg/kg. *Afzelia africana* at doses used in this study reduced the cataleptic score and increased time spent in the beam walking. Treatment with *A, africana* reduced rotenone elevated levels of alanine transaminase, alkaline phosphatase, aspartate transaminase in the livers of test animals. *A. africana* also reduced urea and creatinine levels increased by rotenone. Findings from this that the ethanol extract of *Afzelia africana* has nephroprotective and hepatoprotective potential.

CHAPTER ONE

INTRODUCTION

The occurrence of Parkinson's disease (PD) has more than doubled worldwide over the last 25 years. In 2019, it was estimated that over 8.5 million people were living with PD. The rates of disability and fatalities associated with PD are rising more rapidly than those of any other neurological condition. Current figures indicate that in 2019, PD was responsible for 5.8 million disability-adjusted life years, marking an 81% increase since the year 2000, and it led to 329,000 deaths, more than doubling since 2000 (World Health Organization, 2023).

Parkinson’s disease is a neurological disorder marked by classic motor symptoms of Parkinsonism, linked to Lewy bodies and a reduction in dopaminergic neurons in the substantia nigra (Samaila and Hayatudeen, 2019).

Parkinson's disease (PD) is a progressive neurodegenerative condition marked by both motor and non-motor symptoms. Over two centuries after it was first clinically identified, Parkinson's disease continues to be a significant health issue that impacts an increasing segment of the population. Existing treatments only alleviate symptoms; there is still no cure available that addresses the underlying neurodegenerative mechanisms or alters the progression of the disease. (Dovonou, 2023).

Parkinson’s disease majorly affects the basal ganglia, a cluster of nuclei situated at the base of the forebrain. Two principal neuropathological features of the disease are: the damage of pigmented dopaminergic neurons in the substantia nigra pars compacta (SNpc), and the appearance of Lewy bodies that lead to the formation of Lewy neurites, which are normal neuropathological outcomes in Parkinson's disease (Samaila and Hayatudeen, 2019).

Age is a significant risk factor: both prevalence and incidence increase with age. The peak rates are often in older age-groups (e.g., 70 - 80+ years). Males also have higher burden than

females: many studies show higher incidence and prevalence in men. Other contributing factors include population aging, growth, and increases in the underlying risk including environmental exposures (Luo, 2025). The reduced mobility elevates risk of falls, fractures, hospitalization, thus increasing health-care costs and resource needs in many countries.

1.2 Etiology of Parkinson's Disease

The exact cause of Parkinson's disease remains unclear, though it is believed to arise from intricate interactions between genetic and environmental factors that influence key cellular processes. To date, no environmental factors have been definitively linked to Parkinson's disease. Currently known genetic factors account for approximately 10% of the hereditary aspects of the condition (Hauser *et al*., 2014).

1. Environmental risk factors include: chronic pesticide exposure, previous head injury, and chronic use of beta-blockers (Kalia and Lang, 2015).

2. Genetic risk factors: occur as a result of some genes, such as the beta-glucocerobrosidase gene (GBA), leucine-rich repeat kinase 2 (LRRK2), and the synuclein alpha gene (SNCA). Changes in these genes lead to the development of Lewy bodies (Kalia and Lang, 2015).

Mutations in the LRRK2 gene are frequently linked to genetic forms of Parkinson's disease, making up around 4% of familial cases and about 15% of sporadic ones. On the other hand, Mutations in the parkin gene are the leading cause of autosomal recessive Parkinson's disease. These parkin mutations are often found in individuals who develop Parkinson's disease before the age of 45. In fact, parkin mutations can account for roughly 50% of familial cases and around 15% of sporadic cases (Jankovic *et al*., 1990; Corti *et al*., 2011).

However, the primary genetic risk factor for developing Parkinson's disease is a mutation in the GBA gene, which encodes beta-glucocerebrosidase, a lysosomal enzyme (Sidransky and Lopez, 2012).

1.3 Pathophysiology of Parkinson's Disease

Parkinson's disease (PD) primarily involves the gradual loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), a midbrain region that produces dopamine and helps control motor function. When these neurons degenerate, dopamine levels in the striatum drop significantly, upsetting the balance of excitatory and inhibitory signals in the basal ganglia circuit (Radhakrishnan and Goyal, 2018; Marino *et al*., 2020). This disturbance hampers voluntary movement coordination and leads to the key motor symptoms of PD: resting tremor, rigidity, bradykinesia, and postural instability (Bloem *et al*., 2021).

1.3.1 Degeneration of Dopaminergic Neurons in the Substantia Nigra:

Anatomically, the substantia nigra has two parts: the pars compacta, containing dopaminergic neurons, and the pars reticulata, mainly made up of GABAergic neurons. In Parkinson's disease (PD), the pars compacta undergo targeted neurodegeneration, while the pars reticulata remains largely intact (Bloem *et al*., 2021). This loss of dopamine in the striatum then alters activity in connected nuclei, such as the globus pallidus and subthalamic nucleus, leading to increased inhibitory signals to the thalamus and decreased cortical stimulation, which affects motor control (Marino *et al*., 2020). At the cellular level, the degeneration of dopaminergic neurons is closely associated with Lewy bodies, which are intraneuronal inclusions primarily composed of misfolded α-synuclein. The accumulation of α-synuclein disrupts synaptic vesicle trafficking, impairs neuronal communication, and interferes with normal protein degradation pathways (Vázquez-Vélez and Zoghbi, 2021). As the disease advances, degeneration spreads beyond the nigrostriatal pathway to other brain regions, such as the locus coeruleus, dorsal motor nucleus of the vagus, and cerebral cortex, contributing to the development of non-motor symptoms like cognitive decline, sleep issues, and autonomic problems (Radhakrishnan and Goyal, 2018; Marino *et al*., 2020).

1.3.2 Role of Oxidative Stress in Parkinson's disease

Oxidative stress is one of the key pathogenic mechanisms implicated in the progressive degeneration of dopaminergic neurons in Parkinson's disease (PD). The substantia nigra is particularly susceptible to oxidative damage because of its high oxygen use and abundant lipids content, and dopamine metabolism, which naturally generates reactive oxygen species (ROS) (Radhakrishnan and Goyal, 2018; Marino *et al*., 2020). When the brain's antioxidant defenses are overwhelmed, excessive ROS accumulation leads to oxidative modification of lipids, proteins, and DNA, ultimately impairing neuronal function and survival (Chang and Chen, 2020; Guo *et al*., 2018).

These free radicals further activate microglial cells and trigger pro-inflammatory cytokine release, amplifying neurodegeneration in a vicious cycle (Bloem *et al*., 2021).

Moreover, the oxidative metabolism of dopamine produces hydrogen peroxide and quinones, which, in the presence of iron, can form highly reactive hydroxyl radicals that exacerbate neuronal damage. This cumulative oxidative burden contributes to α-synuclein misfolding and aggregation, a hallmark of PD pathology (Chang and Chen, 2020; Marino *et al*., 2020).

1.3.3 Mitochondrial dysfunction

Mitochondrial dysfunction represents one of the most consistent and defining pathological features of Parkinson's disease (PD). Mitochondria are vital for neuronal survival due to their central role in energy metabolism, calcium homeostasis, and regulation of apoptotic pathways. In PD, impaired mitochondrial activity (particularly at the level of complex I of the electron transport chain) leads to reduced ATP synthesis, excessive production of reactive oxygen species (ROS), and activation of cell death cascades that compromise dopaminergic neurons in the substantia nigra (Bose and Beal, 2016; Hu and Wang, 2016). These mitochondrial impairments not only disrupt oxidative phosphorylation but also trigger oxidative stress,

which further damages mitochondrial DNA, proteins, and lipids, perpetuating a vicious cycle of neurodegeneration (Henrich *et al*., 2023; Marino *et al*., 2020).

Genetic studies have also highlighted the role of mitochondrial pathways in PD. Mutations in genes such as PINK1, DJ-1, and Parkin, which regulate mitochondrial quality control and mitophagy, have been associated with familial forms of the disease. Dysfunctional PINK1-Parkin signaling impairs the removal of damaged mitochondria, leading to their accumulation and increased susceptibility to oxidative injury (Park *et al*., 2018; Vázquez-Vélez and Zoghbi, 2021). Additionally, α-synuclein aggregates have been shown to interact with mitochondrial membranes, altering permeability and promoting cytochrome c release, a key trigger of apoptosis (Hu and Wang, 2016; Henrich *et al*., 2023). It serves as both a trigger and amplifier of dopaminergic neuronal death in PD, interlinking with oxidative stress and protein aggregation to drive disease progression.

1.3.4 **Neuroinflammation**:

Neuroinflammation is a key factor in the development and progression of Parkinson's disease (PD). It acts as both a driving force and a result of the loss of dopamine-producing neurons. When glial cells, especially microglia and astrocytes, become chronically activated, they release a host of pro-inflammatory substances, including tumor necrosis factor-alpha (TNF-α) and various interleukins, like IL-1β and IL-6, along with nitric oxide. These inflammatory agents can lead to oxidative stress, impair the functioning of mitochondria, and trigger neuronal death in the substantia nigra, a critical region that suffers damage in PD(Wang *et al*., 2015).

Microglial activation, while initially a protective mechanism to clear damaged neurons and misfolded proteins, becomes detrimental when sustained. Prolonged inflammatory signaling amplifies neuronal damage through feedback loops involving α-synuclein aggregation, which

further stimulates microglial reactivity (Vivekanantham *et al*., 2015). This interplay between neuroinflammation and proteinopathy accelerates neurodegeneration and exacerbates motor dysfunction.

Recent studies have identified potential biomarkers of neuroinflammation in PD, including elevated levels of cytokines, chemokines, and inflammatory enzymes in cerebrospinal fluid and peripheral blood. These markers provide valuable insight into disease progression and may guide early diagnosis and therapeutic interventions (Liu *et al*., 2022).

1.3.5 Protein Aggregation:

A key feature of Parkinson's disease (PD) pathology is the abnormal aggregation of the neuronal protein α-synuclein, leading to the formation of Lewy bodies and Lewy neurites within dopaminergic neurons. α-Synuclein is a protein found in presynaptic terminals involved in synaptic vesicle trafficking and neurotransmitter release under physiological conditions. However, under pathological circumstances such as oxidative stress, mitochondrial dysfunction, or genetic mutations, the protein misfolds and aggregates into insoluble fibrils (Srinivasan *et al*., 2021).

The aggregation of α-synuclein follows a complex multistep process involving the conversion of monomeric α-synuclein into oligomers, protofibrils, and finally mature fibrils that accumulate in the cytoplasm (Leitão *et al*., 2021). These oligomeric intermediates are considered particularly neurotoxic, as they can disrupt membrane integrity, impair synaptic function, and trigger neuroinflammation. Over time, these aggregates coalesce into Lewy bodies, which are eosinophilic inclusions that serve as neuropathological signatures of PD (Gómez-Benito *et al*., 2020).

The exact mechanisms driving α-synuclein aggregation are multifactorial, involving post-translational modifications, metal ion interactions, oxidative stress, and genetic mutations in the SNCA gene encoding α-synuclein (Singh *et al*., 2025).

Overall, α-synuclein aggregation and Lewy body formation represent key molecular events that link cellular dysfunction, oxidative damage, and neuronal death in PD.

Understanding these processes is essential for identifying potential therapeutic strategies to modulate protein aggregation and prevent dopaminergic neurodegeneration.

1.4 Clinical Features of Parkinson's Disease

1.4.1 Motor Symptoms:

PD is classically characterized by the Parkinsonian triad: resting tremor, bradykinesia, and rigidity (Opara *et al*., 2017). Tremors usually begin unilaterally, often in the hands, while bradykinesia manifests as slowness in voluntary movements, and rigidity causes stiffness and resistance to passive movement. Postural instability develops later, increasing fall risk (Sheta *et al*., 2024).

1.4.2 Non-Motor Symptoms:

Non-motor features are increasingly recognized for their prodromal and clinical significance, sometimes appearing years before motor symptoms. These include sleep disturbances, cognitive impairment, mood disorders, autonomic dysfunction, and anxiety (Pellicano *et al*., 2007; Zambetta *et al*., 2025). Such symptoms can significantly reduce the quality of life and are crucial for early diagnosis.

1.5 Pharmacological management of Parkinson's disease

This primarily focuses on restoring dopaminergic activity and addressing both motor and non-motor symptoms.

a) Levodopa remains the gold standard therapy, as it is converted to dopamine in the brain, directly compensating for dopaminergic neuronal loss in the substantia nigra. It is typically combined with carbidopa or benserazide to inhibit peripheral decarboxylation, thereby increasing central availability and reducing side effects such as nausea and hypotension (Armstrong and Okun, 2020).

b) Dopamine agonists (e.g., pramipexole, ropinirole) stimulate dopamine receptors directly, providing symptomatic relief with a lower risk of motor fluctuations in early disease stages, though they are often associated with neuropsychiatric side effects (Oertel and Schulz, 2016).

c) Monoamine oxidase-B (MAO-B) inhibitors, such as selegiline and rasagiline, reduce dopamine breakdown, thereby extending dopamine's synaptic activity (Stoker, Torsney, and Barker, 2018).

d) Catechol-O-methyltransferase (COMT) inhibitors like entacapone and tolcapone further enhance levodopa's efficacy by preventing peripheral dopamine metabolism (Lang and Espay, 2018).

In more advanced stages, adjuvant therapies such as amantadine, an NMDA receptor antagonist help manage levodopa-induced dyskinesia by modulating glutamatergic transmission (Stoker and Barker, 2020).

1.6 Ethnopharmacology

Ethnopharmacology is essentially a scientific field focused on exploring how various substances used by humans can affect biology. This includes studying their potential benefits, toxic effects, or other pharmacological impacts. Rather than just cataloging traditional or local uses of these substances, it involves a comprehensive examination that blends anthropology with pharmacology and toxicology to understand their wider implications (Heinrich *et al*., 2009).

Ethnopharmacology uncovers numerous potential drug candidates by exploring how different cultures use plants and natural products medicinally. This compelling method expands the horizons of drug discovery, emphasizing the importance of combining traditional knowledge with contemporary scientific research to invigorate the development of new medicines field (Aswar, 2025).

The ultimate goal of ethnopharmacology should be to identify drugs to alleviate human illness via a thorough analysis of plants alleged to be useful in human cultures throughout the world (Farnsworth, 2007). Ethnopharmacology, by highlighting the beneficial effects of plants, has laid an early foundation for the therapeutic use of natural compounds. Natural products, whether in their native form or after crude extraction of their active ingredients, have long been used by various populations and have been explored as valuable sources for drug development design (Pirintsos *et al*., 2022).

Ethnopharmacological studies typically start with ethnobotanical investigations surveys. In this process, researchers collaborate with traditional healers and local communities to gather information about how plants are used for medicine, including their preparation methods and where they can be found. Accurate identification of plant species is essential, after which samples are collected for further testing in the lab. Various extraction techniques are applied to isolate the beneficial compounds found in the plants. These extracts are then subjected to bioassays to assess their properties, such as antimicrobial, anti-inflammatory, antidiabetic, or anticancer activities (Oimau, 2025).

Quinine is an alkaloid derived from Cinchona spp. It was the first antimalarial medication and was an effective treatment for this potentially deadly infectious disease during colonial times, enabling European settlement in numerous tropical and subtropical regions (Pirintsos *et al*., 2022). The interest in using plant-based extracts as supplementary or alternative

therapies is rapidly increasing. Among traditional African medicinal plants, *Afzelia africana* (a tree widely used in West Africa) stands out for its known benefits in treating neurological, and metabolic disorders. This study specifically aims to assess the biochemical parameters of an ethanolic extract derived from the stem bark of *Afzelia africana* in a rodent model of rotenone-induced Parkinsonism.

1.7 Background of the plant

Afzelia africana Sm. Ex Pers., is a large deciduous tree commonly found across West and Central Africa. It is appreciated for its durable wood and its various traditional medicinal uses in African medicine (Iliemene, Uju, and Atawodi, 2014). This species grows well in tropical environments, typically in savannah and transition zones between forests and savannahs. Morphologically, it features a straight, cylindrical trunk, grayish-brown bark, and pinnate leaves (Okeke, *et al*., 2021).

1.7.1 Botanical Description

Scientific name: *Afzelia africana* Sm. ex Pers

Family: Leguminosae, Fabaceae

Common names: Afzelia, lucky-bean tree, African oak, African mahogany (English); kawo (Hausa), apa (Yoruba); akpalata (Igbo); gayoki (Fulani); papao (Ghana).

(Heuzé et al., 2019)

1.7.2 Ethnomedicinal uses

1. The stem bark and leaves are traditionally used for managing inflammation, arthritis, and body pains due to their presumed anti-inflammatory properties (Akah *et al*., 2008; Oyedemi *et al*., 2011).

2. Decoctions made from the bark or leaves are administered to treat feverish conditions and malaria (Gérard *et al*., 2011).

3. The bark and seed extracts are applied topically to promote wound healing and treat various skin infections (Vigbedor *et al*., 2023).

4. Traditional healers use stem bark and seeds for the treatment of bacterial and fungal infections, aligning with reports of antimicrobial efficacy in modern studies (Akah *et al*., 2008; Vigbedor *et al*., 2023).

5. Decoctions of the seed or bark are used as antidiarrheal and gastrointestinal remedies (Ndukwe *et al*., 2022).

6. The stem bark extract has been used traditionally to manage diabetes and blood sugar imbalances, a practice supported by animal studies showing antidiabetic effects (Oyedemi *et al*., 2011).

7. Infusions of the bark and leaves are employed in some regions to treat coughs, colds, and related respiratory issues (Gérard *et al*., 2011)

1.7.3 General Uses

1. Provides high-quality hardwood for furniture, flooring, joinery, and construction. Durable, termite- and fungus-resistant, suitable for outdoor structures and heavy carpentry. Also used for boat building, canoes, and veneer production (Gérard and Louppe., 2011)

2. In traditional medicine, the bark and leaves are employed to treat various ailments, with specific conditions differing by region. The gum exuded from the bark is used in local remedies and occasionally in pharmaceutical products (Gérard and Louppe., 2011)

3. The seeds are commonly used as soup thickeners in southeastern Nigeria. They are rich in carbohydrates and proteins, providing valuable nutrients for local diets. Additionally, the seed

oil has potential as an edible oil due to its favorable fatty acid profile (Ejikeme *et al*., 2010; Igwenyi and Akubugwo, 2010)

4. The seed oil is suitable for industrial uses like soap, paint, and cosmetics. The tree's gum serves as an adhesive and in varnish. Wood residues are valuable for crafts, fuel, and charcoal (Ejikeme *et al*., 2010; Gérard and Louppe, 2011).

1.7.4 Phytochemical profile

Studies have shown that the bioactive compounds present in *Afzelia africana* include;

1. Flavonoids, tannins, saponins, alkaloids, terpenoids, steroids, phenolic compounds, and glycosides, which are known to exhibit antioxidant, antimicrobial, and anti-inflammatory activities, support the plant's traditional medicinal applications (Friday *et al*., 2018; Okeke *et al*., 2020).

2. Notably, the bark includes flavanone derivatives such as eriodictyol, known for strong antioxidant activity (Vigbedor *et al*.,2023)

3. Proximate analysis also identifies minerals like magnesium, potassium, and calcium, as well as vitamins like ascorbic acid, highlighting its nutritional and therapeutic potential (Okeke *et al*., 2020).

4. Gas chromatographic fingerprinting of root bark fractions, revealing the presence of multiple phenolic and terpenoid compounds. These phytochemicals exhibited notable antibacterial and antifungal activity, reinforcing earlier reports of the plant's antimicrobial efficacy (Vigbedor *et al*., 2023)

1.7.5 Pharmacological activities of *Afzelia africana*

Afzelia africana has been the focus of several pharmacological studies due to its rich content of bioactive compounds with multiple therapeutic effects. Experimental findings from

different plant parts (bark, roots, and seeds) indicate broad pharmacological potential, validating its ethnomedicinal applications.

1. Anti-inflammatory and Analgesic Activities: The bark extract of *Afzelia africana* has demonstrated potent anti-inflammatory and analgesic properties (Akah, Okpi, and Okoli, 2008).

2. Antioxidant Properties: They have a high total phenolic content in bark and root extracts, indicating significant antioxidant capacity. These antioxidant activities are critical for protecting cells from oxidative damage, which is relevant in diseases involving oxidative stress such as neurodegeneration (Okeke *et al*., 2020; Vigbedor *et al*.,2022).

3. Antimicrobial and Antifungal Effects: Root and seed extracts from *Afzelia africana* have shown Displays broad-spectrum antimicrobial activity against both bacteria and fungi pathogens. Studies reported substantial inhibitory effects against Staphylococcus aureus, Escherichia coli, Candida albicans, and Aspergillus species (Akah *et al*., 2008; Friday and Igwe, 2018, and Vigbedor *et al*.,2022).

4. Antidiabetic and Hematological Activities: Aqueous stem bark extracts of *Afzelia africana* significantly reduced blood glucose levels in streptozotocin-induced diabetic rats while improving hematological indices. The results indicate potential antidiabetic and hematopoietic benefits, possibly linked to its antioxidant and metabolic-modulating constituents (Oyedemi *et al*. 2011)

5. Antidiarrheal and Biosafety Potentials: *Afzelia africana* seed n-hexane extract reported significant inhibition of diarrheal episodes in experimental animals (Ndukwe *et al*. 2022)

Multiple studies collectively confirm that *Afzelia africana* has various biological effects, such as anti-inflammatory, antioxidant, antimicrobial, antidiabetic, and antidiarrheal activities. These pharmacological properties support its traditional medicinal uses and offer a scientific

basis for investigating its neuroprotective potential, particularly in neurological disorders linked to oxidative stress, like Parkinson's disease.

1.8 Animals Models for Parkinsonism Studies

1.8.1 Relevance of animal models in Parkinsonism studies

Animal models play a central role in Parkinson's disease research, offering valuable insights into the mechanisms underlying neuronal degeneration, behavioural deficits, and potential therapeutic interventions. Since direct experimentation on humans is ethically and practically impossible, animal models provide an essential bridge between laboratory findings and clinical application (Gubellini and Kachidian, 2015; Imran *et al*., 2020).

1.8.2 Advantages:

Rodents are the most frequently used species in Parkinson's disease research due to their genetic similarity to humans, well-characterized neuroanatomy, and high responsiveness to dopaminergic neurotoxins. Their relatively short lifespan, ease of handling, and availability of transgenic lines make them highly suitable for studying disease progression and testing pharmacological agents (Konnova and Swanberg, 2018). Moreover, the rodent nigrostriatal system closely resembles that of humans, allowing researchers to assess both motor and non-motor symptoms through standardized behavioural tests (Lee *et al*., 2012).

1.8.3 Limitations:

1. Incomplete Representation: Rodent models fail to fully mimic the complexity of human PD, particularly regarding its gradual progression and non-motor symptoms (Gubellini and Kachidian, 2015).

2. Acute vs. Chronic Degeneration: Models like 6-OHDA and MPTP induce rapid neuronal loss, contrasting with the slow degeneration seen in humans (Imran *et al*., 2020).

3. Species Differences: Variations in brain structure and physiology affect drug metabolism and treatment outcomes (Lee *et al*., 2012).

4. Experimental Variability: Differences in toxin dosage and genetic backgrounds cause inconsistencies in research findings (Konnova and Swanberg, 2018).

5. Need for Combined Models: Using a combination of toxin-based and genetic models can better reflect the multifactorial nature of Parkinson's disease (Chia *et al*., 2020).

1.9 Rotenone-Induced Parkinsonism

1.9.1 Mechanism of Action

Rotenone is a pesticide that occurs naturally and that exerts its neurotoxic effects primarily through the inhibition of mitochondrial complex I in the electron transport chain. This inhibition disrupts ATP synthesis and increases the production of reactive oxygen species (ROS), leading to oxidative stress and neuronal damage (Radad *et al*., 2019). Dopaminergic neurons in the substantia nigra are especially vulnerable to oxidative damage because of their high metabolic activity and limited antioxidant defenses. Consequently, prolonged exposure to rotenone results in the selective loss of these neurons, causing dopamine depletion in the striatum and leading to Parkinsonian motor symptoms (Zeng *et al*., 2018). In addition to oxidative stress, rotenone exposure promotes α-synuclein aggregation and the formation of Lewy body-like inclusions, closely resembling the pathological features of idiopathic Parkinson's disease (Wrangel *et al*., 2015).

1.9.2 Advantages Over Other Models

Compared to other toxin-based models such as 6-hydroxydopamine (6-OHDA) and MPTP, the rotenone model offers several advantages

a) Rotenone is highly lipophilic, allowing it to cross the blood–brain barrier easily and reproduce systemic mitochondrial dysfunction similar to that observed in human Parkinson's disease (Radad *et al*., 2019).

b) Chronic administration of low-dose rotenone induces gradual neurodegeneration, which more accurately mimics the progressive nature of Parkinson's disease than the acute lesions produced by other toxins (Lee *et al*., 2012).

c) Furthermore, rotenone-treated animals develop both behavioural and neuropathological features, including dopaminergic neuronal loss, α-synuclein aggregation, and gliosis, thus providing a comprehensive model to investigate multiple aspects of disease pathology (Wrangel *et al*., 2015).

1.9.3 Limitations

Despite its strengths, the rotenone model presents notable challenges.

a) Variability in neurodegenerative outcomes due to differences in dosage, administration routes, and animal strains (Lee *et al*., 2012).

b) Additionally, rotenone's systemic toxicity can cause peripheral effects such as gastrointestinal dysfunction and weight loss, which may confound behavioural interpretations (Zeng *et al*., 2018).

c) Not all exposed animals consistently develop Parkinsonian symptoms, leading to poor reproducibility across studies (Radad *et al*., 2019).

These factors complicate the model's use for standardized therapeutic screening, although refinements to dosing protocols have improved reliability in recent years.

1.9.4 Other Models used in Parkinsonism

Several experimental models have been developed to reproduce the pathophysiological and behavioural characteristics of Parkinson's disease. These models help elucidate the underlying mechanisms of dopaminergic neurodegeneration and are essential for testing neuroprotective and therapeutic interventions. The most widely used PD models fall into two major categories: neurotoxin-induced models and genetic models.

1. Neurotoxin-Induced Models

Neurotoxin models are designed to selectively destroy dopaminergic neurons, thereby mimicking the neuronal loss observed in human PD. These models are widely used because they reproduce key biochemical, histological, and behavioural features of the disease.

a) 6-Hydroxydopamine (6-OHDA) Model

6-OHDA is a hydroxylated analogue of dopamine that selectively targets catecholaminergic neurons through uptake by dopamine and noradrenaline transporters (Imran *et al*., 2020; Gubellini and Kachidian, 2015).

This model is typically established in rodents by injecting 6-OHDA directly into the medial forebrain bundle, striatum, or substantia nigra, resulting in either unilateral or bilateral lesions. The unilateral lesion model, in particular, allows for easy behavioural assessment through rotation tests in response to dopaminergic agents. Its reliability, reproducibility, and well-characterized lesion pattern make it a gold standard for testing neuroprotective and neurorestorative drugs (Jiang and Dickson, 2018).

However, a limitation of the 6-OHDA model is that it represents an acute lesion rather than a progressive neurodegeneration, thereby not fully mimicking the slow progression of human PD. Additionally, it does not induce Lewy body–like inclusions, which are a hallmark of the human disease (Zeng *et al*., 2018).

b) MPTP (1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine) Model:

MPTP is a lipophilic compound that crosses the blood–brain barrier and is metabolized to MPP+ (1-methyl-4-phenylpyridinium), a potent inhibitor of mitochondrial complex I (Chia *et al*., 2020). The resulting mitochondrial dysfunction leads to dopaminergic neuron degeneration, ATP depletion, oxidative stress, and neuronal death (Zeng *et al*., 2018). This model is primarily used in mice and primates and produces classic motor symptoms, such as rigidity and bradykinesia ((Jiang and Dickson, 2018). The main advantage of the MPTP model is its ability to produce a systemic and selective dopaminergic lesion following peripheral administration, making it useful for studying both acute and sub-chronic degeneration (Lee *et al*., 2012). However, limitations include species-specific variability (rats are notably resistant) and the relatively rapid nature of neuronal loss compared to the gradual course of human PD (Jiang and Dickson, 2018). Despite its reproducibility, MPTP-induced lesions are acute rather than progressive, limiting its use for chronic studies (Imran *et al*., 2020).

c) Paraquat and Maneb Model:

Paraquat (an herbicide) and maneb (a fungicide) are environmental toxins that, when administered together, induce dopaminergic cell death and oxidative stress (Imran *et al*., 2020). This combined exposure model supports the hypothesis that environmental toxins contribute to PD etiology.

2. Genetic Models

Genetic models are developed to replicate familial types of PD resulting from mutations in particular genes. They provide insight into the molecular mechanisms underlying disease progression.

a. α-Synuclein (SNCA) Models

Overexpression or mutation of the SNCA gene (encoding α-synuclein) leads to protein aggregation and Lewy body formation, two central features of PD pathology (Konnova and Swanberg, 2018).

b. Parkin (PARK2), PINK1, and DJ-1 Models

These models replicate mitochondrial dysfunction and oxidative stress observed in familial PD (Imran *et al*., 2020). Although they may not produce substantial dopaminergic neuron loss, they are valuable for understanding mechanisms of cellular stress and neuroprotection (Chia *et al*., 2020).

c. LRRK2 (Leucine-rich repeat kinase 2) Models

Changes in the LRRK2 gene are among the most common causes of autosomal dominant PD. LRRK2 transgenic rodents demonstrate dopaminergic dysfunction, microglial activation, and progressive motor deficits (Gubellini and Kachidian, 2015).

1.10 behavioural Assessment in Experimental Parkinsonism

behavioural assessments are essential tools for evaluating motor and neurofunctional deficits in animal models of Parkinson's disease (PD). These tests provide measurable endpoints that reflect the extent of dopaminergic neuronal loss, helping researchers correlate neurochemical changes with behavioural outcomes (Gubellini and Kachidian, 2015; Konnova and Swanberg, 2018). In rodent models, particularly those induced by neurotoxins such as rotenone, behavioural assays are indispensable for confirming the successful induction of parkinsonian symptoms and for assessing the therapeutic efficacy of potential neuroprotective agents (Jiang and Dickson, 2018; Zeng *et al*., 2018).

1.10.1 Catalepsy test:

The catalepsy test is a widely employed behavioural method for evaluating muscular rigidity and akinesia, two cardinal features of Parkinsonian motor deficits (Frussa-Filho *et al*., 2016).

Catalepsy refers to a state of immobility and maintenance of an externally imposed posture, often observed following dopaminergic blockade or neuronal degeneration. The test operates on the principle that reduced dopaminergic activity in the nigrostriatal pathway prolongs the maintenance of imposed postures, reflecting the motor dysfunction associated with Parkinson's disease (Waku *et al*., 2021; Wrangel *et al*., 2015). The catalepsy test can be performed using two primary methods: the bar test and the grid test. Both methods assess the animal's ability to correct an imposed posture and are sensitive to alterations in motor function due to pharmacological or neurotoxic treatments. It can be repeated at different time intervals following drug or toxin administration to assess the onset, intensity, and recovery of cataleptic effects (Waku *et al*., 2021). Catalepsy duration correlates strongly with the degree of dopaminergic blockade or degeneration in the basal ganglia (Frussa-Filho *et al*., 2016). Increased immobility signifies dopaminergic hypoactivity, while reduced cataleptic time following drug treatment reflects restoration of dopamine function (Yesmin *et al*., 2025). This makes the catalepsy test a valuable indicator of both disease severity and drug efficacy in Parkinson's disease models (Waku *et al*., 2021).

1.10.2 Beam Walking Test:

The beam walking test is a sensitive behavioural method used to evaluate balance, coordination, and fine motor control in rodents. It involves training the animal to traverse a narrow, elevated beam from a starting point to a goal box, and measuring parameters such as latency to cross, number of foot slips, and fall frequency (Allbutt and Henderson, 2007;

Taylor *et al*., 2010). Because successful beam traversal requires precise postural control, even minor impairments in motor coordination led to increased foot slips and prolonged crossing time (Sawers and Ting, 2015). The test is also useful in other neurological conditions, offering a reliable measure of dynamic balance and motor integration (Shultz *et al*., 2020; Hortobágyi *et al*., 2024). In Parkinson's disease (PD) models, the beam walking test effectively detects akinesia, bradykinesia, and postural instability, which arise from dopaminergic degeneration (Taylor *et al*., 2010).

Rodents exposed to toxins such as rotenone or 6-OHDA show delayed beam crossing and more slips, reflecting the gait and coordination impairments typical of PD (Allbutt and Henderson, 2007). Improvement in beam performance following treatment indicates neuroprotection and restored motor control.

1.10.3 Other Complementary Behavioural Tests (e.g., Rotarod, Open Field)

1. Rotarod Test: The rotarod test evaluates motor coordination, balance, and endurance. Rodents are placed on a rotating rod, and the latency to fall is measured. Shorter latency indicates motor dysfunction, while improvements reflect restored motor function (Prasad and Hung, 2020).

2. Open Field Test (OFT): The OFT measures spontaneous locomotion, exploration, and anxiety-like behaviours. Parameters include distance traveled, rearing frequency, and time spent in the center vs. periphery. This test is valuable for assessing both motor deficits (hypokinesia, akinesia) and non-motor symptoms such as anxiety (Sirajo *et al*., 2021; Yousefi *et al*., 2025).

3. Elevated Plus Maze (EPM): The EPM specifically measures anxiety-like behaviours. Rodents' preference for closed versus open arms reflects anxiety levels, which are often altered in PD models (Yousefi *et al*., 2025).

4. Wire Hanging Test: This test assesses muscle strength and neuromuscular coordination. Rodents are suspended on a wire, and the latency to fall is recorded. Reduced hang time indicates motor weakness or neuromuscular deficits, complementing other motor assessments like catalepsy and beam walking (Yousefi *et al*., 2025).

5. Pole Test: The pole test evaluates bradykinesia and coordination deficits. Rodents are placed head-up on a vertical pole, and the time to turn and descend is measured (Prasad and Hung, 2020).

6. Cylinder Test: It is used to evaluate asymmetry in forelimb use during vertical exploration. Reduced use of one forelimb reflects unilateral motor deficits, relevant in toxin-induced PD models (Prasad and Hung, 2020).

7. Stepping Test: The stepping test measures forelimb akinesia. While restraining the hind limbs, the frequency of forelimb stepping is recorded. Reduced stepping indicates bradykinesia (Opara *et al*., 2017).

8. Gait and Grip Strength Assessments: Gait analysis (stride length, paw placement) and grip strength tests assess motor coordination, postural stability, and muscular strength, providing further insight into motor impairment severity (Opara *et al*., 2017).

These tests collectively allow a comprehensive evaluation of motor and non-motor deficits in Parkinsonian rodents. Catalepsy and beam walking are prioritized in this study for their sensitivity to rotenone-induced motor impairments (Bidgood *et al*., 2024).

1.11 Biochemical Analysis in Toxicity Studies

Biochemical analysis serves as a critical component in the evaluation of toxicological studies, providing insight into the physiological and metabolic state of experimental animals following exposure to chemical or plant-based substances. It involves the measurement of various serum or plasma biomarkers that reflect the functional integrity of key organs such as

the liver and kidney. Through such biochemical assessments, researchers can detect early indications of tissue injury, oxidative imbalance, or metabolic disturbances that may not be immediately visible through histological or behavioural examinations (Dzoyem *et al*., 2014).

1.11.1 Significance of Biochemical Parameters

In toxicity studies, biochemical parameters are essential indicators of systemic effects resulting from exposure to test compounds. In the liver, enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are routinely analyzed to assess hepatic function, as elevated serum levels often reflect hepatocellular injury or membrane permeability alteration. Similarly, in the kidney, urea and creatinine concentrations are measured to evaluate renal function, since they are waste products cleared through glomerular filtration. Disturbances in their levels indicate impaired kidney function (Dzoyem *et al*., 2014). Beyond organ-specific enzymes, biochemical analysis can also reveal systemic metabolic responses such as changes in total protein, albumin, cholesterol, and electrolyte levels, which may indicate disruptions in homeostasis or biosynthetic pathways. These parameters collectively help determine the safety profile of plant extracts or synthetic compounds, distinguishing between therapeutic and toxic doses (Hamada, 2018). Biochemical analysis provides vital insights into the systemic and metabolic alterations associated with rotenone-induced Parkinsonism. Rotenone, a pesticide and mitochondrial inhibitor, is widely used to reproduce Parkinson's disease (PD)-like pathology in experimental models due to its ability to induce dopaminergic degeneration and mimic peripheral metabolic disturbances characteristic of human PD. Assessing biochemical parameters in this model allows researchers to explore the interplay between neurodegeneration and peripheral organ dysfunction. Recent findings demonstrate that lipid

metabolism plays a crucial role in the pathophysiology of Parkinsonism. Rotenone exposure triggers significant lipidomic alterations, indicating a system-wide metabolic imbalance closely linked to neuronal vulnerability and disease progression (Tiwari *et al*., 2025). Similarly, clinical studies have reported abnormal serum lipid profiles among PD patients, suggesting that dyslipidemia may serve as both a biomarker and a contributing factor to neurodegeneration (Macías-García *et al*., 2021; Saedi *et al*., 2021). These biochemical changes in lipid components—such as cholesterol and triglycerides—reflect disrupted energy metabolism and may influence membrane composition, signaling pathways, and neuroinflammatory responses. Hence, evaluating lipid profiles in rotenone-induced models provides valuable data for understanding disease mechanisms and testing metabolic-targeted interventions. In addition to lipid alterations, hepatic and renal biochemical assessments reveal the systemic impact of rotenone-induced neurotoxicity. Research indicates that Parkinson's disease affects more than just the central nervous system and may involves metabolic crosstalk with peripheral organs.

Experimental evidence indicates that rotenone can impair liver function, as shown by altered enzyme activities and metabolic dysregulation (Vairetti *et al*., 2012; Liu *et al*., 2025).

This disruption of hepatic homeostasis may aggravate neurodegenerative processes through inflammatory mediators and altered detoxification capacity, supporting the hypothesis of a bidirectional liver-brain axis. Likewise, renal function is increasingly recognized as a determinant of PD risk and progression.

Clinical research demonstrates that reduced kidney function is associated with a higher likelihood of developing PD (Peng *et al*., 2024), while rotenone-treated rodents display histopathological and biochemical evidence of nephrotoxicity, including changes in urea and creatinine levels (Jiang *et al*., 2017).

Biochemical analysis in rotenone-induced Parkinsonism extends beyond neurological assessment. it provides an integrative perspective on metabolic, hepatic, and renal alterations.

In phytochemical and ethnopharmacological research, biochemical analysis is essential for assessing the biosafety of medicinal plants. For instance, evaluating herbal extracts like *Afzelia africana* involves analyzing biochemical parameters as indicators of potential toxicity or therapeutic effects. These assessments help determine safe dosages and support traditional medicine claims with scientific toxicology evidence (Dzoyem *et al*., 2014).

Therefore, biochemical analyses offer mechanistic understanding of toxic responses and serve as a basis for creating safe and effective therapeutics derived from plants.

1.12 Rationale and Justification of the Study

Neurodegenerative diseases like Parkinson's disease (PD) are a major health concern because of the loss of dopaminergic neurons and limited treatments. Growing interest in medicinal plants with antioxidant and neuroprotective properties provides a promising, sustainable alternative (Oladele *et al*., 2025). *Afzelia africana*, a medicinal plant used in African ethnomedicine, has bioactive compounds with antioxidant, anti-inflammatory, and hepatoprotective activities that may combat oxidative stress involved in PD. Studying its behavioural and biochemical effects in models provides evidence supporting its traditional use and potential as a neuroprotective, nephroprotective and hepatoprotective agent.

1.13 Aims and Objectives of the Study

Aim/ General Objective: To evaluate the behavioural and biochemical effects of ethanol extract of *Afzelia africana* stem bark in rotenone-induced Parkinsonism in rodents.

Specific Objectives:

a) To assess the acute toxicity profile (LD50 determination) of the ethanol extract of *Afzelia africana* stem bark

b) To evaluate the effects of *Afzelia africana* extract on neurological impairments in rats treated with rotenone using the beam walking and catalepsy tests.

c) To evaluate the effects of *Afzelia africana* on liver and kidney enzymes in rats treated with rotenone

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials, Chemicals and Drugs

Powdered stem bark of *Afzelia africana,* rotenone, ethanol, tween 80, beaker, gloves, extraction jar, orogastric tube, cotton wool, methylated spirit, stop watches, marker, animal cages, animal feed, experimental animals (Wistar rats), distilled water, olive oil, dimethyl sulfoxide (DMSO), weighing balance, mortar and pestle, syringes, plain tubes, horizontal bar and narrow beam.

2.2 Method

2.2.1 Plant collection

Fresh leaves and stem barks of *Afzelia africana* collected from Okeigbo in Ondo State were identified and authenticated by Mr. Kehinde Adeniyi of the Forestry Research Institute of Nigeria (FRIN) where a voucher specimen (FH112824) was kept for future reference and study.

2.2.2 Plant Extraction

The collected stem barks were cleaned to remove any dirt, dried over several days, ground into powder and stored in an airtight container. A total of 600 g of the powdered bark was macerated in 1500 ml of absolute ethanol (99.9%) for 72 hours, with occasional stirring. Afterward, the mixture was filtered, and the resulting filtrate was evaporated to dryness in an oven set at 40°C. The extract which had a brown color and a sticky texture was stored in a freezer at 4^0C. The percentage yield from the extraction was 1.62%. The plant extract was prepared by mixing 0.1 ml of Tween 80 with 9 ml of water to create a homogeneous solution, which was then

administered orally at doses of 250 mg/kg and 500 mg/kg. A new extract was prepared daily for administration.

2.2.3 Rotenone Preparation

10 mg of rotenone was weighed and dissolved in 1 ml of pure dimethyl sulfoxide (DMSO) and added to 9 ml of olive oil, resulting in a 10 ml solution with a final concentration of 1 mg/ml. A new stock solution was prepared prior to beginning the experiment.

2.2.4 Animals

Male Wistar rats (230-300 g) were sourced from the Department of Pharmacology and Toxicology at the University of Ibadan, Nigeria, for the study. The animals were placed in clean polypropylene cages and kept at the animal house of the Department of Pharmacology and Toxicology, University of Benin, Nigeria, under standard environmental conditions, with access to standard laboratory animal feed (grower pellets) and water *ad libitium*. Proper hygiene practices were carried out through regular cleaning of the cages. The animals were housed in conditions that provided natural lighting and maintained a room temperature between 24-28 °C. They were handled according to established protocols for laboratory animals, as outlined by the National Institutes of Health (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). Ethical approval for the study was obtained from the Ethics Committee of the Faculty of Pharmacy at the University of Benin, Nigeria, prior to the initiation of the research.

2.2.5 Phytochemical screening

The screening was conducted according to the standard protocol outlined by Trease and Evans (1989). The ethanol extract from the stem bark of *Afzelia africana* was tested for the presence of

various phytoconstituents, such as alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins, terpenoids, reducing sugars, and glycosides.

2.2.6 Acute Toxicity study in mice

The acute toxicity of *Afzelia africana* was assessed using Swiss Albino mice adapted from Lorke (1983). The study had two phases. Initially, nine mice were divided into three groups of three. The extract was given at 10, 100, and 1000 mg/kg body weight to groups 1, 2, and 3, respectively, to determine the dose range that might elicit toxic effects. The animals were observed for symptoms such as writhing, diarrhoea, tremors, and mortality over 24 hours. Following the results from the first phase, a new group of animals (n=3 per group) received higher doses of the extract (1600, 2900, and 5000 mg/kg) and were monitored for signs of toxicity, including tremors, diarrhea, and mortality for 24 hours. The median lethal dose (LD50) was estimated as the geometric mean of the lowest dose that caused mortality (the lowest lethal dose) and the highest dose that did not cause fatalities (the non-lethal dose).

2.2.7 Induction and Treatment

The study was done using Wistar rats, which were randomly divided into five groups, with each group consisting of six animals. Each group were assigned different treatments as follows: Group 1- No treatment, Group 2- Vehicle only, Group 3-Rotenone at a dose of 1 mg/kg combined with the vehicle, Group 4-Rotenone at 1 mg/kg combined with a low dose of *Afzelia africana* extract (250 mg/kg), Group 5- Rotenone at 1 mg/kg combined with a high dose of *Afzelia africana* extract (500 mg/kg). The volume of the extract and rotenone administered to each animal was calculated based on their weight. True parkinsonism was induced by administering rotenone (1 mg/kg) intraperitoneally on days 1, 4, 7, and 10. Neuro behavioural tests were conducted on days

0 (before the start of the study), days 5 and 10. The animals were weighed on days 1, 4, 7, 10, and 11, while the extract and vehicle were given orally on a daily basis. The neuro behavioural assessments included catalepsy test to evaluate muscle rigidity and a beam walking test to assess motor coordination. On day 11, the animals were sacrificed under ketamine anaesthesia. Blood samples were collected for biochemical assays.

2.2.8 Neuro behavioural Tests

1. Beam Walking Test

a) The animal was placed gently at the starting point of the beam

b) The timer was started immediately after the animal was placed on the beam

c) The latency of the animal to cross or fall was recorded within a three-minute duration

Delayed beam crossing and lack of balance due to a fall indicate reduced motor coordination.

2. Catalepsy

a) The animal was placed gently on a flat surface to acclimate.

b) The animal's forepaws were then lifted and place them carefully on a horizontal bar positioned approximately 11 cm above the surface.

c) The timer was started immediately after positioning the animal.

d) The duration (in seconds) for which the animal maintains this position without moving its forepaws within a three-minute duration was recorded

A longer duration of immobility indicates higher cataleptic behaviour and greater motor rigidity

2.2.9 Biochemical Analysis

1. Sample collection and preparation

The blood was collected for biochemical analysis through the abdominal aorta. It was then processed to obtain serum for biochemical assays.

Serum Preparation: The blood was collected in a tube without an anticoagulant and allowed to clot for approximately 30 minutes at room temperature. After the sample had clotted, it was centrifuged at 4000rpm for ten minutes. The serum (the resulting supernatant) was carefully transferred into a clean polypropylene tube for biochemical analysis.

2. Biochemical Parameters Assayed

The serum was analyzed for specific biomarkers of liver and kidney function. This is done to assess organ function and overall health. The parameters that were analyzed for kidney function tests are Creatinine, urea, and electrolytes such as bicarbonate, sodium, potassium, and chloride. For liver function tests, the following parameters were analyzed: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), conjugated bilirubin, total bilirubin, total protein and albumin. Lipid profile test parameters comprising of total cholesterol, triglycerides, low density lipoprotein (LDL) and high-density lipoprotein (HDL) were also analyzed.

2.2.10 Statistical analysis

For statistical significance, the results were assessed using one-way ANOVA, followed by the Tukey test to compare group differences. Findings are reported as the mean and standard error of the mean (SEM). A p-value of greater than 0.05 was considered statistically significant, and all analyses were conducted using Sigma Stat version 14.0.

CHAPTER THREE

RESULTS

3.1 Phytochemical Test

Phytochemical screening of the ethanol extract of *Afzelia africana* stem bark shows the presence of various bioactive phytoconstituents. The extract tested positive for saponins, glycosides, reducing sugars, terpenoids, alkaloids, tannins, and cardiac glycosides, while steroids were absent. The results are summarized in Table 3.1

Table 3.1: Phytochemicals Present in Ethanol Extract of *Afzelia africana* Stem Bark

Phytoconstituents	
Saponin	Present
Reducing sugar	Present
Glycosides	Present
Terpenoids	Present
Alkaloids	Present
Tanin	Present
Steroids	Absent
Cardiac glycosides	Present

3.2 Acute Oral Toxicity (LD50) Evaluation

The study was conducted in two phases, and in both phases, no mortality was recorded. The results are presented in Table 3.2

Table 3.2: Oral lethal dose (LD50) of Ethanol Extract of *Afzelia africana* Stem Bark

	Dose (mg/kg)	Writhing	Diarrhoea	Tremor	Death
Phase I	10	0/3	0/3	0/3	0/3
	100	0/3	0/3	0/3	0/3
	1000	0/3	0/3	0/3	0/3
Phase II	1600	0/3	0/3	0/3	0/3
	2900	0/3	0/3	0/3	0/3
	5000	0/3	0/3	0/3	0/3

3.3 Neurobehavioural tests

For catalepsy, the rotenone group had a higher cataleptic score than the control group ($p<0.05$), whereas the extract group had a lower score. The results are presented in Figure 3.1.

The results for beam walking are presented in Figure 3.2, showing a significant decrease in time spent on the beam for the rotenone group compared to the control group ($p<0.05$) and improved time spent on the beam for the extract group.

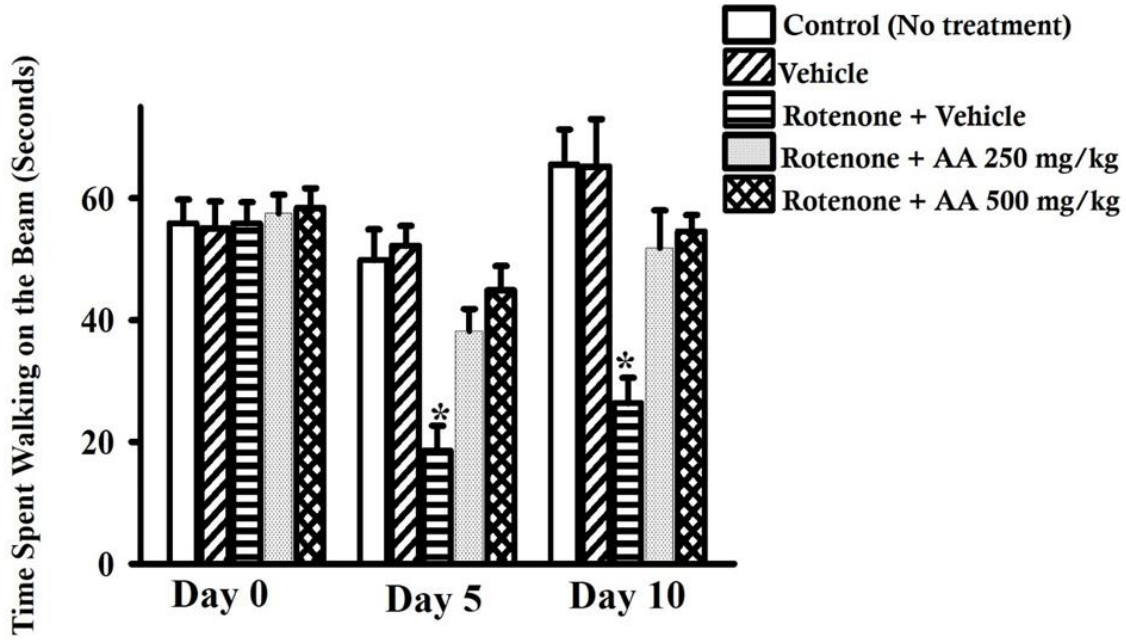

Figure 3.1 Effect of *Afzelia africana* in the beam walking test of Rotenone Treated Animals

*p<0.05 compared to control. n=6 per group

Results are expressed as mean ± standard error of mean.

AA- *Afzelia africana*

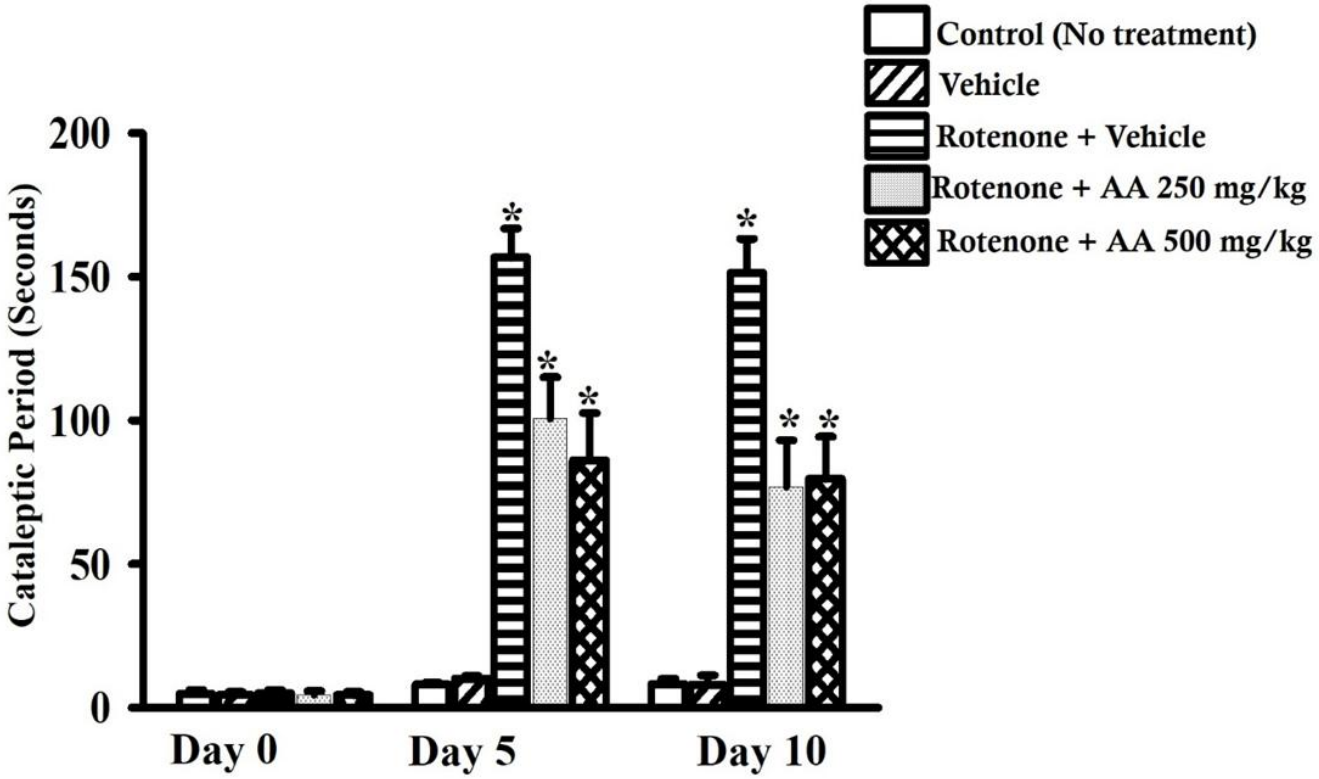

Figure 3.2 Effect of *Afzelia africana* in the Catalepsy test of Rotenone Treated Animals

*p<0.05 compared to control. n=6 per group.

Results are expressed as mean ± standard error of mean.

AA- *Afzelia africana*

3.4 Biochemical Analysis

3.4.1 Kidney Function Tests

The results for the effects of the extract on kidney function indices are summarized in Table 3.3. The levels of urea and creatinine were significantly higher (though not significantly different) in the rotenone-treated animals compared to the control the extract treated groups.

Table 3.3: Effect of ethanol extract of *A. africana* Stem Bark on Renal Indices in Rotenone Treated Animals

	Control	Vehicle	Rot + Vehicle	Rot + AA 250 mg/kg	Rot + AA 500 mg/kg
Urea	26.67± 1.83	32.67 ± 2.97	27.00 ± 1.00	27.67 ± 0.56	26.00 ± 1.10
Sodium	138.67 ± 0.67	138.67 ±0.56	139.33 ±0.42	138.33 ±0.61	139.33 ± 0.34
Potassium	3.73 ± 0.09	3.92 ± 0.12	3.78 ± 0.07	3.65 ± 0.06	3.75 ± 0.07
Bicarbonate	18.83 ± 0.48	18.83 ± 0.60	19.33 ± 0.62	19.33 ± 0.33	18.50 ± 0.67
Chloride	101.50 ± 0.72	100.33 ± 0.81	100.83 ± 0.80	100.67 ± 0.96	101.33 ± 0.81
Creatinine	0.67 ± 0.04	0.65 ± 0.02	0.81 ± 0.04	0.68 ± 0.05	0.62 ± 0.03

Results are expressed as mean ± standard error of mean. AA: *Afzelia africana*

3.4.2 Liver Function Tests

The results for the effects of the extract on liver function indices are summarized in Table 3.4. The level of ALT, AST and ALP in the vehicle groups were significantly different form the control groups while there was no significant difference in the lipid profile across all the treatment groups.

Table 3.4: Effect of ethanol extract of A. africana Stem Bark on Hepatic Indices in Rotenone Treated Animals

	Control	Vehicle	Rot + Vehicle	Rot + AA 250 mg/kg	Rot + AA 500 mg/kg
ALP	14.67± 1.31	14.33 ± 0.56	20.67 ± 1.34*	15.83 ± 0.95	13.67 ± 1.29
AST	52.00± 4.81	53.33± 4.95	72.50 ± 5.92*	56.50 ± 4.19	54.67 ± 4.34
ALT	23.83 ± 1.58	22.17 ± 1.40	29.83 ± 2.32*	23.17 ± 2.46	24.83 ± 2.30
TP	5.88 ± 0.19	5.87 ± 0.24	6.20 ± 0.10	5.97 ± 0.25	6.02 ± 0.20
TB	0.60 ± 0.01	0.46 ± 0.02	0.50 ± 0.03	0.58 ± 0.02	0.54 ± 0.05
CB	0.27 ± 0.03	0.23 ± 0.03	0.18 ± 0.03	0.23 ± 0.02	0.27 ± 0.04
TCHOL	109.50 ± 4.50	113.83 ± 2.99	106.50 ± 3.25	105.67 ± 4.83	107.17 ± 2.84
HDL CHOL	52.83 ± 3.64	57.17 ± 4.33	51.83 ± 1.54	55.67 ± 2.45	56.83 ± 4.32
LDL CHOL	41.17 ± 2.24	40.50 ± 5.89	40.50 ± 1.55	39.17 ± 1.71	34.33 ± 2.39
TRIG	85.67 ± 5.35	82.17 ± 9.11	72.83 ± 7.05	78.83 ± 8.03	77.50 ± 10.90

Keywords: ALP- Alkaline phosphatase, AST- Aspartate transaminase, ALT- Alanine Transaminase, TP- Total protein, TB- Total bilirubin, CB- Conjugated bilirubin, TCHOL- Total cholesterol, HDL CHOL- High density lipoprotein cholesterol, LDL CHOL- Low density lipoprotein cholesterol, TRIG - Triglycerides.

CHAPTER FOUR

DISCUSSION

4.1 Phytochemical screening

Phytochemical screening is a scientific method used to analyze, examine, extract, and identify various phytoconstituents found in different parts of a plant. This process aids in the discovery of drugs, allowing the active components to be further investigated and researched (Sharma., *et al*, 2020)

Medicinal plants are vital species recognized for their therapeutic properties, supported by traditional medicine and contemporary scientific research. They play a significant role in alleviating illnesses and enhancing overall human health. These plants are considered valuable sources of compounds that can be utilized in the development and formulation of medications (Oladeji *et al.,* 2019). Natural products and their derivatives tend to have fewer side effects and greater effectiveness compared to synthetic alternatives. Components derived from plants, such as flavonoids, quinine, and terpenoids, perform specific biological functions that boost therapeutic activities, including anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidant effects (Batiha *et al.,* 2020).

The results for the phytochemical screening of *Afzelia africana* revealed the presence of tannins, reducing sugar, glycosides, saponins, terpenoids, alkaloids, and cardiac glycosides, with steroids being absent.

These phytoconstituents possess pharmacological activities that can be linked to studies conducted.

Tannins act as potent antioxidants, neutralizing free radicals and protecting biomolecules from oxidative stress (Maugeri *et al.,* 2022). In addition, tannins exhibit anti-inflammatory and antimicrobial properties, helping to reduce inflammation and inhibit the growth of bacteria and fungi (Akah *et al.,* 2008; Vigbedor *et al.,* 2023). Their hepatoprotective and neuroprotective effects have also been reported, as they help maintain cellular integrity in the liver, kidney, and nervous system through the modulation of oxidative and inflammatory pathways (Maugeri *et al*., 2022; Ndukwe *et al.,* 2022).

Alkaloids present in *A. africana* have been associated with analgesic, antimicrobial, and anti-inflammatory actions, consistent with their bioactivity in other medicinal plants (Friday *et al.,* 2018; Awuchi, 2020).

Saponins exhibit hepatoprotective and anti-inflammatory effects, supporting the plant's traditional use in treating inflammatory and hepatic disorders (Okeke *et al.,* 2020).

Terpenoids also contribute to cytoprotective and antioxidant activities, reinforcing the plant's therapeutic value in protecting tissues such as the liver and kidneys (Awuchi, 2020; Ejikeme *et al.,* 2010; Okeke *et al.,* 2020). Cardiac glycosides are naturally occurring compounds well known for their potent cardiotonic activity and for their role in the management of congestive heart failure and certain arrhythmias. They display a broad spectrum of pharmacological activities,

including anti-inflammatory, and anticancer effects. These actions are attributed to their ability to influence cellular signaling pathways, regulate ion balance, and induce apoptosis in malignant cells. Furthermore, they demonstrate antimicrobial and immunomodulatory properties, broadening their therapeutic potential (Morsy, 2017).

The presence of these phytoconstituents suggests that *Afzelia africana* has potential for neuroprotection in the CNS.

4.2 Acute Oral Toxicity (LD_{50}) Evaluation

This evaluation assesses the possible harmful effects of the plants and confirms their safety profile. The oral acute toxicity study revealed no mortality at doses up to 5000 mg/kg, indicating a high safety margin for the ethanol extract of *Afzelia africana*. There were no significant behavioural and physiological changes observed in the animals, indicating that *Afzelia africana* is tolerated at high doses and has a good safety profile.

4.3 Neuro behavioural Test Evaluation

Neuro behavioural tests are critical tools used to assess cognitive function and behaviour, especially in cases of neurological disorders. In this study, catalepsy and beam walking were used to assess the neurological effects of rotenone and *Afzelia africana.*

Following rotenone administration, animals consistently exhibit significant deficits in the rotenone groups, as a reduction in time spent on the beam balance and increased time in the rod for catalepsy was observed, confirming that Parkinsonism was induced. The Beam Walking Test is a highly effective sensorimotor assessment for rodent models of PD, primarily because it detects subtle changes in walking, balance and coordination (Sawers and Ting, 2015). In the

rotenone model, beam walking performance serves as a measurable, behavioural readout of the disease's progression.

The rotenone group were observed to have spent less time on the beam compared to the results of the control while the animals treated with rotenone and the stem bark extract of *Afzelia africana* were observed to have improved time spent on the beam on day 5 and day 10.

This is indicative of the effects of *Afzelia africana* as an antioxidant inhibiting the oxidative mechanism of rotenone and thereby alleviating the effects of rotenone and hence parkinsonism.

Catalepsy is a state characterized by a failure to correct an externally imposed posture, often described as an absence of spontaneous movement and is essential in modeling the rigidity and bradykinesia seen in human Parkinson's Disease (PD) (Prasad and Hung, 2020).

The rotenone and vehicle group demonstrated a significant increase in cataleptic period on day 5 and day 10 compared to the control, confirming that chronic rotenone induced Parkinsonism. Both doses of *Afzelia africana* attenuated rotenone-induced catalepsy, as animals that received the plant extract reduced the cataleptic score. Therefore, a significant increase in time spent suggests that the extract exerts neuroprotective effects against rotenone-induced motor deficits and has anti-Parkinsonian properties.

4.4 Biochemical analysis

Biochemical indices are measurable indicators of the effects of chemicals in biological fluids and tissues. Rotenone is mainly investigated for its neurotoxic effects in Parkinson's disease (PD) models. However, as a systemic toxin, its primary mechanism (inhibiting mitochondrial complex I) also significantly harms other organs with high metabolic rates, such as the liver and kidneys (Radad *et al.,* 2019).

Biochemical analysis of these peripheral organs is essential for tracking systemic toxicity and assessing protective measures. The consistent factor behind rotenone's toxicity in the brain, liver, and kidney is oxidative stress. The resulting oxidative stress overwhelms the body's natural defenses, causing depletion of antioxidants and cellular damage.

4.4.1 Effect of Ethanol Extract of *Afzelia africana* Stem Bark in Liver Function

The liver plays a key role in metabolizing and detoxifying rotenone. Rotenone is metabolized by hepatic microsomal enzymes, yielding metabolites that can serve as exposure biomarkers (Radad *et al.,* 2019).

In this study, biochemical analysis of liver function showed minor variations in the levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphatase (ALP) in the rotenone group compared to the control, indicative of possible hepatocellular injury while the extract group demonstrated no significant elevation and suggests that the plant may possess hepatoprotective potential. However, no significant difference was observed for the lipid profile parameters.

4.4.2 Effect of Ethanol Extract of *Afzelia africana* Stem Bark in Kidney Function

The kidney's function in clearance makes it a key target for systemic toxins. Similar to the liver, the most consistent biochemical evidence of renal damage is oxidative imbalance. Elevated levels of urea and creatinine were observed in the rotenone group compared with the control, indicating nephrotoxicity, whereas no changes were observed in animals treated with the extract, suggesting nephroprotective potential. This confirms that the systemic oxidative stress caused by the toxin (the same mechanism driving neurodegeneration) is active in peripheral organs.

CHAPTER FIVE

CONCLUSION

This study finds that ethanolic extract of *Afzelia africana* stem bark has neuroprotective effects against rotenone-induced Parkinsonism in rodents. The extract improved motor function, confirmed by catalepsy and beam walking tests, and normalized biochemical indicators. These effects may stem from phytochemicals like flavonoids, tannins, and phenolic compounds, which enhance antioxidants, protect dopaminergic neurons, and support liver and kidney health. The research identifies *Afzelia africana* as a potential neuroprotective source, advancing understanding of phytochemicals in neurodegenerative disease treatment. Further studies should explore its molecular mechanisms and effects on neuroprotection pathways disorders.

REFERENCES

Ahmadu, B. S. (2020). Effects of fermentation on the nutritional status of *Afzelia africana* Sm. seed and its potentiality as aqua feedstuff. Nigerian Journal of Animal Production, 46(2): 111–117. https://ija.oauife.edu.ng/index.php/ija/article/view/158

Allbutt, H. N., and Henderson, J. M. (2007). Use of the narrow beam test in the rat, 6-hydroxydopamine model of Parkinson's disease. Journal of Neuroscience Methods, 159(2): 195-202.

Amusa, T. O. (2011). Effects of three pre-treatment techniques on dormancy and germination of seeds of *Afzelia africana* (Sm. Ex pers). Journal of Horticulture and Forestry, 3(4): 96–103. http://www.academicjournals.org/JHF/PDF/pdf2011/April/Amusa.pdf

Armstrong, M. J., and Okun, M. S. (2020). Diagnosis and treatment of Parkinson disease: a review. JAMA, 323(6): 548-560.

Batiha, G. E., and Beshbishy, A. M. (2020). Gas chromatography-mass spectrometry analysis, phytochemical screening and anti-protozoal effects of the methanolic Viola tricolor and acetonic Laurus nobilis extracts. BMC Complementary Medicine and Therapies, 20(87). https://doi.org/10.1186/s12906-020-2848-2

Bhushan Patwardhan, and Urmila Aswar. (2025). Harnessing the potential of ethnopharmacology for future medicines. Journal of Ethnopharmacology, 353(A), 120359. https://doi.org/10.1016/j.jep.2025.120359

Bidgood, R., Zubelzu, M., Ruiz-Ortega, J. A., and Morera-Herreras, T. (2024). Automated procedure to detect subtle motor alterations in the balance beam test in a mouse model of early Parkinson’s disease. Scientific Reports, 14(1): 862.

Bose, A., and Beal, M. F. (2016). Mitochondrial dysfunction in Parkinson's disease. Journal of Neurochemistry, 139: 216-231.

Chia, S. J., Tan, E. K., and Chao, Y. X. (2020). Historical perspective: models of Parkinson’s disease. International Journal of Molecular Sciences, 21(7): 2464.

Chang, K. H., and Chen, C. M. (2020). The role of oxidative stress in Parkinson’s disease. Antioxidants, 9(7): 597.

Corti, O., Lesage, S., and Brice, A. (2011). What genetics tells us about the causes and mechanisms of Parkinson's disease. Physiological Reviews, 91(4): 1161-1218.

Cui, Y., L. Y. L. Y. Z. B., (2022). Modeling Mechanisms and Applications of Parkinson's Disease Animal Models. J Alzheimers Dis Parkinsonism, 12, 550. https://doi.org/10.4172/2161-0460.1000550

Donkpegan, A. S. L., Doucet, J.-L., Dainou, K., and Hardy, O. J. (2015). Microsatellite development and flow cytometry in the African tree genus Afzelia (Fabaceae, Caesalpinioideae) reveal a polyploid complex. Applications in Plant Sciences, 3(1): 1400097. https://doi.org/10.3732/apps.1400097

Dovonou, A., B. C. S. L. V. G. C. P. I. M. L. M., (2023). Animal models of Parkinson's disease: bridging the gap between disease hallmarks and research questions. Translational Neurogeneration, 12(1): 36.

Dzoyem, J. P., Kuete, V., and Eloff, J. N. (2014). Biochemical Parameters in Toxicological Studies in Africa: Significance, Principle of Methods, Data Interpretation, and Use in Plant Screenings.

Ejikeme, P. N., Obasi, L. N., and Egbuonu, A. C. C. (2010). Physico-chemical and toxicological studies on *Afzelia africana* seed and oil. African Journal of Biotechnology, 9(13): 1959-1963.

Farnsworth, N. R. (2007). The Role of Ethnopharmacology in Drug Development. In Ciba Foundation Symposium 154 - Bioactive Compounds from Plants (eds D.J. Chadwick and J. Marsh). https://doi.org/10.1002/9780470514009.ch2

Frussa-Filho, R., Fukushiro, D. F., Patti, C. D. L., Chinen, C. C., Kameda, S. R., and Carvalho, R. D. C. (2016). Assessment of motor function in rodents: behavioural models sharing simplicity and multifaceted applicability: Part 2: The catalepsy test. In Rodent model as tools in ethical biomedical research (pp. 459-485). Cham: Springer International Publishing.

Friday, C., Akwada, U., and Igwe, O. U. (2018). Phytochemical screening and antimicrobial studies of *Afzelia africana* and Detarium microcarpum seeds. Chemistry International, 4(3): 170-176.

Gérard, J., and Louppe, D. (2011). *Afzelia africana* Sm. ex Pers. In: Lemmens, R. H. M. J., Louppe, D., and Oteng-Amoako, A. A. (Eds). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale).

Gómez-Benito, M., Granado, N., García-Sanz, P., Michel, A., Dumoulin, M., and Moratalla, R. (2020). Modeling Parkinson's disease with the alpha-synuclein protein. Frontiers in Pharmacology, 11, 356.

Guo, J. D., Zhao, X., Li, Y., Li, G. R., and Liu, X. L. (2018). Damage to dopaminergic neurons by oxidative stress in Parkinson's disease. International Journal of Molecular Medicine, 41(4): 1817-1825.

Gubellini, P., and Kachidian, P. (2015). Animal models of Parkinson's disease: An updated overview. Revue Neurologique, 171(11): 750-761.

Hamada, C. (2018). Statistical analysis for toxicity studies. Journal of Toxicologic Pathology, 31(1): 15–22. https://doi.org/10.1293/tox.2017-0050

Hauser, R. A., Benbadis, S. R., Lyons, K. E., McClain, T. A., and Pahwa, R. (2014). Parkinson Disease, Medscape. Updated, June 2018.

Heinrich, M., Edwards, S., Moerman, D. E., and Leonti, M. (2009). Ethnopharmacological field studies: a critical assessment of their conceptual basis and methods. Journal of Ethnopharmacology, 124: 1-17. https://doi.org/10.1016/j.jep.2009.03.043

Henrich, M. T., Oertel, W. H., Surmeier, D. J., and Geibl, F. F. (2023). Mitochondrial dysfunction in Parkinson's disease–a key disease hallmark with therapeutic potential. Molecular Neurodegeneration, 18(1):83.

Heuzé, V., Tran, G., Edouard, N., and Lebas, F. (2019). Afzelia (*Afzelia africana*). Feedipedia, INRAE, CIRAD, AFZ and FAO. Retrieved July 1, 2019, from https://www.feedipedia.org/node/24689

Hortobágyi, T., Vetrovsky, T., Uematsu, A., Sanders, L., da Silva Costa, A. A., Batistela, R. A., ... and Tollár, J. (2024). Walking on a balance beam as a new measure of dynamic balance to predict falls in older adults and patients with neurological conditions. Sports Medicine-Open, 10(1), 59.

Iliemene, D., Uju, I., and Atawodi, S. E.-O. (2014). Some biochemical effects of methanolic extract of *Afzelia africana* seed in rats following single or repeated carbon tetrachloride intoxication. Biotechnology Journal International, 4(12):1272–1282. https://journalbji.com/index.php/BJI/article/view/586

Jankovic, J., McDermott, M., Carter, J., Gauthier, S., Goetz, C., Golbe, L., and Stern, M. (1990). Variable expression of Parkinson's disease. A base-line analysis of the DAT ATOP cohort. Neurology, 40(10): 1529-1529.

Jiang, P., and Dickson, D. W. (2018). Parkinson's disease: experimental models and reality. Acta Neuropathologica, 135(1): 13-32.

Konnova, E. A., and Swanberg, M. (2018). Animal models of Parkinson's disease. Exon Publications, 83-106.

Lamula, S. Q. N., Bhanisa, T., Wium, M., Paccez, J. D., Zerbini, L. F., and Buwa-Komoreng, L. V. (2025). Phytochemical Analysis, Antioxidant Activity, and Anticancer Potential of Afzelia quanzensis Welw—Bark Extract: A Traditional Remedy Utilized by Indigenous Communities in KwaZulu-Natal and Eastern Cape Provinces of South Africa. International Journal of Molecular Sciences, 26(15): 7623.

Lang, A. E., and Espay, A. J. (2018). Disease modification in Parkinson's disease: current approaches, challenges, and future considerations. Movement Disorders, 33(5): 660-677.

Lee, Y., Dawson, V. L., and Dawson, T. M. (2012). Animal models of Parkinson's disease: vertebrate genetics. Cold Spring Harbor Perspectives in Medicine, 2(10), a009324. doi:10.1101/cshperspect.a009324

Leitão, A. D., Rudolffi-Soto, P., Chappard, A., Bhumkar, A., Lau, D., Hunter, D. J., ... and Sierecki, E. (2021). Selectivity of Lewy body protein interactions along the aggregation pathway of α-synuclein. Communications Biology, 4(1): 1124.

Liu, T. W., Chen, C. M., and Chang, K. H. (2022). Biomarker of neuroinflammation in Parkinson's disease. International Journal of Molecular Sciences, 23(8): 4148.

Luo, Y., Q. L. L. M. W. X. Z. W. L. X., (2025). Global, regional, national epidemiology and trends of Parkinson's disease from 1990 to 2021: findings from the Global Burden of Disease Study 2021. Frontiers in Aging Neuroscience, 16.

Marino, B. L., de Souza, L. R., Sousa, K. P., Ferreira, J. V., Padilha, E. C., da Silva, C. H., ... and Hage-Melim, L. I. (2020). Parkinson's disease: a review from pathophysiology to treatment. Mini Reviews in Medicinal Chemistry, 20(9): 754-767.

Maugeri, A., Lombardo, G. E., Cirmi, S., *et al*. (2022). Pharmacology and toxicology of tannins. Archives of Toxicology, 96:1257–1277. https://doi.org/10.1007/s00204-022-03250-0

Mathilde, P. B. A., Wandji, Y. F., Wona, J. P., and Wouessidjewe, D. (2021). Evaluation of the anti-inflammatory activity of a gel based on *Afzelia africana* (Fabaceae) leaves. GSC Biological and Pharmaceutical Sciences, 16(3):041–048. https://gsconlinepress.com/journals/gscbps/sites/default/files/GSCBPS-2021-0262.pdf

Moon, H. E., and Paek, S. H. (2015). Mitochondrial dysfunction in Parkinson's disease. Experimental Neurobiology, 24(2), 103.

Ndukwe, G. I., Okhiku, J. O., Obomanu, F. G., and Benjamin, G. O. (2022). Biosafety, Antioxidant and Antidiarrhoeal Potentials of *Afzelia africana* Seed n-Hexane Extract. Journal of Applied Sciences and Environmental Management, 26(4).

Oimau, A. (2025). Role of Ethnopharmacology in Integrating Traditional Medicine with Evidence-Based Healthcare. Journal of Clinical and Experimental Pharmacology, 15, 477. https://doi.org/10.35248/2161-1459.25.15.477

Oertel, W., and Schulz, J. B. (2016). Current and experimental treatments of Parkinson disease: A guide for neuroscientists. Journal of Neurochemistry, 139: 325-337.

Okeke, C. U., Okeke, P. N., Iroka, F. C., and Orji, M. E. (2021). Biochemical studies of different parts of *Afzelia africana* Smith. African Journal of Agriculture and Food Science, 4(4): 11–24. https://abjournals.org/ajafs/papers/volume-4/issue-4/biochemical-studies-of-different-parts-of-afzelia-africana/

Okeke, C. U., Okeke, P. N., Iroka, F. C., and Orji, M. E. (2020). Biochemical Studies of Different Parts of *Afzelia africana* Smith. Food Science, 4(4): 11-24.

Oladeji, O. S., Odelade, K. A., and Oloke, K. (2019). Phytochemical screening and anti-microbial investigation of Moringa oleifera leaf extract. African Journal of Science and Technology, Innovation, and Development, 12(1): 79-84. https://doi.org/10.1080/20421338.2019.1589082

Oladele, J. O., Ajayi, E. I., Flores-López, M. L., and Okoro, O. E. (Eds.). (2025). Sustainable Use of Plants and Their Products in Neurodegenerative Diseases Management. CRC Press

Park, J. S., Davis, R. L., and Sue, C. M. (2018). Mitochondrial dysfunction in Parkinson's disease: new mechanistic insights and therapeutic perspectives. Current Neurology and Neuroscience Reports, 18(5): 21.

Pellicano, C., Benincasa, D., Pisani, V., Buttarelli, F. R., Giovannelli, M., and Pontieri, F. E. (2007). Prodromal non-motor symptoms of Parkinson's disease. Neuropsychiatric Disease and Treatment, 3(1): 145–152. https://doi.org/10.2147/nedt.2007.3.1.145

Pirintsos, S., Panagiotopoulos, A., Bariotakis, M., Daskalakis, V., Lionis, C., Sourvinos, G., Karakasiliotis, I., Kampa, M., and Castanas, E. (2022). From Traditional Ethnopharmacology to Modern Natural Drug Discovery: A Methodology Discussion and Specific Examples. Molecules, 27(13): 4060. https://doi.org/10.3390/molecules27134060

Prasad, E. M., and Hung, S. Y. (2020). behavioural tests in neurotoxin-induced animal models of Parkinson's disease. Antioxidants, 9(10): 1007.

Rasheed, M. Z., Andrabi, S. S., Salman, M., Tabassum, H., Shaquiquzzaman, M., Parveen, S., and Parvez, S. (2018). Melatonin improves behavioural and biochemical outcomes in a rotenone-induced rat model of Parkinson's disease. Journal of Environmental Pathology, Toxicology and Oncology, 37(2).

Radad, K., Al-Shraim, M., Al-Emam, A., Wang, F., Kranner, B., Rausch, W. D., and Moldzio, R. (2019). Rotenone: from modelling to implication in Parkinson's disease. Folia Neuropathologica, 57(4): 317–326.

Sawers, A., and Ting, L. H. (2015). Beam walking can detect differences in walking balance proficiency across a range of sensorimotor abilities. Gait and Posture, 41(2): 619-623.

Sarrafchi, A., Bahmani, M., Shirzad, H., and Rafieian-Kopaei, M. (2016). Oxidative stress and Parkinson's disease: new hopes in treatment with herbal antioxidants. Current Pharmaceutical Design, 22(2): 238-246.

Samaila, A., and Hayatudeen, M. (2019). Pathophysiology and management of Parkinson disease: a review. Bayero Journal of Pure and Applied Sciences, 11(1), 238–243. https://doi.org/10.4314/bajopas.v11i1.39S

Sharma, T., Pandey, B., Shrestha, B. K., Koju, G. M., Thusa, R., and Karki, N. (2020). Phytochemical Screening of Medicinal Plants and Study of the Effect of Phytoconstituents in Seed Germination. Tribhuvan University Journal, 35(2): 1–11. https://doi.org/10.3126/tuj.v35i2.36183

Shultz, S. R., McDonald, S. J., Corrigan, F., Semple, B. D., Salberg, S., Zamani, A., ... and Mychasiuk, R. (2020). Clinical relevance of behaviour testing in animal models of traumatic brain injury. Journal of Neurotrauma, 37(22): 2381-2400.

Sheta, R., Berard, M., Musiol, D., Martínez-Drudis, L., and Oueslati, A. (2024). behavioural analysis of motor and non-motor impairment in rodent models of Parkinson's disease. Frontiers in Aging Neuroscience, 16, 1464706. doi:10.3389/fnagi.2024.1464706

Sidransky, E., and Lopez, G. (2012). The link between the GBA gene and Parkinsonism. The Lancet Neurology, 11(11): 986-998.

Srinivasan, E., Chandrasekhar, G., Chandrasekar, P., Anbarasu, K., Vickram, A. S., Karunakaran, R., ... and Srikumar, P. S. (2021). Alpha-synuclein aggregation in Parkinson's disease. Frontiers in Medicine, 8, 736978.

Stoker, T. B., and Barker, R. A. (2020). Recent developments in the treatment of Parkinson's Disease. F1000Research, 9, F1000-Faculty.

Stoker, T. B., Torsney, K. M., and Barker, R. A. (2018). Emerging treatment approaches for Parkinson's disease. Frontiers in Neuroscience, 12, 693.

Sun, C., and Armstrong, M. J. (2021). Treatment of Parkinson's disease with cognitive impairment: current approaches and future directions. behavioural Sciences, 11(4), 54.

Su, D., *et al*. (2025). Projections for prevalence of Parkinson's disease and its driving factors in 195 countries and territories to 2050: modelling study of Global Burden of Disease Study 2021. BMJ.

Taylor, T. N., Greene, J. G., and Miller, G. W. (2010). behavioural phenotyping of mouse models of Parkinson's disease. Behavioural Brain Research, 211(1):1–10. https://doi.org/10.1016/j.bbr.2010.03.004

Vázquez-Vélez, G. E., and Zoghbi, H. Y. (2021). Parkinson's disease genetics and pathophysiology. Annual Review of Neuroscience, 44(1): 87-108.

Vigbedor, B. Y., *et al*. (2023). Isolation and identification of flavanone derivative eriodictyol from the methanol extract of *Afzelia africana* bark and its antimicrobial and antioxidant activities. Journal of Ethnopharmacology. https://pubmed.ncbi.nlm.nih.gov/37200890

von Wrangel, C., Schwabe, K., John, N., Krauss, J. K., and Alam, M. (2015). The rotenone-induced rat model of Parkinson's disease: behavioural and electrophysiological findings. Behavioural Brain Research, 279, 52-61.

Waku, I., Magalhaes, M. S., Alves, C. O., and de Oliveira, A. R. (2021). Haloperidol-induced catalepsy as an animal model for parkinsonism: A systematic review of experimental studies. European Journal of Neuroscience, 53(11): 3743-3767.

Wang, Q., Liu, Y., and Zhou, J. (2015). Neuroinflammation in Parkinson's disease and its potential as therapeutic target. Translational Neurodegeneration, 4(1): 19.

Wei, Z., Li, X., Li, X., Liu, Q., and Cheng, Y. (2018). Oxidative stress in Parkinson's disease: a systematic review and meta-analysis. Frontiers in Molecular Neuroscience, 11, 236.

Yesmin, S., Kombo, H. Z., Singh, L., and Singh, G. (2025, July). Experimental models and behavioural parameters for evaluation of Parkinson disease. In AIP Conference Proceedings (Vol. 2807, No. 1, p. 040007). AIP Publishing LLC.

Zambetta, M. L., Fernandes, E. B., Kim, A., Russo, T. L., and Gianlorenço, A. C. (2025). Preclinical Parkinson's Disease Models for Non-Motor Symptoms: Research Recommendations from a Systematic Review. Life (Basel, Switzerland), 15(7): 1034. https://doi.org/10.3390/life15071034

Zeng, X. S., Geng, W. S., and Jia, J. J. (2018). Neurotoxin-induced animal models of Parkinson disease: pathogenic mechanism and assessment. ASN Neuro, 10, 1759091418777438