ACUTE TOXICITY STUDIES AND ANALGESIC EFFECTS OF THE ETHANOL EXTRACT OF *MORINGA OLEIFERA* ROOT BARK 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 **OSAIGBOVO ESOHE NANCY** with matriculation number **PHA1908582**, 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-PLAGIARISM CERTIFICATION

We the undersigned certify attest and declare that the thesis of **OSAIGBOVO ESOHE NANCY** titled ACUTE TOXICITY STUDIES AND ANALGESIC EFFECTS OF THE ETHANOL EXTRACT OF THE ROOT BARK OF *MORINGA OLEIFERA* IN MICE has successfully done the anti-plagiarism 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 Hon Mr. and Mrs. Osaigbovo and my amazing siblings for their love, grace, guidance. and support through the period of my university education.

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ABSTRACT

This study used mouse models to assess the acute toxicity and analgesic effects of *Moringa oleifera* extract. Acute toxicity was tested by giving different oral dose up to 5000 mg/kg, which resulted in no mortality, showing relative safety. The analgesic efficacy was assessed using acetic acid-induced writhing and formalin-induced paw licking assays. The extract considerably reduced writhing behaviors in a dose-dependent manner ($p < 0.05$), indicating peripheral analgesic effects. In the formalin test, the extract significantly reduced paw licking time in both neurogenic (early) and inflammatory (late) phases, with significant effects at moderate and high dosages ($p < 0.05$), indicating wide analgesic and anti-inflammatory activities. These data confirm *Moringa oleifera* extract's potential as a safe and effective analgesic agent.

Moringa oleifera's phytochemical components, which include flavonoids, alkaloids, saponins, tannins, and phenolic acids, are thought to work together to provide analgesic and anti-inflammatory benefits. The extract's ability to attenuate nociceptive behaviors in established experimental models backs up its longstanding use in folk medicine to treat pain and inflammation. The findings of this work give experimental confirmation for *Moringa oleifera* root bark as a promising natural analgesic with a wide range of efficacy, prompting further investigation into its pharmacological mechanisms and possible clinical applications. The extract's analgesic efficacy and safety profile make it a promising low-risk pain management option.

CHAPTER ONE

INTRODUCTION

1.1 PAIN AND ANALGESIA

Pain is a complex sensory and emotional experience that reflects actual or potential tissue damage and functions as an important protective mechanism (Katz & Melzack, 1999). It encompasses a variety of processes, including the detection and transmission of noxious stimuli, as well as the perception and management of pain in the central nervous system. Pain management is crucial to improving patient comfort and quality of life (Shah et al., 2019).

Analgesics are drugs that reduce pain without causing loss of consciousness (Bonica, 1990). They work by blocking pain impulses or altering the brain's perception of pain. Non-opioid analgesics, such as nonsteroidal anti-inflammatory drugs (NSAIDs), treat pain by blocking cyclooxygenase enzymes (COX-1 and COX-2), which lowers the production of prostaglandins that produce inflammation and nociception. Vane and Botting (1998), Shah et al. (2019). Acetaminophen (paracetamol) works through both peripheral and central mechanisms, including indirect inhibition of prostaglandin synthesis and alteration of pain pathways in the brain. (Shah et al. 2019).

Opioid analgesics function centrally by binding to opioid receptors in the brain and spinal cord, altering pain perception and emotional reaction (Fields, 2004; StatPearls, 2023). Adjuvant analgesics, such as antidepressants and anticonvulsants, are also utilized to control neurotransmitter pathways, particularly in chronic and neuropathic pain. (Stat Pearls, 2023). Analgesics target many areas in the pain pathway to provide symptomatic relief, making them useful in the treatment of both acute and chronic pain (Katz and Melzack, 1999; Shah et al., 2019).

1.1.1 PHYSIOLOGY OF PAIN

Pain physiology consists of complex processes for detecting, transmitting, and regulating painful signals. The detection of noxious stimuli begins with peripheral nociceptors, which

are specialized free nerve endings that respond to mechanical, thermal, or chemical damage (Costigan et al. 2009). These nociceptors convey signals via short myelinated A-delta fibers, which transport strong, localized pain, and unmyelinated C fibers, which communicate slower, dull, and burning pain sensations (Purves et al., 2018).

Pain physiology involves complex processes for detecting, transmitting, and regulating painful signals. The initial detection of painful stimuli occurs at peripheral nociceptors, which are free nerve endings that respond to mechanical, thermal, or chemical damage (Costigan et al. 2009). These nociceptors communicate via short myelinated A-delta fibers that carry strong, localized pain, as well as unmyelinated C fibers that transmit slower, dull, and burning pain sensations (Purves et al., 2018).

Pain is regulated by descending inhibitory channels from the brainstem that release neurotransmitters such serotonin, norepinephrine, and endogenous opioids that block pain transmission at the spinal level (Millan, 1999). Furthermore, peripheral and central sensitization pathways exacerbate pain in injured tissues, resulting in hyperalgesia and chronic pain states (Woolf and Salter 2000).

These processes transduction, transmission, perception, and modulation form the physiological basis of pain, allowing for both acute and chronic pain (Costigan et al., 2009; Treede et al., 2015).

1.1.2 THE PAIN PROCESS

Pain comprises four connected stages: transduction, transmission, perception, and modulation (Bear et al., 2020).

Transduction is the first stage in which unpleasant stimuli, such as mechanical, thermal, or chemical damage, activate peripheral nociceptors and turn them into electrical impulses (Costigan et al., 2009). These impulses are then transported by A-delta and C fibers to the dorsal horn of the spinal cord, where the second stage of transmission occurs (Treede et al., 2015). Neurotransmitters such as glutamate and substance P aid in the transmission of these

signals.

Perception is the cortical processing of pain signals in brain regions such as the somatosensory cortex, insula, and anterior cingulate cortex, which integrate sensory and affective aspects of the pain experience (Tracey & Mantyh, 2007). The subjective aspect of pain becomes apparent here, influenced by factors such as attention, mood, and prior experience.

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1.1.3 TYPES OF PAIN

Pain is divided into five types:

1. Nociceptive pain
2. Neuropathic pain
3. Deafferentation pain
4. Psychogenic pain
5. Inflammatory pain

(Demir, 2023; IASP, 2024).

1. Nociceptive pain is characterized by the activation of peripheral nociceptors in response to tissue injury or inflammation, and it is often described as acute, severe, or throbbing. It frequently affects the skin, muscles, joints, and visceral organs. This type of discomfort serves as a protective mechanism, signaling potential or actual danger (Demir, 2023).
2. Neuropathic pain is caused by damage or dysfunction of the nervous system, whether peripheral or central. It produces scorching, shooting, or electric-like sensations and is frequently accompanied by sensory abnormalities such as numbness or tingling

(Demir, 2023).

3. Deafferentation pain is a type of neuropathic pain caused by the loss or interruption of afferent nerve input from peripheral nerve transection or central lesions such as spinal cord injury. This results in persistent pain that is often resistant to traditional analgesics and can be associated with illnesses such as thalamic pain syndrome (Demir, 2023).
4. Psychogenic pain is characterized as pain that is influenced or exacerbated by psychological factors such as stress, worry, or depression but does not have evident physical or neurological causes. Such pain is just as real and unpleasant as other types, and it frequently coexists with chronic pain issues (Demir, 2023; IASP, 2024).
5. Tissue inflammation causes inflammatory pain because it releases chemical mediators that sensitize nociceptors, resulting in increased pain sensitivity (hyperalgesia). It is commonly seen in autoimmune diseases and infections (IASP, 2024).

These pain types may coexist or overlap in a number of clinical settings, making diagnosis and therapy more complex. Understanding these interactions is crucial for effective pain management and personalized treatment (Demir, 2023; IASP, 2024).

1.1.4 ANALGESIA

Analgesia is the reduction or elimination of pain by various medications known as analgesics. Non-opioid analgesics (e.g., NSAIDs and acetaminophen) act peripherally to reduce inflammation and pain signals, whereas opioid analgesics act centrally on opioid receptors to relieve moderate to severe pain. Adjuvant analgesics, such as antidepressants and anticonvulsants, are used specifically for neuropathic pain, and local anesthetics block nerve conduction locally. Longdom et al. (2024); Medical News Today (2022); Britannica (2025); Stat Pearls (2023). Multimodal analgesia combines various approaches to give effective pain relief while reducing undesirable side effects.

1.1.5 ANALGESICS

Analgesics are pain-relieving medications classified as non-opioid, opioid, adjuvant, or local anesthetic. Non-opioid analgesics like acetaminophen and NSAIDs reduce inflammation,

which relieves mild to moderate pain (Longdom et al., 2024). Opioid analgesics alleviate moderate to severe pain by acting centrally on opioid receptors; nevertheless, they have risks such as dependence (Medical News Today, 2022). Adjuvant analgesics, such as antidepressants and anticonvulsants, are generally used to treat neuropathic pain, whereas local anesthetics block nerve conduction and provide temporary relief (Britannica, 2025; StatPearls, 2023). A combination of these approaches, known as multimodal analgesia, typically leads to better pain control with fewer adverse effects (Longdom et al., 2024).

1.1.6 MECHANISM OF ANALGESIA

Analgesia occurs through a variety of mechanisms, depending on the type of analgesic used. Non-opioid analgesics, such as NSAIDs, primarily block cyclooxygenase (COX) enzymes, thereby decreasing the generation of prostaglandins that promote inflammation and pain sensitization (Cashman, 1996). When compared to nonselective NSAIDs, selective COX-2 inhibition provides pain relief while having less gastrointestinal side effects.

Opioid analgesics alleviate pain by binding to opioid receptors, primarily mu receptors, in the brain and spine. They inhibit presynaptic neurotransmitter release by blocking calcium channels and increase postsynaptic potassium channel activity, resulting in decreased neuronal excitability and pain transmission (StatPearls, 2023; Osmosis, 2025).

Antidepressants enhance descending inhibitory pathways by adjusting serotonin and norepinephrine levels, whereas anticonvulsants reinforce neuronal membranes to reduce abnormal excitability.

Adjuvant analgesics, such as anticonvulsants, stabilize neuronal membranes to reduce abnormal excitability, whereas antidepressants strengthen descending inhibitory pathways by modulating serotonin and noradrenaline. Local anesthetics function by blocking sodium channels in neurons, preventing action potential propagation and nerve signal transmission (Britannica, 2025).

Overall, analgesics work through both peripheral and central mechanisms to reduce pain

perception and increase patient comfort.

1.2 ANIMAL MODELS

Animal models are commonly used in pain research to investigate both nociceptive and spontaneous pain behaviors. Reflexive tests, such as paw licking or withdrawal, tail flicking, hot plate, and von Frey filament assays, assess the animal's response to thermal, mechanical, or chemical stimuli and are widely used to assess acute and chronic pain states (Deuis et al., 2017; Mogil, 2009).

Here is a list of common animal behavioral models used for pain testing:

- Paw licking or paw withdrawal test
- Tail flick test
- Hot plate test
- Von Frey filament test (mechanical sensitivity)
- Hargreaves test (thermal sensitivity)
- Formalin test (chemical-induced pain behavior)
- Acetone test (cold sensitivity) Conditioned place preference (CPP)
- Conditioned place avoidance (CPA)
- Escape/avoidance behavior test

1.2.1 ACETIC ACID INDUCED WRITHING TEST

The acetic acid-induced writhing test is a prominent mouse model for assessing peripheral pain and analgesic activity. In this test, a mild acetic acid solution is injected intraperitoneally, causing irritation and inflammation that leads in characteristic writhing responses such as belly contraction, stretching, and hind leg extension. Pain is quantified by the frequency with which these writhes occur over a specific time period.

Test pain is caused by endogenous mediators such prostaglandins, which sensitize nociceptors and generate nociceptive responses. Analgesic medicines reduce the amount of writhes, implying analgesic or anti-inflammatory qualities (Ashok et al., 2006; Sharma and Sharma, 1997).

The acetic acid writhing test remains popular due to its simplicity, sensitivity, and inexpensive cost, despite ethical concerns regarding the severity of the pain produced. It is especially useful when analyzing nonsteroidal anti-inflammatory drugs (NSAIDs) and natural analgesics.

1.2.2 PAW LICKING (FORMALIN TEST)

The formalin test is a prominent animal paradigm for researching pain and analgesia. It involves injecting a small amount of dilute formalin (typically 2.5-5%) subcutaneously into the rodent's hind paw. This causes a biphasic pain response, including paw licking, biting, and flinching (Hunskaar and Hole, 1987; Mogil, 2009).

The early phase (Phase I), which lasts between 0 and 5 minutes after injection, is distinguished by acute nociceptor activation and significant discomfort. Inflammatory processes and cerebral sensitization cause the late phase (Phase II), which lasts 15-30 minutes following a brief period of quiescence. Recording the duration and frequency of paw licking or biting enables an objective assessment of pain intensity and analgesic efficacy.

Opioids and other centrally acting analgesics frequently suppress both stages, but anti-inflammatory medications mostly reduce the late phase (Hunskaar and Hole, 1987; Mogil, 2009). The formalin test's capacity to distinguish between these phases makes it useful for evaluating a variety of analgesic mechanisms.

1.2.3 TAIL FLICK TEST

A thermal stimulation (often a focussed beam of heat) is administered to the animal's tail. The time it takes for the animal to flick or withdraw its tail from the heat source is measured as a measure of pain perception. Increased delay suggests analgesic activity involving spinal reflexes and central pathways (Hunskaar, 2004).

1.2.4 HOT PLATE TEST

Animals are placed on a hot surface, and their latency to respond (licking, jumping, or paw withdrawal) is recorded. It evaluates central pain processes and the effectiveness of central analgesics such as opioids. Longer response times imply higher analgesic activity (D'Amour

& Smith, 1941).

1.2.5 VON FREY FILAMENT TEST (MECHANICAL SENSITIVITY)

This test determines mechanical pain sensitivity by introducing calibrated filaments with different forces to the paw or other body parts until a withdrawal response is recorded. It is useful for measuring mechanical allodynia and hyperalgesia, particularly in neuropathic pain models (Djouhri et al., 2003).

1.2.6 HARGREAVES TEST (THERMAL SENSITIVITY)

Similar to the tail flick and hot plate experiments, this involves introducing a concentrated heat source to the paw or plantar area and measuring withdrawal delay. It is a sensitive method for assessing thermal pain thresholds and analgesic efficacy (Hargreaves et al., 1988).

1.2.7 FORMALIN TEST (CHEMICAL-INDUCED PAIN BEHAVIOR)

This test involves injecting formalin into the paw, which causes a biphasic pain response: the initial neurogenic phase (0-5 minutes) involves direct nerve stimulation, while the late inflammatory phase (15-30 minutes) involves the release of inflammatory mediators. Reduced paw licking or flinching in either phase suggests analgesic activity (Tjolsen et al., 1992).

1.2.8 ACETONE TEST (COLD SENSITIVITY)

Acetone is administered to the paw, generating a chilling sensation and eliciting withdrawal behaviors such as paw licking. It assesses cold allodynia, which is beneficial in neuropathic or inflammatory pain models (Devor 1999).

1.2.9 CONDITIONED PLACE PREFERENCE (CPP)

This behavioral test measures the rewarding effects of analgesics. Animals are trained to identify a specific chamber with medicine administration. The preference for that chamber implies pain alleviation and analgesic efficacy (Lacay et al., 2014).

1.2.10 CONDITIONED PLACE AVOIDANCE (CPA)

In contrast, CPA evaluates the disagreeable or unpleasant effects of pain or pharmacological

side effects. Animals learn to avoid chambers that cause discomfort or illness (Bingel et al., 2007).

1.2.11 ESCAPE/AVOIDANCE BEHAVIOR TEST

Animals are exposed to a noxious stimulus with the option to escape or avoid it, often through operant conditioning. The latency or frequency of avoidance responses indicates pain perception or relief (Kostov and McIntosh, 2012)

1.3 PRINCIPLES AND METHODS OF TOXICITY EVALUATION IN MEDICINAL PLANTS

Opioids and other centrally acting analgesics frequently inhibit both phases, but anti-inflammatory medications mostly reduce the late phase. The formalin test's capacity to distinguish between different phases makes it valuable for evaluating various analgesic processes.

In vitro cytotoxicity assays with cell cultures evaluate plant extract effects on cell viability and function, providing early warning signs of toxicity (Itharat et al. 2004). Genotoxicity and mutagenicity testing reveal the potential for DNA harm. Phytochemical profiling identifies bioactive and potentially dangerous chemicals, which helps with risk assessment (Chan et al., 2016).

Overall, toxicity evaluation uses a variety of approaches to adequately explain the safety of medicinal plants before clinical use, in compliance with regulatory standards and Good Laboratory Practices (Mukinda and Syce, 2007; OECD, 2001).

1.4 HERBAL MEDICINE

Herbal medicine, commonly referred to as herbalism or phytotherapy, is the use of whole plants or plant extracts to promote health and treat illness. It is based on fundamental concepts such working with nature, addressing root causes rather than symptoms, restoring body balance, and personalizing treatment to the particular patient (Heritage School of Herbal Medicine, 2024). Unlike conventional pharmacology, herbal treatment usually uses whole plants or herb mixes, which can have synergistic effects while lowering toxicity (National

Center for Complementary and Integrative Health, 1998; Britannica, 2025).

Herbal medicine is one of the oldest healing modalities and is still widely practiced around the world, notably in Asia and Africa, as a supplement to modern healthcare (WHO, 2025). Infusions, decoctions, tinctures, poultices, and ointments are all types of preparations. Diagnosis and therapy are based on holistic approaches that take into account the patient's lifestyle, constitution, and environmental factors (Clinical Gate, 2025).

1.4.1 MEDICINAL PLANTS FOR ANALGESIA

Medicinal herbs have a long history of use as analgesics, and numerous species have been clinically proven to relieve pain. These plants include bioactive compounds such flavonoids, alkaloids, terpenoids, and phenolics, which relieve pain via anti-inflammatory, antioxidant, and opioid receptor-related pathways (Raza et al., 2007; Jlassi et al., 2020).

The Lamiaceae family is extensively studied for its analgesic effects. Stachys lavandulifolia extracts have been shown to dramatically reduce acute pain in rats, including formalin and acetic acid writhing tests, perhaps by interaction with inflammatory mediators (Miraj et al. 2018). Similarly, Origanum vulgare provides analgesia through antioxidant activity and opioid receptor modulation (Eidi et al., 2019).

Several plants, including Eugenia caryophyllata, Platostoma africanum, Tribulus terrestris, Lavandula angustifolia, and Nigella sativa, have been used to treat pain in ethnobotanical studies from Shahrekord, Iran. Their active molecules are thought to influence both peripheral and central pain pathways, such as neurotransmitter regulation and inflammatory cytokine suppression (Iheanacho et al., 2024).

In preclinical studies, Moroccan medicinal plants such as Clerodendrum phlomidis and Curcuma longa (turmeric) have shown significant analgesic and anti-inflammatory properties. Curcuminoids and phenolic components inhibit prostaglandin production and oxidative stress (Bouyahya et al., 2021).

Other notable examples include:

- *Bubonium graveolens* reduces pain behavior in mice comparable to diclofenac (Jlassi

et al., 2020).

- *Alafia barteri* and *Capparis decidua showing* analgesic properties in rodent models (Okafor et al., 2023).
- *Zingiber officinale* (ginger), which inhibits peripheral release of pro-inflammatory cytokines and prostaglandins (Sharifi-Rad et al., 2020).
- *Tanacetum polycephalum* and *Mentha piperita* with central and peripheral analgesic effects through anti-inflammatory and neurotransmitter balancing activities (Miraj et al., 2018).

Phytochemical synergy in multi-herbal formulations can improve analgesic efficacy while minimizing the side effects commonly associated with synthetic medications (Pattanayak et al., 2010). However, important challenges remain, such as extract standardization, dose optimization, and rigorous clinical studies to prove efficacy and safety in people (Jlassi et al., 2020; Iheanacho et al., 2024).

Medicinal plants thus make fascinating alternative agents in pain management, particularly in locations with limited access to conventional pharmaceuticals, supporting integrative healthcare approaches.

1.5 THE FAMILY MORINGACEAE

The Moringaceae family, led by the Moringa genus (*Moringa oleifera* and *Moringa peregrina*), is well-known for its therapeutic properties, including analgesic effects. These herbs are indigenous to tropical and subtropical regions and have long been used to treat pain, inflammation, muscle spasms, and other ailments (Al-Asmari et al., 2015; Rachana et al., 2018).

Moringa oleifera (drumstick tree) includes bioactive compounds such as flavonoids, alkaloids, saponins, tannins, and phenolic acids, which contribute to its anti-inflammatory, antioxidant, analgesic, and antipyretic effects. Extracts of M. oleifera leaves, seeds, and roots have been shown in studies to reduce pain in animals by regulating inflammation, scavenging free radicals, and modulating neurochemical pathways (Parvathy and Umamaheswari, 2019;

Patwardhan et al., 2014).

Moringa peregrina, a plant native to the Arabian Peninsula, has long been used to alleviate muscle aches, labor pain, fever, and wound healing. Pharmacological investigations reveal that it has analgesic, antispasmodic, and anti-inflammatory activities by suppressing ileal contractions and decreasing inflammatory cytokines (Alqahtani et al., 2018; Khan et al., 2017). Alqahtani et al. (2018) observed that the seeds contain isothiocyanates and glycosides, which are strong analgesics.

Other Moringaceae species exhibit analgesic characteristics, which are often linked to the synergistic action of polyphenols, flavonoids, and other secondary metabolites that inhibit cyclooxygenase enzymes and reduce prostaglandin synthesis (Rachana et al., 2018; Al-Asmari et al., 2015).

These traditional and scientific evidences support the use of Moringaceae plants as supplementary analgesics, albeit standardized extracts and clinical trials are required to optimize their medicinal efficacy and safety.

1.6 LITERATURE REVIEW OF MORINGA OLEIFERA

Scientific Classification:

Family: Moringaceae

Subfamily: (not commonly subdivided)

Synonyms: None widely accepted (varies by source)

Authority: Lam.

Genus: Moringa

Species Name: *Moringa oleifera Lam*.

Frequency: Common

Status: Native to the Indian subcontinent; widely cultivated and naturalized globally

Worldwide distribution: Native to northern India and Pakistan; widely cultivated and naturalized in tropical and subtropical regions of Africa, Asia, the Middle East, and the Americas

English name: Moringa, Drumstick tree, Horseradish tree, Ben oil tree

Local names (in Nigeria): Zogale (Hausa), Ewe Igbale (Yoruba), Okwe Oyibo (Igbo), Agunmu (Yoruba), Ewe Ilu (Yoruba), Kombu (Kanuri), Bayuya (Fulani), Ufu-igwu (Igede tribe of Benue State), Usi(Edo).

1.6.1 MORPHOLOGICAL DESCRIPTION

Morphological Description of *Moringa oleifera*

General Habit:

Moringa oleifera is a rapidly growing deciduous tree that can reach a height of 10 to 12 meters (33 to 39 feet) with a trunk diameter of 46 cm (18 inches). It features a large, spreading, umbrella-shaped crown and fragile branches. The bark is pale gray and smooth or somewhat rough (Razis et al., 2014; Parvathy and Umamaheswari, 2019).

Leaves:

The leaves are bipinnate or tripinnate, growing up to 45 cm in length. The leaflets are small (1-2 cm), hairy on the underside, and almost smooth on the upper surface. The leaves are airy and vary in hue from bright to dark green. Both sides exhibit unicellular non-glandular hairs with curved tips, and anomocytic stomata are concentrated on the lower surface (Parvathy and Umamaheswari, 2019; Singh et al., 2020).

Flowers:

The fragrant, bisexual blooms are pale yellowish-white, measuring 0.7 to 1 cm long and 2 cm wide. They are arranged in spreading or drooping axillary panicles 10-25 cm long. They contain five unequal spatulate petals, five stamens with five smaller sterile filaments, and a pistil composed of a single-celled ovary and a slender style (Parvathy and Umamaheswari, 2019).

Fruits:

Fruits are triangular, long, pendulous pods (sometimes called drumsticks) that are 30 to 120 cm long and 1.8 cm wide. They are green as juveniles and subsequently mature into brown pods with three valves. Each pod contains about 26 seeds (Parvathy & Umamaheswari, 2019).

Seeds:

The seeds are spherical, measuring about 1 cm in diameter, with a brownish semi-permeable seed coat and three papery wings. Seed color changes with viability, ranging from brown to black to white. Seeds typically germinate in two weeks, and a mature tree can produce 15,000 to 25,000 seeds annually (Parvathy and Umamaheswari, 2019).

Roots and Stem:

Roots include alkaloids such as moringine and have unique secondary metabolites. The stem is round, smooth to light brown, and has a little gritty texture. The wood exhibits reticulate thickening in xylem arteries, phloem fibers, and mucilage canals (Singh et al., 2020).

1.6.2 HABITAT AND GEOGRAPHICAL DISTRIBUTION

Habitat and Geographical Distribution of *Moringa oleifera*;

Moringa oleifera is a tropical and subtropical tree that grows in the sub-Himalayan regions of northern India and Pakistan. It thrives in warm climates with temperatures ranging from 25 to 35°C and can tolerate drought. The tree prefers well-drained sandy or loamy soils with a pH ranging from 5 to 9, however it cannot live in wet circumstances. It may grow at altitudes ranging from sea level to around 2,000 meters (Razis et al., 2014; Parvathy and Umamaheswari, 2019).

The plant is commonly grown and naturalized in Asia, Africa (especially Nigeria), the Caribbean, Central and South America, Oceania, and parts of the United States, such as Florida and Hawaii. It can grow in a wide range of agroecological zones, making it an important multipurpose tree for food, medicine, and environmental sustainability (Wikipedia, 2025; Razis et al., 2014; Bosch, 2004).

1.6.3 ETHNOMEDICAL USES

Ethnomedical Uses of Moringa oleifera

Moringa oleifera, also known as the "miracle tree," it has long been used in traditional medicine to treat a range of diseases. Plant parts such as leaves, roots, bark, seeds, flowers, and gum are utilized to cure a wide range of ailments (Razis et al., 2014; Ahmad et al., 2023).

Leaves: Powdered leaves are used as a vitamin and mineral supplement, as well as to treat wounds, insomnia, and overall health, particularly in malnourished infants and nursing mothers (Ahmad et al., 2023; Razis et al., 2014).

Roots and Bark: Traditionally used to cure toothaches, ulcers, inflammation, kidney stones, and ear problems. The bark is also used to treat skin infections and wounds (Ahmad et al., 2023).

Seeds: It is used as a laxative and to treat cancer, prostate, bladder, and arthritis by lowering oxidative stress and inflammation (Ahmad et al., 2023).

Flowers: Employed for treating ulcers and enlarged spleen and as aphrodisiacs (Ahmad et al., 2023).

Gum: Traditional uses include fever treatment and, in some situations, abortion induction (Ahmad et al., 2023).

Other traditional applications include treating diabetes, hypertension, diarrhea, asthma, colitis, headaches, and inflammatory illnesses. Leaf poultices are utilized to relieve glandular irritation and bronchitis (Ahmad et al., 2023).

While many traditional applications are supported by pharmacological research (anti-inflammatory, antioxidant, and antibacterial), others require further scientific validation (Ahmad et al., 2023; Razis et al., 2014).

1.6.4 CHEMICAL COMPOSITION

Chemical Composition of Moringa oleifera

Moringa oleifera includes a variety of bioactive chemicals throughout its sections. Fatty acids, phenolics, flavonoids, alkaloids, vitamins, and minerals are among the most important classes.

Essential Oils: The leaf essential oil is mostly hydrocarbons (91.1%), with hexacosane (13.9%), pentacosane (13.3%), and heptacosane (11.4%). Oxygenated monoterpenes like linalool and α-terpineol are present in trace amounts. The leaves also include flavonoids as quercetin and luteolin (Paulo et al., 2013).

Fatty Acids: The seed oil is high in unsaturated fatty acids (about 78%), particularly oleic acid (22.5%), palmitic acid (10.6%), stearic acid (6.1%), and linoleic acid. This makes the oil advantageous in both nutritional and industrial applications (Ige et al., 2016).

Phenolic and Flavonoid Content: Polyphenols found in the leaves and other parts of the plant include rutin, kaempferol, quercetin, and tannins, which contribute to antioxidant activity as well as anti-inflammatory and antibacterial characteristics (Azraida et al., 2025).

Other Constituents: Moringa contains vitamins A, C, E, calcium, potassium, and iron, indicating its nutritional value (Razis et al. 2014).

1.7 SOME SCIENTIFIC WORK DONE FOR MORINGA OLEIFERA

Hepatoprotective effects: In animal models with induced liver damage, administering an ethanolic bark extract of *Moringa oleifera* at a dose of 250-500 mg/kg body weight significantly improved liver enzyme levels (JPRI, 2019).

Cardioprotective potential: Leaf extracts at 150-400 mg/kg protected cardiac tissues from oxidative stress in preclinical investigations (Razis et al., 2014).

Anti-inflammatory mechanisms: Oral treatment of *Moringa oleifera* leaf extracts at 100-400 mg/kg reduced pro-inflammatory cytokines and decreased NF-κB activation (PMC, 2023).

Anticancer activity: Nano-formulated *Moringa oleifera* extracts shown cytotoxic effects against cancer cell lines at doses ranging from 40 to 320 µg/mL (Ahmad et al., 2023).

Antihypertensive effects: Clinical research indicated that 1-4 grams of leaf powder per day reduced blood pressure in hypertensive patients (Razis et al., 2014).

Nutritional supplementation: Daily doses of 1-8 grams of leaf powder are commonly used for nutritional support and antioxidant effects (Stohs & Hartman, 2015).

Anti-asthmatic properties: Ahmad et al. (2023) observed that bioactive compounds in leaves effectively reduce infections at MICs ranging from 25 to 200 µg/mL.

Immunomodulatory effects: Extract dosages of 200-600 mg/kg improved immune function in immunodeficiency models (Ahmad et al., 2023).

Antiparasitic synergy: Administering 500 mg/kg Moringa oleifera extract with praziquantel enhanced anti-parasitic activity (Ahmad et al., 2023).

Broad-spectrum antimicrobial effects: In animal models with caused liver injury, providing an ethanolic bark extract of Moringa oleifera at a concentration of 250-500 mg/kg body weight dramatically increased liver enzyme levels.

Antioxidant activity: In animal studies, oral dosages ranging from 100 to 400 mg/kg decreased oxidative damage (Razis et al. 2014).

Antidiabetic efficacy: Leaf extracts of 200-500 mg/kg improved glucose metabolism in diabetic rats (Razis et al., 2014).

Neuroprotective properties: Extracts administered at 50-100 mg/kg reduced brain damage in neurodegenerative disease models (Ahmad et al., 2023).

Anti-obesity effects: Leaf powder at 4 g/day improved lipid profiles and promoted weight loss in animal experiments (Rana et al., 2025).

Agricultural bio-stimulant: Moringa formulations at doses ranging from 0.5 to 2% increased crop growth and pest resistance (Ahmad et al., 2023).

1.8 AIM AND OBJECTIVE OF THE STUDY

AIM: To determine the analgesic effect of the ethanol extract of *moringa oleifera* root on pain using animal models

Specific objectives are:

1. Determine the active toxicity profile of the ethanol root bark extract of *moringa oleifera*
2. Investigate the analgesic activity of the ethanol root bark extract of *moringa oleifera*

CHAPTER TWO

MATERIALS AND METHODS

2.1 DRUGS AND CHEMICALS

Diclofenac sodium (Voltaren, Dicloflex), Pentazocine (Talwin, Fortwin), Acetic acid (Sigma-Aldrich or Merck, lab grade), Formalin (Sigma-Aldrich formaldehyde solution), Acacia gum powder (food or pharmaceutical grade from Sigma-Aldrich or local suppliers), and Distilled water (lab grade distilled water).

2.2 COLLECTION AND PROCESSING OF PLANT MATERIAL

The plant was collected Obe Quarters, Benin City, Edo State, Nigeria and identified by Prof. Akinnibosun Henry Adewale at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin (UNIBEN), Benin City, Edo State, Nigeria, with the voucher number UBH-M340. It was washed, diced into bits, shade-dried, and then oven-dried at 65℃. It was then powdered using a milling machine. The resulting powder weighed 654.2g and was stored in an airtight container for further analysis.

2.3 EXTRACTION OF PLANT MATERIAL

The plant was collected at Obe Quarters, Benin City, Edo State, Nigeria, and identified by Prof. Akinnibosun Henry Adewale at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin (UNIBEN), Benin City, Edo State, Nigeria, with the voucher number UBH-M340. The roots were washed, chopped into bits, shade-dried for 14 days, and further oven-dried at 60°C for 45 minutes. Thereafter, it was pulverized using a British milling machine. The Soxhlet extraction technique was employed to obtain bioactive constituents from *Moringa oleifera* roots. A total of 610g of dried powdered roots was loaded into a cellulose thimble and placed in the main chamber of a Soxhlet extractor. The apparatus was mounted on a round-bottom flask containing 95% ethanol, which served as the extraction solvent, and fitted with a condenser.

It was heated in the solvent to reflux, at about 90°C, and vapours formed which rose through the distillation arm and later condensed in the condenser. The solvent was condensed into the

chamber where the plant material was. Once the chamber had been filled, it was emptied automatically through a siphon side arm, which gives the solvent a significant chance to get back into the distillation flask. This was a cyclic process that guaranteed effective extraction of soluble phytochemicals in powdered leaves.

After the extraction procedure was done, the solution was dried by means of a rotary evaporator at low pressure. The concentrated extract was dried further in an oven to get rid of the solvent. The dried extract obtained weighed 15.8g; the extraction yield was 2.6%.

2.4 PREPARATION OF LABORATORY ANIMALS AND SAMPLES FOR ASSAY

Swiss mice weighing between 16 and 35 g of male sex were purchased from the Animal House of the Department of Pharmacology and Toxicology, College of Pharmacy, University of Benin, Edo State, Nigeria. They were housed in regular environmental circumstances, with free access to producers' mash and tap water. The animals were acclimated for at least two weeks and fasted overnight before to the studies.

Aqueous extract solutions were created by homogenizing measured amounts in a 5% aqueous suspension of acacia powder before dissolving them in appropriate volumes of distilled water. Similarly, diclofenac was homogenized with the needed distilled water.

Both the extract and the drug were administered to the animals according to body weight (mg/kg)

2.5 ACUTE TOXICITY TEST

Adult male Swiss albino mice weighing 17-23 g were fasted overnight with free access to water and randomly assigned to groups of five animals each. The groups were given single oral doses of *Moringa oleifera* ethanol root bark extract at 500 mg/kg, 1000 mg/kg, 2500 mg/kg, and 5000 mg/kg body weight, respectively, whereas the control group was given 3 mL/kg of distilled water. Later the animals were observed for 24 h (0.5, 1, 3, 6 and 12 h) and daily until day 14 after dosing. (OECD, 2008).

2.6 PHARMACOLOGICAL SCREENING FOR ANALGESIC ACTIVITY OF THE EXTRACT

2.6.1 ACETIC ACID-INDUCED MOUSE WRITHING TEST

The experiment was carried out using the Koster et al. (1959) approach, with 25 mice divided into five groups of five each. The first group, which served as the negative control, was given 10ml/kg of distilled water. The second group, received diclofenac sodium at a dose of 100 mg/kg. The third, fourth, and fifth groups received root bark extract at doses of 150mg/kg, 300mg/kg, and 600mg/kg, respectively. All treatments were administered 60 minutes prior to an intraperitoneal injection of acetic acid (0.6%v/v in distilled water) at a dose of 10mL/kg body weight. The number of writhes in each group was counted at 5-minute intervals for 30 minutes. Writhing was characterized as abdominal stretching along with the extension of at least one hind limb. The mean number of writhes per group was obtained to evaluate the analgesic impact of the medication (Igbe et al., 2013).

2.6.2 FORMALIN-INDUCED PAW LICKING TEST

Mice were split into groups and orally given ethanol root bark extract of *Moringa oleifera* at doses of 150 mg/kg, 300 mg/kg, and 600 mg/kg. A standard reference group received 30 mg/kg of pentazocine intraperitoneally, while the control group was given 10ml/kg of distilled water.

After 60 minutes of treatment, each mouse received a subcutaneous injection of 20 µL of 2.5% formalin solution into the plantar surface of its left hind paw. The mice were then kept in separate clear cages for 30 minutes. The time spent licking or biting the injected paw was divided into two phases: early (0-5 minutes post-injection) reflecting neurogenic pain and late (25-30 minutes post-injection) representing inflammatory pain.

The analgesic efficacy was determined by comparing the total paw licking time of the treatment and control groups over both stages, with significant decreases suggesting effective analgesia. (Hunskaar and Hole 1987)

2.7 STATISTICAL ANALYSIS

The statistical study used mean ± SEM for continuous variables and one-way ANOVA with Tukey post hoc test to assess analgesic effects between groups. Analyses were conducted using appropriate statistical tools with a significance level of $p < 0.05$.

CHAPTER THREE

RESULTS

3.1 ACUTE TOXICITY TEST

The acute toxicity research table in table 1 below demonstrated that oral treatment of *Moringa oleifera* extract caused dose-dependent mild drowsiness, diarrhea, and tremors in mice, but no mortality was detected even at the highest tested dose (5000 mg/kg).

3.2 ANALGESIC EFFECT

3.2.1 Acetic acid-induced mouse writing

Figure 1 depicts the results, which show that the extract (*Moringa oleifera*) significantly ($p<0.05$) decreased writhing at the highest dose of 600 mg/kg when compared to the control. This shows that analgesic activity of the extract was dose-dependent.

Table 3.1: Acute oral toxicity of ethanol root bark of *M. oleifera* extract in mice.

Treatments	Convulsion	Sedation	Diarrhea	Tremors	Mortality
Control	0/5	0/5	0/5	0/5	0/5
MO 500 mg/kg	0/5	0/5	0/5	0/5	0/5
MO 1000 mg/kg	0/5	0/5	0/5	0/5	0/5
MO 2500 mg/kg	0/5	0/5	0/5	0/5	0/5
MO 5000 mg/kg	1/5	0/5	0/5	0/5	0/5

0/5 = number of observed animals/ total number of animals in the group. *MO; M. oleifera*

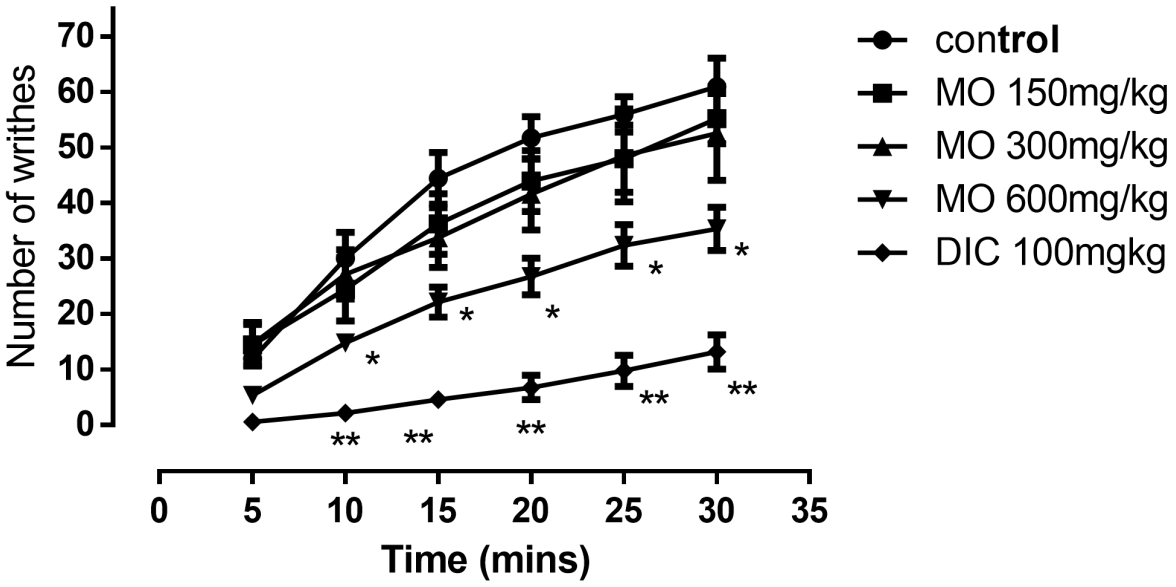


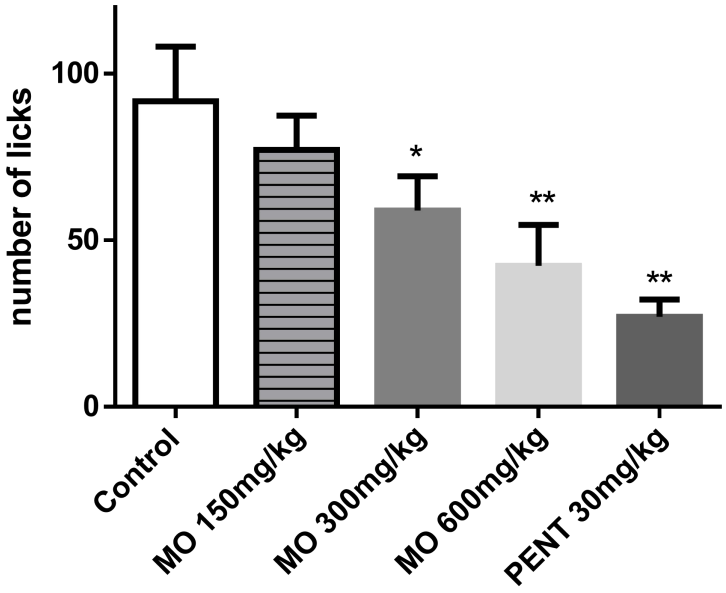
Figure 3.1: . Effect of ethanol extract of *M. oleifera* on acetic acid-induced writhing in mice. MO = *M. oleifera*, DIC = Diclofenac sodium. *p < 0.05, **p < 0.01 as compared to the control (n = 5 for each group)

3.2.2 Formalin Induced Paw Licking Test

In the early phase (Fig 3.2 A), the control group exhibited a mean paw licking duration of 91.74±18.38 seconds. Treatment with M. oleifera extract resulted in a dose-dependent reduction in paw licking time: 77.11 ± 10.38s (150 mg/kg), 58.99 ± 10.24s(300 mg/kg), and 42.36 ± 12.33s(600 mg/kg). Pentazocine showed the most pronounced effect, reducing licking time to 27.05 ± 5.22 seconds.

In the late inflammatory phase (Fig 3.2B), the control group showed a licking duration of 90.29±27.37 seconds. However, the 300 mg/kg dose markedly inhibited paw licking to 5.24 ±3.07seconds, and the 600 mg/kg dose produced a moderate reduction to58.96±11.76 seconds. Pentazocine also significantly reduced the licking time in this phase to 6.83±3.61 seconds.

A



B

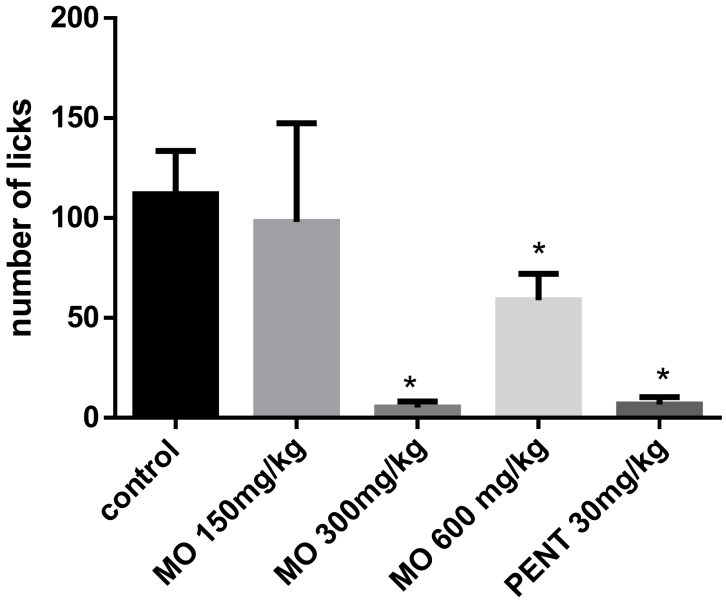


Figure 3.2: Effect of ethanol extract of *M. oleifera* in formalin induced paw-licking test on first phase (A) and second phase (B) in mice.

Each column represents the mean ± SEM of 5 mice. *$p < 0.05$; **$p < 0.01$ statistically significant compared to the control group. MO = *M. oleifera,* PENT = Pentazocin

CHAPTER 4

DISCUSSION

The acute toxicity evaluation of Moringa oleifera's ethanol root bark extract in mice revealed no death up to the highest dose tested (5000 mg/kg), indicating a high safety margin and an LD50 value greater than this dose. According to OECD rules, compounds with an LD50 greater than 5000 mg/kg are deemed practically non-toxic (OECD, 2001). Nonetheless, modest symptoms such as drowsiness, diarrhea, and tremors were noticed as doses increased, demonstrating dose-dependent mild toxic effects that were not deadly. These findings are consistent with earlier Moringa oleifera research, which have reported substantial safety margins. Kasolo et al. (2010) discovered that methanol extracts of Moringa roots have LD50 values much above 5000 mg/kg, demonstrating their low toxicity. Similarly, Igbe et al. (2020) found no mortality at large dosages of methanol extracts, but did observe modest behavioral and physiological alterations such as CNS depression and mild GI discomfort.

The absence of mortality, combined with minor reversible toxic symptoms, confirms *Moringa oleifera's* traditional use and implies that it is safe to administer orally at therapeutic levels.

The absence of mortality reveals that the ethanol extract's LD50 (median lethal dose) exceeds 5000 mg/kg, making it practically non-toxic under worldwide toxicity standards (OECD, 2001).

In this work, the analgesic effects of Moringa oleifera ethanol root bark extract were assessed using two experimental models, which commonly include the acetic acid-induced writhing test and the formalin or tail-flick test. These approaches evaluate peripheral and central analgesic actions, respectively.

The acetic acid-induced writhing test primarily reflects the peripheral analgesic effect, which causes the release of pain mediators such as prostaglandins and cytokines, resulting in characteristic writhing responses in mice. The extract significantly reduced writhing responses in a dose-dependent manner, demonstrating that it has effective peripheral

analgesic qualities. This action is most likely caused by suppression of prostaglandin synthesis or interference with peripheral nociceptive pathways, as evidenced by the extract's content of bioactive components such as flavonoids, saponins, and tannins (Kumbhare and Sivakumar, 2011; Ngueguim et al., 2017).

The second method (the formalin or tail-flick test) evaluates central and peripheral analgesic effect by evaluating pain response latency, which is frequently associated with central nervous system regulation via opioid receptors or other neurotransmitter systems. The root bark extracts increased response latency and shown central analgesic effects, perhaps through interactions with opioid receptors or CNS depression caused by phytochemicals such as alkaloids and flavonoids (Gupta et al., 1999; Leone et al., 2015). These findings are consistent with previous studies of Moringa oleifera exhibiting strong CNS depressive and analgesic activity in animal models.

The combined use of these models demonstrates that the extract has both central and peripheral analgesic effects, implying that it could be useful as a broad-spectrum painkiller. This dual activity increases the therapeutic usefulness of Moringa oleifera root bark in traditional medicine to treat pain and inflammation (Bhattacharya et al., 2018).

In terms of analgesic effects, the root bark extract showed considerable pain-relieving activity in mouse models, which is consistent with Moringa oleifera's established anti-inflammatory and analgesic properties in other part of the plant (Gopalakrishnan et al., 2016). Bioactive secondary metabolites such as flavonoids, saponins, and tannins are thought to contribute to analgesic qualities by influencing inflammatory pathways and nociceptor activation (Ngueguim et al., 2017). The extract's efficacy suggests that it could be used as a natural pain reliever, especially in resource-limited areas where conventional analgesics are less accessible or economical.

Overall, the study supports the traditional usage of Moringa oleifera root bark to manage pain and emphasizes its low toxicity risk in acute exposure. More research on chronic toxicity, precise mechanisms of analgesic action, and clinical trials in people are needed to properly define safety and efficacy profiles.

CHAPTER FIVE

5.1 CONCLUSION

This study shows that the root bark extract of *M.Oleifera* possesses analgesic properties via peripheral and central inhibitory pathways. This lends credence to its long-standing application in pain management.

Furthermore, the extract demonstrated minimal toxicity even at high doses, indicating that it is suitable for medicinal usage. These findings highlight the potential benefits for human health and promote additional investigation into its effectiveness and safety.

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APPENDIX

TEST IN MICE

		0-5 Mins (Early phase)	15-30 Mins (Late phase)
Group 1	Control	91.74 ± 18.38	90.29 ± 27.37
Group 2	M.Oleifera 150mg\kg	77.11 ± 10.38	146.08 ± 25.01
Group 3	M.Oleifera 300mg\kg	58.99 ± 10.24	5.24 ± 3.07
Group 4	M.Oleifera 600mg\kg	42.36 ± 12.33	58.96 ± 11.76
Group 5	Pentazocine 30mg\kg	27.05 ± 5.22	6.83 ± 3.61

THE EFFECT OF ETHANOL EXTRACT OF MORINGA OLEIFERA USING THE FORMALIN-INDUCED PAW LICKING

Values are mean number of paw licking ±SEM (n=5 animals per group)