EVALUATION OF THE NEUROPROTECTIVE AND HISTOPATHOLOGICAL EFFECTS OF THE ETHANOLIC EXTRACT OF *AFZELIA AFRICANA* IN ROTENONE-INDUCED PARKINSONISM IN WISTAR RATS

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CERTIFICATION

We certify that this work was carried out by **Oronsaye, Esosa Jessica**, with matriculation number **PHA1908579** in the Department of Pharmacology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria.

…….. **……**

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(Project Supervisor)

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(*Ag.* Head of Department)

DEDICATION

This project work is dedicated to God Almighty, who has been my pillar and rock from the beginning till date.

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ABSTRACT

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the gradual loss of dopaminergic neurons within the substantia nigra pars compacta, leading to dopamine depletion, oxidative stress, and motor dysfunction. Despite advances in neuropharmacology, current treatment options remain largely symptomatic and fail to halt disease progression. This has intensified the search for novel neuroprotective agents, particularly from natural sources. *Afzelia africana*, a medicinal plant traditionally used across West Africa for the management of inflammatory and oxidative stress-related conditions, contains phytochemicals such as flavonoids, tannins, and terpenoids that may possess neuroprotective properties.

This study investigated the neuroprotective and histopathological effects of the ethanolic stem bark extract of *Afzelia africana* in a rotenone-induced Parkinsonism model using male Wistar rats. The plant material was extracted by cold maceration in ethanol and subjected to qualitative phytochemical screening. Acute toxicity was evaluated using a modified Lorke's method. The animals used for this study were randomly distributed in 5 different treatment groups, namely: control (no treatment), vehicle alone, rotenone and vehicle, rotenone and 250 mg/kg *Afzelia africana* extract and rotenone and 500 mg/kg *Afzelia africana* extract. Animals in all groups received the different drug treatments daily while those in the last three groups received 1 mg/kg of rotenone on days 1,4,7 and 10. Neurobehavioural performance was assessed using the wire hanging and elevated plus maze tests on days 0, 5 and 10. On the 11^{th} day, animals were sacrificed via ketamine injection, organs were harvested, weighed and preserved in formalin and used for histopathological studies.

The phytochemical screening revealed the presence of saponins, glycosides, terpenoids, alkaloids, tannins, and phenolic compounds; the LD_{50} was estimated to be than 5000 mg/kg. Rotenone administration caused marked impairment in motor coordination and increased anxiety-like behaviour; however, treatment with *A. africana* extract significantly improved ($p<0.05$) motor strength, prolonged latency to fall in the wire hanging test, and enhanced open-arm exploration in the elevated plus maze. Histopathological examination of the striatum showed that *A. africana* attenuated neuronal atrophy and pyknosis, while also ameliorating hepatic and renal alterations induced by rotenone exposure. The findings demonstrate that the ethanolic stem bark extract of *Afzelia africana* possesses neuroprotective and anxiolytic activities suggesting its potential as a promising natural therapeutic candidate drug discovery.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.0 Background of Study

Parkinson's disease is a neurological degenerative illness that often manifests as stiffness, slowness, and tremors of movement. Additionally, the substantia nigra and other brain regions are affected, leading to gradual neurodegeneration. Non-motor symptoms including dysautonomia and dementia often manifest as the illness progresses. Reduced mitochondrial complex-1, a number of cell-damaging mechanisms, including protein aggregation, apoptosis, calcium homeostasis, inflammation, excitotoxicity, atypical energy metabolism, and well-established links between genetics and environmental interactions, all contribute to the development or triggering of Parkinson's disease (Soumya, 2022). Parkinson's disease is primarily caused by oxidative stress, which interferes with dopamine metabolism. Reactive oxygen species (ROS) are produced as a consequence of this oxidative damage, which kills neurons. A decrease in the quantities of spontaneous antioxidant molecules demonstrated this. These findings indicate that using antioxidants as a successful therapy for Parkinson's disease in addition to other preventive measures is now crucial (Duarte *et al.,* 2022).

The goal of the *Afzelia africana* ethanolic extract was to lessen oxidative stress in the substantia nigra. Because it includes flavonoids, which are effective against oxidative stress and exhibit antioxidant activity, the ethanolic extract of *Afzelia africana* has antioxidant properties (Pacifici *et al.,* 2021). Dopamine auto-oxidation in dopaminergic neurons, a major source of reactive oxygen species that results in neuronal oxidative stress, is the mechanism behind Parkinson's disease. Neuroinflammation, which may accelerate pathogenic damage to the substantia nigra, can be brought on by microglia and astrocytes. A reduction in striatal dopamine is the mechanism underlying Parkinson's disease that results in bradykinesia, stiffness, and tremors. One of the mental illnesses that causes tremors, stiffness, and bradykinesia is Parkinson's

disease, a neurodegenerative condition of the central nervous system. The disease's progression is influenced by several variables. Increased oxidative stress is the primary cause of dopaminergic neuron dementia in the substantia nigra (Sayyaed *et al.,* 2023).

Since ancient times, people have used plants as a source of medicine. In order to sustain the life cycle and improve human well-being, plants are essential. They have helped humanity in a variety of ways, including flavourings, cosmetic ingredients in medications, colours, and drinks. Plant science has been more popular in recent years, and extensive data sets have been examined to show the immense potential of medicinal plants employed in a variety of alternative medical procedures.

1.1 Parkinson's Disease: An Empirical Overview

Parkinson's disease (PD) is a chronic, progressive neurodegenerative condition predominantly defined by the destruction of dopaminergic neurons in the substantia nigra pars compacta, resulting in a depletion of dopamine in the striatum. The neurotransmitter imbalance leads to the characteristic motor symptoms of Parkinson's disease, including resting tremor, bradykinesia, stiffness, and postural instability (Bloem *et al.,* 2021). Non-motor symptoms, including cognitive impairment, depression, sleep difficulties, and autonomic dysfunction, significantly impact patients' quality of life (Schapira *et al.,* 2017).

Parkinson's disease presents with both motor and non-motor symptoms that progressively worsen and lead to functional impairment. Motor symptoms include resting tremor, which is usually asymmetrical and affects the hands. Bradykinesia, defined by diminished movement speed and decreased facial expressiveness, Rigidity, characterized by muscular rigidity and resistance to passive motion, Postural instability, leading in decreased balance and increased risk of falls (Marogianni *et al.,* 2020).

Non-motor symptoms are becoming acknowledged as significant factors in disease burden. These include: Sleep abnormalities, such as insomnia or REM sleep behaviour disorder,

Cognitive impairment, ranging from moderate executive dysfunction to dementia, Autonomic dysfunction, including constipation, orthostatic hypotension, and urine problems (Okunoye *et al.,* 2023).

Disease development is generally tiered using the Hoehn and Yahr scale, which grades severity from early unilateral involvement (Stage 1) to advanced, debilitating symptoms with significant postural instability (Stage 5) (Kalia and Lang, 2015). The clinical course is very varied, with some individuals suffering fast advancement while others have gradual functional decline.

1.1.1 Epidemiology of Parkinson's Disease

Parkinson's disease is the second most widespread neurodegenerative ailment after Alzheimer's disease, affecting around 1–2% of adults over 65 years of age worldwide (Pringsheim *et al.,* 2024). The prevalence rises with age, and it is estimated that the worldwide burden of PD will double by 2040 owing to increasing life expectancy and population aging. Although PD usually affects older persons, early-onset instances have been documented in individuals under 50 years, generally linked with genetic abnormalities (Kalia and Lang, 2015). The cardinal symptoms of PD include akinesia, bradykinesia, resting tremor, stiffness, postural instability, and is commonly linked with cognitive impairment with disease progression. The neuropathological characteristic of PD is the specific and gradual loss of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc), resulting in dopamine deficit in the striatum.

According to WHO 2023, the worldwide burden of Parkinson's disease was about 11.77 million individuals in 2021, which grew by 273% compared to 1990. Disability-Adjusted life years (DALYs) attributable to PD in 2021 was 7.47 million. PD is rising as a worldwide public health burden, and its incidence has increased in the previous decades.

1.1.2 The Etiology of Parkinson's disease

The genesis of Parkinson's disease is multifaceted, including intricate interactions between genetic predispositions and environmental influences. Several genes, including SNCA, LRRK2, PINK1, and PARK2, have been linked in familial and sporadic variants of the condition (Bridi and Hirth, 2018). Environmental pollutants such as pesticides, heavy metals, and some solvents have been associated to increased PD risk owing to their capacity to promote mitochondrial dysfunction and oxidative stress (Ball *et al.,* 2019). Rotenone, a naturally occurring insecticide, has been frequently utilized experimentally to recreate Parkinsonian symptoms in animal models because of its propensity to block mitochondrial complex I and drive dopaminergic neurodegeneration.

Though the genetic and environmental variables constitute the major causes, additional factors such as reactive oxygen species-induced damage, excitotoxicity, mitochondrial dysfunctions, and inflammation-mediated cell injury have also been implicated in the genesis of PD. Over the decades, experimental data has focused on mitochondrial dysfunction as a major pathogenic component of PD (Duarte *et al.,*2022). Dopaminergic neurons are more vulnerable to oxidative stress. An increase in the metabolism of dopamine creates large quantities of Reactive Oxygen free radicals that cause cellular damage. Rotenone, a natural insecticide, is a highly lipophilic and selective mitochondrial complex-I inhibitor. Therefore, it has been frequently employed as a mitochondrial toxin model.

1.1.3 The pathophysiology of Parkinson's disease

The pathology of Parkinson's disease (PD) involves a wide variety of neuropathological, biochemical and molecular abnormalities that explain its motor and non-motor symptoms. The neuropathological characteristic of PD is the specific and gradual degradation of dopaminergic neurons in the SNpc, resulting in dopamine deficit in the striatum

The pathogenesis of PD is predominantly connected with oxidative stress, mitochondrial dysfunction, neuroinflammation, and protein aggregation. Mitochondrial malfunction leads to excessive formation of reactive oxygen species (ROS), which, in turn, destroys cellular lipids, proteins, and DNA, leading to neuronal death. Another feature of PD is the buildup of misfolded α-synuclein proteins creating Lewy bodies inside neurons, which further disturb cellular homeostasis (Bloem *et al.,* 2021). Neuroinflammatory mechanisms involving activated microglia also accelerate dopaminergic neuronal loss via the production of pro-inflammatory cytokines and nitric oxide (Tansey and Romero-Ramos, 2019).

Current pharmaceutical care of PD focuses mostly on symptomatic alleviation using dopaminergic medications such as levodopa, dopamine agonists, and monoamine oxidase-B inhibitors (Connolly and Lang, 2014). However, these therapies may not arrest disease development and may be linked with severe consequences during long-term usage.

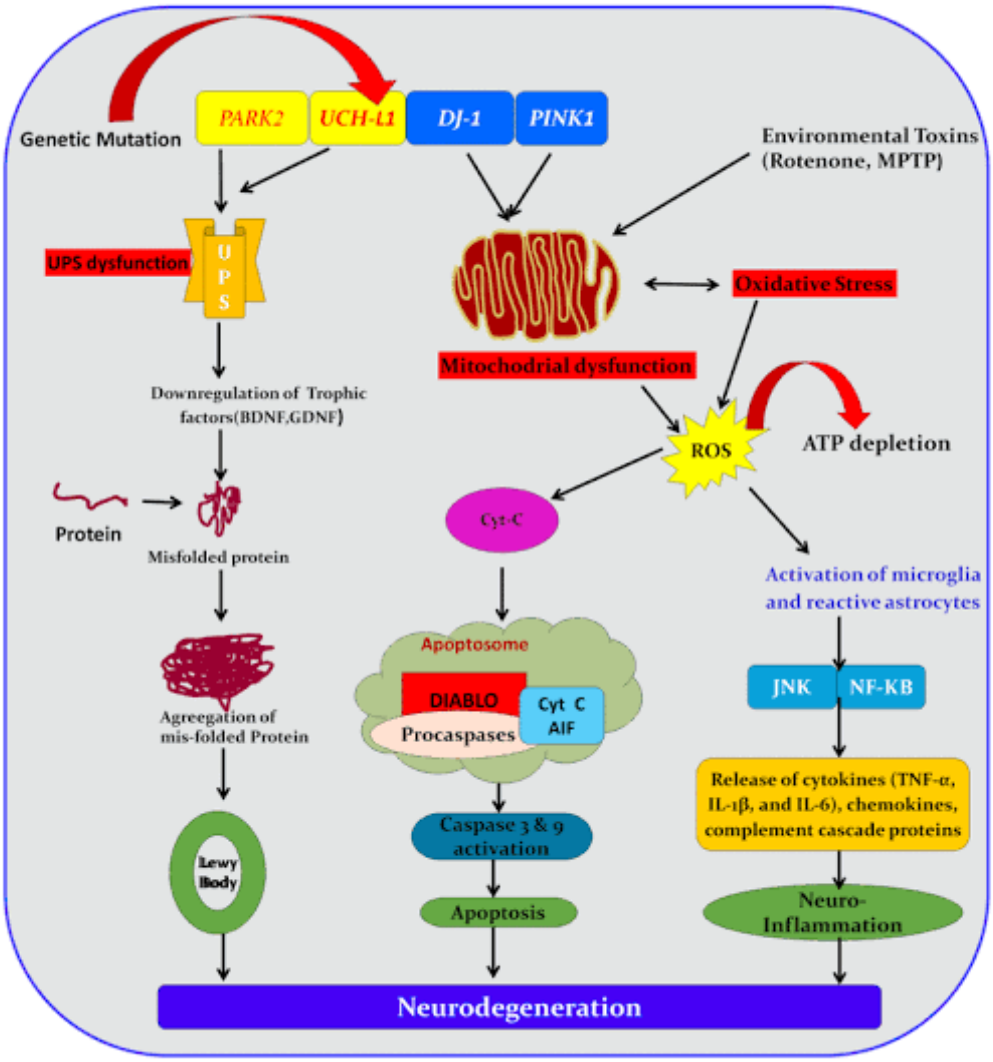

Figure 1. 1: Pathophysiology of Parkinson's disease (Desai et al., 2020)

1.2 Rotenone-Induced Parkinsonism Model

Mechanism of Rotenone Toxicity

Rotenone, a naturally occurring lipophilic chemical generated from the roots and stems of plants belonging to the Derris and Lonchocarpus species, is well recognized as a strong mitochondrial toxin. It has been widely employed as an experimental drug to cause Parkinson's-like pathology in animal models owing to its ability to mimic several important aspects of human Parkinson's disease (PD) (Betarbet *et al.,* 2000). The principal mechanism of rotenone-induced neurotoxicity includes the inhibition of mitochondrial complex I (NADH: ubiquinone oxidoreductase) of the electron transport chain (Sherer *et al.,* 2003).

Complex I inhibition leads to decreased electron transport from NADH to ubiquinone, resulting in diminished ATP generation and the buildup of electrons inside the mitochondrial matrix. These extra electrons combine with molecular oxygen to form reactive oxygen species (ROS) such as superoxide anions and hydrogen peroxide, which in turn induce oxidative damage to cellular macromolecules, including lipids, proteins, and DNA (Fang and Beattie, 2003; Testa *et al.,* 2005). This oxidative stress induces mitochondrial malfunction, loss of membrane potential, and ultimately, neuronal death via the activation of caspase-dependent pathways (Greenamyre *et al.,* 2021).

In addition to oxidative stress, rotenone stimulates α-synuclein aggregation and development of Lewy body-like inclusions inside dopaminergic neurons (Betarbet *et al.,* 2000). These protein aggregates interfere with normal cellular processes such as vesicular trafficking and autophagy, further accelerating neuronal degeneration (Sherer *et al.,* 2003). Furthermore, rotenone-induced mitochondrial dysfunction leads to microglial activation and production of pro-inflammatory cytokines, so producing a neuroinflammatory milieu that exacerbates dopaminergic neuron loss in the substantia nigra pars compacta (SNpc) (Gao *et al.,* 2023).

1.2.1 Advantages and Limitations of the Rotenone Model

One of the primary benefits of the rotenone-induced Parkinsonism model is its ability to recapitulate important neuropathological and behavioural aspects of genuine PD. It promotes selective degeneration of dopaminergic neurons in the SNpc and leads in motor impairments such as stiffness, bradykinesia, and tremor in mice (Betarbet *et al.,* 2020; Cannon *et al.,* 2023). Additionally, rotenone causes Lewy body-like inclusions containing α-synuclein, closely matching the characteristic histopathology of PD (Sherer *et al.,* 2023). Because rotenone is extremely lipophilic, it quickly penetrates the blood–brain barrier and accumulates inside mitochondria, allowing for systemic administration and successful induction of central neurotoxicity (Greenamyre *et al.,* 2001). This model also offers a good platform for researching oxidative stress-mediated pathways and evaluating antioxidant-based neuroprotective treatments (Tieu, 2021).

However, despite its benefits, the rotenone model has significant drawbacks. The toxin demonstrates nonspecific systemic toxicity, which may lead to significant fatality rates and variability in outcomes across animals (Höglinger *et al.,* 2023). Moreover, since rotenone affects several mitochondrial-dependent organs, including the liver and heart, systemic treatment might create peripheral effects that confound interpretation of neurological results (Johnson and Bobrovskaya, 2015). The repeatability of this model may also vary based on aspects such as dose, duration, method of administration, and species employed. Additionally, although it efficiently replicates dopaminergic neuronal loss and Lewy body development, it does not entirely reproduce the chronic and progressive character of real PD, which occurs over decades (Cannon *et al.,* 2023).

Despite these drawbacks, the rotenone-induced Parkinsonism model remains one of the most dependable and commonly used environmental toxin-based models for researching PD etiology. It provides a robust experimental framework for testing the neuroprotective potential

of natural substances, such as the ethanolic extract of *Afzelia africana*, which may reverse oxidative stress and mitochondrial dysfunction associated with dopaminergic dementia.

1.3 Oxidative Stress, Mitochondrial dysfunction and Neurodegeneration in Parkinson's disease

Oxidative stress and mitochondrial dysfunction play essential roles in the development and progression of Parkinson's disease (PD), contributing considerably to dopaminergic neuronal loss in the substantia nigra pars compacta. The brain, owing to its high oxygen consumption and relatively limited antioxidant defenses, is especially prone to oxidative injury (Subramaniam and Chesselet, 2013). Oxidative stress refers to an imbalance between the formation of reactive oxygen species (ROS) and the capacity of the body's antioxidant mechanisms to neutralize them (Puspita *et al.,* 2017). In PD, excessive ROS such as superoxide anions, hydroxyl radicals, and hydrogen peroxide are formed, leading to lipid peroxidation, DNA damage, and protein oxidation, which together impair neuronal function and survival (Dias *et al.,* 2013).

The dopaminergic neurons of the substantia nigra are particularly sensitive to oxidative stress because dopamine metabolism itself may create reactive intermediates. During dopamine oxidation, quinones and hydrogen peroxide are produced, which may further react with cellular macromolecules, increasing mitochondrial damage and cell death (Rees *et al.,* 2018). Moreover, iron buildup in the substantia nigra of PD patients catalyzes Fenton-type reactions, boosting hydroxyl radical production and oxidative damage (Poewe *et al.,* 2017). This oxidative assault is worsened by the loss of essential endogenous antioxidants such as reduced glutathione (GSH) and enzymes like superoxide dismutase (SOD) and catalase (CAT), which typically protect neurons from ROS-induced injury (Blesa *et al.,* 2015).

Mitochondrial dysfunction is another essential pathogenic characteristic of PD and a significant source of ROS production. Mitochondria serve as the major location of aerobic respiration and

ATP synthesis; however, blockage of the electron transport chain, especially at complex I, leads to electron leakage and subsequent creation of superoxide radicals (Schapira, 2012). Several environmental toxins and neurotoxins such as rotenone and 1-methyl-4-phenylpyridinium (MPP^+) are known to mimic PD-like symptoms by blocking mitochondrial complex I, resulting in ATP depletion and neuronal death (Sherer *et al.,* 2003). Postmortem investigations of PD patients have demonstrated a considerable decline in mitochondrial complex I activity in the substantia nigra, further demonstrating the relationship between mitochondrial dysfunction and neurodegeneration (Schapira *et al.,* 1990).

Mitochondrial failure generates a cascade of detrimental events including calcium dysregulation, release of pro-apoptotic substances such as cytochrome c, and activation of caspase-mediated cell death pathways (Exner *et al.,* 2012). Additionally, mitochondrial DNA (mtDNA) is especially sensitive to oxidative damage owing to its closeness to the electron transport chain and absence of protective histones, leading to mutations that further degrade mitochondrial function (Blesa *et al.,* 2015). This vicious loop of ROS production and mitochondrial damage maintains neuronal degeneration in PD.

Neurodegeneration in Parkinson's disease is therefore a complex process caused by the interaction between oxidative stress, mitochondrial failure, and protein aggregation. The aggregation of misfolded α-synuclein forms Lewy bodies inside dopaminergic neurons alters mitochondrial integrity and hampers autophagic clearance pathways (Bridi and Hirth, 2018). Activated microglia in the substantia nigra emit pro-inflammatory cytokines and nitric oxide, which further worsen oxidative stress and neuronal death (Tansey and Romero-Ramos, 2019). Over time, these pathogenic processes combine to induce selective death of dopaminergic neurons, dopamine depletion in the striatum, and the typical motor and cognitive symptoms of PD (Bloem *et al.,* 2021).

Given this intricate relationship, treatment methods targeting oxidative stress and

mitochondrial dysfunction have attracted increased interest in PD therapy. Antioxidants and natural substances with free radical scavenging characteristics might possibly restore redox equilibrium, retain mitochondrial function, and provide neuroprotection (Bishnoi *et al.,* 2020).

1.3.2 Role of Oxidative Stress in Neurodegeneration

Oxidative stress is a central and recurrent theme in the pathology of many neurodegenerative disorders, including Parkinson's disease (PD), Alzheimer's disease, and amyotrophic lateral sclerosis. At its core, oxidative stress denotes an imbalance between the production of reactive oxygen species (ROS) and the capacity of cellular antioxidant defenses to detoxify these reactive intermediates (Puspita *et al.,* 2017). In neurons-which have high metabolic rates, abundant polyunsaturated fatty acids, and relatively low antioxidant reserves-this imbalance readily precipitates molecular damage that impairs function and promotes cell death (Subramaniam and Chesselet, 2023).

Major endogenous sources of ROS in the nervous system include mitochondrial respiration, monoamine (e.g., dopamine) metabolism, and the activity of enzyme systems such as NADPH oxidases. Under normal conditions mitochondria generate ROS as by-products of electron transport, primarily when electrons leak from complexes I and III and reduce molecular oxygen to form superoxide anion (O2•−) (Schapira, 2022). In pathologic states-such as when complex I is inhibited by toxins like rotenone-electron leakage increases, producing higher ROS flux that overwhelms cellular defenses (Sherer *et al.,* 2023). Dopaminergic neurons face an additional ROS burden because dopamine oxidation yields reactive quinones and hydrogen peroxide, which can further react via Fenton chemistry in the presence of iron to form highly damaging hydroxyl radicals (Rees *et al.,* 2018; Poewe *et al.,* 2017). Activated microglia also contribute to ROS production through inducible nitric oxide synthase and NADPH oxidase during neuroinflammation, compounding oxidative injury (Gao *et al.,* 2023).

One of the most deleterious consequences of excessive ROS is lipid peroxidation, a chain reaction that targets polyunsaturated fatty acids in cell membranes. Initiation occurs when ROS abstract a hydrogen atom from a lipid, producing a lipid radical that reacts with oxygen to form lipid peroxyl radicals; these propagate the chain, yielding lipid hydroperoxides and secondary reactive aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) (Butterfield and Boyd-Kimball, 2018). These lipid peroxidation products disrupt membrane integrity, alter membrane-bound enzyme activities, and form adducts with proteins and DNA, thereby impairing synaptic transmission and mitochondrial function-processes critically important for neuronal survival (Pike, 2021). Elevated levels of MDA and 4-HNE are frequently reported in postmortem brain tissue and biofluids from patients with neurodegenerative diseases, underscoring lipid peroxidation's role as both marker and mediator of neuronal injury (Blesa *et al.,* 2015).

To counteract ROS and repair oxidative damage, cells rely on a coordinated antioxidant defense system composed of enzymatic and non-enzymatic elements. Key enzymatic antioxidants include superoxide dismutases (SODs), which catalyze the dismutation of superoxide into hydrogen peroxide; catalase (CAT), which converts hydrogen peroxide into water and oxygen; and glutathione peroxidases (GPx), which reduce hydrogen peroxide and lipid hydroperoxides using reduced glutathione (GSH) as an electron donor (Perry *et al.,* 2022). Non-enzymatic antioxidants-such as GSH itself, vitamin E (α-tocopherol), vitamin C (ascorbate), and various dietary polyphenols-scavenge free radicals and regenerate oxidized antioxidant enzymes (Bishop and Darley-Usmar, 2012). In PD and related disorders, multiple studies report decreased GSH levels and compromised activity of SOD, CAT, and GPx in affected brain regions, indicating a failure of antioxidant defenses that contributes to progressive neuronal loss (Blesa *et al.,* 2015; Subramaniam and Chesselet, 2023).

When antioxidant systems are overwhelmed, oxidative modifications accumulate on lipids, proteins (carbonylation, nitration), and nucleic acids (8-oxo-2′-deoxyguanosine), leading to dysfunctional proteins, impaired proteostasis, mitochondrial DNA mutations, and activation of cell death pathways (Dias *et al.,* 2013). Importantly, oxidative stress is not only downstream of primary insults such as mitochondrial dysfunction or protein aggregation (e.g., α-synuclein) but can also act upstream to promote these pathogenic events-creating a feed-forward loop that accelerates neurodegeneration (Bridi and Hirth, 2018).

1.4 Medicinal Plants with Neuroprotective Effects

Natural products and medicinal plants have attracted considerable attention as potential sources of neuroprotective agents for Parkinson's disease (PD) because many contain multiple bioactive compounds that target oxidative stress, inflammation, mitochondrial dysfunction, and protein aggregation-pathways central to PD pathogenesis (Bishnoi, Chopra, and Kulkarni, 2020). Below is a concise review of several plants and phytochemicals that have demonstrated antioxidant or dopaminergic neuroprotective properties in preclinical studies and, in some cases, early clinical work.

Mucuna pruriens (velvet bean).

Mucuna pruriens seeds are a natural source of L-dopa, the immediate precursor of dopamine, and have been used in traditional Ayurvedic medicine for parkinsonian symptoms. Clinical and experimental studies indicate that *Mucuna* preparations can improve motor function similarly to standard levodopa formulations, while some animal studies suggest additional antioxidant and neuroprotective effects beyond simple dopamine replacement (Katzenschlager *et al.,* 2004; Bishnoi *et al.,* 2020). Because *Mucuna* contains other phytochemicals (e.g., antioxidants and trace elements), it is hypothesized to confer adjunctive protection of dopaminergic neurons.

***Curcuma longa* (curcumin).**

Curcumin, the principal polyphenol of *Curcuma longa*, exhibits potent antioxidant and anti-inflammatory activities and modulates multiple cell-survival pathways (e.g., Nrf2, NF-κB, and mitochondrial cascades). In rotenone, MPTP, and 6-OHDA animal models, curcumin reduces oxidative markers, inhibits α-synuclein aggregation, preserves mitochondrial function, and improves behavioural outcomes-supporting its candidacy as a multi-target neuroprotective agent (Rajput, Zhang, and Wang, 2022; Tiwari, Singh, and Singh, 2019).

Ginkgo biloba.

Extracts of *Ginkgo biloba* (standardized EGb 761) contain flavonoids and terpenoids that scavenge free radicals and inhibit lipid peroxidation. Preclinical research has shown that *Ginkgo* extracts attenuate dopaminergic neuron loss, reduce oxidative damage, and modulate mitochondrial function and inflammation in various toxin-based PD models. Although clinical evidence for disease modification is limited, *Ginkgo* remains widely studied for symptomatic and neuroprotective potential (Ahlemeyer and Krieglstein, 2003; Bishnoi *et al.,* 2020).

***Camellia sinensis* (epigallocatechin-3-gallate, EGCG).**

Green tea polyphenols-especially EGCG-have demonstrated strong free-radical scavenging activity, metal-chelating capacity, and the ability to modulate cell signaling pathways implicated in neuronal survival. EGCG has been protective in MPTP and rotenone models, reducing oxidative stress, inhibiting α-synuclein fibrillization, and improving motor deficits in rodents (Singh, Srivastava, and Shukla, 2021; Lee, Park, and Lee, 2019).

***Panax ginseng* (ginsenosides).**

Ginsenosides from *Panax ginseng* exert antioxidant, anti-inflammatory, and anti-apoptotic effects, and several studies report preservation of dopaminergic neurons and motor function in toxin-induced PD models. Mechanistically, ginsenosides enhance mitochondrial function,

reduce oxidative damage, and modulate neurotrophic signaling-actions that support their neuroprotective profile (Khan, Marya, and Nabavi, 2019).

***Withania somnifera* (ashwagandha).**

Withania somnifera (ashwagandha) contains withanolides that show neuroprotective effects in preclinical studies by reducing oxidative stress, attenuating neuroinflammation, and stimulating antioxidant defense systems. In PD models, ashwagandha extracts have been reported to preserve dopaminergic neurons and improve behavioural outcomes (Bishnoi *et al.,* 2020).

***Bacopa monnieri* and other cognitive herbs.**

Bacopa monnieri (brahmi) and other traditional nootropics possess antioxidant and anti-apoptotic properties. In experimental PD models, *Bacopa* extracts reduce lipid peroxidation, restore antioxidant enzyme activities, and ameliorate motor and cognitive deficits (Tiwari *et al.,* 2019).

Resveratrol and grape polyphenols.

Resveratrol (a stilbene present in grapes and berries) activates SIRT1 and Nrf2 pathways, improves mitochondrial biogenesis, reduces oxidative stress, and inhibits apoptosis in neuronal models. In toxin-based PD models resveratrol has shown neuroprotective efficacy, supporting interest in polyphenol-rich diets or supplements (Rajput *et al.,* 2022).

1.5.1 The Plant: *Afzelia africana*

The Botanical and Ethnomedicinal description of the Plant

Afzelia africana Sm., commonly known as African mahogany, Afzelia, or “papao” in some regions of West Africa, is a tropical deciduous tree belonging to the family Fabaceae

(Leguminosae**)**, subfamily Caesalpinioideae (Orwa *et al.,* 2023). It is widely distributed across tropical Africa, occurring predominantly in the Sudanian and Guinean savannah zones, from Senegal and Sudan to Uganda (Keay, 1989). The leaves are compound and alternately arranged, consisting of 2–5 pairs of opposite leaflets. Each leaflet is broadly elliptical or oblong, measuring approximately 8–15 cm long and 4–7 cm wide, with a smooth, glossy surface and entire margins (Keay, 1989). The petiole and rachis are stout, and the leaflets exhibit a characteristic asymmetrical base with rounded apices. The root system is deep and extensive, providing stability in arid and semi-arid environments. The bark contains tannins and gum and has traditional medicinal applications for treating diarrhea, wounds, and other ailments (Burkill, 1995).

The ethnomedicinal applications of *A. africana* are extensive and vary across different regions of Africa. In Nigeria, Ghana, Senegal, Benin, and Cameroon, it is traditionally used for the treatment of inflammatory diseases, infections, gastrointestinal disorders, anemia, and wounds (Adedapo *et al.,* 2024). The bark, leaves, and roots are the most commonly utilized parts due to their abundance of bioactive compounds, including flavonoids, tannins, saponins, and alkaloids, which contribute to its medicinal efficacy (Oluwole *et al.,* 2020).

Figure 1. 2: Morphology of the fruit (pod) of Afzelia africana (Sm.) Pers. (Heuzé et al., 2019)

1.5.2 The Phytochemical constituents of the Plant

These bioactive compounds occur in varying concentrations in the bark, leaves, seeds, and roots, and they confer multiple pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective effects.

1. Flavonoids

Flavonoids are a major class of secondary metabolites present in *Afzelia africana*. They are polyphenolic compounds known for their antioxidant, anti-inflammatory, and antimicrobial activities (Lamien-Meda *et al.,* 2020). The leaves and bark of the plant are particularly rich in flavonoids such as quercetin, kaempferol, and luteolin, which act as free radical scavengers and protect biological tissues from oxidative damage (Adedapo *et al.,* 2014). These compounds have also been linked to the plant's antidiarrheal and hepatoprotective properties, as they stabilize cellular membranes and prevent lipid peroxidation.

2. Alkaloids

Alkaloids are nitrogen-containing organic compounds that play a critical role in the plant's pharmacological profile. In *Afzelia africana*, the presence of alkaloids has been reported in the bark, roots, and seeds (Adewumi *et al.,* 2021). These compounds exhibit analgesic, antimalarial, and antimicrobial properties. Some alkaloids may also act on the central nervous system, contributing to the plant's traditional use in treating pain and fever (Akinmoladun *et al.,* 2016). Furthermore, the alkaloid fraction is thought to contribute to the **a**ntiplasmodial and antipyretic effects of bark decoctions used in local medicine.

3. Tannins

Tannins are high-molecular-weight polyphenols that have astringent and antimicrobial properties. They are present in large amounts in the bark and leaves of *Afzelia africana* (Burkill, 1995). Their astringent property explains the traditional use of bark extracts in the treatment of diarrhea, wounds, and skin infections. Tannins precipitate proteins on cell surfaces, forming protective layers that help in wound healing and reduce inflammation (Orwa *et al.,* 2023). Moreover, tannins contribute to the antioxidant capacity of the plant, neutralizing free radicals and protecting against oxidative stress-induced tissue damage.

4. Saponins

Saponins are glycosidic compounds known for their foaming property and diverse biological activities, including anti-inflammatory, antifungal, and immune-modulatory effects. Studies have confirmed the presence of saponins in both the leaves and seeds of *A. africana* (Oluwole *et al.,* 2020). They are believed to contribute to the plant's hypocholesterolemic and antidiabetic properties, as they bind cholesterol and reduce its intestinal absorption. In addition, saponins enhance the permeability of microbial cell membranes, explaining the antimicrobial activity of the plant extracts (Adewumi *et al.,* 2021).

5. Glycosides

Glycosides are bioactive compounds in which sugar molecules are bound to a non-carbohydrate moiety, often conferring pharmacological activity. *Afzelia africana* contains several cardiac and flavonoid glycosides, which may account for its anti-inflammatory and diuretic effects (Akinmoladun *et al.,* 2016). These compounds play essential roles in the

regulation of blood pressure, stimulation of cardiac activity, and control of inflammation, further supporting traditional uses in the treatment of hypertension and rheumatism.

6. Terpenoids and Steroids

Terpenoids and steroids are among the most therapeutically significant classes of secondary metabolites in *A. africana*. They have been identified in methanolic and ethanolic extracts of the leaves and bark (Lamien-Meda *et al.,* 2010). These compounds are known for their anti-inflammatory, analgesic, and hepatoprotective properties. Steroidal compounds such as β-sitosterol and stigmasterol may also contribute to the plant's anti-arthritic and antiulcer activities (Adewumi *et al.,* 2021). Terpenoids, on the other hand, are often associated with antimicrobial and anticancer potentials due to their ability to disrupt microbial cell membranes and induce apoptosis in cancer cells.

7. Phenolic Compounds

Phenolic compounds, including phenolic acids and simple polyphenols, are widely distributed in *A. africana* and account for its antioxidant and free radical scavenging activities (Lamien-Meda *et al.,* 2010). These compounds can chelate metal ions, inhibit lipid peroxidation, and protect against oxidative stress-related diseases. The high total phenolic content (TPC) of *A. africana* extracts correlates strongly with their observed antioxidant and cytoprotective activities (Adedapo *et al.,* 2014).

8. Anthraquinones

Anthraquinones are less abundant but have been detected in the bark and roots of *Afzelia africana*. These compounds exhibit laxative, antimicrobial, and anti-inflammatory effects

(Burkill, 1995). Their presence supports the traditional use of bark infusions in managing constipation and gastrointestinal disorders.

Summary of Major Phytochemicals in *Afzelia africana*

Phytochemical Class	Principal Location in Plant	Pharmacological Role
Flavonoids	Leaves, bark	Antioxidant, anti-inflammatory, hepatoprotective
Alkaloids	Bark, roots	Analgesic, antimalarial, antimicrobial
Tannins	Bark, leaves	Astringent, wound-healing, antimicrobial
Saponins	Leaves, seeds	Hypocholesterolemic, antifungal, immunomodulatory
Glycosides	Leaves, bark	Anti-inflammatory, diuretic
Terpenoids/Steroids	Bark, leaves	Analgesic, anti-arthritic, hepatoprotective
Phenolic compounds	All parts	Antioxidant, cytoprotective
Anthraquinones	Roots, bark	Laxative, antimicrobial
Coumarins	Leaves	Anticoagulant, antifungal

Figure 1. 3: Characteristic seeds of Afzelia africana (Sm.) Pers

1.5.3 Pharmacological and Biological Activities of *Afzelia africana*

Afzelia africana Sm. (family: Fabaceae) is an important African medicinal plant widely utilized in ethnomedicine for the treatment of numerous diseases, including fever, inflammation,

diarrhea, and infections. Scientific investigations have revealed that its pharmacological activities are closely linked to its rich phytochemical composition, particularly flavonoids, alkaloids, tannins, saponins, glycosides, phenolic acids, and terpenoids (Akinmoladun *et al.,* 2016; Adedapo *et al.,* 2024). Extracts from its bark, leaves, roots, and seeds have been evaluated through both in vitro and in vivo studies, revealing broad-spectrum pharmacological and biological properties.

1. Antioxidant Activity

The antioxidant potential of *Afzelia africana* is among its most extensively documented biological effects. Both ethanolic and aqueous extracts of the bark and leaves have shown significant free radical scavenging activity, which is attributed to their high levels of flavonoids and phenolic compounds (Lamien-Meda *et al.,* 2020). These compounds neutralize reactive oxygen species (ROS) such as superoxide radicals and hydrogen peroxide, thus preventing oxidative damage to lipids, proteins, and DNA (Adedapo *et al.,* 2014). The antioxidant capacity contributes to the plant's hepatoprotective**,** anti-inflammatory, and anti-aging properties, supporting its traditional use in treating chronic diseases linked to oxidative stress.

2. Anti-inflammatory and Analgesic Activity

Extracts of *Afzelia africana* exhibit potent anti-inflammatory and analgesic effects, validated through several experimental studies. Akinmoladun et al. (2016) demonstrated that methanolic stem bark extract significantly reduced carrageenan-induced paw edema and acetic acid-induced writhing in rodents, indicating both anti-inflammatory and peripheral analgesic actions. The bioactivity is largely due to flavonoids, tannins, and triterpenoids that inhibit the synthesis of pro-inflammatory mediators such as prostaglandins, histamine, and cytokines (Adewumi *et al.,* 2021). These findings correlate with the plant's traditional use for relieving rheumatism, muscle pain, and fever in African medicine.

3. Antimicrobial Activity

Numerous studies have demonstrated the broad-spectrum antimicrobial potential of *Afzelia africana* extracts against both bacterial and fungal pathogens. The bark and leaf extracts show inhibitory effects against Escherichia coli, Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae, Candida albicans, and Aspergillus niger (Oluwole *et al.,* 2020). These antimicrobial activities are linked to the presence of alkaloids, tannins, and saponins that disrupt microbial cell walls and inhibit nucleic acid synthesis (Adewumi *et al.,* 2021). The strong antibacterial properties justify its traditional use in treating gastrointestinal infections, diarrhea, wounds, and skin diseases**.**

4. Antidiarrheal and Gastroprotective Activity

The antidiarrheal activity of *A. africana* has been scientifically validated. Adedapo et al. (2024) reported that methanolic leaf extract significantly reduced castor oil–induced diarrhea and intestinal motility in rats. The mechanism is believed to involve reduction in intestinal secretions and inhibition of smooth muscle contraction, mediated by tannins and flavonoids. Additionally, the gastroprotective effect of the bark extract is associated with increased

mucosal defense, decreased gastric acid secretion, and antioxidant protection of the gastric lining (Akinmoladun *et al.,* 2016). This supports its ethnomedicinal application in treating stomach upset and dysentery**.**

Other include: Antipyretic and Antimalarial Activity, Hepatoprotective Activity, Antidiabetic and Hypoglycemic Activity, Antifertility and Aphrodisiac Effects, Cytotoxic and Anticancer Activity, Hematological and Immunomodulatory Effects.

1.6 Significance of the Study

Parkinson's disease (PD) is one of the most prevalent and devastating neurodegenerative illnesses, second only to Alzheimer's disease in frequency. It is characterized by the gradual death of dopaminergic neurons in the substantia nigra pars compacta, resulting to lower dopamine levels in the striatum and concomitant deterioration of motor coordination, muscular stiffness, and tremor. Despite improvements in neuroscience, the current pharmaceutical care of Parkinson's disease remains essentially symptomatic, focused on dopamine replacement using medications such as Levodopa, dopamine agonists, or monoamine oxidase-B inhibitors. However, these medications do not prevent or cure neuronal loss, and long-term usage is generally accompanied with significant consequences, including dyskinesia, mental disorders, and motor irregularities. This underlines the essential need for innovative neuroprotective drugs capable of reducing or preventing neuronal damage rather than only easing symptoms.

1) Neuroprotective and histopathological advantages of the ethanolic extract of the stem bark of *Afzelia africana*-a medicinal plant extensively utilized in African traditional medicine. The plant is regarded for its antioxidant, anti-inflammatory, antibacterial, and wound-healing qualities, although its neuroprotective potential remains largely unknown. Evaluating its effects in a rotenone-induced model of Parkinsonism offers a good scientific basis to evaluate and expand the ethnomedicinal claims around the herb. Since oxidative stress and mitochondrial

dysfunction are central to the pathogenesis of PD, the phytochemicals present in *A. africana*-such as flavonoids, phenolic compounds, tannins, and terpenoids-may offer significant neuroprotection by scavenging reactive oxygen species (ROS), stabilizing mitochondrial activity, and modulating neuroinflammatory responses.

2) Scientific and pharmacological standpoint, this work adds to the developing body of information on plant-based neurotherapeutics. Identifying plants with significant antioxidant and neuroprotective characteristics may serve as a basis for medication discovery and development in the treatment of neurodegenerative illnesses. The investigation may identify bioactive molecules that can be isolated, purified, and developed into lead compounds for pharmaceutical formulation. Moreover, it highlights the necessity of bioprospecting indigenous African flora, harmonizing with worldwide tendencies toward integrating traditional medicine into contemporary health care systems. By providing empirical proof of the neuroprotective properties of *A. africana*, the study might drive additional investigations into its mechanism of action, safety profile, and therapeutic uses.

3) Toxicological and histopathological importance, as it will help evaluate if the ethanolic extract of *A. africana* affords structural protection to dopaminergic neurons and protects brain integrity in the substantia nigra and striatum after rotenone exposure. Histopathological investigation gives actual visual proof of neuronal survival and recovery, providing scientific weight to biochemical results. Demonstrating a beneficial histopathological impact would bolster the assumption that *A. africana* contains actual restorative or neuroprotective ability, rather from just modifying biochemical markers.

4) Societal and public health level: The study is relevant because neurodegenerative disorders such as Parkinson's are on the increase internationally, even in low- and middle-income nations where access to traditional treatments is restricted and frequently pricey. Natural plant-based alternatives might give cost-effective, culturally acceptable, and locally accessible medicinal

choices. Validating the neuroprotective properties of *A. africana* consequently accords with the World Health Organization's support for the scientific study of traditional medicinal plants as possible sources of safe and efficacious pharmaceuticals.

1.7 Research Questions or Hypotheses

- Does *Afzelia africana* extract have neuroprotective effects in rotenone-induced PD rats?
- Is there a significant improvement in motor and biochemical parameters compared to the control?

1.8 Aim and objectives

The study aimed to evaluate the histopathologic effects that can be observed in acute administration of ethanolic extract of *Afzelia africana* to rats

Specific objectives include:

1. To evaluate the neuroprotective effects of the ethanol extract of the stem bark of *Afzelia africana* in a rotenone-induced model of Parkinson's disease in rats.
2. To determine the effect of the extract on motor coordination and behavioural changes in rotenone-induced Parkinsonian rats.
3. To examine the histopathological changes in the substantia nigra and striatum of the brain of the rats following treatment with the extract.

1.9 Justification of study

Ultimately, the findings from this study will contribute to the scientific validation and conservation of *Afzelia africana* as a valuable medicinal plant. Should the extract demonstrate significant neuroprotective and histopathological benefits, it would justify further

phytochemical characterization, isolation of active compounds, and mechanistic studies aimed at elucidating its therapeutic pathways. Such outcomes could pave the way for the development of new neuroprotective agents from indigenous African plants, promoting innovation in neuropharmacology and enhancing global scientific recognition of African medicinal resources.

This work provides an educational platform for students and researchers in pharmacology, biochemistry, and toxicology to deepen their understanding of experimental models of Parkinsonism**,** behavioural and biochemical assessments, and histopathological evaluation techniques**.** It also offers an opportunity to bridge the gap between traditional medicine and modern biomedical research, fostering interdisciplinary collaboration among pharmacognosists, neuroscientists, and pathologists.

Therefore, evaluating the neuroprotective and histopathological effects of the ethanolic extract of the stem bark of *Afzelia africana* in rotenone-induced model of Parkinsonism in Wistar rats. The findings from this study will help contribute to the discovery of new plant-based agents capable of mitigating neurodegeneration and improving therapeutic options for Parkinson's disease.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Drugs, Chemicals and Materials

Afzelia africana stem bark, 1g of rotenone powder, 99.9% ethanol, methylated spirit, 10% Dimethyl sulfoxide (DMSO), 10% tween 80, olive oil, distilled water, measuring cylinders, beakers, extraction jars, orogastric tube, laboratory scale, funnel, filter paper, labelling markers, syringes, stop watch, hand gloves.

2.2 Plant Collection, Identification and Authentication

The fresh stem bark of *Afzelia africana* was obtained from Mr. Okeigbo in Ondo State, they were identified and authenticated by Mr. Kehinde Adeniji of The Forestry Research Institute of Nigeria (FRIN), where a voucher specimen (FHI 112824) is deposited.

2.3 Preparation of *Afzelia africana* Extract

The stem bark of *Afzelia africana* were air dried for several days and milled into coarse powder. 600g of the stem bark powder was coldly macerated in 1500ml of ethanol, with intermittent agitation over a 72hour period. The mixture was filtered and concentrated to dryness in an oven at 40°C.

The percentage yield was calculated using the formular below;

$$\frac{Weight\ of\ extract}{Weight\ of\ plant\ material\ used} \times \frac{100}{1}$$

The percentage yield from the extract was 1.83%

2.3.1 Preparation of Rotenone

10 mg of Rotenone was weighed using a standard analytical weighing balance and dissolved in 1 mL 100% dimethyl sulfoxide (DMSO). About 1 mL of DMSO mixture was added to 9 mL

olive oil to get a solution of 10 mL, with the final concentration of rotenone 1 mg/mL. Fresh stock solutions of rotenone were prepared before the commencement of a new experiment.

2.4 Animals

Male Wistar rats (250-300 g) were obtained through from the animal house in the department of Pharmacology and Toxicology in University of Ibadan, Ibadan, Nigeria. The rodents were housed in the animal house of the department of Pharmacology and Toxicology in University of Benin, Benin city. They were kept in clean plastic cages and allowed to acclimatize for two weeks before the commencement of the experiments. There was consistent access to clean water daily, the beddings were replaced and the cages washed on alternate days. Ethical approval was obtained from the Ethics Committee of the Faculty of Pharmacy, University of Benin, Nigeria before the commencement of the study.

2.5 Phytochemical Screening

The ethanolic extract of the plant *Afzelia africana* was screened for the presence of various chemical compositions using standard screening procedures. Conventional protocol for detecting the presence of alkaloids, glycosides, saponins, and carbohydrates was used.

2.6 Acute toxicity

The acute oral toxicity of the *Afzelia africana* extract was evaluated using a modified version of Lorke's method (Lorke, 1983). The study was carried out in two phases. In the first phase, nine mice were randomly divided into three groups of three and administered 10, 100, and 1000 mg/kg of the extract orally to establish a suitable dose range capable of inducing toxic effects. The animals were closely monitored for 24 hours for behavioural and physiological signs of

toxicity, including tremors, writhing, diarrhoea, and mortality. Based on the observations from this phase, the second phase was conducted using fresh groups of mice (n = 3 per group), which received higher oral doses of 1600, 2900, and 5000 mg/kg of the extract. The animals were again observed for 24 hours for signs of toxicity and death. The median lethal dose (LD_{50}) was then calculated as the geometric mean of the lowest dose producing mortality and the highest dose that did not cause death.

2.7 Induction of Parkinsonism and Treatment

The study was conducted using Male Wistar rats, which were randomly placed into five groups, with each group comprising of six animals;

Group 1- No treatment

Group 2- Vehicle only

Group 3-Rotenone at a dosage of 1 mg/kg combined with vehicle

Group 4-Rotenone at 1 mg/kg combined with a low dose of *A. africana* extract (250 mg/kg) and Group 5- Rotenone at 1 mg/kg with a high dose of *A. africana* extract (500 mg/kg).

The administered volumes of extract and rotenone were determined based on individual animal weights. Parkinsonism was induced with rotenone (1 mg/kg, intraperitoneally) on days 1, 4, 7, and 10 while the extract and vehicle were given daily via oral route. Neurobehavioural assessments were carried out on days 0 (before the start of the study), 5, and 10. The weights of the animals were measured on days 1, 4, 7, 10, and 11. The neurobehavioural assessments used in this study were wire hanging to assess motor coordination and muscle strength, and elevated plus maze to assess anxiety. On day 11, the animals were sacrificed, with ketamine used as the anaesthetizing agent, and the organs (brain, kidney, liver) were weighed to calculate the organ-to-body weight ratio, which was recorded accordingly.

2.8 Neurobehavioural Tests

2.8.1 Wire hanging

Wire hanging is a test used to measure muscle coordination and endurance in rodents.

Method:

Each animal was gently placed so that it could grasp a horizontal stainless-steel wire positioned approximately 30–35 cm above a padded surface to prevent injury in the event of a fall. The forepaws were allowed to grip the wire naturally and immediately upon release, a timer was started. The latency (in seconds) until the animal released its hold and fell was recorded, with a maximum cut-off time of 120 seconds. If the animal remained suspended for the full 120 s, the value was recorded as 120 s. Each animal underwent up to three trials, with at least five minutes of rest between trials. Hanging durations closer to 0 s were interpreted as indicative of diminished neuromuscular strength or endurance, reflecting motor impairment (Klein et al., 2012; Hoffman and Winder, 2016).

2.8.2 Elevated plus maze

The elevated plus maze apparatus consisted of two open arms and two closed arms elevated 50 cm above the floor. Each animal was gently placed at the central platform facing an open arm, and the timer was started immediately. The duration of time spent in the open versus closed arms over a maximum observation period of 180 seconds was recorded. Reduced exploration of open arms was interpreted as increased anxiety-like behaviour, while increased open-arm activity indicated reduced anxiety (Shi *et al.,* 2021).

2.9 Histopathological studies

After 10 days of chronic administration, the animals were humanely anaesthetized by using ketamine injections at doses calculated according to their weights. Once the death of the rat

had been confirmed, tissue collection began immediately to minimize post-mortem changes and preserve the integrity of the tissue. Then, following proper dissection protocol and using sterile equipment and techniques, the brain, kidney, liver, and spleen were correctly excised. The tissues were handled carefully to avoid contamination. Then it was weighed and duly recorded. Then, for proper preservation, they were placed in a formalin preparation and labeled appropriately.

The histopathological analysis was performed at the University of Benin, Department of Anatomy, at the Histochemistry laboratory. The tissues were analyzed for tissue morphological changes, necrosis, inflammation, and cellular morphology.

2.10 Statistical analysis

For statistical significance, the results were analyzed using one-way ANOVA followed by the Tukey test. The results are expressed as mean ± standard error of the mean (SEM).

At $p < 0.05$, a difference was considered statistically significant and all statistical analysis were done using Sigma Stat version 14.0

CHAPTER THREE

RESULTS

3.1 Phytochemical Constituents of *Afzelia africana*

Phytochemical screening of the ethanol extract of *Afzelia africana* stem bark showed the presence of saponins, glycosides, reducing sugars, terpenoids, alkaloids, tannins and cardiac glycosides, while steroids were absent. The results are presented in Table 3.1

3.2 Acute Toxicity Screening

This aspect of studies describes the median lethal dose (LD_{50}) to be 5000 mg/kg and this is presented in Table 3.2

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

Phytoconstituents	
Saponin	+
Glycoside	+
Reducing sugar	+
Terpenoid	+
Alkaloid	+
Tannin	+
Steroid	-
Cardiac glycoside	+

+ = present, - = absent

Table 3.2: Oral lethal dose (LD_{50}) of Ethanol Extract of Afzelia 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 Evaluation of Neuro-behavioural tests

Wire hanging test

The result of the effect of treatment with the ethanolic extract of the stem bark of *Afzelia africana* on wire hanging test is depicted in Figure 3.14. The results show that the rotenone group significantly ($p < 0.05$) reduced time spent in the wire hanging test in comparison to the control.

Elevated plus maze analysis

The result of the effect of treatment with the ethanolic extract of the stem bark of *Afzelia africana* on time spent in each arm of the apparatus and number of entries is shown in Tables 3.3 and 3.4 respectively. The number of entries into the open arm increased in the extract group as compared to the control. Also, in the rotenone group, the time spent in the closed arm is significantly different from the control ($p <0.05$).

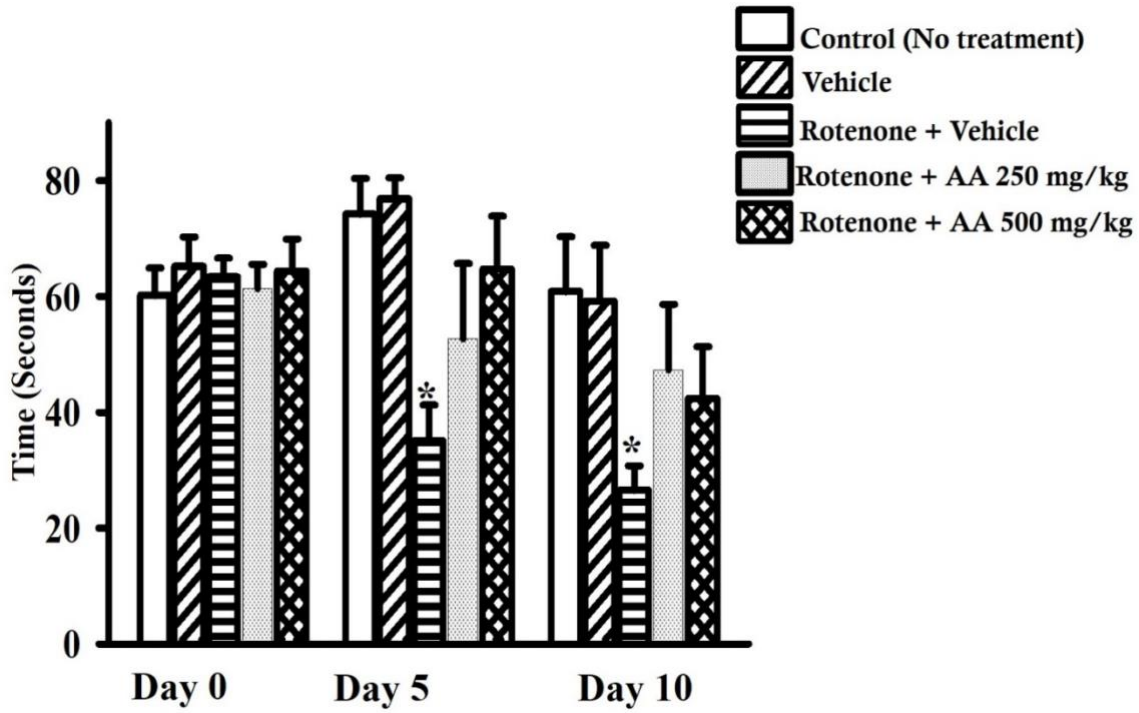

Figure 3. 1: Effect of Afzelia africana in the Wire Hanging Test of Rotenone Treated Animals. *p<0.05compared to control. n=6 per group. AA- Afzelia africana

Table 3.3: Effect of ethanol extract of *A. africana* stem bark on Time Spent in Both Arms of the Elevated Plus Maze

Time spent in each arm	Day 0 Open Arms	Day 0 Closed Arms	Day 5 Open Arms	Day 5 Closed Arms	Day 10 Open Arms	Day 10 Closed Arms
No Treatment (Control)	283.50 ± 1.86	5.33 ± 1.76	259.00 ± 21.86	32.67 ± 21.54	268.83 ± 15.39	25.50 ± 15.01
Vehicle	290.33 ± 2.64	2.00 ± 0.86	267.67 ± 29.54	32.50 ± 27.32	267.83 ± 17.97	27.83 ± 17.20
Rotenone + Vehicle	295.00 ± 1.59	3.17 ± 1.19	135.33 ± 15.49*	148.00 ± 15.93*	104.67 ± 19.14*	191.17 ± 19.27*
Rotenone + AA 250 mg/kg	282.33 ± 4.69	5.00 ± 1.48	210.50 ± 19.64	82.33 ± 17.74	240.17 ± 18.86	53.00 ± 18.27
Rotenone + AA 500 mg/kg	287.33 ± 2.68	3.00 ± 1.51	205.50 ± 21.82	86.83 ± 21.29	225.33 ± 27.73	69.67 ± 27.18

* Significantly different from the control. $p < 0.05$. Results are expressed as mean ± standard error of mean. AA: *Afzelia africana*

Table 3.4: Effect of ethanol extract of A. africana Stem Bark on Total Number of Entries into Both Arms of the Elevated Plus Maze

	Day 0		Day 5		Day 10	
	Open Arms	**Closed Arms**	**Open Arms**	**Closed Arms**	**Open Arms**	**Closed Arms**
No Treatment (Control)	1.14 ± 0.15	1.14 ± 0.15	1.17 ± 0.17	1.50 ±0.76	1.17 ± 0.17	0.17 ± 0.17
Vehicle	1.14 ± 0.15	0.00 ± 0.00	1.33 ± 0.33	1.33 ±0.80	1.33 ± 0.33	0.83 ± 0.40
Rotenone + Vehicle	1.14 ± 0.15	0.00 ± 0.00	1.67 ± 0.33	3.50 ±0.85	1.50 ± 0.34	3.67 ± 0.88
Rotenone + AA 250 mg/kg	1.14 ± 0.15	0.00 ± 0.00	2.17 ± 0.60	1.33 0±.42	1.33 ± 0.21	1.67 ± 0.33
Rotenone + AA 500 mg/kg	1.14 ±0 .15	0.14 ± 0.15	2.33 ± 0.99	2.17 ±0.48	1.33 ± 0.33	2.17 ± 0.54

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

3.5 HISTOPATHOLOGICAL ANALYSIS

The brain, liver and kidney of the rats treated with control, vehicle, rotenone and vehicle, rotenone and 250 mg/kg, and, rotenone and 500mg/kg doses of the *A. africana* extract daily for the duration of the study showed various mild changes in different organs and slight neurological damage in the rotenone group was observed upon examination of the striata of the brain. Images of these organs are found in Figures 3.2 - 3.4

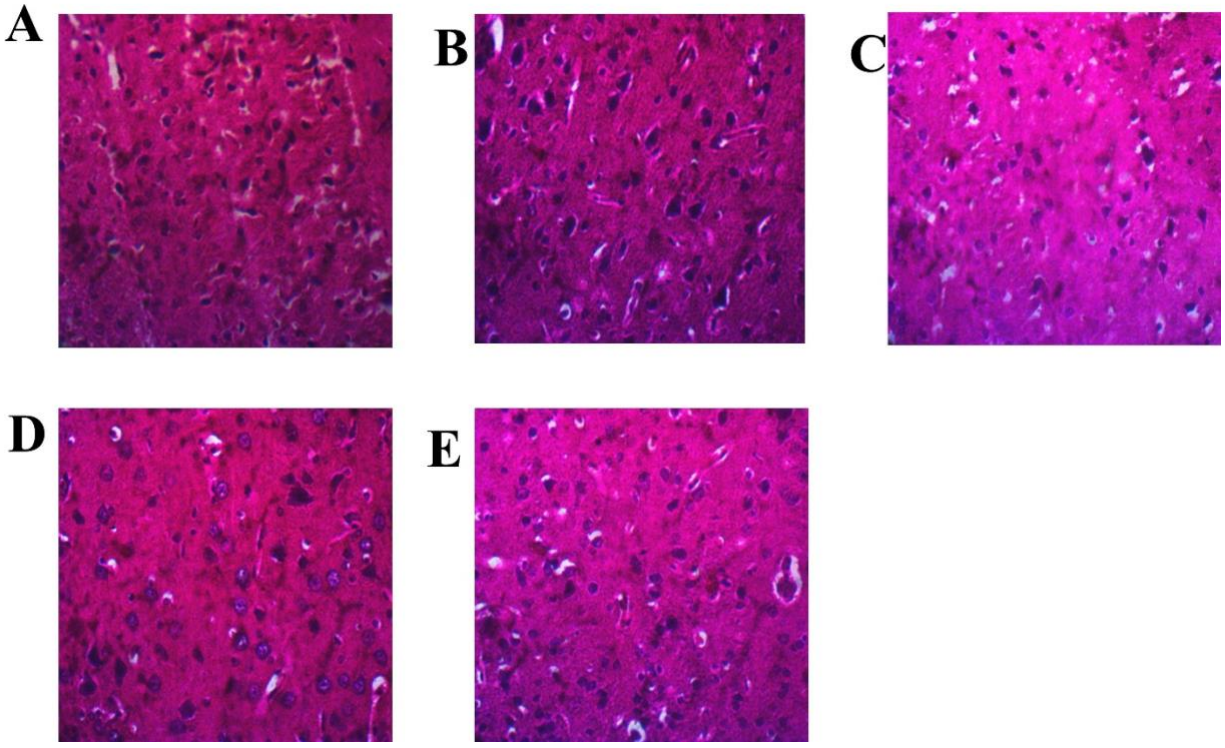

Figure 3. 2: Representative Photomicrographs showing the effect of Afzelia africana in the straita of rats.

No lesions were observed in the control and vehicle-treated animals (A and B). Atrophy and pyknosis of neurons occurred in animals treated with rotenone and vehicle (C). Slight atrophy of neurons, histopathological changes were noticed in animals treated with 250 and 500 mg/kg of *A. africana* (D and E)

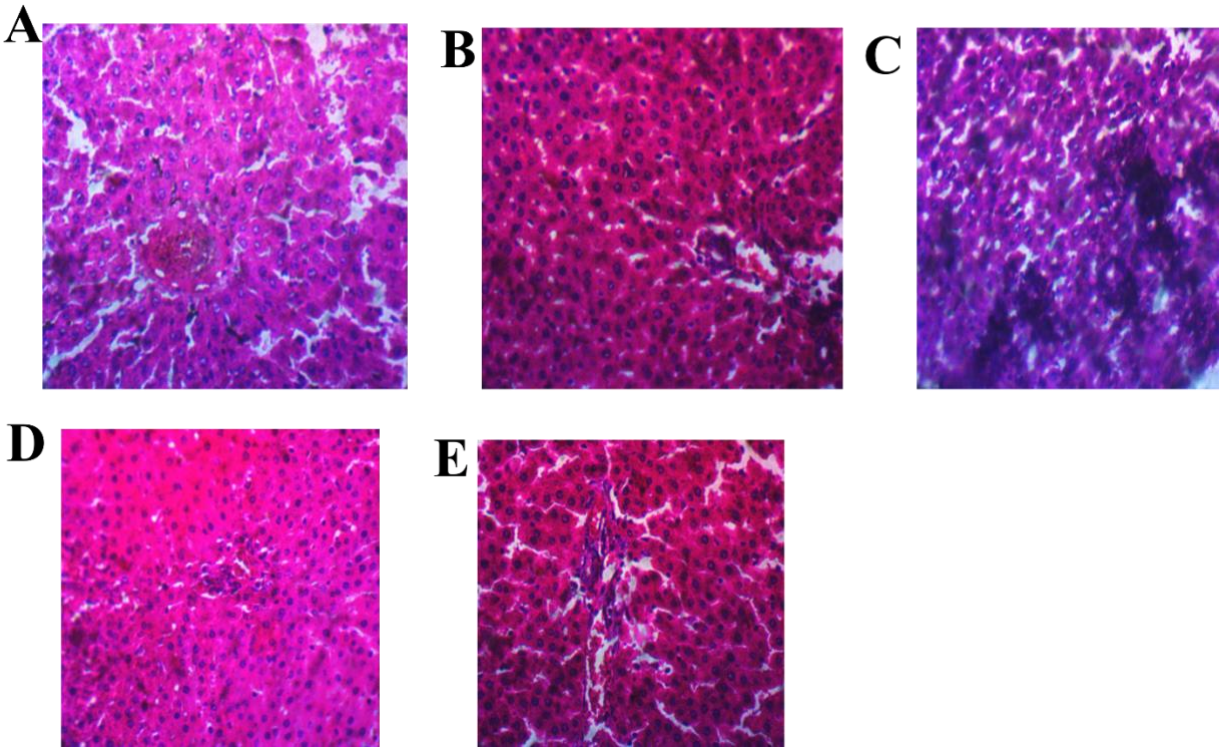

Figure 3. 3: Representative Photomicrographs showing the effect of Afzelia africana in the liver cells of rats**.**

In control animals, no observable lesions (control and vehicle-treated groups (A and B)). Diffuse hepatocellular atrophy was observed in the rotenone-treated group (C), but no observable lesion was noticed in *A. africana* treated animals (D and E).

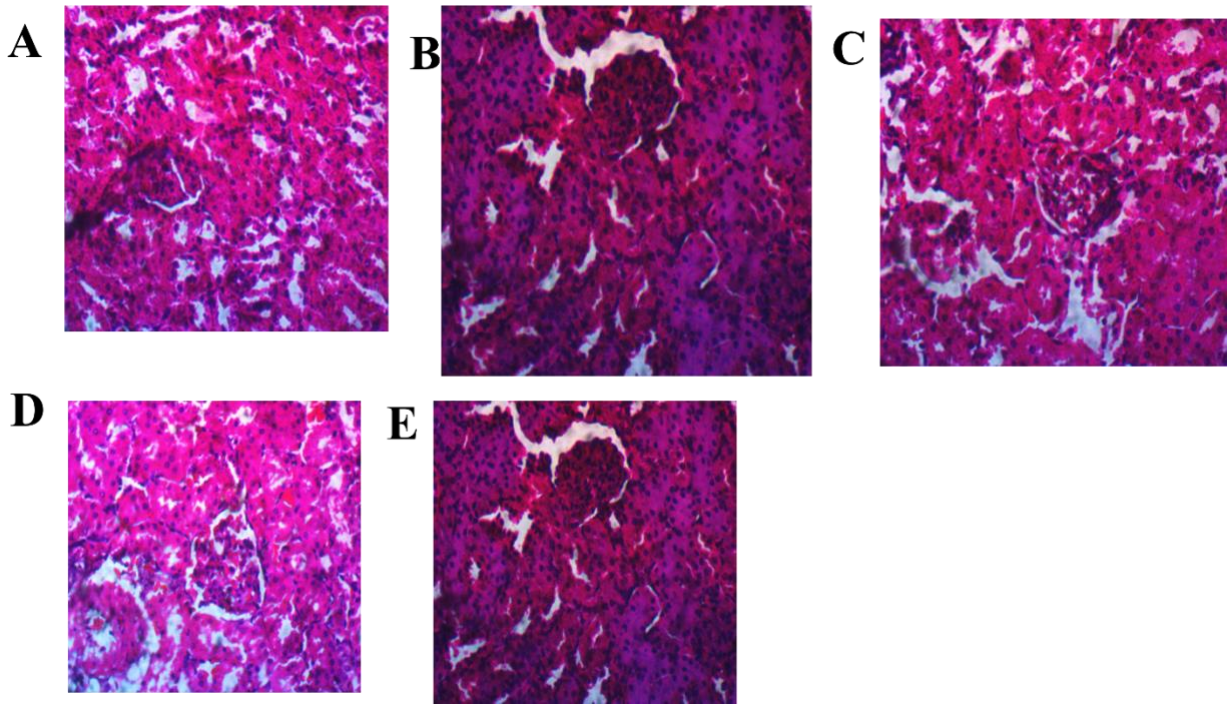

Figure 3. 4: Representative Photomicrographs showing the effect of Afzelia africana in the renal cells of rats.

Photomicrographs of A-E representing the effects that the control, vehicle, rotenone and vehicle, rotenone and 250 mg/kg, and rotenone and 500mg/kg doses of the *A. africana* extract respectively, had on the renal cells. Patchy tubular epithelial necrosis and inflammation, as well as atrophy of glomeruli and tubules, were observed.

CHAPTER FOUR

DISCUSSION

4.1 Phytochemical Constituents of *Afzelia africana*

The preliminary phytochemical screening of the ethanol extract of *Afzelia africana* stem bark revealed the presence of several key secondary metabolites: saponins, glycosides, reducing sugars, terpenoids, alkaloids, tannins, and cardiac glycosides. The presence of these diverse compounds, particularly alkaloids and terpenoids, provides a strong chemical basis for investigating the plant's neuropharmacological activities. Alkaloids are widely known for their ability to cross the blood-brain barrier and modulate neurotransmitter systems, while terpenoids often exhibit significant anti-inflammatory and antioxidant properties, both crucial mechanisms for neuroprotection (Ahlemeyer and Krieglstein, 2003)

The confirmation of tannins and saponins further supports the plant's traditional use, often linked to antimicrobial and general health-boosting effects. Notably, the extract tested negative for steroids

4.2 Acute Toxicity

Acute toxicity studies are designed to determine the short-term adverse effects of a substance when administered either as a single dose or multiple doses within 24 hours in laboratory animals. These studies help to establish the median lethal dose (LD_{50}), which is defined as that amount of chemical required to kill 50% of the test animals in a group over 24 hours, while also monitoring for clinical signs of toxicity such as excessive salivation, tearing, diarrhoea, lethargy, diminished exploratory activity, stereotypic behaviours, tremors, piloerection, and redness.

Using Lorke's (1983) protocol for evaluating oral acute toxicity, administration of the ethanolic stem bark extract of *Afzelia africana* up to 5000 mg/kg resulted in no deaths or observable toxic

symptoms in mice, indicating high tolerability and safety of the extract at the tested doses. These findings are consistent with the traditional use of the plant and support its potential safety for future human studies (Lorke, 1983).

4.3 Neurobehavioural analysis

4.3.1 Wire Hanging Test (Motor Coordination)

The wire hanging test is a fundamental assay used to assess motor coordination and muscle strength, which are typically impaired in Parkinson's Disease (PD) models due to dopaminergic neurodegeneration in the striatum. The results demonstrated a significant reduction ($p < 0.05$) in the time spent hanging in the rotenone and vehicle group compared to the control group on both Day 5 and Day 10. This confirms that rotenone, a potent mitochondrial complex I inhibitor, successfully induced a motor deficit characteristic of PD (Sherer, Betarbet and Greenamyre, 2003; Exner *et al.,* 2012).

In contrast, rats treated with the *Afzelia africana* extract, particularly at doses of 250 mg/kg and 500 mg/kg, showed a restoration of time spent to control levels on Days 5 and 10. While the result does not specify if the treatment groups reached statistical significance compared to the rotenone group, the observable trend suggests the extract provided a protective or ameliorative effect against the rotenone-induced motor impairment.

4.3.2 Elevated Plus Maze (Anxiety and Locomotor Activity)

The elevated plus maze (EPM) is used to assess anxiety-like behaviour and general locomotor activity (Schapira, Chaudhuri and Jenner, 2017; Puspita, Chung and Shim, 2017). Anxiety and cognitive deficits are common non-motor symptoms associated with PD progression. On Days 5 and 10, the rotenone and vehicle treated groups spent significantly less time in the open arms and significantly more time in the closed arms compared to the control group ($p < 0.05$). This

shift, moving away from the anxiogenic open arms toward the sheltered closed arms, indicates that rotenone induced a significant increase in anxiety-like behaviour.

Rats treated with Rotenone and *A. africana*, particularly at 250 mg/kg showed a clear amelioration of this anxious behaviour, spending a longer time in the open arms and a shorter time in the closed arms compared to the rotenone and vehicle group. The ability of the extract to counteract the rotenone-induced anxiety suggests the presence of bioactive compounds, likely alkaloids or terpenoids, capable of modulating the central nervous system (CNS) to exert an anxiolytic or neurostabilizing effect (Zeng *et al.,* 2021). Furthermore, the general increase in the number of entries into the open arm in the extract groups also suggests a potentially beneficial effect on locomotor function (Shi *et al.,* 2021).

4.4 Histopathological Findings

Histopathological screening entails the microscopic assessment of animal tissues and organs to detect disease by evaluating morphological alterations that may be linked to the effects of drug extracts (Gurcan, 2009).

For this study, three important organs were selected for histological examinations, namely; brain, liver and kidney. The rationale behind their selection is due to the known mechanisms of Rotenone, which is a chemical known to exert hepatotoxic, renotoxic, and neurotoxic effects. These organs are the most susceptible during chronic toxicity studies, and structural and functional impairments within them can have severe consequences on the entire organism.

Results of histopathology analysis revealed changes in the specified organs, suggesting systemic toxicity induced by rotenone and the extract's protective impact on systemic function.

Striata: The control and vehicle groups showed no lesions, indicating a healthy baseline for the rat models.

However, the administration of rotenone (Group C) caused significant atrophy and pyknosis of neurons. This result is consistent with the known mechanism of rotenone, which acts as a lipophilic mitochondrial complex I inhibitor. By blocking the electron transport chain, rotenone induces the accumulation of electrons and the subsequent production of reactive oxygen species (ROS), leading to oxidative stress and neuronal death. The observed pyknosis (irreversible condensation of chromatin) is a classic indicator of apoptosis or necrosis in these neurons.

Notably, groups treated with 250 and 500 mg/kg of *A. africana* (Groups D and E) exhibited only slight atrophy and fewer histopathological changes. This suggests that the extract successfully mitigated the severe neurotoxic effects of rotenone.

Liver: Interestingly, no observable lesions were noticed in the AA-treated animals (250 and 500 mg/kg). This finding indicates that *Afzelia africana* not only protects the brain but also provides a systemic safety profile, shielding the liver from oxidative assault and metabolic stress induced by the toxin. This aligns with the traditional use of the plant in treating inflammatory and gastrointestinal disorders.

Kidney: From the renal histopathology results, the changes observed highlight the vulnerability of the kidneys to oxidative stress and inflammation induced by rotenone. The protective effect seen in the extract-treated groups further corroborates the role of *A. africana* in modulating neuroinflammatory and oxidative pathways. By reducing tubular necrosis and glomerular atrophy, the extract demonstrates its potential as a broad-spectrum cytoprotective agent.

CHAPTER FIVE

CONCLUSION

The ethanolic stem bark extract of *Afzelia africana* (AA) demonstrated significant neuroprotective and anxiolytic efficacy in rats. This was evidenced by the extract's ability to attenuate the rotenone-induced motor deficits (increased latency to fall in the Wire Hanging Test) and, most notably, cause the significant reversal of anxiety-like behaviour (decreased time spent in the closed arm of the Elevated Plus Maze). These findings validate the potential therapeutic role of *Afzelia africana* in managing both the motor and non-motor symptoms associated with neurodegenerative disorders.

From the histopathological analysis, it was revealed that the extract provided neuroprotective effects against cellular and systemic damage, mitigating atrophy and pyknosis of neurons while offering relief from the systemic toxicity observed in the liver and kidney.

Given the established oral LD_{50} of 5000 mg/kg and the rich profile of alkaloids and tannins, the extract appears to possess a favourable safety margin and potential antioxidant capacity that warrants further translational research.

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