Afzelia africana ATTENUATES ROTENONE INDUCED NEUROBEHAVIOURAL, HAEMATOLOGICAL AND NEURONDEGENERATIVE CHANGES IN RODENTS

.

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN, NIGERIA IN PARTIAL FULFILLMENT OF THE AWARD OF DOCTOR OF PHARMACY (PHARMD) DEGREE.

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CERTIFICATION

This is to certify that this study was conducted by HENRY-UZOR, OGHENEFEGO with matriculation number PHA1908506, in the Department of Pharmacology, Faculty of Pharmacy, University of Benin, Benin-city, Edo State, Nigeria.

_____ _____

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_____ _____

Dr. A. P. Uchendu Date
(*Ag.* Head of Department)

DEDICATION

I dedicate this work to my God Almighty for His guidance, to my parents, Mr. and Mrs. Henry-Uzor, to my big mummy and daddy, Mr. and Mrs. Sunday Edoge, and also my brothers and sister, Oke, Keno and Ewoma, for their continuous support, care, and prayers throughout my academic journey in the University of Benin

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ABSTRACT

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by loss of dopaminergic neurones, motor dysfunction and accumulation of misfolded alpha-synuclein protein in the brains of affected individuals. There is currently no cure for this disorder which is prevalent in African and Hispanic men. Drugs currently in use only alleviate symptoms but do not reverse underlying neurodegeneration, thus the need for novel therapies cannot be over emphasized. *Afzelia africana,* a plant used in Ethnomedicine for a host of diseases is credited with neuroprotective properties. This study evaluated the effect of the ethanol extract of *Afzelia africana* stem bark in neurobehaviour, haemotological indices and the presence of misfolded alpha synuclein protein in rotenone-induced PD in Wistar rats.

Phytochemical screening was conducted to determine the bioactive constituents present in the extract. Acute toxicity (LD_{50}) was assessed in mice using the modified Lorke's method, while the neurobehaviour was evaluated in rats using the beam walking and catalepsy tests. Thirty male rats randomly distributed into five groups were used for the second phase of this study. There were either untreated (control), treated with the vehicle only, 250 or 500 mg/kg *A. Africana* daily for 10 days (Groups 1-5). Animals in groups 3-5 received 1 mg/kg of rotenone on days 1, 4, 7 and 10. All animals were subjected to the beam walking assay and catalepsy tests on days 0,5 and 10. On the 11^{th} day, animals were sacrificed with ketamine, the brains and blood of test animals were collected and used for assay of misfolded alpha synuclein and haematological analysis respectively.

The phytochemical analysis revealed the presence of saponins, glycosides, reducing sugars, terpenoids, alkaloids, tannins and cardiac glycosides while steroids were absent. Acute toxicity studies showed no mortality at doses up to 5000 mg/kg, indicating a high safety margin. Treatment with both doses of *A. africana* significantly ($p<0.05$) increased time spent in the beam walking assay and reduced the cataleptic score following treatment with rotenone. Haematological indices were also significantly increased at both doses while only the low dose caused a decrease in the expression of misfolded alpha synuclein post treatment with rotenone. The ethanol extract of *Afzelia africana* exhibited a favourable safety profile, reduced neurological deficits, improved haematological indices and reduced expression of misfolded alpha synuclein protein, and thus may be a candidate in the search for agents useful in the amelioration of PD

CHAPTER ONE

INTRODUCTION

Neurodegenerative diseases are characterized by the progressive loss of neuronal structure and function, often leading to debilitating motor, cognitive, and behavioural impairments. Globally, these disorders represent a major public health burden, affecting millions of individuals and posing significant challenges to healthcare systems and caregivers (Sharma *et al.,* 2023; Gupta and Sharma, 2025). The increasing prevalence of neurodegenerative diseases is closely linked to population aging, environmental exposures, and genetic susceptibility, emphasizing the need for early diagnosis and effective therapeutic interventions (Zahra *et al.,* 2019).

Among these conditions, Parkinson's Disease (PD) serves as a prototypical neurodegenerative disorder, with hallmark dopaminergic neuronal loss in the substantia nigra pars compacta and striatal dopamine depletion (Oluwole *et al.,* 2019). PD manifests clinically with both motor symptom - such as tremor, rigidity, bradykinesia, and postural instability - and a range of non-motor features including cognitive decline, sleep disturbances, and autonomic dysfunction (Marogianni *et al.,* 2020; Okunoye *et al.,* 2023). The chronic and progressive nature of PD contributes to functional disability, reduced quality of life, and increased caregiver burden, highlighting the urgency of advancing both mechanistic understanding and therapeutic strategies (Kelechi *et al.,* 2025).

Studying PD pathogenesis is critical for the identification of novel neuroprotective interventions. Experimental models play a pivotal role in this endeavor, enabling the replication of key pathological features of human PD in controlled laboratory settings. Among these, the rotenone-induced rodent model has gained prominence due to its

ability to mimic mitochondrial dysfunction, oxidative stress, dopaminergic neuronal loss, and α-synuclein aggregation observed in PD (Von Wrangel *et al.,* 2015; Ibarra-Gutiérrez *et al.,* 2023). The model provides an important platform for evaluating potential therapeutic agents and for elucidating the molecular pathways driving neurodegeneration.

Natural products, particularly plant-derived phytochemicals, have shown promising neuroprotective properties in various PD models due to their antioxidant, anti-inflammatory, and anti-apoptotic activities (Pohl and Kong Thoo Lin, 2018; Javed *et al.,* 2019). One plant of growing interest is *Afzelia africana*, a widely used medicinal species in Africa with documented pharmacological activities, including antioxidant, anti-inflammatory, and neuroprotective effects (Vigbedor *et al.,* 2022; Bamigboye *et al.,* 2024). The stem bark extract of *Afzelia africana* contains bioactive constituents such as flavonoids, tannins, saponins, and phenolics, which may mitigate oxidative stress, modulate neuroinflammation, and support neuronal survival in PD models (Daffalha and Mona, 2015; Foutse *et al.,* 2023). Evaluating this extract within the rotenone-induced PD model is therefore justified, as it could provide novel insights into natural, plant-based neuroprotective strategies for PD.

In summary, the study of PD using relevant experimental models and potential natural therapeutic agents is essential for addressing the growing global burden of neurodegenerative diseases. By focusing on the rotenone model and *Afzelia africana* stem bark extract, this research aims to explore mechanisms of neuroprotection and identify interventions that could attenuate dopaminergic neurodegeneration and improve patient outcomes.

1.1 Parkinson's Disease: An Overview

Parkinson's Disease (PD) is a progressive neurodegenerative disorder primarily characterized by motor dysfunction but also encompassing a spectrum of non-motor symptoms. It represents one of the most prevalent neurodegenerative diseases worldwide, imposing significant healthcare, socioeconomic, and caregiving challenges. Understanding the epidemiology, clinical manifestations, and underlying risk factors is crucial for informing research priorities, early diagnosis, and therapeutic development (Oluwole *et al.,* 2019; Kelechi *et al.,* 2025).

1.1.1 Epidemiology and Global Burden

PD affects millions of individuals globally, with prevalence and incidence steadily increasing in parallel with population aging. Epidemiological studies estimate that approximately 6 million people worldwide are living with PD, with higher prevalence in populations aged 60 years and above (Onohuean *et al.,* 2022). In sub-Saharan Africa, including Nigeria, PD prevalence is underreported due to limited diagnostic capacity and a lack of robust population-based studies, although emerging data suggest a growing recognition of the disease in both urban and rural communities (Williams *et al.,* 2018; Okunoye *et al.,* 2023).

The disease demonstrates clear age and gender-related patterns. Risk increases significantly after 60 years, with men consistently exhibiting higher incidence rates than women, potentially due to neuroprotective effects of oestrogen and differential exposure to environmental toxins (Hindle, 2010; Okubadejo *et al.,* 2010).

From a socioeconomic perspective, PD imposes a substantial burden. Patients require long-term medical care, pharmacological therapy, and rehabilitative support, while

caregivers provide extensive, often unpaid assistance (Kelechi *et al.,* 2025). In regions with limited healthcare infrastructure, these demands are magnified, resulting in delayed diagnosis, suboptimal management, and increased disability (Oluwole *et al.,* 2019; Zahra *et al.,* 2019).

1.1.2 Clinical Features of Parkinson's Disease

PD manifests with both motor and non-motor symptoms, which progress over time and contribute to functional impairment.

Motor symptoms include:

- Resting tremor, typically asymmetrical and affecting the hands.
- Bradykinesia, characterized by slowed movement and reduced facial expression.
- Rigidity, with stiffness of muscles and resistance to passive movement.
- Postural instability, resulting in impaired balance and increased risk of falls (Kalia and Lang, 2015; Marogianni *et al.,* 2020).

Non-motor symptoms are increasingly recognized as critical contributors to disease burden. These include:

- Sleep disturbances, such as insomnia or REM sleep behaviour disorder.
- Cognitive impairment, ranging from mild executive dysfunction to dementia.
- Autonomic dysfunction, including constipation, orthostatic hypotension, and urinary disturbances (Marogianni *et al.,* 2020; Okunoye *et al.,* 2023).

Disease progression is often staged using the Hoehn and Yahr scale, which classifies severity from early unilateral involvement (Stage 1) to advanced, debilitating symptoms with severe postural instability (Stage 5) (Kalia and Lang, 2015). The clinical course is highly variable, with some patients experiencing rapid progression while others have slower functional decline.

1.1.3 Etiology and Risk Factors

The etiology of PD is multifactorial, involving the interplay of genetic, environmental, and lifestyle factors.

Genetic factors: Mutations in genes such as SNCA, LRRK2, PARK2, and PINK1 contribute to familial forms of PD and influence protein aggregation, mitochondrial function, and cellular stress responses (Vázquez-Vélez and Zoghbi, 2021). Even in sporadic PD, genetic susceptibility may modulate the impact of environmental exposures.

Environmental toxins: Exposure to pesticides, herbicides, and heavy metals is linked to increased PD risk. Notably, rotenone, a mitochondrial complex I inhibitor, has been widely used to model PD due to its ability to reproduce dopaminergic neurodegeneration and α-synuclein aggregation seen in humans (Raza and Anjum, 2019; Ibarra-Gutiérrez *et al.,* 2023).

Lifestyle and comorbid factors: Protective factors such as regular physical activity and certain dietary patterns may reduce PD risk, while comorbidities, oxidative stress, and chronic inflammation can exacerbate disease onset and progression (Lees *et al.,* 2009; Marino *et al.,* 2020). PD is a complex, age-related neurodegenerative disorder with multifaceted clinical manifestations and increasing global prevalence. Its motor and

non-motor features, coupled with the influence of genetic, environmental, and lifestyle risk factors, underscore the necessity of integrated research approaches (Lees *et al.,* 2009; Marino *et al.,* 2020). Understanding epidemiology, clinical presentation, and etiological determinants provides a foundation for exploring experimental models and potential therapeutic interventions, including natural products like *Afzelia africana*.

1.2 Pathophysiology of Parkinson's Disease

The pathophysiology of Parkinson's Disease (PD) is complex and multifactorial, involving dopaminergic neurodegeneration, neuroinflammation, and protein aggregation, particularly of α-synuclein. Understanding these mechanisms is crucial for identifying therapeutic targets and elucidating disease progression (Dugger and Dickson, 2017; Marino *et al.,* 2020).

1.2.1 Dopaminergic Neurodegeneration

A hallmark feature of PD is the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), leading to striatal dopamine depletion and the emergence of characteristic motor symptoms (Kalia and Lang, 2015; Latif *et al.,* 2021). The striatum relies on dopamine for normal motor control, and its deficiency disrupts basal ganglia circuitry, manifesting as tremor, rigidity, bradykinesia, and postural instability (Dirkx and Bologna, 2022).

Several molecular mechanisms drive dopaminergic neuronal loss. Mitochondrial dysfunction impairs ATP production, increases reactive oxygen species (ROS) formation, and reduces neuronal energy reserves, rendering neurons susceptible to oxidative stress (Pozo Devoto and Falzone, 2017; Marino *et al.,* 2020). Oxidative stress further damages lipids, proteins, and DNA, amplifying cellular injury and promoting

apoptosis (Medeiros *et al.,* 2016; Barmaki *et al.,* 2021). Collectively, these pathways create a feed-forward cycle of neuronal degeneration, which underlies the progressive motor deficits observed in PD.

1.2.2 Neuroinflammation

Neuroinflammation plays a critical role in PD pathogenesis. The activation of microglia and astrocytes contributes to the release of pro-inflammatory mediators, which exacerbate neuronal injury (Lee *et al.,* 2019; Marogianni *et al.,* 2020). Key cytokines implicated in PD include tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), which can induce oxidative stress, compromise mitochondrial function, and trigger apoptotic pathways in dopaminergic neurons (Mehta and Tanner, 2016; Arena *et al.,* 2022).

Chronic neuroinflammation forms a self-perpetuating loop, whereby neuronal damage stimulates glial activation, which in turn amplifies inflammation and accelerates neurodegeneration (Vivekanantham *et al.,* 2015; Liu *et al.,* 2022). This mechanism links the central nervous system's immune response to both disease progression and the manifestation of non-motor symptoms in PD.

1.2.3 Protein Aggregation and Alpha-Synuclein Pathobiology

α-Synuclein is a small presynaptic protein involved in synaptic vesicle trafficking and neurotransmitter release (Bendor *et al.,* 2013; Sulzer and Edwards, 2019). In PD, α-synuclein undergoes misfolding and abnormal aggregation, forming oligomers and insoluble fibrils, which accumulate as Lewy bodies within dopaminergic neurons (Stefanis, 2012; Du *et al.,* 2020). These inclusions contribute to neuronal dysfunction and death through mechanisms including mitochondrial impairment, endoplasmic

reticulum stress, and disruption of protein degradation pathways (Dehay *et al.,* 2015; Calabresi *et al.,* 2023).

Furthermore, α-synuclein exhibits "prion-like" propagation, whereby misfolded proteins spread from cell to cell, amplifying neurodegeneration across interconnected neural networks (Serratos *et al.,* 2022; Burré *et al.,* 2024). Given its central role in PD pathophysiology, α-synuclein is considered a key biomarker for disease diagnosis and progression, detectable in cerebrospinal fluid, blood, and other peripheral tissues (Henderson *et al.,* 2019; Du *et al.,* 2021). PD pathophysiology reflects a complex interplay of dopaminergic neuronal loss, neuroinflammatory processes, and pathological protein aggregation. Mitochondrial dysfunction and oxidative stress initiate and amplify neuronal injury, while glial activation and pro-inflammatory cytokines exacerbate degeneration. Misfolded α-synuclein aggregates and propagates through neural circuits, driving the hallmark clinical manifestations of PD and serving as a critical biomarker for experimental and clinical studies. Understanding these mechanisms provides a foundation for evaluating potential neuroprotective interventions, including natural compounds such as *Afzelia africana* stem bark extract.

1.3 Experimental Models of Parkinson's Disease

Experimental models are indispensable tools for elucidating Parkinson's Disease (PD) pathogenesis, testing neuroprotective strategies, and evaluating potential therapeutic interventions. These models aim to recapitulate the hallmarks of PD, including dopaminergic neuronal loss, α-synuclein aggregation, oxidative stress, neuroinflammation, and motor dysfunction (Jiang and Dickson, 2018; Ke *et al.,* 2021). The careful selection of an appropriate model is critical for ensuring translational relevance to human PD.

1.3.1 Overview of PD Animal Models

PD animal models can be broadly categorized into genetic, pharmacological, and toxin-induced models, each with unique advantages and limitations.

- Genetic models focus on manipulating PD-associated genes, including SNCA, LRRK2, PARK2, PINK1, and DJ-1, to mimic familial PD forms (Vázquez-Vélez and Zoghbi, 2021). These models are valuable for studying molecular mechanisms of α-synuclein aggregation, mitochondrial dysfunction, and protein degradation pathways. However, they often fail to reproduce the full spectrum of progressive neurodegeneration and motor deficits seen in sporadic PD, which represents the majority of human cases (Jiang and Dickson, 2018; Ke *et al.,* 2021).

- Pharmacological models employ compounds such as reserpine or tetrabenazine, which deplete dopamine to induce motor impairments (Lama *et al.,* 2021). These models are advantageous for rapid assessment of symptomatic therapies but do not replicate chronic neurodegeneration or pathological protein aggregation. Their transient nature limits their utility for studying disease-modifying interventions.

- Toxin-induced models include the use of 6-hydroxydopamine (6-OHDA), MPTP, and rotenone, which selectively target dopaminergic neurons to reproduce PD-like neurodegeneration (Blandini and Armentero, 2012; Ke *et al.,* 2021). These models are particularly relevant for investigating oxidative stress, mitochondrial dysfunction, and inflammatory responses, as they closely mimic cellular and behavioural hallmarks of PD. Among toxin models, rotenone is unique in reproducing both central and peripheral PD features, including α-

synuclein pathology, making it highly suitable for translational research (Ibarra-Gutiérrez *et al.,* 2023).

Comparative analysis of models underscores the trade-offs inherent in each system. Genetic models provide mechanistic insights but limited phenotypic fidelity; pharmacological models allow rapid testing but lack progressive neurodegeneration; toxin-based models replicate key pathological features but can vary in reproducibility and systemic toxicity (Bezard *et al.,* 2013; Jiang and Dickson, 2018). Consequently, the choice of model must be guided by the specific research question, desired outcome measures, and translational objectives.

1.3.2 Rotenone-Induced Parkinsonism Model

The rotenone-induced PD model is widely used due to its ability to mimic multiple aspects of human PD, including dopaminergic neuron loss, α-synuclein aggregation, mitochondrial dysfunction, oxidative stress, and neuroinflammation (von Wrangel *et al.,* 2015; Ibarra-Gutiérrez *et al.,* 2023). Rotenone is a lipophilic pesticide that inhibits mitochondrial complex I, impairing ATP production and generating reactive oxygen species (ROS), which contribute to oxidative damage and apoptotic neuronal death (Pozo Devoto and Falzone, 2017; Raza and Anjum, 2019).

Behavioural studies show that rotenone-treated rodents develop motor deficits including bradykinesia, tremor, postural instability, and impaired coordination, closely resembling PD in humans (Angeline *et al.,* 2012; Zhang *et al.,* 2017). Biochemical analyses reveal dopamine depletion in the striatum, increased lipid peroxidation, and elevated oxidative stress markers, while histopathological examination demonstrates loss of dopaminergic neurons in the substantia nigra and formation of α-synuclein-positive inclusions (von Wrangel *et al.,* 2015; Ibarra-Gutiérrez *et al.,* 2023).

Beyond reproducing central nervous system pathology, rotenone also induces systemic effects, reflecting peripheral manifestations of PD, such as altered gastrointestinal motility and oxidative stress in peripheral tissues, enhancing its translational relevance (Lama *et al.,* 2021).

Strengths of the rotenone model include its ability to induce chronic, progressive dopaminergic neurodegeneration, α-synuclein aggregation, and mitochondrial dysfunction, making it ideal for studying both pathogenesis and neuroprotective interventions (Blandini and Armentero, 2012). However, limitations include high variability in systemic toxicity, animal mortality, and reproducibility, which necessitate careful dose titration and monitoring (von Wrangel *et al.,* 2015; Kelechi *et al.,* 2021). Despite these challenges, the rotenone model remains a preferred experimental platform for testing neuroprotective compounds, such as *Afzelia africana* stem bark extract.

Animal models of PD are critical for understanding disease mechanisms and evaluating therapeutic interventions. The rotenone-induced model stands out for its robust replication of human PD pathology, including dopaminergic degeneration, oxidative stress, and α-synuclein aggregation. The careful use of this model enables preclinical testing of novel neuroprotective agents and offers translational insights into disease progression and potential interventions.

1.4 Oxidative Stress, Neuroinflammation, and Haematological Indices in PD

Parkinson's Disease (PD) is characterized by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to the classical motor and non-motor symptoms of the disorder. Beyond neuronal loss, PD is increasingly recognized as a systemic disease, with peripheral alterations that reflect central pathology. Two

key interconnected mechanisms—oxidative stress and neuroinflammation—play pivotal roles in driving both central neurodegeneration and systemic changes, including alterations in haematological indices (Medeiros *et al.,* 2016; Barmaki *et al.,* 2021). Understanding these pathways provides a foundation for identifying biomarkers of disease progression and for evaluating potential therapeutic interventions.

1.4.1 Role of Oxidative Stress in Neurodegeneration and Systemic Alterations

Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and the capacity of antioxidant defenses to neutralize them. In PD, dopaminergic neurons are particularly susceptible to oxidative damage due to their high metabolic demand, low antioxidant capacity, and dopamine metabolism, which generates ROS as a byproduct (Medeiros *et al.,* 2016; Pozo Devoto and Falzone, 2017). Excessive ROS accumulation leads to lipid peroxidation, protein oxidation, DNA damage, and mitochondrial dysfunction, ultimately activating apoptotic pathways that culminate in neuronal death (Marino *et al.,* 2020; Barmaki *et al.,* 2021).

Peripheral oxidative stress is also observed in PD patients and experimental models. Elevated markers of systemic oxidative damage, including malondialdehyde (MDA), advanced oxidation protein products (AOPP), and depleted antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase), have been reported in plasma and erythrocytes (Nikolova and Mancheva, 2013; Barmaki *et al.,* 2021). These systemic alterations suggest that oxidative stress is not confined to the CNS but extends to peripheral tissues, linking central neurodegeneration with systemic pathology. Such peripheral changes offer accessible biomarkers for disease monitoring and therapeutic evaluation.

1.4.2 Relationship Between Neuroinflammation and Peripheral Haematological Changes

Neuroinflammation is a hallmark of PD pathogenesis, characterized by the activation of microglia and astrocytes in the substantia nigra and other affected brain regions. These glial cells release pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, which exacerbate dopaminergic neuronal loss and promote α-synuclein aggregation (Lee *et al.,* 2019; Marogianni *et al.,* 2020). Importantly, neuroinflammatory signals can extend beyond the CNS, influencing peripheral immune responses.

Studies have demonstrated that PD patients and animal models exhibit altered peripheral haematological indices, reflecting systemic inflammation. For example, increased neutrophil-to-lymphocyte ratio (NLR), lymphopenia, and elevated monocyte counts are commonly observed and correlate with disease severity and progression (Stanca *et al.,* 2022; Grillo *et al.,* 2023; Kim *et al.,* 2023). These findings suggest a bidirectional communication between central neuroinflammation and peripheral immune responses, emphasizing the potential of haematological parameters as surrogate markers of neurodegeneration.

In toxin-induced PD models, such as rotenone-treated rodents, elevated peripheral inflammatory markers parallel CNS inflammation, including microglial activation and cytokine upregulation (Kavuri and Sivanesan, 2019; Raza and Anjum, 2019). This alignment underscores the relevance of haematological assessments for tracking systemic manifestations of PD in preclinical studies.

1.4.3 Significance of Haematological Indices as Biomarkers in PD Models

Peripheral haematological indices—including red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb) levels, and platelets—are increasingly recognized as accessible and non-invasive biomarkers for systemic oxidative stress and inflammation in PD.

- RBC and Hb levels: Decreases in RBC count or hemoglobin reflect oxidative damage and impaired oxygen transport, which may exacerbate neuronal vulnerability and contribute to disease progression (Deng *et al.,* 2017; Umehara *et al.,* 2020).

- WBC counts and differentials: Alterations in neutrophils and lymphocytes serve as indicators of systemic inflammation and correlate with central neurodegeneration (Stanca *et al.,* 2022; Kim *et al.,* 2023).

- Platelet counts: Platelets are sensitive to oxidative stress and inflammatory mediators; platelet dysfunction or changes in number can provide insights into systemic oxidative and inflammatory status (Kavuri and Sivanesan, 2019).

Experimental studies have shown that rotenone-treated rodents exhibit significant alterations in RBC, WBC, Hb, and platelet levels, reflecting both systemic toxicity and PD-like pathology (Kavuri and Sivanesan, 2019). Monitoring these indices is valuable for evaluating therapeutic interventions, such as neuroprotective phytochemicals, by providing a peripheral measure of their efficacy in mitigating oxidative stress and inflammation.

Furthermore, integrating haematological indices with CNS biomarkers (e.g., α-synuclein, dopamine levels) enhances our understanding of the systemic impact of PD

and offers a multidimensional approach for assessing disease-modifying strategies. Oxidative stress and neuroinflammation are central to PD pathogenesis, driving both dopaminergic neuronal loss and systemic alterations. Peripheral haematological indices serve as readily accessible biomarkers, reflecting systemic oxidative and inflammatory status, and correlating with central neurodegenerative processes. Their inclusion in PD experimental models provides valuable insights into disease progression and facilitates the preclinical evaluation of neuroprotective interventions, including plant-derived compounds such as *Afzelia africana* stem bark extract.

1.5 Natural Products in Neuroprotection

The search for novel neuroprotective strategies in Parkinson's Disease (PD) has increasingly focused on natural products, particularly phytochemicals derived from medicinal plants. Phytochemicals are bioactive secondary metabolites that possess antioxidant, anti-inflammatory, and anti-apoptotic properties, offering multi-targeted approaches to mitigate PD pathology (Pohl and Kong Thoo Lin, 2018; Velmurugan *et al.,* 2018).

1.5.1 Role of Phytochemicals in Neurodegenerative Diseases

Phytochemicals have emerged as promising agents in the management of neurodegenerative disorders due to their ability to modulate key pathological processes. In PD, oxidative stress, neuroinflammation, mitochondrial dysfunction, and protein aggregation are central mechanisms driving neuronal loss. Phytochemicals such as flavonoids, phenolics, alkaloids, and saponins can interact with these pathways, offering neuroprotection at multiple levels (B Mythri *et al.,* 2012; Ogidi and Ajoko, 2024).

Mechanistically, phytochemicals:

1. Scavenge reactive oxygen species (ROS), reducing oxidative damage in dopaminergic neurons (Farooqui and Farooqui, 2017).
2. Modulate neuroinflammatory pathways by downregulating pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and inhibiting microglial activation (Elahi *et al.,* 2025).
3. Prevent neuronal apoptosis through regulation of mitochondrial integrity and anti-apoptotic signaling pathways (Velmurugan *et al.,* 2018).

Experimental studies using animal models of PD, particularly toxin-induced models, have demonstrated the efficacy of phytochemicals in ameliorating behavioural deficits, restoring neurotransmitter levels, and reducing oxidative and inflammatory markers (Javed *et al.,* 2019; Aijaz *et al.,* 2024).

1.5.2 Overview of Antioxidant, Anti-Inflammatory and Anti-Apoptotic Phytochemicals

Several medicinal plants have been studied extensively in PD models for their neuroprotective effects:

- Curcuma longa (Turmeric): Curcumin, the active compound, exhibits potent antioxidant and anti-inflammatory effects, reduces α-synuclein aggregation, and protects dopaminergic neurons in rotenone and 6-OHDA models (B Mythri *et al.,* 2012).

- Ginkgo biloba: Flavonoids and terpenoids in Ginkgo extract attenuate oxidative stress, inhibit apoptosis, and improve motor function in PD models (Velmurugan *et al.,* 2018).

- Mucuna pruriens: Rich in L-DOPA, it replenishes striatal dopamine, while also exerting antioxidant and anti-apoptotic effects in neurotoxin-induced PD models (Aijaz *et al.,* 2024).

These studies highlight the multi-targeted potential of phytochemicals, making them attractive candidates for complementary or alternative therapies in PD. Importantly, their ability to interact with oxidative, inflammatory, and apoptotic pathways simultaneously aligns with the complex and multifactorial pathogenesis of PD (Farooqui and Farooqui, 2017; Ogidi and Ajoko, 2024).

The success of these natural products in preclinical PD models provides a strong rationale for exploring other medicinal plants with similar bioactive compounds, such as *Afzelia africana*, whose phytochemical richness and pharmacological activities suggest potential neuroprotective effects (Vigbedor *et al.,* 2022; Bamigboye *et al.,* 2024).

Phytochemicals derived from medicinal plants offer multi-mechanistic neuroprotection in PD by mitigating oxidative stress, neuroinflammation, and apoptosis. Studies in experimental models, including rotenone-induced PD, have demonstrated improved behavioural, biochemical, and histopathological outcomes, underscoring the potential of natural products as adjunctive or standalone therapeutic agents. This lays the foundation for investigating *Afzelia africana* stem bark extract as a candidate neuroprotective compound in PD.

1.5.2 *Afzelia africana*: Ethnopharmacological and Biological Profile

Afzelia africana, a member of the Fabaceae family, is a leguminous tree widely distributed in West Africa, particularly in Nigeria. Locally known for its medicinal and timber value, the species has been traditionally employed in the management of fever, infections, diabetes, and inflammation, reflecting its broad ethnopharmacological significance (Bamigboye *et al.,* 2024). Its stem bark, seeds, and roots are used in various forms, including decoctions and extracts, to treat ailments ranging from gastrointestinal disturbances to neurological complaints, suggesting multi-target therapeutic potential (Vigbedor *et al.,* 2022; Foutse *et al.,* 2023).

Phytochemical Constituents

Phytochemical investigations of *Afzelia africana* stem bark reveal a rich profile of bioactive secondary metabolites, including:

- Flavonoids – potent antioxidants capable of scavenging reactive oxygen species (Vigbedor *et al.,* 2022).
- Tannins – with anti-inflammatory and anti-apoptotic properties, contributing to tissue protection (Foutse *et al.,* 2023).
- Alkaloids – implicated in neuroprotection and modulation of neurotransmitter activity (Daffalha and Mona, 2015).
- Saponins and phenolics – providing both antioxidant and hepatoprotective effects (Oyedemi *et al.,* 2011; Daffalha and Mona, 2015).

This phytochemical richness underscores its capacity to interact with multiple pathogenic mechanisms involved in PD, including oxidative stress, inflammation, and apoptosis.

Reported Pharmacological Activities

Experimental studies have demonstrated several biological activities of *Afzelia africana*, supporting its potential as a neuroprotective agent:

1. Antioxidant activity: Both *in-vitro* and *in-vivo* studies indicate significant free radical scavenging, reducing oxidative stress in tissues exposed to toxic insults (Daffalha and Mona, 2015; Vigbedor *et al.,* 2022).

2. Anti-inflammatory effects: Extracts have been shown to downregulate pro-inflammatory mediators, aligning with the need to modulate neuroinflammation in PD (Vigbedor *et al.,* 2022).

3. Hepatoprotective activity: Protection against chemically induced liver injury has been observed, reflecting systemic safety and organ-protective potential (Oyedemi *et al.,* 2011).

4. Neuroprotective potential: Preliminary evidence from rodent studies demonstrates improvements in cognitive and behavioural outcomes, highlighting its relevance in neurodegenerative models (Bamigboye *et al.,* 2024).

These pharmacological properties suggest that *Afzelia africana* stem bark extract can simultaneously target oxidative stress, neuroinflammation, and apoptotic pathways, making it a compelling candidate for PD research.

Justification for Exploring *Afzelia africana* in Rotenone-Induced Parkinsonism

Rotenone-induced PD models replicate key aspects of human PD, including dopaminergic neuronal loss, α-synuclein aggregation, oxidative stress, and

neuroinflammation (von Wrangel *et al.,* 2015; Ibarra-Gutiérrez *et al.,* 2023). Given the antioxidant and anti-inflammatory profile of *Afzelia africana*, its extract offers the potential to mitigate rotenone-induced oxidative and inflammatory damage, restore neuronal function, and modulate α-synuclein expression.

Furthermore, preclinical studies in other neurotoxic models suggest that plant extracts rich in flavonoids, tannins, and phenolics can improve behavioural outcomes and biochemical indices associated with PD pathology (B Mythri *et al.,* 2012; Velmurugan *et al.,* 2018). Therefore, investigating *Afzelia africana* in rotenone-induced Parkinsonism aligns with the growing interest in natural products as multi-target neuroprotective agents, addressing the limitations of current therapeutic approaches and providing a scientifically justified rationale for its evaluation. *Afzelia africana* is a phytochemically rich plant with documented antioxidant, anti-inflammatory, hepatoprotective, and neuroprotective properties. Its traditional medicinal use, coupled with preclinical evidence, provides a strong rationale for exploring its stem bark extract in rotenone-induced PD models, with the aim of mitigating neurodegeneration, modulating α-synuclein, and improving systemic and behavioural outcomes.

1.6 Biomarkers in Parkinson's Disease: Focus on Alpha-Synuclein

Biomarkers are critical tools for understanding the pathophysiology, progression, and therapeutic response in Parkinson's Disease (PD). Among molecular biomarkers, α-synuclein has emerged as a central player due to its involvement in protein aggregation, neurodegeneration, and clinical manifestations (Du *et al.,* 2021; Burré *et al.,* 2024).

1.6.1 Overview of Molecular Biomarkers in PD

Multiple molecular biomarkers have been studied in PD, reflecting the multifactorial nature of the disease. These include:

- α-Synuclein: A presynaptic neuronal protein, whose abnormal aggregation forms Lewy bodies, a hallmark of PD pathology (Sulzer and Edwards, 2019; Serratos *et al.,* 2022).
- DJ-1: A protein involved in oxidative stress response, mitochondrial function, and neuroprotection; mutations are linked to familial PD (Du *et al.,* 2021).
- Tau protein: Microtubule-associated protein implicated in neuronal cytoskeletal integrity and co-pathology with α-synuclein in PD dementia (Pan *et al.,* 2021).
- Other emerging markers include neurofilament light chain (NfL), inflammatory cytokines, and oxidative stress indicators (Arena *et al.,* 2022; Liu *et al.,* 2022).

Collectively, these biomarkers offer insights into neuronal integrity, protein misfolding, inflammation, and oxidative stress, making them invaluable for preclinical and clinical studies.

1.6.2 Alpha-Synuclein as a Diagnostic and Prognostic Biomarker

α-Synuclein plays a pivotal role in PD pathogenesis. Physiologically, it regulates synaptic vesicle trafficking and neurotransmitter release, but its misfolding and oligomerization lead to neuronal toxicity (Bendor *et al.,* 2013; Pozo Devoto and Falzone, 2017).

- Diagnostic relevance: Elevated or misfolded α-synuclein in cerebrospinal fluid (CSF), blood, or saliva correlates with PD onset and progression (Devine *et al.,* 2011; Du *et al.,* 2021).

- Prognostic significance: Levels of α-synuclein oligomers may predict disease severity, rate of motor decline, and cognitive dysfunction, providing a potential tool for patient stratification and monitoring therapeutic responses (Henderson *et al.,* 2019; Burré *et al.,* 2024).

1.6.3 Peripheral Detection and Relevance in Experimental Studies

Peripheral detection of α-synuclein enables less invasive monitoring in both clinical and preclinical settings:

- Blood and plasma: Reflect systemic α-synuclein changes associated with neuronal loss and oxidative stress (Du *et al.,* 2021).
- Cerebrospinal fluid (CSF): Provides a direct measure of central α-synuclein pathology and correlates strongly with disease stage (Burré *et al.,* 2024).
- Saliva: Emerging as a convenient medium for early detection, though standardization is ongoing (Serratos *et al.,* 2022).

In experimental PD models, including rotenone-induced parkinsonism, α-synuclein measurement in brain tissue and peripheral fluids serves as a reliable biomarker to evaluate neuroprotective interventions, including plant extracts (Ibarra-Gutiérrez *et al.,* 2023).

1.6.4 Interaction Between Alpha-Synuclein, Oxidative Stress, and Neuroinflammation

A self-perpetuating loop links α-synuclein, oxidative stress, and neuroinflammation:

1. α-Synuclein aggregation increases reactive oxygen species (ROS) production and mitochondrial dysfunction (Stefanis, 2012; Pozo Devoto and Falzone, 2017).

2. Oxidative stress promotes further misfolding and oligomerization of α-synuclein (Du *et al.,* 2020).

3. Neuroinflammation, mediated by microglia and astrocytes, is triggered by α-synuclein aggregates, leading to the release of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and exacerbating neuronal death (Marogianni *et al.,* 2020; Arena *et al.,* 2022).

This interconnected pathway underscores the potential of interventions targeting oxidative stress and inflammation - such as phytochemical-rich plant extracts like *Afzelia africana* - to modulate α-synuclein pathology and improve neuronal survival in PD models.

α-Synuclein remains a central biomarker in PD due to its direct involvement in neuronal dysfunction and aggregation pathology. Its peripheral detection enables non-invasive monitoring, while understanding its interplay with oxidative stress and neuroinflammation provides a mechanistic basis for testing neuroprotective agents, including phytochemicals and natural plant extracts, in both preclinical and clinical studies.

1.7 Neurobehavioural Assessment in Parkinson's Disease

Neurobehavioural evaluations play a crucial role in experimental models of Parkinson's disease (PD), offering functional insights that complement biochemical and histological findings. These behavioural assays provide quantifiable measures of motor

coordination, muscular rigidity, and balance, which are cardinal features of PD resulting from dopaminergic neurodegeneration in the nigrostriatal pathway (Blandini and Armentero, 2012; Ibarra-Gutiérrez *et al.,* 2023). In experimental studies, such assessments are indispensable for determining both disease progression and the neuroprotective efficacy of therapeutic agents, including natural products.

1.7.1 Overview of Neurobehavioural Tests in Experimental PD Models

Several behavioural paradigms have been standardized for assessing PD-related motor dysfunctions in rodents. These include the open field test (for spontaneous locomotion), the rotarod test (for motor coordination and endurance), the catalepsy test (for muscular rigidity and akinesia), and the beam walking test (for postural balance and fine motor control) (Blandini and Armentero, 2012; Chang *et al.,* 2020). Among these, catalepsy and beam walking tests are particularly sensitive for detecting the motor deficits associated with rotenone-induced Parkinsonism, where mitochondrial inhibition and oxidative stress lead to loss of dopaminergic neurons in the substantia nigra pars compacta (Ibarra-Gutiérrez *et al.,* 2023).

1.7.2 Catalepsy Test

The catalepsy test evaluates muscular rigidity and the inability to correct imposed postures—hallmark signs of extrapyramidal dysfunction in PD (Blandini and Armentero, 2012). Typically, a rat's forepaws are placed on an elevated horizontal bar, and the duration for which the posture is maintained before correction is recorded. A prolonged cataleptic response indicates impaired dopaminergic neurotransmission, while a reduction following treatment suggests recovery of motor function (Uttara *et al.,* 2009; Ogidi and Ajoko, 2024).

In rotenone-induced PD models, the cataleptic response arises from oxidative stress, mitochondrial dysfunction, and subsequent dopaminergic neuron loss (Ibarra-Gutiérrez *et al.,* 2023). Thus, a decrease in cataleptic duration after administration of antioxidant-rich plant extracts - such as *Afzelia africana* - is indicative of neuroprotective and motor-restorative effects (Ogidi and Ajoko, 2024).

1.7.3 Beam Walking Test

The beam walking test is widely employed to assess motor coordination, balance, and postural stability in rodent models of PD (Fernagut and Chesselet, 2004; Chang *et al.,* 2020). The task involves training rats to traverse a narrow elevated beam toward a home platform, with crossing time, number of foot slips, and falls recorded as indices of performance.

In rotenone-treated rats, deficits such as increased traversal latency and frequent slips reflect striatal dopamine depletion and motor circuit disruption (von Wrangel *et al.,* 2015). Conversely, improved performance following treatment indicates the restoration of nigrostriatal integrity and functional motor recovery. Importantly, this test closely parallels human PD symptoms, such as postural instability and gait disturbances, reinforcing its translational validity (Bloem *et al.,* 2021).

1.7.4 Integrative Value of Behavioural Assessment

The inclusion of both catalepsy and beam walking assessments in experimental PD models provides complementary insights into motor dysfunction. Behavioural outcomes frequently correlate with biochemical markers—such as reduced lipid peroxidation, enhanced antioxidant enzyme activity, and normalized neurotransmitter

levels—reflecting functional recovery alongside molecular restoration (Marogianni *et al.,* 2020; Ibarra-Gutiérrez *et al.,* 2023).

Therefore, integrating these neurobehavioural paradigms with molecular and haematological analyses yields a comprehensive assessment framework for evaluating neuroprotective interventions. This integrative approach is particularly relevant for studies examining *Afzelia africana* stem bark extract, where improvements in behavioural parameters can substantiate its antioxidant and anti-inflammatory potential in mitigating rotenone-induced dopaminergic dysfunction.

1.8 Knowledge Gaps Identified from Literature

Despite significant advances in Parkinson's Disease (PD) research, several critical gaps persist, limiting the translation of preclinical findings to clinical applications.

1. Limitations of Current PD Models: While various animal models, including genetic, toxin-induced, and pharmacological approaches, have contributed to understanding PD pathophysiology, they often fail to fully recapitulate the human disease. For instance, the rotenone model replicates dopaminergic neuronal loss, oxidative stress, and α-synuclein aggregation, but does not consistently reproduce all motor and non-motor features of PD seen in humans (von Wrangel *et al.,* 2015; Ibarra-Gutiérrez *et al.,* 2023). Such limitations underscore the need for refined experimental approaches that more accurately reflect disease progression, neurodegeneration patterns, and therapeutic responsiveness.

2. Lack of Studies Linking *Afzelia africana* Extract with α-Synuclein Modulation: Although *Afzelia africana* exhibits antioxidant, anti-inflammatory, and

neuroprotective properties, current literature lacks studies directly assessing its effect on α-synuclein expression or aggregation in PD models. Given the central role of α-synuclein in PD pathogenesis, evaluating the extract's capacity to modulate α-synuclein could provide mechanistic insight into its neuroprotective potential and inform the development of multi-target natural therapies (Vigbedor *et al.,* 2022; Bamigboye *et al.,* 2024).

3. Limited Research on Haematological Alterations in Rotenone-Induced PD Treated with plant extract: Emerging evidence suggests that peripheral haematological indices, such as RBC, WBC, hemoglobin, and platelets, may reflect systemic inflammation and oxidative stress in PD (Kavuri and Sivanesan, 2019; Grillo *et al.,* 2023). However, there is a paucity of studies evaluating the impact of neuroprotective plant extracts, including *Afzelia africana*, on these parameters in rotenone-induced PD models. Addressing this gap could provide valuable biomarkers for monitoring systemic effects of natural therapies alongside central neuroprotective outcomes.

While rotenone models, α-synuclein biomarkers, and phytochemicals have been individually studied, there remains a clear need for integrative investigations linking *Afzelia africana* extract, α-synuclein modulation, oxidative stress, neuroinflammation, and haematological indices. Addressing these gaps will enhance mechanistic understanding and potentially inform translational strategies for PD management.

1.9 Summary of Literature Review

This literature review has highlighted the multifaceted nature of Parkinson's Disease (PD), encompassing epidemiological, clinical, molecular, and experimental dimensions. PD is a progressive neurodegenerative disorder with global prevalence

increasing with aging populations, and it presents a substantial socioeconomic and healthcare burden (Erkkinen *et al.,* 2018; Kelechi *et al.,* 2025). Clinically, PD is characterized by motor symptoms such as tremor, bradykinesia, rigidity, and postural instability, alongside a broad spectrum of non-motor symptoms including sleep disturbances, cognitive impairment, and autonomic dysfunction (Kalia and Lang, 2015; Lees *et al.,* 2009).

At the molecular and cellular levels, PD pathophysiology involves dopaminergic neurodegeneration, α-synuclein aggregation, mitochondrial dysfunction, oxidative stress, and neuroinflammation (Stefanis, 2012; Marogianni *et al.,* 2020). The interplay between these mechanisms contributes to neuronal loss and disease progression, while highlighting potential targets for therapeutic intervention. Among these, α-synuclein has emerged as a central biomarker, providing diagnostic and prognostic value and serving as a mechanistic link between oxidative stress, inflammation, and neurodegeneration (Du *et al.,* 2021; Burré *et al.,* 2024).

Experimental models, particularly rotenone-induced Parkinsonism in rodents, offer valuable platforms for studying PD pathogenesis and testing neuroprotective strategies (von Wrangel *et al.,* 2015; Ibarra-Gutiérrez *et al.,* 2023;). These models replicate key features of human PD, including dopaminergic neuron loss, oxidative stress, and α-synuclein pathology, though limitations remain in fully recapitulating the disease's clinical complexity. Peripheral markers, such as haematological indices, may further complement CNS-based assessments by reflecting systemic oxidative and inflammatory changes (Kavuri and Sivanesan, 2019; Grillo *et al.,* 2023).

Natural products, particularly phytochemicals, have demonstrated antioxidant, anti-inflammatory, and anti-apoptotic properties that may counteract PD-related

neurodegeneration (Velmurugan *et al.,* 2018; Ogidi and Ajoko, 2024). Among these, *Afzelia africana* stem bark extract presents a compelling candidate for neuroprotective research due to its ethnopharmacological significance, phytochemical richness, and preliminary evidence of antioxidant and anti-inflammatory activity (Vigbedor *et al.,* 2022; Bamigboye *et al.,* 2024). However, critical gaps remain regarding its effect on α-synuclein modulation and haematological alterations in PD models.

Taken together, the literature underscores the importance of a multifactorial investigative approach that integrates behavioural, biochemical, and molecular assessments in PD research. This framework supports the present study, which aims to evaluate the neuroprotective potential of *Afzelia africana* stem bark extract in a rotenone-induced Parkinsonism model, with a focus on α-synuclein expression, oxidative stress, neuroinflammation, and haematological indices. By addressing existing gaps, this study has the potential to advance understanding of natural product-based interventions and provide a basis for future translational applications in PD management.

1.10 Rationale for the study

In recent times, PD has been associated with rising prevalence and drugs currently in use only alleviate symptoms but do not reverse underlying neurodegeneration, thus the need for novel therapies cannot be over emphasized. *Afzelia africana,* a plant used in Ethnopharmacolopgy for management of several disorders is credited with antioxidant, anti-inflammatory and neuroprotective properties

This study is thus aimed at evaluating the potential of *A. africana* - if any- in ameliorating rotenone induced neurological damage in rats

1.11 Aim of The Study

To evaluate the safety profile of ethanol extract of *Afzelia africana* stem bark and its cytoprotective properties in rotenone induced damage in rodents.

1.12 Objectives of The Study

- To assess the acute toxicity profile (LD_{50} determination) of the ethanol extract of *Afzelia africana* stem bark.
- To investigate the effects of *A. africana* on beam walking and catalepsy tests following treatment with rotenone
- To evaluate effects of *A. africana* in haematological indices in rotenone treated animals.
- To assay levels of misfolded alpha synuclein in brains of rats treated with effects of *A. africana* rotenone administration.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials, Chemicals, and Equipment

The materials, chemicals, and equipment used for this study are powdered stem bark of *Afzelia africana*, rotenone, ethanol, Tween 80, distilled water, DMSO, beakers, measuring cylinders, conical flasks, gloves, extraction bottles, orogastric tubes, funnels, cotton wool, methylated spirit, laboratory scale, stopwatches, cylindrical bowls, suspended bars, masking tape, markers, and plastic cages.

2.2 Plant Collection

Fresh stem bark of *Afzelia africana* was collected from Okeigbo, Ondo State. The plant material was identified and authenticated by Mr. Kehinde Adeniji of the Forestry Research Institute of Nigeria (FRIN), and a voucher specimen (FHI 112824) was deposited for future reference.

2.3 Plant Extraction

The collected stem bark was carefully cleaned to remove dirt, air-dried, and ground into a fine powder. A total of 600 g of powdered bark was macerated in 1500 ml of 96% ethanol for 72 hours using a cold maceration technique with intermittent stirring. The mixture was filtered, and the filtrate was concentrated under reduced pressure using a rotary evaporator to remove the solvent. The resulting gel-like residue was oven-dried at 40°C, yielding a brown-colored extract. The percentage yield was calculated using the formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material used}} \times 100$$

For oral administration, stock solutions were prepared by dissolving 250 mg, and 500 mg of extract in 0.5 ml of Tween 80, then diluted with 4.5 ml of distilled water to form a homogeneous solution. The doses administered to the animals were 250 mg/kg, and 500 mg/kg body weight.

2.4 Phytochemical Analysis

Preliminary phytochemical screening of the stem bark extract was conducted following the methods described by Trease and Evans (1983) with modifications. The tests were performed to detect the presence of saponins, flavonoids, tannins, glycosides, terpenoids, steroids, alkaloids, cardiac glycosides, and reducing sugars.

2.5 Experimental Animals

Wistar rats (200 - 300 g) obtained from the Department of Pharmacology and Toxicology, University of Ibadan, Nigeria were acclimatized for two weeks. The animals were housed in polypropylene cages with a maximum of seven rats per cage. They were maintained under natural light conditions at a temperature of 25 ± 1°C and fed daily with standard rodent pellets, with *ad libitum* access to clean water. Beddings (wood shavings) were replaced every other day; cages were also cleaned every other day using ethanol and cotton wool and washed weekly.

All experiments were conducted in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (2015). Ethical approval was obtained from the Faculty of Pharmacy Ethics Committee.

2.6 Acute Oral Toxicity

Acute oral toxicity testing of the ethanolic extract of *A. africana* stem bark was performed using the Lorke's method with slight modifications. The procedure consisted of two phases:

Phase 1: Nine male mice weighing 22 - 30 g were divided into three groups of three mice each. Each group received different doses of the extract (10 mg/kg, 100 mg/kg, and 1000 mg/kg) and were observed for 24 hours for any signs of toxicity or mortality.

Phase 2: Another set of nine mice divided into three groups, received higher doses of 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg, respectively. Animals were similarly observed for 24 hours for toxicity signs and mortality.

2.7 Rotenone Induced Study

Rotenone Induced toxicity evaluation was carried out using Wistar rats that were randomly assigned into five groups, each comprising six animals. The treatment regimen for each group was as follows:

- **Group 1:** Control (no treatment)
- **Group 2:** Vehicle only
- **Group 3:** Rotenone (1 mg/kg) and with vehicle
- **Group 4:** Rotenone (1 mg/kg) and 250 mg/kg *Afzelia africana* extract
- **Group 5:** Rotenone (1 mg/kg) and 500 mg/kg *Afzelia africana* extract

The volume of extract and rotenone administered to each animal was adjusted according to individual body weight. Parkinsonism was induced by intraperitoneal administration of rotenone at a dose of 1 mg/kg on days 1, 4, 7, and 10.

Neurobehavioural assessments were performed on days 0 (baseline, prior to induction), 5, and 10.

Body weights of all animals were recorded on days 1, 4, 7, 10, and 11. The extract and vehicle treatments were administered orally once daily throughout the study period. Neurobehavioural evaluations carried out were the beam walking test, used to evaluate motor coordination and balance, and the catalepsy test, which assessed muscle rigidity.

On day 11, the animals were humanely sacrificed under ketamine anaesthesia. Blood samples were collected for biochemical analyses, and vital organs were excised and weighed to determine the organ-to-body weight ratios, which were subsequently recorded.

2.8 Neurobehavioural Tests

2.8.1 Catalepsy Test

Method:

Each animal was gently placed on a flat surface to allow for acclimatization. The forepaws were then carefully positioned on a horizontal bar located approximately 11 cm above the surface. Immediately after positioning, a timer was started, and the duration (in seconds) for which the animal maintained this posture without moving its forepaws was recorded within a three-minute observation period.

A longer duration of immobility was interpreted as increased cataleptic behaviour, reflecting heightened motor rigidity and reduced dopaminergic function (Wang *et al.,* 2016).

2.8.2 Beam Walking Test

Method:

Each animal was gently placed at the starting point of a narrow beam, and the timer was activated immediately. The latency time taken by the animal to traverse the beam or to fall off was recorded within a three-minute observation period.

An extended latency time or inability to maintain balance, leading to a fall, was considered evidence of impaired motor coordination.

2.9 Blood Collection and Analysis

On the 11th day of the study, the experimental rats were weighed and anaesthetized humanely using ketamine before being sacrificed. Blood samples were obtained from the abdominal artery using sterile techniques and transferred into well-labeled ethylenediaminetetraacetic acid (EDTA) anticoagulant bottles.

The haematological parameters evaluated are red blood cell (RBC) count, white blood cell (WBC) count, lymphocyte count, haemoglobin concentration, platelet count, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW), plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW). All analyses were carried out by qualified laboratory scientists from the Department of Haematology, University of Benin Teaching Hospital, using standard hematological procedures in accordance with established guidelines (Lewis *et al.,* 2016).

2.10 Biomarker analysis

On the 11th day of the study, the experimental rats were weighed and anaesthetized humanely using ketamine before being sacrificed. The *straita* were carefully isolated from the whole brains harvested and placed in Eppendorf tubes filled with triazole and immediately transferred to a deep freezer and stored at 80^0C. These were used for gene expression studies for expression of misfolded alpha synuclein protein.

2.10.1 Gene expression Study

Isolation of Total RNA

Total RNA was isolated from rats' tissue samples with Quick-RNA MiniPrep™ Kit (Zymo Research). The DNA contaminant was removed following DNAse I (NEB, Cat: M0303S) treatment. The RNA was quantified at 260 nm and the purity confirmed at 260 nm and 280 nm using AandE Spectrophotometer (AandE Lab. UK).

cDNA conversion

One (1 µg) of DNA-free RNA was converted to cDNA by reverse transcriptase reaction with the aid of cDNA synthesis kit based on ProtoScript II first-strand technology (New England BioLabs) in a condition of 3-step reaction: 65 °C for 5 min, 42 °C for 1 h, and 80 °C for 5 min (Olumegbon *et al.,* 2020).

PCR Amplification and Agarose Gel Electrophoresis

Polymerase chain reaction (PCR) for the amplification of gene of interest was carried out with OneTaqR2X Master Mix (NEB) using the following primers (Inqaba Biotec, Hatfield, South Africa) see table below. PCR amplification was performed in a total of 25 µl volume reaction mixture containing cDNA, primer (forward and reverse) and

Ready Mix Taq PCR master mix. Under the following condition: Initial denaturation at 95 ◦C for 5 min, followed by 30 cycles of amplification (denaturation at 95 ◦C for 30 s, annealing for 30 s and extension at 72 ◦C for 60 s) and ending with final extension at 72 ◦C for 10 min. The amplicons were resolved on 1.0% agarose gel. The GAPDH gene was used to normalize the relative level of expression of each gene, and quantification of band intensity was done using "image J" software (Elekofehinti *et al.,* 2020).

Synuclein alpha (Snca)

Forward: AGGTGTTCTTCCATGGCGTA

Reverse: ACAAGAGCCTGCTACCATG

GAPDH

Forward: GCAAGGATACTGAGAGCAAGAG

Reverse: CATCTCCCTCACAATTCCATCC

2.11 Statistical Analysis

All data were expressed as mean ± standard error of mean (SEM). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons or Student - Newman - Keuls (SNK) . Analyses were conducted using Sigma® Stat software version 14.0, and differences were considered statistically significant at $p < 0.05$. The statistical approach followed the recommendations for biomedical data analysis as outlined by Zar (2010).

CHAPTER THREE

RESULTS

3.1. Phytochemical Test The results of the phytochemical test of the ethanol extracts of the stem bark of *A. africana* showed the presence of saponins, glycosides, reducing sugar, terpenoids, alkaloids, and cardiac glycosides and absence of saponins as shown in Table 3.1.

Table 3.1: Results of the phytochemical screening of the ethanol extract of *A. africana* stem bark.

Phytoconstituents	
Saponins	Present
Glycosides	Present
Reducing sugars	Present
Terpenoids	Present
Alkaloids	Present
Tannins	Present
Steroids	Absent
Cardiac glycosides	Present

3.2 Oral Acute Toxicity Test

No deaths were observed in both phases of the acute toxicity test. These findings are presented in Table 2.

Table 2: Oral lethal dose (LD_{50}) of ethanol extracts *A. africana stem bark*

	Dose (mg/kg)	Writhing	Diarrhoea	Tremors	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 Test

3.3.1 Beam Walking Test

Administration of all doses of *Afzelia africana* improved time spent in the beam walking assay following treatment with rotenone, this was significantly different ($p<0.05$) from control. Data is presented in Fig 3.1.

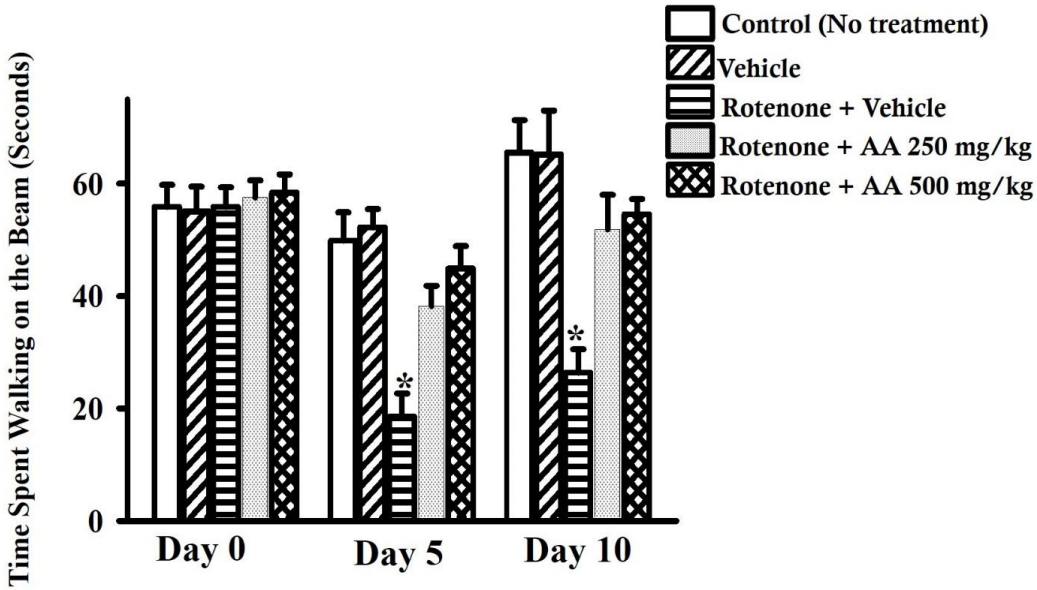

Figure 3.1. Effect of *Afzelia africana* in the Beam Walking Assay of Rotenone Treated Animals. *p<0.05compared to control. n=6 per group. Results are expressed as mean ± standard error of mean. AA- *Afzelia africana*

3.3.2 Catalepsy Test

Administration of all doses of *Afzelia africana* improved time spent in the catalepsy assay following treatment with rotenone, this was significantly different ($p<0.05$) from the control. Data is presented in Fig 3.2.

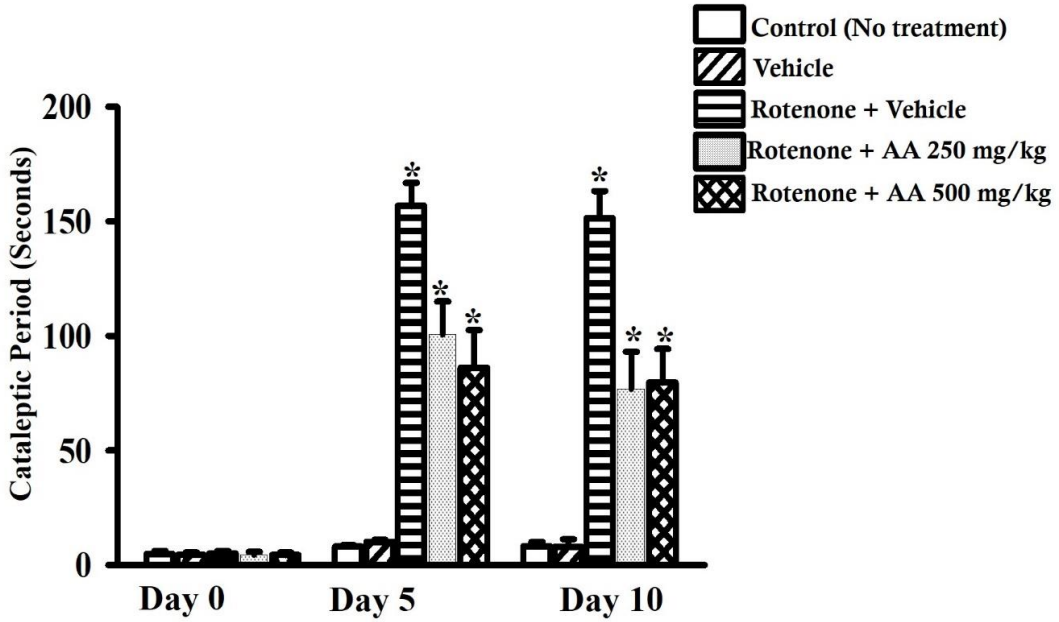

Figure 3.2. Effect of *Afzelia africana* in the Catalepsy test of Rotenone Treated Animals. *p<0.05 compared to control. n=6 per group. Results are expressed as mean ± standard error of mean. AA- *Afzelia africana*

Table 3: Effect of ethanol extract of *A. africana* Stem Bark on Haematological Indices in Rotenone Treated Animals

	Control	Vehicle	Rot + Vehicle	Rot + AA 250 mg/kg	Rot + AA 500 mg/kg
RBC	9.01 ± 0.66	8.12. ± 0.91	5.43 ± 1.87*	7.59 ± 1.62	8.02 ± 0.46
HB	13.15 ± 0.01	14.01 ± 0.13	10.8 ± 0.11	12.95 ± 2.57	14.0 ± 0.24
PCV	44.23 ± 0.31	43.09 ± 2.03	31.6 ± 3.55	39.99 ± 0.97	41.0 ± 0.87
PLT	398.22 ± 55.72	388.83 ± 45.08	291.47 ± 57.99*	314.45 ± 52.3	348.93 ± 52.06
PCT	0.90 ± 0.01	0.85± 0.10	0.44 ± 0.01*	0.63 ± 0.01	0.73 ± 0.01
MCV	65.59 ± 5.17	63.4 ± 6.52	41.3 ± 7.1*	51.37 ±11.33	55.83 ± 2.38
MCH	21.35 ± 1.48	19.99 ± 0.18	12.8 ± 0.44*	16.7 ± 2.52	14.66 ± 0.36
MCHC	34.7 ± 0.63	35.7 ± 0.36	23.37 ± 1.09*	33.39 ± 0.74	31.14 ± 0.85
RDW	19.91 ± 1.22	18.59 ± 0.69	11.65 ± 0.21*	19.62 ± 0.8	16.71 ± 1.31
PDW	36.88 ± 0.54	36.55 ± 0.46	26.38 ± 1.94*	31.92 ± 2.4	38.77 ± 0.81
MPV	6.8 ± 0.61	6.38 ± 0.42	3.75 ± 0.51*	5.55 ± 0.71	6.01 ± 0.13
WBC	10.29 ± 0.01	9.93 ± 0.42	7.04 ± 0.44*	8.18 ± 2.94	9.6 ± 0.9
LYM	8.70 ± 0.66	7.9 ± 0.61	5.36 ± 0.51*	6.95.1 ± 0.67	7.68 ± 0.16
GRA	1.69 ± 0.03	2.00 ± 0.42	1.14 ± 0.34*	1.22 ± 0.83	1.99 ± 1.02
MID	1.41 ± 0.02	1.21 ± 0.13	0.61 ± 0.22*	0.96 ± 0.1	1.13 ± 0.12

***** Significantly different from the control. $p < 0.05$. Results are expressed as mean ± standard error of mean. AA: *Afzelia africana.* RBC Red blood cells, HB Haemoglobin, PCV Packed cell volume. PLT Platelet, PCT Plateletcrit, MCV Mean Corpuscular Volume, MCH Mean Corpuscular Hemoglobin MCHC Mean Corpuscular Hemoglobin Concentration, RDW Red Blood Cell distribution width, PDW Platelet Distribution Width, MPV Mean Platelet Volume, WBC white blood cell, LYM Lymphocytes, GRA Granulocytes, MID Mixed cells

3.3.3 Alpha Synuclein Test

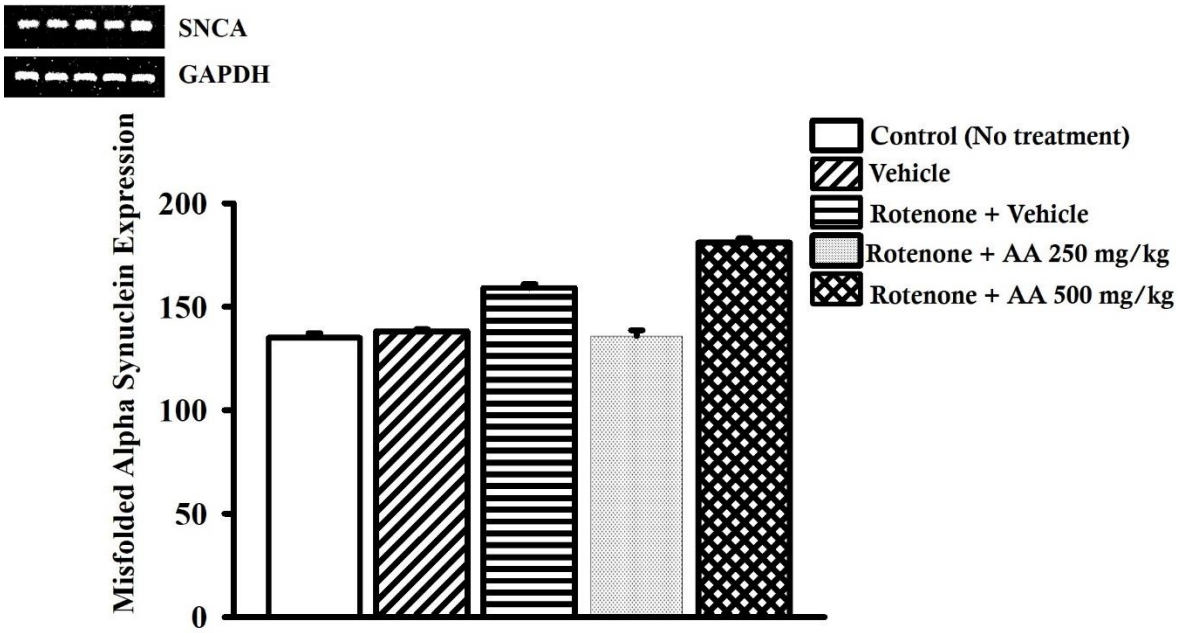

Figure 3.3. Effect of *Afzelia africana* on Misfolded Alpha synuclein levels of Rotenone Treated and Control Animals. n=6 per group. AA- *Afzelia africana*

CHAPTER FOUR

DISCUSSION

4.1 Phytochemical Constituents

The phytochemical screening revealed that the ethanol extract of *A. africana* stem bark contained saponins, glycosides, reducing sugars, terpenoids, alkaloids, tannins, and cardiac glycosides, but not steroids. The presence of these bioactive compounds may underpin the neuroprotective effects observed. For instance, flavonoids and terpenoids are known for their antioxidant and anti-inflammatory properties, which can mitigate oxidative stress and neuroinflammation, central mechanisms in dopaminergic neuronal loss in Parkinson's disease (Trease and Evans, 1983; Olumegbon *et al.,* 2020).

4.2 Acute Toxicity

The oral acute toxicity evaluation demonstrated that *A. africana* extract was safe at doses up to 5000 mg/kg, as no mortality or adverse signs were observed. This indicates a wide safety margin, consistent with earlier studies on plant-based extracts showing low toxicity in rodents (Lewis *et al.,* 2016). The absence of acute toxic effects supports its potential suitability for chronic administration in neurodegenerative disease models.

4.3 Neurobehavioural Effects

Neurobehavioural assessments are central to evaluating functional outcomes in experimental models of Parkinson's disease. Motor coordination, balance, and muscle rigidity are hallmark symptoms of PD resulting from dopaminergic neuronal loss in the substantia nigra pars compacta (SNpc) and subsequent striatal dopamine depletion (Jankovic, 2008). The beam walking test evaluates fine motor coordination and balance, which are impaired in PD models due to oxidative stress and neuroinflammation (Wang

et al., 2016). The catalepsy test measures muscle rigidity and akinesia, providing insight into basal ganglia dysfunction and dopaminergic system integrity (Olumegbon *et al.,* 2020). Improvements in these behavioural parameters indicate potential neuroprotective effects of interventions at the functional level.

Rotenone administration induced significant motor deficits, as evidenced by impaired performance in the beam walking and catalepsy tests. These results are consistent with the well-documented ability of rotenone to mimic Parkinsonian motor dysfunction by selectively targeting dopaminergic neurons in the substantia nigra (Wang *et al.,* 2016).

Treatment with *A. africana* extract improved motor coordination and reduced cataleptic behaviour in a dose-dependent manner. This improvement may be attributed to the antioxidant and anti-inflammatory actions of its phytoconstituents, which likely attenuate dopaminergic neuron damage and restore motor function. The neurobehavioural improvements align with previous reports, where plant extracts containing flavonoids and alkaloids have demonstrated protective effects in neurotoxin-induced Parkinson's disease models (Elekofehinti *et al.,* 2020).

4.4 Haematological Indices

Hematological parameters are valuable indicators of systemic toxicity, oxidative stress, and inflammation (Lewis *et al.,* 2016). Neurotoxins such as rotenone can induce haematotoxicity by generating free radicals, disrupting bone marrow function, and promoting apoptosis in circulating blood cells. Evaluating red blood cells (RBC), hemoglobin, packed cell volume (PCV), and platelets provides insight into the systemic effects of neurotoxic insult and the protective potential of therapeutic agents.

Rotenone exposure resulted in significant reductions in RBC, Hb, PCV, and other haematological indices, indicating systemic stress and possible haematotoxicity. Oral administration of *A. africana* extract ameliorated these alterations, particularly at the 500 mg/kg dose. The restoration of haematological parameters may reflect the extract's antioxidant capacity, which protects against rotenone-induced oxidative damage to hematopoietic tissues (Lewis *et al.,* 2016).

4.5 Alpha-Synuclein

Alpha-Synuclein (SNCA) plays a central role in the pathogenesis of Parkinson's disease. Overexpression or aggregation of this protein leads to dopaminergic neuron degeneration and formation of Lewy bodies, hallmarks of PD pathology (Spillantini *et al.,* 1998). Modulation of SNCA expression provides a molecular-level indicator of neuroprotective efficacy. Therapies that reduce α-synuclein accumulation can potentially slow neurodegeneration and ameliorate motor deficits Javed *et al., 2018,* Amro *et al.,* 2019; Olumegbon *et al*., 2020; Elekofehinti *et al.,* 2020).).

Rotenone upregulated SNCA expression, consistent with dopaminergic neuronal stress. Low dose (250 mg/kg) but not high dose (550 mg/kg) of *A. africana* extract reduced SNCA expression, suggesting that the extract may protect against molecular processes underlying neurodegeneration. The discrepancies in the effect of high dose may be as a result of experimental errors, though further analysis will be carried out to validate or refute these results.

Overall, the molecular effect complements behavioural and haematological improvements, indicating that *A. africana* provides neuroprotection at both functional and cellular levels.

CHAPTER FIVE

CONCLUSION

The ethanol extract of *Afzelia africana* improved time spent in the beam walking assay, improved rotenone induced haematological changes, and reduced levels of misfolded alpha synuclein proteins in the brains of rats treated with rotenone.

Taken together, these results are indicative of cytoprotective properties of *Afzelia africana* in rotenone induced toxicity and may be a potential candidate for drug development

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