ACUTE TOXICITY STUDIES AND ANALGESIC EFFECTS OF ETHANOL EXTRACT OF THE STEM BARK OF *FADOGIA CIENKOWSKII SCHWEINF.* IN MICE

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

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CERTIFICATION

This is to certify that this work was successfully carried out by **NKECHI JOYCE OSIEGBU** with matriculation number **PHA1808455,** Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, in partial fulfillment of the requirement for the award of the degree of Doctor of Pharmacy (Pharm.D) of the University of Benin, Benin City, Edo State, Nigeria.

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ANTI-PLAGARISM CERTIFICATION

We the undersigned certify attest and declare that the thesis of Nkechi Joyce Osiegbu titled ACUTE TOXICITY STUDIES AND ANALGESIC EFFECTS OF ETHANOL EXTRACT OF THE STEM BARK OF *FADOGIA CIENKOWSKII SCHWEINF*. IN MICE has successfully passe the anti-plagarism test and does not violate any copyright regulations.

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DEDICATION

This work is wholly dedicated to God Almighty and to my lovely parents Mr and Mrs J.K Osiegbu and my amazing siblings for their love, grace, guidance, and support through the period of my university education.

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ABSTRACT

Fadogia cienkowskii is a medicinal plant traditionally used for pain relief and other therapeutic purposes. Despite its widespread ethnobotanical use, scientific validation of its safety and analgesic properties remains limited. This research investigated the acute toxicity and analgesic properties of the ethanol extract from *F. cienkowskii* stem bark in mice.

The acute toxicity study was conducted using Lorke's method, with oral administration of the extract at doses up to 5000 mg/kg to assess toxicity signs and determine the median lethal dose (LD_{50}). The analgesic activity was evaluated using two pain models: the acetic acid-induced writhing test and the tail immersion test. Mice were treated with *F. cienkowskii* extract at doses of 100, 200, and 400 mg/kg, and their responses were compared to those of control and standard drug groups.

The result of the acute toxicity study revealed no mortality or significant behavioral changes, indicating that the extract is well-tolerated at 5000 mg/kg. In the mice writhing test, the extract significantly reduced the number of writhes in a dose-dependent manner, suggesting a potent peripheral analgesic activity. However, in the tail immersion test, the extract did not significantly prolong pain response latency, unlike morphine, indicating a lack of central analgesic action.

In summary, the results indicate that *F. cienkowskii* stem bark extract has strong peripheral pain-relieving effects, possibly by inhibiting prostaglandin synthesis, but lacks central analgesic activity. Furthermore, the extract demonstrated a favorable safety profile. These results support the plant's traditional use for pain relief and warrant further studies to isolate and characterize its active compounds.

CHAPTER ONE

INTRODUCTION

1.1 PAIN AND ANALGESIA

The International Association for the Study of Pain defines pain as: "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Several literatures define pain as: "An unpleasant sensation, occurring in varying degrees of severity as a consequence of injury, disease, or emotional disorder." Pain is an unpleasant sensation that signals a potential issue within the body. It can be described in various ways, such as throbbing, stabbing, aching, or pinching. While some pain, like a mild headache, is tolerable, severe pain can be debilitating *(Moini et al., 2023).* As a subjective experience, pain cannot be objectively measured, and its intensity does not always align with the nociceptive signals triggering it. The same nociceptive stimulus may be easily ignored in one context but unbearable in another *(Buschmann, 2002).* Pain is generally categorized into acute pain, chronic pain, and pain associated with malignancy. It plays a crucial role in the nervous system by signaling potential or actual bodily harm. Additionally, pain is not just a physical sensation—it is also influenced by psychological factors such as past experiences, beliefs, fear, and anxiety.

1.1.1 The physiology of pain

The physiology of pain is a complex process that involves the transmission of nociceptive signals, modulation at different levels, and the integration of physiological and psychological factors. When the body encounters a harmful stimulus, nociceptors in the peripheral nervous system are activated. These receptors transmit signals via Aδ and C nerve fibers to the spinal cord, which

then relays them to the brain *(Institute of Medicine (US) Committee on Pain., 1987).* In the brain, pain perception involves multiple regions. The somatosensory cortex processes the sensory aspects, while the anterior cingulate cortex and insula contribute to the emotional experience of pain. The brain interprets these signals, forming a perception of pain influenced by both physical sensations and psychological factors.

Several theories explain pain perception and modulation. The Specificity Theory suggests distinct receptors and pathways for pain, while the Pattern Theory argues that pain perception depends on nerve activity patterns rather than specific receptors. The Gate Control Theory proposes that non-painful stimuli can "close gates" in the spinal cord, reducing pain perception. Moreover, the brain has built-in pain control mechanisms. Descending inhibitory pathways release neurotransmitters like serotonin and norepinephrine, which help regulate and suppress pain sensations.

1.1.2 The pain process

The pain process is a complex interaction of physiological and psychological factors, occurring in four main stages: transduction, transmission, perception, and modulation.

1. Transduction: This is the first step, where a harmful stimulus—such as heat, chemical irritation, or mechanical injury—is converted into an electrical signal. Nociceptors in peripheral tissues detect these stimuli and generate action potentials, initiating the pain signal that travels toward the spinal cord.

2. Transmission: In this phase, the pain signal moves from peripheral nerves to the spinal cord and then to the brain. It travels via Aδ and C fibers—Aδ fibers conduct sharp, localized pain quickly, while C fibers carry dull, aching pain more slowly. Neurotransmitters like substance P

and glutamate help transmit the signal across synapses to secondary neurons, which then send it through pathways such as the spinothalamic tract to the brain.

3. Perception: When the signal reaches the brain, pain is consciously recognized. This experience varies among individuals, depending on pain thresholds, emotions, and psychological factors. Different brain regions contribute to processing pain: the somatosensory cortex determines location and intensity, the limbic system regulates emotional responses, and the frontal cortex handles cognitive aspects of pain.

4. Modulation: The body has natural mechanisms to regulate pain perception. Descending pathways from the brain can suppress pain signals at the spinal cord level, reducing pain intensity. Neurotransmitters like endorphins, serotonin, and norepinephrine play key roles in dampening pain signals. Factors such as stress, mood, and past experiences can influence pain modulation, making the experience highly individualized.

Pain perception is influenced by genetic, environmental, and psychological factors, making it a unique and complex process for each person.

1.1.3 Types of Pain

Pain can be classified into different types based on its origin and physiological mechanisms.

1. Nociceptive pain

Nociceptive pain occurs when nociceptors, which are sensory neurons that detect harmful stimuli, are activated. It is usually linked to tissue damage and can be further divided into:

- Somatic pain: Originates from the skin, muscles, or joints and is often described as sharp or aching. It is usually well-localized.

- Visceral pain: Comes from internal organs and is often described as deep, squeezing, or diffuse. It can be harder to pinpoint.

2. Inflammatory pain

This type of pain results from tissue injury or infection, leading to inflammation. The body releases pro-inflammatory substances like cytokines and chemokines, which sensitize nociceptors and enhance pain perception. Inflammatory pain plays a protective role by signaling the body to begin the healing process.

3. Neuropathic pain

Neuropathic pain is caused by damage to the nervous system, either in the peripheral or central regions. It is often described as burning, shooting, or tingling sensations. Unlike nociceptive pain, it can occur even without an external stimulus due to abnormal nerve activity. Conditions such as diabetic neuropathy and post-herpetic neuralgia are common examples.

4. Pathological pain

Pathological pain occurs when changes in the nervous system lead to pain without a clear cause. This includes:

- Central sensitization: An increased sensitivity of the central nervous system to stimuli, causing pain even from normally non-painful triggers.
- Chronic pain syndromes: Conditions like fibromyalgia, where pain continues long after the initial injury has healed, often due to changes in how pain is processed by the brain and spinal cord.

5. Radicular pain

Radicular pain happens when a spinal nerve root is compressed or inflamed, often due to issues like herniated discs or spinal stenosis. It follows the path of the affected nerve and is commonly seen in conditions such as sciatica, where pain radiates from the lower back down the leg.

1.1.4 Analgesia

Analgesia refers to the absence of pain even when exposed to a stimulus that would typically cause pain. It can occur at the peripheral level (at the site of tissue damage, receptors, or nerves) or centrally (within the spinal cord or brain). Generally, nonsteroidal anti-inflammatory drugs (NSAIDs) and other mild analgesics work mainly at the site of tissue injury, while opioids and certain adjuvant medications primarily act on the spinal cord or brain.

1.1.5 Analgesics

Analgesics are medications that help reduce or relieve pain and are commonly used to manage discomfort in different medical conditions. They are broadly classified into two main categories: opioids, which primarily act on the central nervous system (CNS), and nonopioids, which mainly target the peripheral nervous system.

1.1.6 Mechanism of analgesia

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of medications used for pain relief, fever reduction, and inflammation control. Their primary mechanism of action involves inhibiting the cyclooxygenase (COX) enzyme, which plays a key role in converting arachidonic acid into thromboxanes, prostaglandins, and prostacyclins. By reducing these eicosanoids, NSAIDs provide their therapeutic effects. Thromboxanes aid in platelet adhesion, prostaglandins contribute to vasodilation, help regulate body temperature in the hypothalamus, and play a role in pain perception.

There are two COX isoenzymes: COX-1 and COX-2. COX-1 is continuously present in the body and supports functions such as protecting the stomach lining, maintaining kidney function, and promoting platelet aggregation. COX-2, on the other hand, is mainly produced during inflammation. Most NSAIDs are nonselective, inhibiting both COX-1 and COX-2, while COX-2 selective NSAIDs (such as celecoxib) specifically target COX-2. This selective inhibition aims to provide anti-inflammatory benefits while minimizing stomach-related side effects, as COX-1 plays a protective role in the gastrointestinal tract.

Opioid analgesics are among the most effective medications for managing severe pain. They act through G protein-coupled receptors and mimic the effects of natural opioid peptides. Morphine serves as the standard example of an exogenous opioid analgesic. Opioid agonists activate receptors in both the central and peripheral nervous systems, with three main receptor types identified: mu, delta, and kappa. Opioids function by binding to these receptors, which are linked to G1 proteins, leading to inhibition of cellular activity. They achieve this by closing N-type voltage-gated calcium channels and opening inwardly rectifying potassium channels, which depend on calcium. This results in hyperpolarization, reducing neuronal excitability. Additionally, opioids lower intracellular cAMP levels, which helps regulate the release of pain-related neurotransmitters like substance P, ultimately reducing pain perception.

1.2 ANIMAL MODELS

Animal models are essential tools for studying pain mechanisms and assessing the effectiveness of analgesic agents. These models help control factors such as the type and intensity of pain stimuli, the administration of potential analgesics, and the animals' responses.

Here are some common animal models used for pain testing:

1. Tail-Flick Test

2. Formalin Test

3. Acetic Acid-Induced Writhing Test

4. Randall-Selitto Test

5. Capsaicin-Induced Pain

1.2.1 Acetic acid-induced Writhing test

The acetic acid writhing model is a technique used to investigate pain receptors in the peritoneal lining by observing spontaneous pain responses. In this method, a 0.6% v/v solution of acetic acid is administered through intraperitoneal injection, and the resulting writhing behaviors—such as stretching, retraction, or pressing the abdomen against the floor—are recorded. Following the injection of acetic acid, various inflammatory mediators, including substance P, bradykinin, serotonin, histamine, and prostaglandins, are released. These mediators contribute to increased abdominal contractions and pain sensations. A decrease in the number of writhing responses suggests the analgesic effect of the test compound.

1.2.2 Tail-Immersion Test

The tail immersion test is a technique used to assess central analgesic activity in animal models by applying heat to induce pain *(Bhuiyan et al., 2020)*. This method is particularly effective for detecting analgesia produced by opioid-based analgesics. In this test, the distal portion of an animal's tail is submerged in a heated water bath, and the time it takes for the animal to withdraw its tail is measured. The withdrawal latency serves as an indicator of nociceptive sensitivity or the analgesic effect of a test compound.

1.3 PRINCIPLES AND METHODS OF TOXICITY EVALUATION IN MEDICINAL PLANTS

Acute toxicity studies play a crucial role in evaluating the short-term toxic effects of chemicals, drugs, and other substances. These studies are essential in drug development as they help identify the immediate risks associated with exposure to a substance. Acute toxicity refers to harmful effects that appear within a short time after administering a single dose or multiple doses within 24 hours. The severity of these effects varies depending on the dose and the nature of the toxic substance. To quantify toxicity, acute toxicity studies often use the median lethal dose (LD50), which represents the dose that causes death in 50% of the test animals. Over time, the LD50 value has been widely used in toxicology, though its application has evolved with advancements in alternative testing methods *(Saganuwan, 2017).* These studies serve multiple purposes in safety evaluation and regulatory compliance. By monitoring short-term effects, researchers can determine whether a chemical poses an immediate health risk. Establishing the acute toxic dose also helps define safety thresholds for human exposure *(Saganuwan, 2017).* Additionally, by analyzing animal data, researchers can predict potential toxic effects in humans, ensuring a more comprehensive safety assessment.

1.4 HERBAL MEDICINE

Herbal medicine has been instrumental in the development of modern pharmaceuticals, as many drugs in use today trace their origins to traditional plant-based remedies. For centuries, people have relied on medicinal herbs to treat various ailments, and scientific advancements have allowed researchers to identify and isolate the active compounds responsible for their therapeutic effects. These discoveries have led to the development of numerous prescription drugs, either directly extracted from plants or synthetically modified from natural compounds. Currently, an estimated 25–28% of modern medications are derived, either directly or indirectly, from higher

plants, highlighting the vast medicinal potential of botanicals—a knowledge recognized in traditional medicine for thousands of years *(Fridlender et al., 2015).*

One notable example is artemisinin, a powerful anti-malarial drug obtained from the *Artemisia annua* plant, commonly known as sweet wormwood. This plant has been used in Traditional Chinese Medicine for over two thousand years to treat fevers. In the 1970s, researchers identified artemisinin as an effective malaria treatment *(Wang et al., 2019).* Its discovery has saved millions of lives and earned Tu Youyou the Nobel Prize in Physiology or Medicine. Similarly, plant-derived compounds have played a crucial role in cancer treatment. Paclitaxel (Taxol), extracted from the bark of the Pacific yew tree *(Taxus brevifolia),* and vincristine, obtained from the Madagascar periwinkle *(Catharanthus roseus),* are widely used chemotherapy agents *(Fridlender et al., 2015).* These drugs have been instrumental in treating various cancers, including breast cancer, ovarian cancer, and leukemia.

Modern pharmaceutical research continues to explore plant-derived compounds due to their medicinal potential. Advances in biotechnology and synthetic biology have enabled the controlled production of these bioactive molecules, marking significant progress in drug development *(Ausländer et al., 2017).* One emerging example is cannabidiol (CBD), a compound extracted from the cannabis plant, which is currently undergoing clinical trials. Research has demonstrated its anti-inflammatory, analgesic, and anti-anxiety properties, making it a promising candidate for treating epilepsy, anxiety, and chronic pain *(Chaachouaya et al., 2023).*

Additionally, volatile compounds from *Salacia reticulata Wight.* have been identified through GC-MS analysis. Two compounds (CID-240051 and CID-533471) were found to strongly inhibit human maltase-glucoamylase, an enzyme involved in carbohydrate digestion. Their binding

affinities were comparable to or even stronger than standard α-glucosidase inhibitors, suggesting their potential as natural anti-diabetic agents *(Chakravarty and Ramasamy, 2023).*

Despite these advancements, the use of herbal medicine as a drug source presents several challenges. Identifying the active components in complex plant mixtures can be difficult *(Wachtel-Galor and Benzie, 2011)*, and once isolated, these compounds must undergo extensive testing to ensure their efficacy and safety *(Wachtel-Galor and Benzie, 2011).* Additionally, natural compounds are often present in small quantities, making large-scale production difficult for lead optimization, drug development, and clinical trials *(Wachtel-Galor and Benzie, 2011)*. Conservation and sustainability also pose concerns, as the large-scale harvesting of medicinal plants can threaten wild populations and ecosystems. Implementing sustainable harvesting practices is crucial to prevent overexploitation and protect biodiversity *(Wachtel-Galor and Benzie, 2011)*.

1.4.1 Medicinal plants for Analgesia

Plant-based medicines have been used for centuries across different cultures to manage various health conditions, including pain relief. In developing countries, an estimated 70–95% of the population still depends on traditional medicine *(Fridlender et al., 2015),* largely due to its perceived effectiveness and fewer side effects compared to conventional drugs *(Jahromi et al., 2021).* In Shahrekord, Iran, traditional healers commonly use several medicinal plants for pain relief. *Eugenia caryophylata* (clove) is frequently utilized due to its eugenol content, which acts as a local anesthetic and reduces inflammation, making it effective for toothaches and muscle pain. *Alhagi maurorum* (camel thorn) is also widely used for joint pain and headaches because of its anti-inflammatory properties. Additionally, *Zingiber officinale* (ginger) alleviates pain by blocking pain mediators, making it beneficial for menstrual pain and joint inflammation *(Basati*

et al., 2019). Similarly, Moroccan researchers have identified several plants with significant analgesic properties. *Syzygium aromaticum* (clove) was found to reduce pain and inflammation by inhibiting prostaglandin synthesis and blocking inflammatory mediators. *Papaver rhoeas* (poppy) demonstrated pain-relieving effects similar to morphine, likely due to its alkaloid content. *Melissa officinalis* (lemon balm) contains essential oils that act on the nervous system, reducing pain sensitivity and inflammation. *Marrubium vulgare* (horehound) exhibited moderate analgesic effects, particularly in inflammatory pain models. Furthermore, *Argania spinosa* (argan) oil was noted for its pain-relieving and anti-inflammatory properties, which were attributed to its flavonoid and polyphenol content (*Bouyahya et al., 2022*).

A review of medicinal plants with analgesic and anti-inflammatory properties highlighted numerous species with significant pain-relieving effects. *Acacia hydaspica* was recognized for its potent analgesic activity, primarily due to its flavonoid and catechin content. *Alstonia scholaris* contained alkaloids such as picrinine and vallesamine, which demonstrated strong analgesic and anti-inflammatory effects. *Angelica pubescens* was noted for its high levels of osthole and caffeic acid, compounds that contribute to pain relief and inflammation reduction *(Lisa et al., 2020).*

Other plants with notable analgesic effects include *Annona squamosa*, which exhibited both central and peripheral pain relief due to its caryophyllene oxide content. Eucalyptus species displayed strong analgesic and anti-inflammatory properties, attributed to essential oils that inhibit inflammatory mediators. *Moringa oleifera* was found to have long-lasting pain-relieving effects, primarily due to its flavonoid-rich extracts that help manage pain and inflammation *(Lisa et al., 2020).* These medicinal plants offer potential alternatives to conventional analgesics, targeting pain through multiple mechanisms.

Research on South African medicinal plants identified several species traditionally used to treat pain and inflammation. *Ricinus communis* was frequently mentioned for its topical application in pain relief, while its oil was used to manage inflammation. *Aloe ferox* was taken orally for stomach pain and arthritis, and its infusion was used as a gargle for toothaches. *Pentanisia prunelloides* was commonly applied as a poultice to treat headaches and muscle pain (*Aremu and Pendota, 2021*). Additional South African plants used for pain relief include *Dodonaea viscosa*, which was prepared as a leaf decoction for arthritis and back pain, and *Ruta graveolens*, commonly infused to alleviate menstrual cramps and general pain. *Solanum aculeastrum* was traditionally applied to treat inflammation and toothaches, while *Artemisia afra* was used in infusions or topical applications for headaches, rheumatism, and stomach pain (*Aremu and Pendota, 2021*).

Although many traditional remedies lack extensive scientific validation, some have been supported by research. For instance, *Garcinia kola*, a commonly used local pain remedy, has demonstrated clinically significant analgesic and anti-inflammatory effects in patients with knee osteoarthritis (*Adegbehingbe et al., 2008*).

Despite their potential, herbal medicines are often used without medical supervision. Understanding their active compounds and mechanisms of action is essential for ensuring their appropriate use, predicting possible toxicities, and preventing harmful interactions with conventional medications.

1.5 THE FAMILY RUBIACEAE

The Rubiaceae family, also known as the coffee, madder, or bedstraw family, is a diverse group of flowering plants *(Karou et al., 2011).* It comprises approximately 13,000 species across about 630 genera, making it the fourth-largest family of angiosperms. Rubiaceae plants are

characterized by their simple, opposite leaves with interpetiolar stipules *(Mehriardestani et al., 2017).* With over 600 genera, the family has a cosmopolitan distribution, thriving in various regions worldwide, including a significant presence in Saharan Africa *(Karou et al., 2011).* Various parts of Rubiaceae plants, including leaves, bark, roots, and fruits, are widely used in traditional medicine. In some cases, the entire plant is utilized, including its roots. Among these, leaves are the most frequently used, followed by bark, stems, and roots. Medicinal preparations are typically made in the form of powders, concoctions, and decoctions.

1.6 LITERATURE REVIEW OF *FADOGIA CIENKOWSKII* SCHWEINF.

Scientific Classification

Family: Rubiaceae

Subfamily: Ixoroideae

Synonyms: Fadogia agrestis

Authority: Schweinf.

Genus: Fadogia

Species Name: *Fadogia cienkowskii* Schweinf.

Frequency: Common

Status: Native

Worldwide distribution: Found predominantly in tropical Africa, particularly in regions like Nigeria, Uganda, and Kenya.

English name: No common English name.

Local names (in Nigeria):

- Bakin-haggai (Hausa)

-Ufu-ewureje (Igede tribe of Benue State)

-Ogwu-agu (Igbo)

1.6.1 Morphological Description

Fadogia cienkowskii is a small, upright shrub that can grow up to 2 meters tall. Its leaves are simple, arranged in opposite pairs, and typically lance-shaped, measuring between 3 to 8 cm in length. The upper surface of the leaves is glossy green, while the underside has a slightly hairy texture. The plant produces small, yellowish-green flowers that grow in clusters (cymes) at the tips of branches *(Bruce et al., 2019).* Flowering generally occurs between July and September, though this may vary depending on local climate conditions. The fruits of *Fadogia cienkowskii* are small, round, and about 1 cm in diameter. They change color from green to black as they ripen. The plant is dioecious, meaning that individual plants are either male or female.

1.6.2 Habitat and Geographical Distribution

This plant is sometimes cultivated for ornamental purposes. Native to tropical Africa (*Chukwube et al., 2018*), it is a sub-shrub or shrub that thrives mainly in seasonally dry tropical regions. It is found in countries such as Angola, Benin, Burkina Faso, Cameroon, Chad, Congo, Ethiopia, Ghana, Guinea, Ivory Coast, Kenya, Mali, Nigeria, Sudan, Tanzania, Togo, and Zimbabwe.

1.6.3 Ethnomedical Uses

Ethnobotanically, the Igede tribe of Benue State, located in Nigeria's Middle Belt, has traditionally used this plant to treat various ailments, including general body weakness, inflammation, diarrhea, fever, and other conditions, particularly in infants *(Chukwube et al., 2018).*

In Enugu State, Nigeria, the plant was first discovered in the Ngwo Udi district at Milken Hill by coal miners. The Nsukka district in Enugu State utilizes the aqueous leaf extract to manage malaria and typhoid fever. The people of Ede Oballa in the Nsukka Local Government Area also use the plant for treating acute malaria and male impotence. *Fadogia cienkowskii* is widely employed in traditional medicine for addressing ailments such as general body weakness, inflammation, diarrhea, fever, and malaria.

1.6.4 Chemical composition

Microscopic examination identified calcium oxalate crystals, starch grains, xylem, phloem, trichomes, epidermal cells, collenchyma cells, paracytic stomata, and reticulate vessels. Chemomicroscopic analysis further confirmed the presence of lignin, starch, cellulose, mucilage, and calcium oxalate crystals *(Bruce et al., 2019).*

Physicochemical analysis determined a moisture content of 4.6%, a total ash value of 1.4%, an acid-insoluble ash value of 0.8%, a water-soluble ash value of 0.4%, a water-soluble extractive value of 7.8%, and an alcohol-soluble extractive value of 9.0%. Phytochemical screening detected tannins (17.6%), saponins (1%), glycosides (2.5%), alkaloids (3.3%), steroids (1.1%), terpenoids (6.6%), phenols (8.8%), and flavonoids (17.7%), with no presence of hydrogen cyanide *(Bruce et al., 2019).* The primary phytochemical compounds included phenolics (tannins and flavonoids), saponins, proteins, and carbohydrates *(Odeghe et al., 2024).*

Figure 2.1 Fadogia cienkowskii Plant (Bruce et al., 2019)

1.7 SOME SCIENTIFIC WORK DONE ON *FADOGIA CIENKOWSKII*

1. The 70% ethanol root extract of *Fadogia cienkowskii* was found to increase sperm count at a 200 mg/kg dose without causing toxicity to the testes and epididymis, although it indicated potential liver and kidney toxicity *(Gotep et al., 2023).*

2. Ethanol root extract and its fractions of *F. cienkowskii* enhanced mating behavior in male rats, improving parameters such as mounting, intromission, ejaculation, and copulation efficiency. Additionally, the ethanol extract increased serum testosterone levels *(Jurbe et al., 2021).*

3. At a concentration of 2500 µg/ml*, F. cienkowskii* exhibited the highest superoxide anion (O_2) inhibitory activity. The extract's ability to scavenge nitric oxide (NO) was concentration-dependent, with 250 µg/ml demonstrating greater effectiveness than α-tocopherol. These findings suggest that the leaf extract of *F. cienkowskii* exerts its medicinal effects through efficient free radical scavenging (*Odeghe et al., 2024*).

4. The methanol extract of *Fadogia cienkowskii Schweinf*. var. cienkowskii leaves was evaluated for its effects on the central and peripheral nervous systems. Oral administration of doses up to 4000 mg/kg in mice caused no mortality or signs of toxicity. The extract significantly prolonged phenobarbitone-induced sleep, indicating central nervous system activity. It also exhibited local anesthetic and analgesic effects by reducing pain responses, albeit with lower potency than lignocaine *(Ode et al., 2015).*

5. In vitro studies have demonstrated the strong antioxidant potential of *F. cienkowskii (Chukwube et al., 2021).*

6. The ethanol extract and its fractions showed varying degrees of antioxidant enzyme inhibition at different doses. The ethanol extract at 300 mg/kg exhibited the highest inhibition of superoxide dismutase (SOD) (22.2%), catalase (CAT) (39.3%), and malondialdehyde (MDA) (9.6%). Among the fractions, the ethyl acetate fraction at 400 mg/kg showed the strongest inhibition, with SOD at 88.83%, CAT at 71.43%, and MDA at 68.97%. These findings suggest that both the ethanol extract and ethyl acetate fraction of *F. cienkowskii* provided significant hepatoprotective effects, surpassing the efficacy of Silymarin (100 mg/kg) against paracetamol-induced toxicity (*Bruce et al., 2023*).

7. Studies on the VLC sub-fractions of *F. cienkowskii* leaves demonstrated a significant ($p < 0.05$) reduction in liver antioxidant enzymes and DPPH free radicals, indicating promising hepatoprotective and antioxidant properties *(Bruce, 2021).*

8. The crude extract of *F. cienkowskii* exhibited notable activity against the chloroquine-sensitive strain of *Plasmodium berghei* in both suppressive and curative models. Its antioxidant content likely contributes to free radical scavenging, reducing electron donation effects (Orabueze et al., n.d.).

1.8 AIMS AND OBJECTIVES OF THE STUDY

Aim: To determine the effect of the extract of *Fadogia cienkowskii* stem bark on pain using various animal models

Specific objectives are:

(a) Determine the acute toxicity profile of the aqueous stem bark extract of *Fadogia cienkowskii*

(b) Investigate the Analgesic activity of the aqueous stem bark extract of the plant.

CHAPTER TWO
MATERIALS AND METHODS

2.1 LABORATORY MATERIALS

2.1.1 Equipment

The following equipment and apparatus were used. Analytical weighing scale (Ohaus Corporation, USA), Digital water bath (Stuart Equipment Co. Ltd United Kingdom), Recirculating Cooler (Stuart Equipment Co. Ltd United Kingdom), Rotary evaporator (Stuart Equipment Co. Ltd United Kingdom), Beakers (various sizes), Porcelain dishes, Measuring cylinders, Syringes, Orogastric tube etc

2.1.2 Drugs and Chemicals

1. Acetylsalicylic acid (Anacin, SKG Pharma Nig)
2. Acetic acid (96%)(Sigma Aldrich, Germany)
3. Distilled water
4. Morphine
5. Acacia gum powder
6. Cotton wool

2.2 COLLECTION AND PROCESSING OF PLANT MATERIAL

The stem bark of *Fadogia cienkowskii* was collected from Bauchi Local Government Area, Bauchi State, in August 2024. It was identified and authenticated by Mr. Joseph Azila at the Federal College of Forestry, Jos, Plateau State, Nigeria. A voucher specimen was deposited in the Federal College of Forestry herbarium under voucher number FHJ 258 for future reference.

The stem bark was cleaned to remove debris, then dried and ground into powder using an electric mill. The resulting powdered material weighed 612g and was stored in an airtight container for further analysis.

2.3 EXTRACTION OF PLANT MATERIAL

The stem bark extracts of *Fadogia cienkowskii* were obtained using the Soxhlet extraction method.

A total of 612g of powdered stem bark was placed in a thimble made from thick filter paper and loaded into the main chamber of the Soxhlet extractor. The extractor was positioned on a round-bottom flask containing 95% ethanol as the extraction solvent and fitted with a condenser. The solvent was heated to 90°C to induce reflux. As the solvent vapor rose through the distillation arm into the condenser, it condensed and dripped back into the chamber containing the solid material. This chamber gradually filled with warm solvent, and once nearly full, it was automatically emptied through a siphon side arm, allowing the solvent to return to the distillation flask for continuous extraction. Following extraction, the solvent was removed using a rotary evaporator. The extract was then concentrated and dried in an oven. The obtained extract was collected, while the non-soluble portion remaining in the thimble was discarded. The final extract was stored in an airtight container in a cool, dry place.

2.4 PREPARATION OF LABORATORY ANIMALS AND SAMPLES FOR ASSAY

Swiss mice, weighing between 16–35 g and including both sexes, were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Edo State, Nigeria. They were housed under standard environmental conditions with free access to grower's mash and tap water. The animals were acclimatized for at least two weeks and fasted overnight before the experiments.

Aqueous extract solutions were prepared by homogenizing measured quantities in a 5% aqueous suspension of acacia powder before dissolving them in appropriate volumes of distilled water. Similarly, acetylsalicylic acid was homogenized in a 5% aqueous acacia suspension before being dissolved in the required volume of water. Both the extract and the drug were administered to the animals according to their body weight (mg/kg).

2.5 ACUTE TOXICITY TEST

The acute toxicity study was conducted following Lorke's method (Lorke, 1983) in two phases, using a total of sixteen male mice.

In the first phase, nine mice were divided into three groups, each containing three mice. Groups 1, 2, and 3 received single oral doses of 10, 100, and 1000 mg/kg body weight of the stem bark extract, respectively, to identify potential toxic dose ranges.

In the second phase, higher doses of 1600, 2900, and 5000 mg/kg body weight were administered orally to individual mice (one per group) to determine the exact LD50 value.

All animals were closely monitored on the day of treatment for signs of toxicity, including sedation, diarrhea, and convulsions. They were further observed for 24 hours to assess mortality.

2.6 PHARMACOLOGICAL SCREENING FOR ANALGESIC ACTIVITY OF THE EXTRACT

2.6.1 Mouse writhing method

The experiment followed the method of *Koster et al. (1959)* and involved 25 mice, which were divided into five groups of five mice each. The first group, serving as the negative control, received distilled water at a dose of 10 ml/kg. The second group, designated as the positive control, was administered acetylsalicylic acid at a dose of 100 mg/kg. The third, fourth, and fifth groups received the stem bark extract at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively. All treatments were given 60 minutes before an intraperitoneal injection of acetic acid (0.6% v/v in distilled water) at a dose of 10 ml/kg body weight. The number of writhes in each group was recorded at 5-minute intervals for 30 minutes. Writhing was defined as abdominal stretching accompanied by the extension of at least one hind limb. The mean number of writhes per group was calculated to evaluate the analgesic effect of the treatments *(Igbe et al., 2013).*

2.6.2 Tail Immersion Method

The tail immersion test was performed according to the method described by *Rabanal et al. (2005).* Before treatment, the terminal 3 cm of each mouse's tail was submerged in hot water maintained at 55 ± 0.5 °C, and the time taken for the mouse to flick its tail was recorded in seconds. Only mice with a pre-treatment reaction time of 3 seconds or less were included in the study. Following baseline latency assessment, groups of five mice received either plant extracts at doses of 100, 200, or 400 mg/kg orally, morphine at 10 mg/kg subcutaneously, or a vehicle at 10 ml/kg orally. Reaction times were measured at 30, 60, 90, and 120 minutes after treatment. To

prevent tissue damage, a maximum cut-off time of 15 seconds was set for tail-flick measurements.

2.7 STATISTICAL ANALYSIS

Data were expressed as the mean ± SEM and "n" signifies the number of rats used per group in each experiment. The data were analyzed using one way analysis of variance (ANOVA) followed by Turkey's post hoc test using Graphpad Prism 6.0, San Diego, USA. Data were considered different at significance level of $p>0.05$.

CHAPTER THREE
RESULT

3.1 ACUTE TOXICITY TEST

The extract was well tolerated by the animals, with no signs of acute toxicity, such as restlessness, dizziness, or excitation, observed after administration. No mortality occurred in the mice at any of the tested doses, including the highest dose of 5000 mg/kg. This indicates that the extract is non-toxic at the administered doses. The mortality results are presented in Table 1 below.

3.2 ANALGESIC EFFECT

3.2.1 Acetic acid induced Writhing

The results of the acetic acid-induced mouse writhing test are presented in Figure 1 below. The *F. cienkowskii* extract at doses of 200 and 400 mg/kg significantly ($p<0.05$) reduced the number of writhes compared to the control group. The inhibitory effect was dose-dependent, with the 400 mg/kg dose showing greater inhibition than the 100 mg/kg dose. The extract's effect was comparable to that of the standard drug, acetylsalicylic acid (100 mg/kg), which also produced a significant ($p<0.05$) reduction in writhing.

Table 3.1: Acute toxicity study of oral administration of extract of F. cienkowskii in mice

PHASE 1

Treatment	Dose	Number of deaths	Mortality	Total number of mice
1	10mg/kg	0 of 3	0	3
2	100mg/kg	0 of 3	0	3
3	1000mg/kg	0 of 3	0	3

PHASE 2

Treatment	Dose	Number of deaths	Mortality	Total number of mice
1	1600mg/kg	0 of 1	0	1
2	2900mg/kg	0 of 1	0	1
3	5000mg/kg	0 of 1	0	1

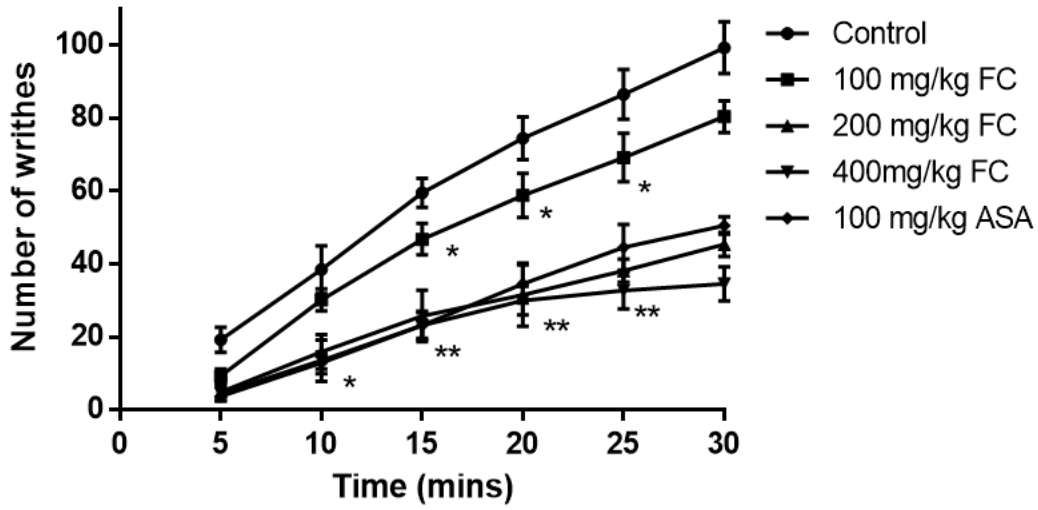

Figure 3.1 Effect of extract of Fadogia cienkowskii on acetic acid-induced mouse writhing.

p < 0.05, **p<0.01 as compared to the control group (distilled water 10ml/kg). n = 5. FC – Fadogia cienkowskii, ASA – Acetylsalicylic acid

3.2.2 Tail immersion

The tail immersion test results are shown in Table 2. The extract (100, 200 and 400 mg/kg) did not produce any significant ($p>0.05$) increase in reaction times when compared to the control. The effect of the standard drug, Morphine (10 mg/kg) was significant (**$p<0.01$), when compared to control (distilled water) at the 60 to 90 minutes

Table 3.2: The effect of extract of F. cienkowskii on reaction times in tail immersion

Treatment	Dose (mg/kg)	Before Treatment	Reaction times				
			0min	30min	60min	90min	120min
Control	-	1.94 ± 0.51	2.03 ± 0.24	3.35 ±0.26	2.12 ± 0.34	3.07 ± 0.03	2.83 ± 0.50
F. cienkowskii	100	2.16 ± 0.43	6.04 ± 1.92	7.42 ± 2.99	7.05 ± 3.23	5.15 ± 1.48	6.56 ± 2.61
F. cienkowskii	200	1.92 ± 0.42	3.68 ± 0.68	4.86 ± 2.13	3.73 ± 1.47	3.27 ± 1.09	2.79 ± 0.44
F. cienkowskii	400	2.06 ± 0.16	2.89 ± 0.46	1.93 ± 0.41	2.26 ± 0.45	2.79 ± 0.62	0.62 ± 0.12
Morphine	10	2.3 ± 0.37	3.82 ± 0.46	3.24 ± 0.74	14.64 ± 3.07**	10.93 ± 4.86**	4.15 ± 0.03

Data are expressed as mean ±SEM of reaction time (secs) (n = 5 animals per group. **p<0.01, when compared to control (distilled water 10 ml/kg)

CHAPTER FOUR
DISCUSSION

Acute toxicity studies are crucial for assessing the safety profile of medicinal plant extracts before their therapeutic use in animals or humans. These studies evaluate the potential toxic effects of a substance by administering a single high dose and monitoring test animals for adverse reactions over a short period, typically 24 hours. The primary goal of acute toxicity testing is to determine the median lethal dose (LD_{50}), which represents the dose required to cause death in 50% of the test population *(Gadaleta et al., 2019).* This information helps identify toxic effects, establish safe dosage ranges, and prevent harmful exposure. Various methods exist for determining acute toxicity, including Lorke's method, which is widely recognized for estimating LD_{50} values of chemical compounds *(Igbe et al., 2013; Lorke, 1983).* In this study, acute toxicity evaluation was conducted in two phases (Phase I and Phase II). Mice treated with the extract showed no physical signs of toxicity, such as seizures, diarrhea, restlessness, or drowsiness. Additionally, no mortality was recorded in either phase of the study.

Since medicinal plants contain both pharmacologically active and potentially toxic compounds, assessing their toxicity before clinical use is essential. Some plant-derived compounds may be harmful if administered improperly, highlighting the importance of thorough toxicological evaluations *(Rehman et al., 2022).* Acute toxicity studies also help determine appropriate doses for further pharmacological screening, ensuring that therapeutic doses remain within safe limits *(Gandhare et al., 2013).* Additionally, they establish a correlation between animal and human responses, aiding in dose extrapolation for future studies.

In this study, the ethanol extract of *Fadogia cienkowskii* stem bark underwent acute toxicity evaluation following established protocols. The extract was administered orally to mice at doses up to 5000 mg/kg body weight, and the animals were monitored for mortality and toxicity signs over 24 hours. The results indicated that:

- No mortality occurred at any of the tested doses.
- No significant behavioral changes, such as seizures, diarrhea, restlessness, or drowsiness, were observed.
- The extract was well tolerated at all dose levels.

These findings suggest that *Fadogia cienkowskii* extract has a high safety margin, as its LD_{50} value exceeds 5000 mg/kg. According to globally accepted toxicity classification scales, substances with LD_{50} values greater than 5000 mg/kg are generally considered non-toxic *(Gadaleta et al., 2019).* The absence of acute toxicity supports the traditional use of this plant in herbal medicine and encourages further pharmacological investigations into its therapeutic potential. Additionally, the use of Lorke's method in this study aligns with widely accepted toxicology research approaches *(Lorke, 1983; Igbe et al., 2013).* The absence of acute toxicity in *Fadogia cienkowskii* extract is a positive indication of its safety for potential therapeutic use. However, while acute toxicity studies provide valuable initial safety data, they do not assess long-term or cumulative toxic effects. Therefore, further studies, including sub-acute and chronic toxicity assessments, are necessary to evaluate any potential delayed toxic effects and ensure long-term safety.

Analgesic effects can be mediated through the central nervous system (CNS), the peripheral nervous system (PNS), or both. To distinguish between these mechanisms, specific tests are

required. The acetic acid-induced writhing test is commonly used to evaluate peripheral analgesics. In this study, the acetic acid-induced writhing test was employed to assess peripheral analgesic activity, while the tail immersion test was used to evaluate central analgesia (*Singh and Majumdar, 1995).* The analgesic properties of *Fadogia cienkowskii* stem bark extract were assessed using the acetic acid-induced writhing test and the tail immersion test in mice. Oral administration of the extract at doses of 200 mg/kg and 400 mg/kg significantly reduced the writhing response caused by a 0.6% acetic acid solution. The acetic acid-induced writhing test is a widely used method for evaluating peripheral analgesic activity in rodents. In this assay, an intraperitoneal injection of acetic acid induces a nociceptive response characterized by abdominal constrictions, commonly referred to as "writhing." These responses result from the release of endogenous substances, such as prostaglandins and bradykinin, which activate nociceptive pathways *(Singh and Majumdar, 1995).* The frequency of writhing episodes serves as an indicator of the nociceptive response, and a reduction in the number of writhes following treatment with a test compound suggests potential analgesic properties *(Koster et al., 1959).*

Writhing is characterized by waves of constriction and elongation along the abdominal wall, accompanied by twisting of the trunk and hind limb extension. This reaction occurs due to the nociceptive effects of acetic acid, which stimulates pain receptors (*Yaghooti and Alimoahmmadi, 2024).* The test is particularly sensitive for detecting the analgesic effects of nonsteroidal anti-inflammatory drugs (NSAIDs) and other compounds that modulate peripheral pain mechanisms.

Intraperitoneal administration of acetic acid releases prostaglandins and inflammatory mediators such as PGE_2 and $PGE_2\alpha$, which sensitize nociceptors and increase pain perception. The inhibition of these mediators is a well-established mechanism of action for many NSAIDs *(Robinson, 1983).* A significant reduction in the number of writhes following drug administration

indicates an analgesic effect, suggesting that the test compound interferes with pain signaling, particularly through the inhibition of prostaglandin synthesis. In this study, the ethanol extract of *Fadogia cienkowskii* stem bark was evaluated for its analgesic potential using the acetic acid-induced writhing test. The extract was administered at doses of 100, 200, and 400 mg/kg body weight. The results demonstrated a significant, dose-dependent reduction in the number of writhes compared to the control group, indicating that the extract possesses notable peripheral analgesic activity.

The observed analgesic effect suggests that the extract may inhibit the synthesis or action of endogenous pain mediators responsible for inducing the writhing response. This aligns with the mechanism of action of many peripheral analgesics, which target inflammatory pathways to alleviate pain. The results showed that the extract effectively reduced pain, performing comparably to aspirin (100 mg/kg) and demonstrating a greater inhibitory effect than the control (water). The results of the writhing test further demonstrated that the stem bark extract significantly inhibited pain in a dose-dependent manner. The 200 mg/kg and 400 mg/kg doses exhibited stronger inhibition compared to aspirin, suggesting that the extract may exert its analgesic effects by reducing the synthesis and release of prostaglandins, which are key mediators of pain. For comparative purposes, standard analgesic drugs are often used in the writhing test to validate the efficacy of test substances. In this study, aspirin, a well-known NSAID, was used as a reference drug.

The tail immersion test is a widely used experimental model for evaluating centrally acting analgesics in rodents. The test involves immersing the distal portion of a rodent's tail in warm water and recording the latency to withdrawal as a measure of pain sensitivity. An increase in

withdrawal latency following drug administration suggests potential analgesic activity *(Janssen et al., 1963).* This method is particularly effective for detecting the analgesic effects of opioids and other CNS-acting drugs, which enhance pain tolerance by modulating descending inhibitory pathways within the CNS *(Ossipov et al., 2010).* Compared to the acetic acid-induced writhing test, which primarily assesses peripheral analgesia, the tail immersion test specifically evaluates centrally mediated pain modulation. The tail-flick response observed in the tail immersion test is a spinally mediated reflex. Heat stimulation activates Aδ and C fibers in the tail, which transmit pain signals to the dorsal horn of the spinal cord. This generates a rapid motor response, leading to tail withdrawal from the heat source. Opioid analgesics, such as morphine, increase tail withdrawal latency by activating μ-opioid receptors in the spinal cord and brainstem, inhibiting pain transmission *(Millan, 2002).*

Morphine (10 mg/kg), a standard central analgesic, significantly increased the pain threshold after administration. However, the extract at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg did not produce a similar effect, as it failed to prolong withdrawal latency in the tail immersion test. This indicates that *Fadogia cienkowskii* extract does not act through central mechanisms but instead provides analgesia primarily through peripheral pathways *(Okpara, 2013).* The findings suggest that while the extract effectively reduces pain via inhibition of peripheral pain mediators such as prostaglandins, it does not influence central pain pathways like opioids.

CHAPTER FIVE
CONCLUSION

5.1 CONCLUSION

This study establishes that the stem bark extract of *F. cienkowskii* has analgesic effects, likely through peripheral inhibitory mechanisms. This supports its traditional use for pain management. Additionally, the extract showed no toxicity even at high doses, suggesting it is safe for therapeutic use. These findings highlight its potential benefits for human health and encourage further research to explore its effectiveness and safety.

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APPENDIX

Table 1: The effect of the extract and Aspirin on Acetic acid induced writhing test

Treatment	Dose	Number of Writhes Per 30 Minutes	Percentage Inhibition
Control	10mg/kg	99.25 ± 6.34	-
F. *cienkowskii*	100mg/kg	56.6 ± 9.42	42.9%
F. *cienkowskii*	200mg/kg	80.4 ± 8.31	18.9%
F. *cienkowskii*	400mg/kg	45.4 ± 12.28	54.2
Aspirin	100mg/kg	34.6 ± 13.25	65.1

Values are mean number of writhes ± SEM (n=5 animals per group). *P< 0.05, **P< 0.01 significantly different from the control group.