ANTINOCICEPTIVE EFFECT OF THE HYDRO-METHANOL LEAF EXTRACT OF *Icacina trichantha oliv*. IN MICE

BY

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DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

(PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES)

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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A PROJECT WORK TO BE SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT FOR THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc) IN SCIENCE LABORATORY TECHNOLOGY (PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES)

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CERTIFICATION

This is to certify that this project work titled "ANTINOCICEPTIVE EFFECT OF THE HYDRO-METHANOL LEAF EXTRACT OF *Icacina trichantha* OLIV. IN MICE" was carried out by Oghomwen Myne OKORO, with matriculation number LSC2007331, of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

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DEDICATION

This research work is dedicated to Jehovah God for giving me the grace, strength, opportunity and courage to complete my undergraduate program.

ACKNOWLEDGEMENT

I am and will forever be grateful to Jehovah God for his love, guidance, infinite mercies, wisdom grace and strength for the completion of this project work and my undergraduate program.

To my supervisor Mrs. E. Okpakpor, I want to thank you for your love, kindness, support throughout the course of this work and ensuring it was a success.

I also express my warm appreciation to the Head of Department, and all the lecturers and staff of the Department of Science Laboratory at the University of Benin for their role in my intellectual journey.

To my wonderful parents Mr. Amenaghawon Okoro and Mrs.Ruth Okoro, my siblings Mary Okoro, Nosarienomwan Okoro and Comfort Okoro, may God continue to bless and reward you for your unfailing devotion and love towards me in my academic journey.

To my extended family who have always been there for me, especially in difficult times, I am so grateful for your love and support, may Jehovah continue to bless you all.

Finally, I extend my love and appreciation to my folks, Lydia, Stanley, Princess, Annabel, Blessing, Sarah and Happy who we've been through thick and thin together and to everyone who supported me during the course of my academic journey.

TABLE OF CONTENTS

LIST OF PLATES

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Pain is a complex sensory and emotional experience managed with analgesics. These drugs which has adverse effects and addiction potential have driven the search for safer alternatives from medicinal plants. *Icacina trichantha oliv.* is a plant used in West African for managing pain and inflammation. However, there is limited scientific evidence supporting such claims. This study evaluates the antinociceptive potential of the hydro-methanol leaf extract of *Icacina trichantha* in mice. Fresh leaves of *I. trichantha* was obtained, air-dried, pulverized and extracted using 1:1 methanol and deionized water to obtain H-MLE and then concentrated to dryness in an oven at 40°C. Phytochemical screening was carried out using standard methods. The antinociceptive experiment was conducted using two murine models: the hot water immersion test for and acetic acid-induced writhing test. Swiss albino mice "25" were divided into five groups of n = 5. Group I was administered deionized water, group II–IV was administered the

extract at graded doses of 100, 200, and 400 mg/kg, and group V, morphine (2mg/kg) for hot water immersion and aspirin, 100 mg/kg for the acetic acid-induced writhing. Phytochemical screening revealed the presence of flavonoids, tannins, saponins, phenolics, alkaloids and carbohydrates. Results obtained revealed that H-MLE had a significant result ($P<0.001$) in the acetic acid-induced writhing test, but had no significant result ($P>0.05$) in the hot water immersion test. This study supports its use in alleviating pain and inflammation in traditional medicine which could be due to the presence of its secondary metabolites.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND TO THE STUDY

One of the most fundamental signals the body relies on to live is Pain. It is more than a mere discomfort, it is the body's early warning system. Pain is defined as a conscious perception. In July 2020, The International Association for the Study of Pain (IASP) revised the definition of Pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage". This definition is very clear: Pain requires subjectivity, which in turn requires consciousness and the ability to evaluate a stimulus/situation (Akparian, 2018). Describing pain as an ‘experience’ separates pain from nociception.

Nociception is the sensory mechanism that allows animals to sense and avoid potentially tissue-damaging stimuli. This process relies on nociceptors, which are specialized neurons that detect and respond to potentially damaging forms of energy, like heat, mechanical and chemical energies in the environment (Daniel, 2017). Although nociception and pain are considered distinct, pain cannot occur without nociception (Sneddon, 2018). With that being established, the key ingredient for pain is the conscious brain, that is, Pain= nociception + consciousness (IASP, 2025).

Pain can be classified into four groups based on its duration, origin, pathophysiology and disease association. Based on duration, IASP describes pain as acute when it lasts from a few seconds to

three months, and is usually associated with actual or threatened tissue injury and chronic pain is pain that lasts or recurs for more than three months, and can last for several years.

On the basis of pathophysiology, pain is grouped into three, namely: Nociceptive pain, Neuropathic pain and Central sensitization (neoplastic pain).

Nociceptive pain typically originates from tissue damage caused by trauma, non-healing injury or inflammatory processes, and can be divided into two categories: somatic pain (injuries of the musculoskeletal system) and visceral pain (internal organ injury, often felt indirectly) (Clauw *et al.,* 2019; Stanos, 2016; Orr, 2017). In response to actual or potentially harmful chemical, mechanical or thermal stimuli, the two types of primary afferent nociceptors, Aδ- and C-fibres, transmit nerve signals to the dorsal horn of the spinal cord and ascending cortical pathways to the brain (Stanos and Greene, 2023; Clauw *et al.,* 2019). If the pain threshold for normal tissue damage is exceeded, peripheral sensitivity to subsequent stimuli increases (Clauw *et al.,* 2019). While sensitisation is only temporary if subsequent stimuli are short in duration, the continuous presence of stimuli and resulting sensitisation may lead to changes to the peripheral nerves and central nervous system (Clauw *et al.,* 2019). Examples of nociceptive pain are acute trauma, peptic ulcer, and arthritis (Yam *et al.* 2018).

Neuropathic pain is a category of pain developed because of nerve damage or nerve injury rather than nociceptors stimulation. The IASP terms neuropathic pain as “pain initiated or caused by a primary lesion or dysfunction of the nervous system” (Hagen and Rekand 2015). It is a pain condition that is generally chronic and occurs because of progressive nerve disease. Neuropathic pain is often described by patients as a burning, squeezing, or shooting painful sensation (Ahd *et al.,* 2023). It can happen due to damage anywhere along the nervous system e r centrally e.g.

pain associated with spinal cord injury and central post-stroke pain, or peripherally e.g. post-herpetic neuralgia and carpal tunnel syndrome (Colloca *et al.,* 2017; Finnerup *et al.,* 2021). The most clinically prevalent peripheral neuropathic pain is that related with diabetes mellitus in which consistent hyperglycemia injures the peripheral nerves throughout the body especially those of the feet and legs (Ahd *et al.,* 2023).

Central sensitization (also known as nociplastic pain, sensory hypersensitivity or central hypersensitivity) manifests as the perception of pain in the absence of pain receptor activation (Clauw *et al.,* 2019; Stanos, 2016). Nociplastic pain disorders are often coupled with other comorbidities, such as sleep disturbances, fatigue, memory dysfunction, and mood problems (Ahd *et al.,* 2023). Examples for nociplastic pain are fibromyalgia and irritable bowel syndrome (Fitzcharles *et al.,* 2021). Central sensitization can explain why many people suffer from chronic non-specific pain in the total lack of nerve or tissue damage and a clear activator of nociceptors (Ahd *et al.,* 2023). The IASP, who was among the first to recognize the CS phenomenon, presented the term "nociplastic pain" in 2017 as the third type of pain, which is distinct from nociceptive and neuropathic pain. Nociplastic pain is described by the IASP as "pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain" (Kosek *et al.,* 2021).

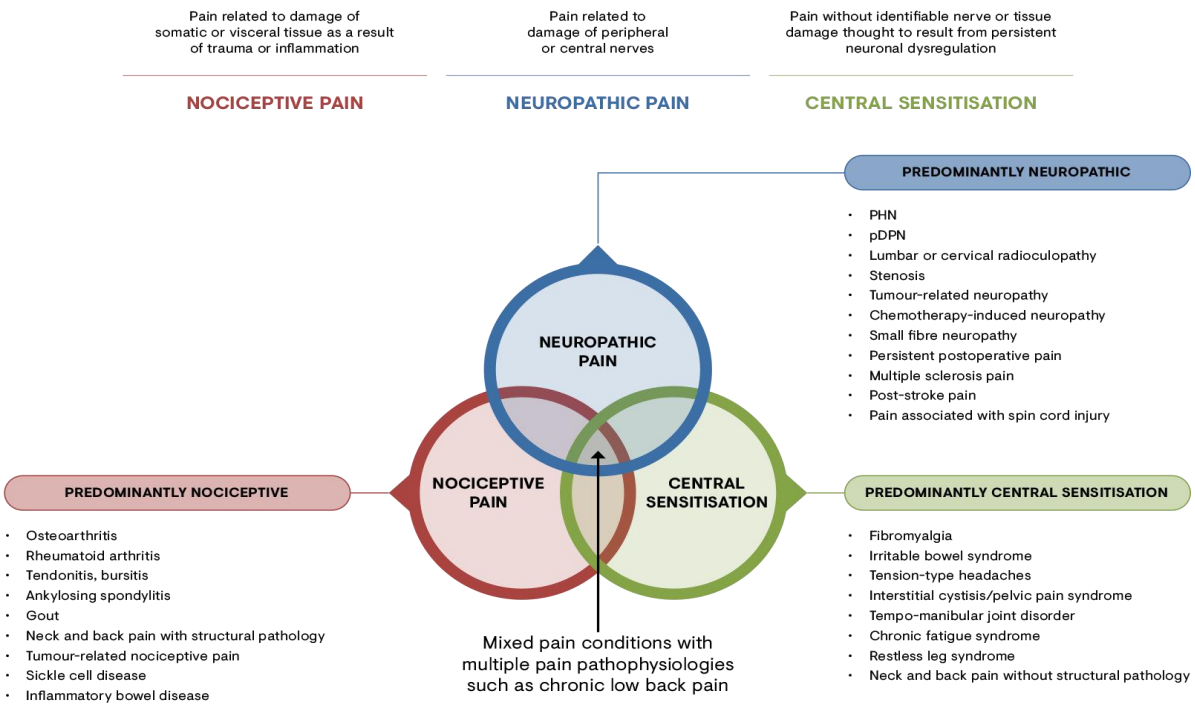

Figure 1: Three types of pain pathophysiology (Stanos *et al.,* 2016)

On the basis of disease association, Chronic Pain comprises the most common clinically relevant disorders. These disorders are divided into 7 groups: chronic primary pain, chronic cancer pain, chronic posttraumatic and postsurgical pain, chronic neuropathic pain, chronic headache and orofacial pain, chronic visceral pain, and chronic musculoskeletal pain (Treede *et al.,* 2015).

Chronic primary pain is pain in 1 or more anatomic regions that persists or recurs for longer than 3 months and is associated with significant emotional distress or significant functional disability (interference with activities of daily life and participation in social roles) and that cannot be better explained by another chronic pain condition (Treede *et al.,* 2015).

Chronic cancer pain includes pain caused by the cancer itself (the primary tumor or metastases) and pain that is caused by the cancer treatment (surgical, chemotherapy, radiotherapy, and others) (Treede *et al.,* 2015).

Chronic postsurgical and posttraumatic pain is defined as pain that develops after a surgical procedure or a tissue injury (involving any trauma, including burns) and persists at least 3 months after surgery or tissue trauma (Treede *et al.,* 2015).

Chronic neuropathic pain is caused by a lesion or disease of the somatosensory nervous system. The somatosensory nervous system provides information about the body including skin, musculoskeletal, and visceral organs.

Chronic headache and chronic orofacial pain is defined as headaches or orofacial pains that occur on at least 50% of the days during at least 3 months.

Chronic visceral pain is persistent or recurrent pain that originates from the internal organs of the head and neck region and the thoracic, abdominal, and pelvic cavities.

Chronic musculoskeletal pain is defined as persistent or recurrent pain that arises as part of a disease process directly affecting bone(s), joint(s), muscle(s), or related soft tissue(s).

1.2 Mechanism of pain (Nociceptive Pathways)

Nociception encompasses four stages: transduction, transmission, modulation, and perception.

During transduction, a physical or chemical stimulus is converted into an electrical signal that can then be transmitted. This process occurs in the periphery at site of cell damage that is caused by noxious stimuli. Cell damage releases excitatory neurotransmitters e.g. substance P (SP), prostaglandins (PG), bradykinin (BK), and histamine (H) which stimulate nociceptors (Ahd *et al.,* 2023).

Transmission refers to the movement of this electrical activity through the nervous system (Karcz *et al.,* 2024). The pain impulse travels along afferent nociceptive fibers from the peripheral site, where cell damage occurs, to the spinal cord's dorsal horn, then up to the brainstem, after that it reaches the thalamus and finally the cerebral cortex (Ahd *et al.,* 2023).

Perception is when somatosensory transmission results in the subjective experience of pain (Karcz *et al.,* 2024). This phase occurs when the person becomes conscious or aware of the pain.

Lastly, Modulation is the alteration of neuronal activity through the pathways of transmission. During this final phase, the brain alters or modulates the pain by releasing inhibitory neurotransmitters e.g. endorphins, norepinephrine (NE) and serotonin (5-HT) that run down to the spinal cord inhibiting the painful impulses transmission (Ahd *et al.,* 2023).

Various physical stimuli in the environment can damage organisms and are detected by nociceptors. These can be activated by extreme heat, intense cold, high-force mechanical pressure, and a range of chemical agents like capsaicin or inflammatory mediators (Alamri *et al.,* 2018; Grzybowski *et al.,* 2022). Nociceptors are broadly classified based on their conduction velocity, axon diameter, myelination status, and the modality of stimulus to which they respond (Bautista *et al.,* 2022).

There are two major classes of nociceptive fibers: Aδ fibers, which are thinly myelinated and conduct impulses rapidly (approximately 5–30 m/s), and C fibers, which are unmyelinated and slower (approximately 0.4–2 m/s) (Grzybowski *et al.,* 2022). Aδ fibers typically mediate the sensation of sharp, localized "first pain," while C fibers mediate dull, burning, or aching "second pain" (Wiebke *et al.,* 2020).

Nociceptors can also be classified based on the range of stimuli they respond to. Polymodal nociceptors, which are predominantly C fibers, can detect multiple noxious stimuli—including mechanical, thermal, and chemical inputs—making them critical to broad-spectrum pain signaling (Alamri *et al.,* 2018). In contrast, mechanically sensitive Aδ nociceptors are more specialized, typically responding to e r mechanical or thermal stimuli alone (Bautista *et al.,* 2022).

A unique subclass of nociceptors known as "silent" nociceptors are normally unresponsive to mechanical stimuli but become active under conditions of inflammation, contributing significantly to inflammatory pain and hyperalgesia (Wiebke *et al.,* 2020).

1.3 Antinociceptive drugs

Antinociceptive drugs are agents that interfere with the transmission or perception of noxious stimuli, thereby reducing or inhibiting the sensation of pain without necessarily affecting the underlying cause. Unlike analgesics, which broadly refer to substances that relieve pain, antinociceptive agents specifically block or suppress the activation of nociceptors or the nociceptive pathways in the central and peripheral nervous systems (Corder *et al.,* 2018; Yaksh and Wallace, 2018). These drugs exert their effects at various stages of the pain signaling process, including peripheral transduction, spinal transmission, and central perception (Chung and Campbell, 2022).

They include non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit prostaglandin synthesis via cyclooxygenase (COX) blockade, opioids that act on μ-opioid receptors to prevent neurotransmitter release, and newer agents like gabapentinoids and antidepressants, which modulate calcium channels and monoaminergic systems to dampen excitatory signaling (Baron

et al., 2021). Importantly, many antinociceptive agents also enhance the body's descending inhibitory pain pathways, such as those involving serotonin, norepinephrine, and endogenous opioids, contributing to a multi-level modulation of pain (Mickle and Gereau, 2018).

1.3.1 Nonsteriodal antiinflammatory drugs

These drugs typically work by blocking cyclooxygenase (COX), an enzyme responsible for the production of prostaglandins (PGs), which are strong inflammatory mediators.Traditional NSAIDs, however, inhibit both COX-1 and COX-2 isoforms and have been linked to serious gastrointestinal adverse effects such as ulcers and an increased risk of bleeding (Arfeen, 2024). For acute mild to moderate pain, first-line treatment options include acetaminophen and NSAIDs, while topical NSAIDs are recommended for non-low back musculoskeletal injuries (Ameachi, *et al.,* 2021). In cases of severe or refractory acute pain, medications that work on opioid and monoamine receptors or acetaminophen/opioid or NSAID/opioid combinations may be used (Anekar and Cascella, 2022). However, NSAIDs are not effective for the management of neuropathic pain (Queremel and Davis, 2022).

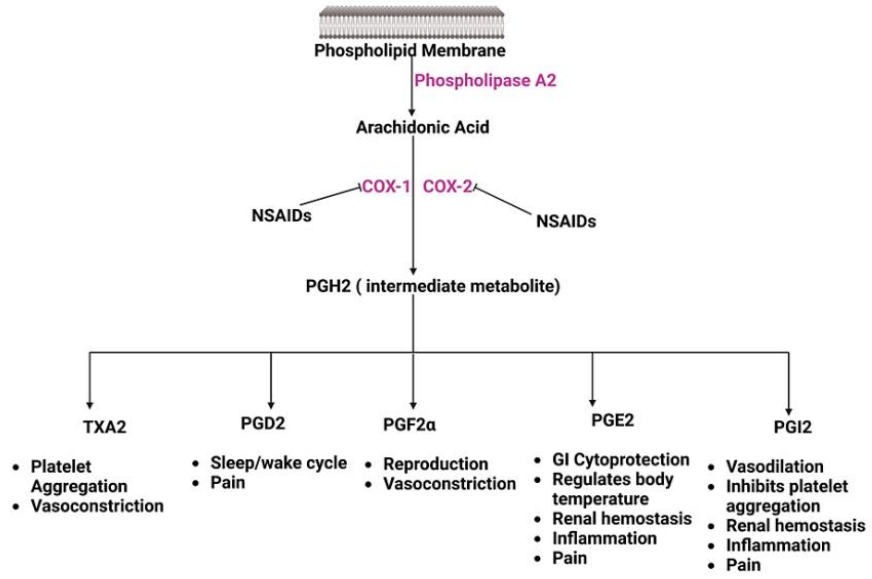

Figure 2: Mechanism of action of NSAIDs (Sohail *et al.,* 2023)

1.3.2 Opioids

Opioids are the oldest and most potent drugs for the treatment of severe pain (Stein, 2016). The National Institute on Drug Abuse (NIDA) highlights opioids as a class of natural, semi-synthetic, and synthetic drugs. These include both prescription medications used to treat pain and illegal drugs like heroin. Natural opioids, such as morphine, codeine, opium, and thebaine are made from the seed pods of the opium poppy plant. These natural substances are also known as opiates. Semi-synthetic opioids, such as heroin and the pain relievers oxycodone, hydrocodone, and oxymorphone, are made in laboratories by chemically processing natural opioids. Synthetic opioids, such as fentanyl are manufactured entirely in laboratories, with no natural ingredients.

Opioids work by binding to specific molecules called opioid receptors on certain nerve cells in the brain, the spinal cord, and throughout the body, that is, the central and peripheral nervous

systems (Corder *et al.,* 2018; Stein, 2016). Opioids exert their effects by binding to and activating three main classes of opioid receptors: mu (μ), kappa (κ), and delta (δ), which are all G-protein-coupled receptors (GPCRs) located throughout the central and peripheral nervous systems (Corder *et al.,* 2018).

The μ-opioid receptor (MOR) is the primary mediator of analgesia. When opioids bind to MORs, they initiate a cascade of intracellular events that include inhibition of adenylyl cyclase, a reduction in cyclic AMP (cAMP) levels, closure of voltage-gated Ca^{2+} channels, and opening of inwardly rectifying K^{+} channels. This leads to hyperpolarization of neurons and inhibition of neurotransmitter release, ultimately reducing the transmission of nociceptive signals (Corder *et al.,* 2018).

In contrast, kappa (κ) receptors (KORs) are found predominantly in the spinal cord and brainstem, and their activation leads to spinal analgesia, sedation, and dysphoria. KOR agonists also reduce the release of substance P and glutamate at the spinal level but are less effective at producing supraspinal analgesia (Bruchas and Roth, 2016).

The δ-Opioid Receptors (DORs) are involved in modulating mood and emotional components of pain. Structural analysis has revealed their high-resolution crystal structure, illustrating how allosteric sodium ions influence receptor conformation, functional selectivity, and downstream signaling bias (Kelly *et al.,* 2023). By targeting these different receptors, opioids not only block the sensation of pain but also influence mood, respiration, gastrointestinal motility, and the reward system, depending on receptor subtype and distribution (Yaksh and Wallace, 2018).

1.3.3 Antidepressants

Antidepressants were originally developed to treat mood disorders, but now, antidepressants particularly tricyclic antidepressants (TCAs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) are now well-established as first-line agents in chronic pain management, especially for neuropathic pain (Finnerup *et al.,* 2021).Antidepressants reduce pain not by altering nociceptive input directly, but by enhancing descending monoaminergic inhibition through elevated synaptic levels of serotonin and norepinephrine (Yekkirala *et al.,* 2017; Finnerup *et al.,* 2021). The TCAs (e.g., amitriptyline, nortriptyline) block both 5-HT and NE reuptake and also inhibit sodium channels, further contributing to analgesia. SNRIs (e.g., duloxetine, venlafaxine) selectively inhibit 5-HT and NE reuptake and have fewer anticholinergic side effects than TCAs. SSRIs (e.g., fluoxetine, sertraline), though effective for depression, are generally less effective for pain unless combined with other agents (Finnerup *et al.,* 2021).

1.4 Experimental models used for this study

In this study, two established models were used: the acetic acid-induced writhing test and the tail flick test.

1.4.1 Acetic acid-induced mouse writhing model

The acetic acid-induced writhing test is a widely accepted model for assessing peripheral antinociceptive activity, particularly in relation to visceral pain. Following intraperitoneal injection of dilute acetic acid, mice exhibit a stereotyped response characterized by abdominal constrictions, known as “writhing.” This nociceptive behavior results from the release of inflammatory mediators, such as prostaglandins and bradykinin, which activate and sensitize peripheral nociceptors (Deuis *et al.,* 2017; Zarei *et al.,* 2022). The writhing model is highly

sensitive to NSAIDs and natural anti-inflammatory agents, making it useful for screening peripherally acting compounds.

1.4.2 Hot water immersion tail flick model

In contrast, the tail immersion test using hot water is a classic model for evaluating thermal nociception mediated through central spinal reflexes. In this method, the distal part of the rodent's tail is immersed in water maintained at a noxious temperature (typically 50–55 °C), and the latency to flick or withdraw the tail is measured. Increased tail withdrawal latency following drug administration is interpreted as an indication of central antinociceptive activity, particularly involving spinal or supraspinal opioid-like pathways (Oliveira *et al.,* 2020; Nwabunike *et al.,* 2021).

1.5 Renewed Interest in Plant-Based Therapeutics

In recent decades, there has been a growing shift toward the use of plant-derived medicines in the search for safer, more affordable, and effective therapies. This resurgence is partly driven by the limitations of conventional drugs, including adverse effects, tolerance, dependence, and high toxicity profiles. In contrast, many medicinal plants are perceived to have lower lethal doses (LD_{50}) and are often associated with better safety margins when used appropriately (Atanasov *et al.,* 2021). Traditional knowledge systems have long utilized these natural products, and recent pharmacological studies are increasingly validating their therapeutic potential through scientific inquiry. Examples of Medicinal Plants and its therapeutic effects are:

1. *Curcuma longa* (Turmeric) used for anti-inflammatory, antioxidant, and anticancer activities. Curcumin is studied in the management of arthritis, neurodegenerative diseases, and metabolic syndromes (Hewlings and Kalman, 2017).

2. *Zingiber officinale* (Ginger) used for managing nausea, motion sickness, and pain relief. Also shows anti-inflammatory properties beneficial in osteoarthritis (Mashhadi *et al.,* 2013).

3. *Cannabis sativa* (Medical Cannabis) used for chronic pain, epilepsy, and multiple sclerosis-related spasticity. Increasingly studied for neuropathic pain and chemotherapy-induced nausea (Whiting *et al.,* 2015).

4. *Moringa oleifera* (Drumstick tree) used as an antioxidant, antidiabetic, and anti-inflammatory effects. Studied in the management of hypertension and metabolic disorders (Leone *et al.,* 2015).

5. *Salix alba* (White willow bark) used for Pain and inflammation. Used traditionally as a natural analgesic and antipyretic, especially in musculoskeletal conditions (Shara and Stohs, 2015).

1.6 AIM AND OBJECTIVES OF THE STUDY

The aim of this study is to evaluate the antinociceptive potential of hydromethanol leaf extract of *Icacina trichantha* in albino mice using hot water immersion tail flick and acetic acid-induced mouse writhing models

The objectives were:

1. To assess the central antinociceptive activity of *Icacina trichantha* extract using the tail flick model in mice;

2. To evaluate the peripheral antinociceptive activity of the extract using the acetic acid-induced writhing test;

3. To compare the extract's effects with a standard analgesic drug morphine and acetylsalicylic acid(Aspirin) and negative control; and,

4. To observe dose-dependent effects of the plant extract in both models.

CHAPTER TWO

LITERATURE REVIEW

2.1 Icacina trichantha

The tropical forest tree family, Icacinaceae, was first recognized by Miers in 1864 and then revised on the basis of DNA sequencing as given by Karehed in 2001. *Icacina trichantha* (false yam), a drought-resistant shrub indigenous to West and Central Africa, is a medicinal plant used by the natives such as the indigenous tribes in Nigeria. It is known as "Urumbia" or "Eriagbo" (referring to its emetic effect) among the Igbos of Nigeria, or "Gbegbe" (meaning to cleanse) by the Yoruba of western Nigeria (Che *et al.,* 2014)

Icacina trichantha is a shrub that can grow up to two meters high (Che *et al.,* 2019). It is characterized by the large, fleshy, yam-like underground tubers, as big as several kilograms in weight, which are often consumed by tribal people. The tuber is rich in starch and can be eaten fresh or processed into flour to make soup, pastes or porridges. The traditional way of preparation involves cleaning, slicing, and soaking in water for several days to soften the mass and leach out bitter components (Wahua and Awogbayila, 2024). After drying under sunlight, the tuber is pulverized and sieved to produce a grayish-white or creamy- yellowish flour (Umoh and Iwe, 2014). It serves as a source of emergency moisture and food energy as a famine-food during long periods of drought. Both the nutrient composition and "anti-nutritional factors" (e.g. the bitter substances such as hydrogen cyanide, oxalates, tannins, phytates and alkaloids) of the flour have been analyzed, showing the presence of not only carbohydrates (mostly starch), lipids and proteins, but also mineral elements such as potassium, sodium and calcium (Okosun *et al.,* 2020).

The fruit of this plant is a drupe with a soft sweet outer pulp which is edible (Wahua and Awogbayila, 2024). In western Nigeria and neighboring areas, *Icacina trichantha* is used as a common household medicine for emergency and first-aid treatment for food poisoning (Che *et al.,* 2019). Tubers and leaves of the plant are allegedly aphrodisiacs. The leaves and seeds, when crushed and macerated in local gin, can be used for the treatment of hypertension and asthma (Che *et al.,* 2019). Its tubers are used by traditional healers to treat various medical conditions including constipation, poisoning, malaria, rheumatism, toothache, as well as to induce emesis and abortion (Che *et al.,* 2019). The tuber juice can be used for treating mumps (Okosun *et al.,* 2020).

2.2 Taxonomy

Kingdom: Plantae

Phylum: Streptophyta

Class: Equisetopsida

Subclass: Magnoliidae

Order: Icacinales

Family: Icacinaceae

Genus: Icacina

Species: *Icacina trichantha*

2.3 Botanical description

Icacina trichantha also known as false yam is a specie in the genus *Icacina* (family Icacinaceae), a small group of drought-tolerant shrubs or small trees known for their large underground tubers. Members of this genus are native to tropical Africa and are recognized for their survival under harsh environmental conditions and their traditional medicinal uses (Wahua and Awogbayila, 2024).

This species is widely distributed in West Africa, especially in Nigeria, Ghana, Benin, Togo, and Cameroon, where it grows naturally in savanna regions and open woodland. It thrives in dry, sandy soils and is often found in areas where few other crops can survive (Burkill, 1985; De Wet *et al.,* 2023).

Icacina trichantha is a perennial shrub or small tree that can grow up to 2 meters tall. It is characterized by its thick, fleshy tuberous roots, which can weigh over 60 kg and are used in times of food scarcity (Wahua and Awogbayila, 2024; Bassey *et al.,* 2024).

LEAVES: The leaves are simple, broadly ovate to lanceolate, with entire margins and pinnate venation. They are arranged alternately and are generally green and glabrous or sparsely hairy, depending on the age and condition of the plant. Anatomically, the leaves exhibit paracytic and diacytic stomata on the abaxial (lower) surface and unicellular glandular trichomes on the adaxial (upper) surface (Wahua and Awogbayila, 2024).

FLOWERS:The plant has broad, simple leaves and produces small, greenish-white flowers. Its flowers are small, bisexual, and greenish-white, arranged in dense axillary clusters (De Wet *et al.,* 2023).

FRUIT: The fruit is an ovoid drupe, about 3 cm in diameter, initially green and covered in soft hairs, turning dark brown to black when mature. The fruits are globose and hairy when young, turning dark brown or black when mature. The tuber, which may weigh several kilograms, is rich in starch and traditionally used as a famine food, though it requires detoxification before consumption (Bassey *et al.,* 2024).

SEED: The seed is hard and smooth with a characteristic reticulate endocarp (De Wet *et al.,* 2023).

TUBER: The fleshy tuber is the storage organ, allowing the plant to endure adverse environmental conditions. Nutritionally, the tuber is rich in carbohydrate but lower in protein and mineral content compared to the leaves. Nonetheless, its high starch content makes it crucial as an emergency food source during droughts (Alawode, 2024).

HABITAT: *Icacina trichantha* grows naturally in savanna ecosystems and open woodlands, particularly in West and Central Africa, including countries like Nigeria, Benin, Ghana, Togo, and Cameroon (Burkill, 1985; POWO, 2024). It thrives in dry, sandy or lateritic soils and is well adapted to drought-prone environments. The plant is typically found in disturbed soils, forest edges, and fallow farmlands. Its deep tuber system allows it to survive long dry seasons, making it an important survival crop in rural communities. It grows best under full sunlight, and while it can tolerate poor soil conditions, it prefers well-drained soils (Wahua and Awogbayila, 2024).

Plate 1: The leaves of *Icacina trichantha* (Wahua and Awogbayila, 2024).

2.4 Phytochemicals Screening

Icacina trichantha is rich in a variety of bioactive phytochemicals that contribute to its medicinal and nutritional properties. Major phytochemical groups identified are:

1. **Alkaloids:** Bassey *et al.* (2024) reported that both tuber and leaf extracts tested positive for alkaloids, flavonoids, saponins, tannins, phenols, and glycosides.

2. **Flavonoids:** Flavonoids are abundant in the leaves contributing to its antioxidant, anti-inflammatory, and hepatoprotective activities (De Wet *et al.,* 2023).

3. **Saponins**: Saponins are present in significant quantities in both fresh and dried parts of the plant and have been linked to immune-modulating and cholesterol-lowering effects (Wahua and Awogbayila, 2024).

4. **Tannins**: This was detected mainly in the tubers, they exhibit astringent and antimicrobial properties.

5**. Phenolics** identified through spectrophotometric analysis contribute to the plant's antioxidant activity.

6. **Terpenoids and Essential Oils**: Essential oil analysis revealed compounds like phytol, oleic acid, and linoleic acid, which have known antimicrobial and antioxidant potential (De Wet *et al.,* 2023).

2.5 Traditional uses of *Icacina trichantha*

The analgesic and anti-inflammatory applications of I. trichantha is one of its most well-documented traditional uses. Contemporary ethnopharmacological surveys conducted in Nigeria and Ghana have confirmed its employment by traditional healers for managing various pain conditions, including headache, dental pain, and musculoskeletal discomfort (Agyare *et al.,* 2021). The plant is typically prepared as a decoction or infusion of leaves for oral administration,

while topical applications involve macerated leaves formulated into poultices for joint inflammation and localized pain (Quazi *et al.,* 2023). This documented use provided the ethnobotanical rationale for your experimental investigation of its antinociceptive properties using contemporary pharmacological models.

Beyond pain management, recent research has documented the plant's continued use in managing febrile conditions. Traditional medical practitioners in endemic regions employ *I. trichantha* preparations as antipyretics, particularly in cases of malaria and other febrile illnesses (Ntie-Kang *et al.,* 2022). The preparation typically involves aqueous extracts administered orally, with practitioners reporting diaphoretic effects. Additionally, gastrointestinal applications remain prevalent, with the plant being used to manage diarrhea and dysentery, likely due to its tannin content which may confer astringent properties (Kuete, 2021).

The plant's dermatological applications have also been documented in recent ethnobotanical surveys. Traditional practitioners utilize leaf preparations for wound care and management of skin infections, suggesting potential antimicrobial and wound-healing properties that warrant further investigation (Adinortey *et al.,* 2022). Furthermore, the tuberous roots continue to be recognized as emergency food sources during periods of food insecurity, though modern nutritional studies have highlighted the need for proper processing to eliminate potential toxic compounds (Sofowora *et al.,* 2022).

2.6 Pharmacological Effects of *Icacina trichantha*

Icacina trichantha has demonstrated several pharmacological activities in both traditional and modern studies. These effects are largely attributed to its rich phytochemical content, including flavonoids, alkaloids, saponins, tannins, phenolics, and essential oils.

1. Anti-inflammatory Effect

Icacina trichantha has demonstrated significant anti-inflammatory activity in both topical and systemic models. In a study using the Croton oil-induced ear edema model in mice, a chloroform fraction of the plant's tuber reduced inflammation with an ID_{50} (dose that inhibits 50% of the response) of 107 µg/cm, which was comparable to the standard anti-inflammatory drug indomethacin (93 µg/cm²) (Asuzu *et al.,* 1999). Furthermore, in carrageenan-induced paw edema in rats—a classic model for studying acute inflammation—the same extract administered orally at doses of 50, 100, and 200 mg/kg resulted in a dose-dependent inhibition of swelling by 15%, 20%, and 34%, respectively, compared to 40% inhibition produced by 10 mg/kg of indomethacin. These findings suggest that the plant possesses both peripherally and centrally acting anti-inflammatory constituents, potentially due to the presence of flavonoids and saponins which modulate inflammatory pathways.

2. Antioxidant Activity

The antioxidant potential of *Icacina trichantha* has been validated through both in vitro and in vivo studies. In a DPPH free radical scavenging assay, the methanol extract of the tuber exhibited 67.3% inhibition at a concentration of 400 µg/mL, which was comparable to the standard antioxidant ascorbic acid (80.3% at the same concentration) (Onakpa *et al.,* 2016). In a ferric reducing antioxidant power (FRAP) assay, the extract showed a reducing capacity of 6.7 µM at 800 µg/mL, confirming its electron-donating potential. In vivo studies involving rats fed with 0.5–1.0 g/kg of the extract for 12 weeks showed a significant increase in antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), with a

concurrent decrease in malondialdehyde (MDA), a marker of oxidative stress. These results confirm the plant's capability to protect biological systems from oxidative damage.

3. Antidiabetic and Organ-protective Activity

The antidiabetic properties of *Icacina trichantha* were evaluated in alloxan-induced diabetic rats, where oral administration of the methanol tuber extract at 200, 400, and 600 mg/kg led to a significant and dose-dependent reduction in fasting blood glucose levels. At the highest dose, blood glucose dropped from around 14.95 mmol/L to \~2.00 mmol/L by day 21 (Onakpa and Asuzu, 2013). Additionally, liver enzyme levels (ALT, AST, ALP) and renal biomarkers (urea and creatinine) were normalized, indicating protection of liver and kidney function. Histological examination of the pancreas revealed regeneration of pancreatic β-cells, especially at doses of 400–600 mg/kg. These results were comparable to those obtained with glibenclamide, a standard antidiabetic drug. The findings suggest that *I. trichantha* not only controls hyperglycemia but also reverses tissue damage associated with diabetes.

4. Cytotoxic and Anticancer Properties

Recent phytochemical studies have isolated several novel 17-norpimarane diterpenoids from the tuber of *Icacina trichantha*, among which humirianthenolide C showed remarkable cytotoxicity. In vitro tests against human cancer cell lines revealed IC_{50} values of 0.66 μM for MDA-MB-435 (melanoma), 0.67 μM for MDA-MB-231 (breast cancer), and 1.05 μM for OVCAR3 (ovarian cancer) (Zhao *et al.,* 2015). These values indicate high potency, comparable to or better than many chemotherapeutic agents. The compounds likely induce apoptosis and inhibit cell proliferation, although the exact molecular mechanism is still under investigation. This positions *I. trichantha* as a potential source of anticancer lead compounds.

5. Antimicrobial Activity

Extracts from the leaves and tubers of *Icacina trichantha* have shown strong antimicrobial activity against a range of pathogens. Ethanol and aqueous extracts were tested using agar well diffusion methods against Escherichia coli, Staphylococcus aureus, Streptococcus spp., Klebsiella pneumoniae, and Candida albicans. The ethanol extract produced zones of inhibition up to 18 mm, especially against S. aureus, suggesting greater efficacy than the aqueous extract (Shagal and Kubmarawa, 2013). The antimicrobial activity is believed to be due to the presence of alkaloids, tannins, phenolics, and saponins, which are known to disrupt microbial membranes and inhibit enzyme function. These findings align with the traditional use of the plant for treating skin infections, wounds, and gastrointestinal disturbances.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant collection and identification

Fresh leaves of *Icacina trichantha* were purchased from the capital in Ovia North East Local Government Area, Benin City, Nigeria. The plant's identification was conducted by Prof. E.I Aigbokhan, and voucher number UBH-1185 was issued by Dr. H.A Akinigbosun, both from the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria. Following collection, the leaves underwent cleaning, drying, pulverization into powder, and were then stored in an airtight container for subsequent use.

3.2 Preparation of plant extract

The fresh leaves of *Icacina trichantha* were cleaned, air-dried in a cool dry room, and was pulverized into powder and was stored in an airtight container. 100 g of the pulverized plant was weighed into a maceration bottle, and a volume of 50:50 of deionized water and methanol was added to the maceration bottle, in volumes it was 600 ml each of both solvents. The sample was left for 72 hours to soak and was shaken vigorously everyday. On the third day, a sieving cloth was used to filter the mixture, the residue was discarded, the filtrate was poured in a container and placed in an oven to concentrate to dryness at 40°C for 36 hours. The concentrated extract was stored in a plain container, labeled and placed in the fridge.

3.3 Experimental animals

Swiss albino mice weighing between 16-27 g were obtained from the animal facility located within the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of

Benin, Benin City, Nigeria. The animals were kept in standard plastic cages and had free access to water and feeds (Livestock feeds Ltd Ibadan Nigeria) and were exposed to natural lighting and room temperature

3.4 Experimental design

The Swiss albino mice were placed in two groups of 25 mice each and left to acclimatize for 14 days. After the 14 days, the mice were placed in 5 groups of 5 mice each for one model.

Group 1 was the negative control group and was administered deionized water only

Group 2, 3 and 4 were administered 100 mg/kg, 200 mg/kg and 400 mg/kg dose of the extract respectively.

Group 5 was administered the standard drug

3.5 Phytochemical Screening

Simple chemical tests were carried out on the crude powdered sample and the methanol extract according to standard procedures to identify the phytochemical constituents (Stahl, 1973; Sofowora, 1982; Harborne, 1998; Evans, 2002).

Approximately 5 g of the crude powdered sample was boiled with 75 mL of distilled water for 30 minutes. The solution was filtered hot and allowed to cool. The filtrate obtained was used to carry out the following tests.

3.5.1 General Tests for Alkaloids

Two drops of Dragendorff's reagent was added to 2 mL of the filtrate.

Two drops of Wagner's reagent was added to 2 mL of the filtrate.

Two drops of Hager's reagent was added to 2 mL of the filtrate.

Two drops of Mayer's reagent was added to 2 mL of the filtrate.

3.5.2 Tests for Carbohydrates

Molisch's Test

To 2 mL of filtrate was added 2 drops of 1% alcoholic naphthol followed by 2 mL of concentrated sulphuric acid at a slanting position.

3.5.3 Tests for Reducing Sugars

Fehling's Test

To 2 mL of filtrate was added 2 drops of Benedict's reagent (a mixture of equal volumes of Fehling's solution A and B). The resulting solution was heated over a boiling water bath for 3 minutes.

Tollen's Test

Dilute ammonium hydroxide solution was added dropwisely to silver nitrate solution containing a few drops of 10% sodium hydroxide until the precipitate of silver oxide almost completely dissolved. Few drops of the filtrate was then added to the mxture above.

Keller Kiliani's Test for Deoxysugars

To 2 mL of filtrate was added few drops of dilute acetic acid containing a trace of 5% ferric chloride. The resulting mixture was transferred to the surface of concentrated sulphuric acid.

3.5.4 Test for Saponins

Frothing Test

The filtrate (1 mL) was diluted with 10 mL distilled water and shaken vigorously for one minute.

Fehling's Test

To 10 mL of the filtrate was added 5 mL of dilute H_2SO_4. The mixture was boiled for 15 min, filtered and cooled. 2.5 mL of the filtrate was made alkaline with 20% NaOH solution and boiled with 0.1 mL each of Fehling's solutions A and B for 2 minutes.

Lieberman Burchard's Test for Steroidal saponins or Phytosterols

sA mixture of 1 mL chloroform and few drops of acetic anhydride was added to 2 mL of the filtrate. To the final mixture was added 2 drops of concentrated sulphuric acid.

3.5.5 Test for Tannins

Gelatin Test

To 2 mL of the filtrate was added 2 mL of 1% gelatin solution in 10% NaCl.

Test for Terpenoids

Salkowski Test

The filtrate (5 mL) was mixed with 2 mL of chloroform and concentrate H_2SO_4 was carefully added (drop wise) to form a layer.

Test for Phenolic compounds

Ferric chloride Test

To 2 mL of filtrate was added 5 mL of distilled water followed by 2 drops of 5% ferric chloride solution. A blank test was done by adding 2 drops of 5% ferric chloride solution to 5 mL of distilled water.

Folin Ciocalteu's Test

To 5 mL of filtrate was added 0.5 mL 10 % folin ciocalteu's phenol reagent followed by 5 mL of 7% Na_2CO_3.

Test for Flavonoids

Alkaline reagent Test

To 2 mL of filtrate was added few drops of 20% sodium hydroxide solution followed by few drops of dilute hydrochloric acid solution.

Lead acetate Test

Few drops of lead acetate solution was added to 2 mL of the filtrate.

Aluminium chloride Test

The filtrate (3 mL) was shaken with 0.1 mL each of 1% $AlCl_3$ solution and 1 M CH_3COOK solution. The mixture was allowed to stand for 30 min.

Test for Anthraquinone Derivatives

Bontreger's Test

The filtrate (2 mL) was shaken with 2 mL of petroleum ether. The ether layer was washed with 2 mL distilled water and then shaken with dilute ammonia solution.

Test for Proteins

Xanthoproteic Test

Few drops of concentrated nitric acid was added to 2 mL of the filtrate.

Ninhydrin Test

Two drops of Ninhydrin solution was added to 2 mL of the filtrate.

3.6 Hot water immersion tail flick model

The mice were weighed, marked and placed in 5 groups of 5 mice each. The necessary calculations were for the concentration and volume to be administered to each mice were done. Morphine (2mg/kg), was administered subcutaneously and the *Icacina trichantha* extract was administered orally. 3-minute interval was taken after each administration and the time of administration was noted. Water was placed in a beaker and was heated up using the double boiling method by placing the beaker of water in a hot water bath at 58°C. 30 minutes after each administration, the mice was picked, its tail relaxed and immersed in the hot water, and the latency time was recorded for each of them. This process was repeated for the next 60, 90 and 120 minutes after administration.

3.7 Acetic acid-induced mouse writhing model

The mice were weighed, marked and placed in 5 groups of 5 mice each. The calculations for the volume and concentration to be administered was done and a 3-minute interval was taken too after each administration. 0.6% acetic acid was gotten from the 96% stock solution. The standard drug, acetylsalicylic acid (Aspirin) (100mg/kg) and the extract were administered orally first, and after 30 minutes of administration, the mice were all induced with 0.2ml of acetic acid interperitoneally and were observed for the number of writhes for 5 minutes each.

3.8 DATA ANALYSIS

The results are presented as mean values with the standard error of mean (SEM), denoted as mean ± SEM with ‘n' indicating the number of experimental animals per group. Group comparisons were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Statistical analysis was performed using GraphPad Prism software version 5 (GraphPad Software, USA). A significance level of $P < 0.05$ was considered indicative of a statistically significant difference between the compared groups.

CHAPTER FOUR

RESULTS

Table 1: Qualitative Phytochemical Constituents of hydro-methanol extract of *I. trichantha*

TEST	INFERENCE
Flavonoid	+
Phenolics	+
Saponins	+
Terpernoids	+
Tannins	+
Alkaloids	+
Carbohydrates	+
Reducing Sugars	+
Assay Sugars	+
Proteins	_

- absent, + present

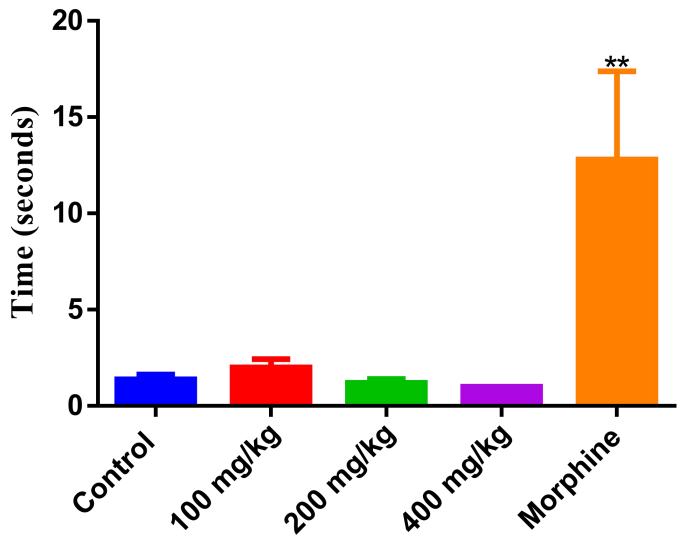

Figure 3: Effect of the hydro-methanol leaf extract of *Icacina trichantha* on tail flick at T_{30} in mice.

No significant difference was observed when compared to the control ($P > 0.05$). Data are represented as mean ± SEM, n = 5.

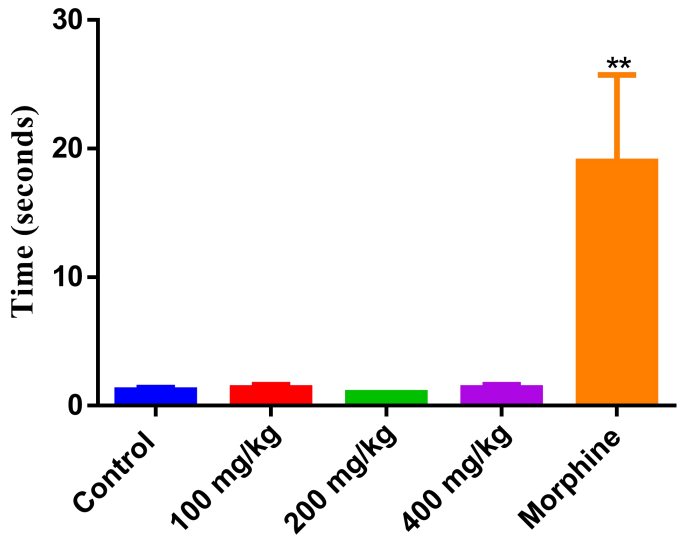

Figure 4: Effect of the hydro-methanol leaf extract of *Icacina trichantha* on tail flick at T_{60} in mice.

No significant difference was observed when compared to the control ($P > 0.05$). Data are represented as mean ± SEM, n = 5.

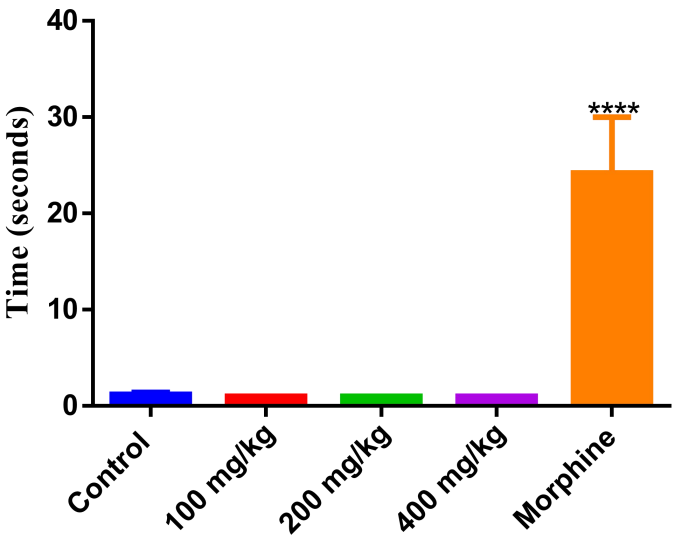

Figure 5: Effect of the hydro-methanol leaf extract of *Icacina trichantha* on tail flick at T_{90} in mice.

No significant difference was observed when compared to the control ($P > 0.05$). Data are represented as mean ± SEM, n = 5.

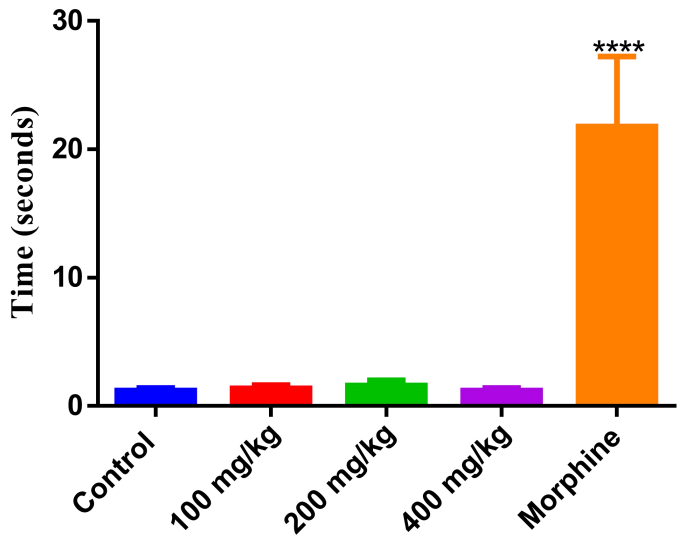

Figure 6: Effect of the hydro-methanol leaf extract of *Icacina trichantha* on tail flick at T_{120} in mice.

No significant difference was observed when compared to the control ($P > 0.05$). Data are represented as mean ± SEM, n = 5.

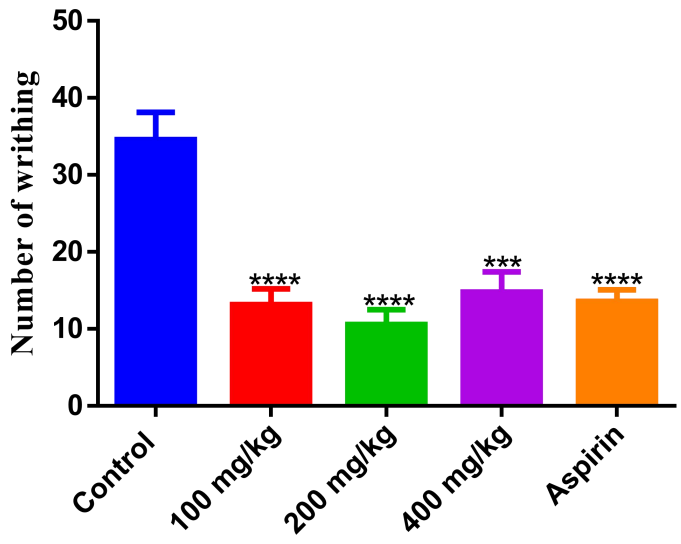

Figure 7: Effect of the hydro-methanol leaf extract of *Icacina trichantha* on acetic acid induced writhing in mice.

There is a significant decrease at 100, 200 and 400mg/kg when compared to control. $P < 0.0001$, $P < 0.0001$ and $P < 0.001$. Data are represented as mean ± SEM, n=5.

5.1 DISCUSSION

Pain is an intricate and subjective sensory and emotional experience associated with actual or potential tissue damage. It serves as a fundamental protective mechanism, alerting an organism to injury and prompting behaviors that facilitate healing. However, when pain becomes chronic or pathological, it transitions from a symptom into a debilitating disease state itself, imposing a severe burden on individual quality of life and global healthcare systems (Cohen *et al.,* 2021). The management of pain, particularly inflammatory and neuropathic pain, remains a significant clinical challenge. Conventional pharmacotherapies, while effective for many, are fraught with limitations. Non-steroidal anti-inflammatory drugs (NSAIDs) can cause gastrointestinal ulceration, renal impairment, and increased cardiovascular risk (Wongrakpanich *et al.,* 2018). Opioids, powerful central analgesics, carry a high risk of tolerance, dependence, respiratory depression, and addiction, a crisis starkly evidenced by the ongoing opioid epidemic in many parts of the world (Volkow and Blanco, 2021).

This pressing need for safer and effective alternatives has catalyzed a renewed scientific interest in ethnopharmacology, that is, the study of traditionally used medicinal plants. Natural products offer a vast repository of chemical diversity, often acting on multiple targets with synergistic effects, which can lead to enhanced efficacy and reduced side effects (Atanasov *et al.,* 2021). *Icacina trichantha*, a plant employed in various traditional medicine systems across West Africa, has been anecdotally reported for its use in managing pain and inflammatory conditions (Che et al., 2019; Nwachukwu et al., 2022). However, such traditional claims require rigorous scientific validation to understand their efficacy, mechanism, and safety. This study aimed to contribute to this validation process by investigating the antinociceptive effects of the hydromethanol leaf

extract of *Icacina trichantha* using two well-established murine models of pain: the hot water tail-immersion test and the acetic acid-induced abdominal writhing test. The distinct outcomes observed a lack of effect in the former and a potent, dose-dependent effect in the latter, which provide critical insights into the specific mechanism of action of this plant extract.

To accurately interpret the pharmacological profile of any substance, it is paramount to understand the specific neurobiological pathways each experimental model engages. Pain models are not created equal; each is designed to probe distinct components of the complex pain pathway, from peripheral transduction to central perception (Muley et al., 2016; Baral et al., 2020)

The hot water tail-immersion (or tail-flick) test is a classic model of acute phasic pain. It involves applying a noxious thermal stimulus (hot water) to a restricted area of the tail, eliciting a rapid, spinally-mediated reflexive withdrawal response. This model is highly sensitive to centrally-acting analgesics, particularly μ-opioid receptor agonists like morphine. These drugs exert their effect by activating descending inhibitory pathways from the periaqueductal grey (PAG) and rostroventromedial medulla (RVM) to the dorsal horn of the spinal cord, thereby raising the threshold for nociceptive transmission and suppressing the reflex (Stein, 2016). The model is notably less sensitive to peripheral analgesics like NSAIDs, which do not significantly alter the response to a brief thermal stimulus unless inflammation is present. Therefore, a compound's efficacy in this test is a strong indicator of a central mechanism, likely involving opioidergic neurotransmission.

In stark contrast, the acetic acid-induced abdominal writhing test is a model of visceral inflammatory pain. The intraperitoneal injection of a dilute solution of acetic acid acts as a potent chemical irritant. It triggers a localized inflammatory response by inducing the release of a plethora of endogenous pro-inflammatory and algesic (pain-producing) mediators. This cascade includes cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukins (IL-1β, IL-6), which in turn stimulate the synthesis and release of prostaglandins (specifically PGE_2 and $PGF_2\alpha$) via the cyclooxygenase (COX) enzymes, bradykinin, serotonin (5-HT), and substance P (Ribeiro *et al.,* 2020). These mediators collectively sensitize peripheral nociceptors (the nerve endings of Aδ and C fibers), leading to a state of peripheral sensitization characterized by a lowered threshold for activation. The behavioral outcome is a stereotypical stretching and writhing response of the abdomen. This model is highly sensitive to drugs that act peripherally to inhibit inflammation, such as NSAIDs (e.g., aspirin, diclofenac), which work primarily by blocking COX enzymes and preventing prostaglandin synthesis (Vane and Botting, 2019). It can also detect compounds that antagonize other mediators like bradykinin or 5-HT.

The central finding of this study is the clear differential effect of : it was ineffective in the hot water tail-immersion test but produced a significant, dose-dependent reduction in wr s in the acetic acid-induced writhing test. This distinction is not a sign of weak or inconsistent activity but rather a powerful indicator of a specific and defined mechanism of action.

The absence of a significant antinociceptive effect in the tail-immersion model strongly suggests that the bioactive compounds within do not exert a major direct influence on the central nervous system pathways that mediate reflexive responses to acute thermal stimuli. Specifically, it indicates that the extract does not have a meaningful agonist activity at μ-opioid receptors, as such activity would have unequivocally increased the latency of the tail withdrawal reflex,

similar to the effect of morphine (Stein, 2016). This finding is crucial as it rules out a central opioidergic mechanism and suggests a low potential for central side effects like sedation or the risk of dependence associated with such a mechanism. It also indicates that the extract does not significantly affect other central monoaminergic pathways (e.g., noradrenergic, serotonergic) that are known to modulate pain perception at the spinal level in this particular model.

The potent, dose-dependent inhibition of acetic acid-induced writhing is a definitive demonstration of peripheral antinociceptive activity. The results align with the effects of standard NSAIDs, pointing towards a mechanism centered on the inhibition of the inflammatory cascade in the periphery. The explanation is that phytoconstituents in interfere with the synthesis, release, or action of the inflammatory mediators that are responsible for sensitizing nociceptors.

The primary mechanism is likely the inhibition of cyclooxygenase (COX) enzymes. Prostaglandins, particularly PGE_2, are key final mediators in the pain and inflammation pathway. They are produced from arachidonic acid by the COX-1 and COX-2 enzymes and directly sensitize nerve endings, dramatically lowering their activation threshold (Smith *et al.,* 2020). By inhibiting these enzymes, would prevent the production of prostaglandins, thereby reducing nociceptor sensitization and the ensuing pain response. This mechanism is the cornerstone of action for aspirin and other NSAIDs, which are highly effective in this model (Vane and Botting, 2019).

However, the complex nature of the writhing response, involving multiple mediators, suggests other potential targets. Bioactive compounds in the extract may also; antagonize the effects of bradykinin: A potent peptide that stimulates nociceptors and promotes vasodilation and plasma

extravasation, inhibit the release or action of serotonin (5-HT) and substance P: Both are key neurotransmitters involved in pain signaling and neurogenic inflammation, suppress the production of pro-inflammatory cytokines: Such as TNF-α and IL-1β, which are upstream initiators of the entire inflammatory mediator cascade (Ribeiro *et al.,* 2020).

The dose-dependent nature of the response is a hallmark of a specific pharmacological interaction and strengthens the validity of the findings, ruling out non-specific or toxic effects as the cause of the reduced writhing. The observed pharmacological activity cannot be seperated from the phytochemical composition of the plant. The preliminary qualitative analysis of revealed the presence of several classes of secondary metabolites, most notably flavonoids, tannins, saponins, and alkaloids (Nwachukwu *et al.,* 2022). The peripheral antinociceptive effect can be robustly attributed to the first two groups.

Flavonoids are polyphenolic compounds extensively documented for their potent anti-inflammatory and analgesic properties (Maleki *et al.,* 2019). Their mechanism is multi-targeted:

1. COX and LOX Inhibition: Many flavonoids, such as quercetin and kaempferol, are known dual inhibitors of cyclooxygenase (COX) and 5-lipoxygenase (LOX) pathways (Rathee *et al.*, 2022). This dual inhibition not only blocks prostaglandin production but also prevents the formation of leukotrienes, another group of pro-inflammatory mediators, offering a broader anti-inflammatory effect than classical NSAIDs.

2. Cytokine Suppression: Flavonoids can downregulate the expression of genes coding for pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6 (Middleton *et al.,* 2021).

3. Antioxidant Activity: The oxidative stress generated during inflammation contributes to tissue damage and pain. The powerful free radical scavenging ability of flavonoids helps mitigate this component (de Cássia da Silveira e Sá *et al.,* 2021).

Tannins (both hydrolysable and condensed) also contribute significantly to the anti-inflammatory and antinociceptive effects. They act through protein denaturation and membrane stabilization, which can prevent the release of inflammatory mediators from mast cells and other immune cells. They can also form complexes with proteins, potentially inhibiting key enzymes like phospholipase A2, which is upstream of COX in the arachidonic acid pathway (Titi-Lartey and Khan, 2023).

While alkaloids are often associated with central effects, some can also possess peripheral anti-inflammatory properties. However, given the lack of central activity in the tail-immersion test, their role here is likely secondary or synergistic, perhaps contributing to the overall effect without dominating the mechanism. The presence of these compounds suggests that the antinociceptive effect of is not due to a single "magic bullet" molecule but is rather the result of a synergistic interplay between multiple bioactive constituents, a common feature of plant extracts that can lead to enhanced efficacy and a superior safety profile compared to isolated single compounds (Wagner and Ulrich-Merzenich, 2021).

5.2 Conclusion

This study concludes that the hydromethanol leaf extract of *Icacina trichantha* possesses flav significant peripheral antinociceptive activity, as evidenced by its dose-dependent reduction of acetic acid-induced writhing in mice.

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