SYNERGISTIC ANTI INFLAMMATORY EFFICACY OF PINEAPPLE AND WATERMELON ON ACUTE AND CHRONIC INFLAMMATION INDUCED IN MICE

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AN UNDERGRADUATE PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.SC.) DEGREE IN SCIENCE LABORATORY TECHNOLOGY

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CERTIFICATION

This is to certify that this research titled "**SYNERGISTIC ANTI INFLAMMATORY EFFICACY OF PINEAPPLE AND WATERMELON ON ACUTE AND CHRONIC INFLAMMATION INDUCED IN MICE**" was carried out by "**Miss Faith EMEFIELE**" with matriculation number "**LSC2009927**" and presented to the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) in Science Laboratory Technology.

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DEDICATION

This project work is dedicated to the Almighty God for his grace and mercies and to my family for their support and love throughout my period of study.

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1 sincerely appreciate my project supervisor Dr. P. O. Obaro for his patience, academic fatherly mentorship, patience and invaluable guidance throughout the period of my project research God bless you.

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I will also like to appreciate my parent: Mr. and Mrs. Emefiele, I will not fail to appreciate my friends and course mates: Jenifer and Lima who has contributed in one way or the other to the success of the work, God bless you all.

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ABSTRACT

Inflammation is a protective biological response, but when uncontrolled it contributes to chronic diseases. Conventional anti-inflammatory drugs such as NSAIDs and corticosteroids, although effective, are often limited by adverse effects with long-term use. This study evaluated the synergistic anti-inflammatory activity of pineapple (*Ananas comosus*) and watermelon (Citrullus lanatus) juice as a potential safer alternative. Fresh juices were prepared and administered to albino mice at doses of 10 and 20 ml/kg after acute toxicity testing. Anti-inflammatory activity was assessed using formalin-induced paw oedema, egg albumin-induced oedema, and carrageenan-induced oedema models. The combination significantly reduced inflammation in a dose-dependent manner across all models, with effects in some cases comparable to ibuprofen and aspirin. No signs of severe toxicity or mortality were observed. The observed activity is attributed to the combined actions of bromelain, lycopene, and L-citrulline, which modulate oxidative stress and inflammatory mediators. These findings suggest that pineapple and watermelon juice may serve as a safe and affordable functional food-based intervention in managing inflammatory conditions

CHAPTER ONE

1.0 INTRODUCTION

Inflammation is a highly regulated and multifaceted biological process that forms a central p art of the body's innate immune defense, triggered by potentially harmful stimuli such as invading pathogens, damaged or dying cells, and environmental irritants. It involves the activation of immune cells, release of inflammatory mediators, and recruitment of leukocytes to the affected site, with the primary aim of eliminating the source of injury, removing necrotic debris, and initiating tissue regeneration (Kumar *et al.,* 2020; Medzhitov*et al.,* 2021). Acute inflammation is a short-term, beneficial response essential for maintaining tissue homeostasis and facilitating recovery. However, when inflammation becomes excessive, dysregulated, or persistent, it can drive the development and progression of numerous chronic diseases, including rheumatoid arthritis, cardiovascular diseases, type 2 diabetes, neurodegenerative disorders such as Alzheimer's disease, and certain cancers (Furman *et al.,* 2019; Rea *et al.,* 2022).

Although conventional anti-inflammatory drugs, particularly non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, remain effective in controlling inflammatory symptoms, their long-term use is often linked to adverse effects such as gastrointestinal ulceration, increased cardiovascular risk, renal impairment, and immune suppression (Bach *et al.,* 2019; Ong*et al.,* 2022). These limitations have intensified scientific interest in exploring plant-derived anti-inflammatory agents,

which are increasingly recognized for their safety, affordability, and potential to modulate inflammation through multiple molecular pathways (Kooti*et al.,* 2020; Arulselvan*et al.,* 2022). As a result, phytochemicals from fruits such as pineapple (*Ananascomosus*) and watermelon (*Citrulluslanatus*) have gained attention for their synergistic potential in attenuating inflammatory processes while minimizing the risks associated with synthetic drugs.

Pineapple (*Ananascomosus*) and watermelon (*Citrullus lanatus*) are widely consumed tropical fruits recognized not only for their sensory appeal but also for their diverse array of bioactive compounds with significant pharmacological potential. Both fruits have been extensively studied for their antioxidant, anti-inflammatory, and immune-modulatory effects, which are largely attributed to their rich content of vitamins, minerals, phenolic compounds, and other phytochemicals (Brito*et al.,* 2020; Liu *et al.,* 2022).

Pineapple contains bromelain, a proteolytic enzyme complex with well-documented therapeutic properties. Bromelain exerts anti-inflammatory effects by downregulating pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), modulating the expression of cyclooxygenase-2 (COX-2), and reducing the synthesis of prostaglandins and thromboxanes involved in inflammation (Kusuma*et al.,* 2020). Additionally, bromelain inhibits platelet aggregation, reduces fibrin formation, and decreases bradykinin levels, collectively contributing to its role in alleviating edema and pain (Secor*et al.,* 2021; Liu *et al.,* 2022).

Watermelon, in contrast, is a rich source of L-citrulline, lycopene, beta-carotene, and phenolic acids, which contribute to its potent antioxidant and anti-inflammatory profile. L-citrulline acts as a precursor for nitric oxide synthesis, improving vascular function and modulating immune cell activity (Jayaprakasha*et al.,* 2019). Lycopene, a carotenoid with high singlet oxygen-quenching ability, protects cellular components from oxidative stress, downregulates nuclear factor-kappa B (NF-κB) signaling, and suppresses the expression of inflammatory enzymes such as inducible nitric oxide synthase (iNOS) (Naz*et al.,* 2020; Oguntibeju, 2021).

When combined, pineapple and watermelon may produce a synergistic anti-inflammatory effect due to the complementary nature of their bioactive compounds. Bromelain primarily targets protein mediators and enzymatic pathways related to inflammation, while lycopene and L-citrulline predominantly function through antioxidant activity and nitric oxide-mediated vascular modulation (Wilhelm, 2020; Tesfaye*et al.,* 2021). This dual-action approach could enhance the overall anti-inflammatory potential, reduce oxidative stress, and improve immune regulation more effectively than either fruit alone. Such synergy highlights the potential of integrating these fruits into functional food formulations or dietary interventions aimed at managing chronic inflammatory conditions.

1.1 BACKGROUND TO THE STUDY

Chronic inflammation has emerged as a major contributor to the global burden of non-communicable diseases, including cardiovascular disorders, diabetes, arthritis, neurodegenerative diseases, and certain cancers (Furman *et al.,* 2019). Unlike acute inflammation, which is self-limiting and essential for healing, chronic inflammation is a prolonged, dysregulated immune response that leads to progressive tissue damage. Modern lifestyle factors such as poor diet, physical inactivity, environmental pollutants, and chronic stress further exacerbate the risk and persistence of inflammatory processes (Calder *et al.,* 2021).

Conventional pharmacological treatments, including non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, remain effective in controlling symptoms but are associated with adverse effects such as gastrointestinal bleeding, cardiovascular complications, renal impairment, and immunosuppression, particularly when used long-term (McCarberg andCryer, 2021). In addition, the high cost of prolonged medication use can limit accessibility, especially in low- and middle-income countries. This has stimulated scientific interest in functional foods and nutraceuticals as safer, more sustainable approaches to inflammation management (Martins *et al.,* 2022).

Watermelon (*Citrullus lanatus*) has been identified as a functional food with significant anti-inflammatory and antioxidant potential. It is a rich source of L-citrullinean amino acid that boosts nitric oxide production leading to vasodilation, improved endothelial function, and reduced oxidative stress (Mao et al., 2021). In

inflammatory bowel conditions, watermelon consumption has been linked to improved colonic health through the nitric oxide-mediated upregulation of peroxisome proliferator-activated receptor gamma (PPAR-γ), a transcription factor that suppresses inflammatory signaling pathways (GSC Biological and Pharmaceutical Sciences, 2020; Patel *et al.,* 2021). Furthermore, watermelon's lycopene and polyphenolic compounds neutralize reactive oxygen species (ROS) and inhibit pro-inflammatory cytokine production, reducing oxidative stress-induced tissue injury (Perkins-Veazie*et al.,* 2021; News-Medical, 2025).

Pineapple (*Ananascomosus*) is equally recognized for its anti-inflammatory potential, largely attributed to bromelaina proteolytic enzyme complex with broad pharmacological activity. Bromelain has demonstrated the ability to downregulate pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), inhibit cyclooxygenase-2 (COX-2) expression, and reduce bradykinin-mediated swelling and pain (Secor*et al.,* 2022; Kansakar*et al.,* 2024). Clinical and preclinical studies have also shown its potential in musculoskeletal conditions, wound healing, and gastrointestinal disorders (StatPearls, 2025; Antioxidants, 2021; Nutrients, 2021). Moreover, novel encapsulation and controlled-release technologies have enhanced bromelain's stability, bioavailability, and therapeutic effect (Kansakar*et al.,* 2024).

When combined, the bioactive compounds in watermelon and pineapple may act synergistically. L-citrulline and lycopene from watermelon primarily function through antioxidant and nitric oxide-mediated signaling, while bromelain from pineapple

modulates inflammation through enzymatic degradation of pro-inflammatory mediators and suppression of key inflammatory pathways. This complementary mechanism could result in enhanced efficacy, making the combination a promising natural dietary intervention for managing both acute and chronic inflammation.

1.2 AIM OF STUDY

To investigate the synergistic anti-inflammatory potential of watermelon and pineapple juice in experimental animal models.

1.3 OBJECTIVES OF STUDY

a. To analyze phytochemical profiles of watermelon and pineapple;

b. To assess in vitro anti-inflammatory activity of each extract individually;

c. To measure the combined effects of the extracts on key inflammatory biomarkers;and

d. To compare their efficacy with standard anti-inflammatory agents (e.g., NSAIDs) in vitro.

1.4 SCOPE OF THE STUDY

This study focuses on the phytochemical analysis and anti-inflammatory evaluation of pineapple and watermelon, both individually and in combination, using appropriate in vitro and/or in vivo models. It will also compare the observed effects with those of a standard anti-inflammatory drug.

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF INFLAMMATION

Inflammation is a fundamental biological response that the body initiates in reaction to harmful stimuli, including pathogens, physical injury, chemical irritants, or damaged cells. This process serves as a protective mechanism of the innate immune system, designed to eliminate the underlying cause of harm, remove injured or dead tissue, and promote healing and regeneration of the affected areas (Denton *et al.,* 2019). The inflammatory response involves a complex and coordinated interaction between immune cells (e.g., macrophages, neutrophils, mast cells), blood vessels, and signaling molecules such as cytokines, chemokines, prostaglandins, and histamine, which together amplify and regulate the immune defense (Chen *et al.,* 2018).

At its core, inflammation is not a disease but rather a physiological process triggered by pathological or immunological disturbances. The immune system detects threats or cellular damage through pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), which recognize specific molecular patterns associated with pathogens (PAMPs) or signals released from damaged tissues (DAMPs) (Liu *et al.,* 2020). Upon activation, these PRRs initiate intracellular signaling pathways that activate the expression and release of a variety of inflammatory mediators, triggering the inflammatory cascade that aims to neutralize the threat and restore tissue

homeostasis (Medzhitov, 2021).

While acute inflammation is typically short-lived and beneficial, playing a crucial role in containing infections and promoting tissue repair, prolonged or poorly regulated inflammation can lead to chronic inflammation, a pathological condition that has been implicated in a range of diseases including rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, and cancer (Rojas *et al.,* 2019). Chronic inflammation can cause ongoing tissue damage by continuously exposing organs to pro-inflammatory mediators, resulting in cumulative damage over time, which can impair the function of the affected tissues (Gupta *et al.,* 2021).

Thus, inflammation operates as both a critical defense mechanism against injury and infection and, when dysregulated or persistent, as a potential source of harm that contributes to the pathogenesis of various chronic diseases. The balance between the beneficial and harmful effects of inflammation is a delicate one, requiring precise regulation to ensure that inflammation serves its protective role without leading to adverse outcomes.

2.2 TYPES OF INFLAMMATION

Inflammation is categorized into two principal types: acute inflammation and chronic inflammation. These two forms differ in terms of duration, the immune responses involved, and the effect on tissues. While acute inflammation typically resolves quickly and is protective, chronic inflammation can last for an extended period, potentially causing long-term tissue damage.

2.2.1 ACUTE INFLAMMATION

Acute inflammation represents the body's immediate immune response to harmful stimuli such as infections, injuries, or chemical irritants. It develops within minutes to hours after the injury and typically resolves within a few days if the stimulus is eliminated or controlled (Kono and Rock, 2008). This type of inflammation occurs in two stages: the vascular response and the cellular response.

The vascular response involves the dilation of blood vessels (vasodilation), which increases blood flow to the affected area. It also increases the permeability of capillaries, allowing plasma proteins and immune cells to exit the bloodstream and enter the affected tissue, where they can neutralize pathogens or clear dead cells (Medzhitov, 2008). The cellular response primarily involves the recruitment of neutrophils, which are the first immune cells to arrive at the site of injury or infection. As the neutrophils work to clear pathogens and debris, macrophages also infiltrate the area to clean up remaining cellular debris and initiate tissue repair (Cao *et al.,* 2016).

The physiological changes that occur during acute inflammation lead to the classic

signs of inflammation: redness (rubor), which results from increased blood flow; heat (calor), due to the increase in blood flow and metabolic activity in the affected tissues; swelling (tumor), which occurs as fluid leaks from the capillaries into surrounding tissues; pain (dolor), caused by the release of inflammatory mediators like bradykinin and prostaglandins, which sensitize nerve endings; and loss of function (functiolaesa), which can be attributed to pain and swelling limiting movement (Cao *et al.,* 2016).

If the inflammatory response is successful in neutralizing the threat, the inflammatory process naturally resolves, and healing begins. This involves tissue regeneration and the restoration of normal function (Medzhitov, 2008).

2.2.2 CHRONIC INFLAMMATION

In contrast to acute inflammation, chronic inflammation develops when the initial inflammatory response is insufficient to eliminate the cause of injury or infection, or when the immune response becomes misdirected. Chronic inflammation can arise due to persistent infections, autoimmune diseases, or prolonged exposure to harmful stimuli, and it can persist for weeks, months, or even years (Nathan and Ding, 2010). This form of inflammation can result in continuous tissue damage and remodeling.

Chronic inflammation is characterized by a different profile of immune cells. While acute inflammation is dominated by neutrophils, chronic inflammation is marked by the presence of macrophages, lymphocytes, and plasma cells. These immune cells contribute to the prolonged production of inflammatory mediators, which continue to drive the inflammatory response, causing sustained tissue destruction and scarring

(Nathan and Ding, 2010). In some cases, granulomas may form clusters of immune cells that attempt to wall off the harmful stimulus but may also lead to further tissue damage, particularly in chronic infections like tuberculosis (Nathan and Ding, 2010).

Conditions associated with chronic inflammation include asthma, where chronic airway inflammation impairs lung function; rheumatoid arthritis, in which persistent joint inflammation leads to joint destruction; inflammatory bowel disease (such as Crohn's disease and ulcerative colitis), which results in ongoing inflammation of the digestive tract; and systemic lupus erythematosus, an autoimmune disorder that causes widespread inflammation and organ damage (Nathan and Ding, 2010). Chronic inflammation also plays a key role in the development of other chronic conditions such as cardiovascular disease, metabolic disorders, and cancer, as prolonged inflammatory signaling can lead to vascular damage, genetic mutations, and insulin resistance (Cao *et al.,* 2016).

As chronic inflammation progresses, the tissue may undergo fibrosis, an accumulation of extracellular matrix proteins such as collagen, which can lead to permanent damage to the organ or tissue. This process of scarring can impair the normal function of the affected tissues and organs, leading to irreversible dysfunction (Kono& Rock, 2008). Therefore, while acute inflammation is necessary for immediate defense and repair, chronic inflammation can be detrimental and often requires intervention to prevent long-term damage.

2.3 CAUSE OF INFLAMMATION

Inflammation can be triggered by a variety of stimuli, ranging from external pathogens to internal disruptions in the body's homeostasis. These triggers include infections by pathogens, physical or chemical tissue injury, foreign bodies, hypersensitivity reactions, autoimmune disorders, and metabolic disturbances. Each of these causes activates specific immune pathways that initiate and sustain the inflammatory response, with the aim of defending the body, repairing tissue, and restoring normal function.

2.3.1 PATHOGENIC MICROORGANISMS

Infections caused by a wide range of pathogens, including bacteria, viruses, fungi, and parasites, are a primary cause of inflammation. The immune system has evolved sophisticated mechanisms to detect these threats, particularly through pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) (Medzhitov, 2021). These receptors are capable of recognizing pathogen-associated molecular patterns (PAMPs), which are unique molecular signatures associated with specific pathogens. Upon recognition, TLRs activate intracellular signaling pathways that lead to the release of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1), which increase vascular permeability and recruit immune cells to the site of infection (Cao *et al.,* 2016). Bacterial infections can trigger complement activation, which enhances immune response and pathogen clearance, while viral infections induce the production of interferons that help inhibit viral

replication and promote immune cell responses (Schneider *et al.,* 2014).

2.3.2 TISSUE INJURY

Tissue damage resulting from physical trauma, chemical exposure, or radiation is another major cause of inflammation. When tissues are damaged, they release cellular contents, including proteins, nucleic acids, and other signaling molecules, which activate nearby immune cells such as mast cells (Huang *et al.,* 2019). Mast cells, upon activation, release histamine and other mediators that promote vasodilation, increase vascular permeability, and facilitate the infiltration of immune cells to the injury site. Additionally, physical injury or radiation can generate reactive oxygen species (ROS), which further contribute to the activation of proinflammatory signaling pathways and perpetuate the inflammatory response (Medzhitov, 2021). This kind of inflammatory response is typically acute, aimed at controlling immediate damage and promoting repair.

2.3.3 FOREIGN BODIES

The introduction of foreign materials into the body, such as splinters, prosthetic devices, or surgical implants, can lead to inflammation. The immune system recognizes these foreign objects as potential threats, even if they are not directly harmful. The inflammatory response initiated by foreign bodies may involve the formation of granulomas—collections of immune cells such as macrophages that attempt to isolate and contain the foreign material (Kono and Rock, 2008). If the foreign material cannot be adequately cleared, granulomatous inflammation can

persist, potentially causing chronic inflammation and tissue damage.

2.3.4 HYPERSENSITIVITY REACTIONS

Hypersensitivity reactions occur when the immune system overreacts to harmless substances, mistaking them for harmful invaders. These reactions can lead to excessive and prolonged inflammation, often seen in allergic responses. For example, in type I hypersensitivity, the immune system reacts to allergens such as pollen, dust, or certain foods by producing antibodies (IgE), which bind to mast cells. This binding triggers the release of histamine and other mediators, leading to symptoms such as swelling, itching, and difficulty breathing (Zhang *et al.,* 2014). Chronic allergic conditions, such as asthma, are characterized by persistent inflammation in the airways, causing breathing difficulties, wheezing, and coughing (Zhang *et al.,* 2014). The inflammation in asthma is often sustained by the infiltration of immune cells, including eosinophils and T-helper 2 cells, which perpetuate the inflammatory cycle.

2.3.5 AUTOIMMUNE DISORDERS

In autoimmune diseases, the immune system erroneously targets and attacks the body's own tissues, resulting in chronic inflammation and tissue destruction. Disorders such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and multiple sclerosis (MS) involve immune-mediated damage to specific organs or tissues. In RA, for example, the immune system attacks the synovial joints, leading to the release of inflammatory cytokines that drive joint inflammation, pain, and swelling. Similarly, in lupus, the immune system attacks various organs such as the

kidneys, skin, and heart, causing widespread inflammation and tissue damage (Nathan and Ding, 2010). In both cases, the inflammation is persistent, and if left uncontrolled, it can lead to irreversible damage and disability.

2.3.6 METABOLIC DISTURBANCES

Metabolic conditions, such as obesity and hyperglycemia, are increasingly recognized as major contributors to chronic low-grade inflammation. In obesity, adipose tissue, particularly visceral fat, secretes a range of proinflammatory cytokines, including TNF-α, interleukin-6 (IL-6), and leptin, into the bloodstream. These cytokines contribute to systemic inflammation, which in turn promotes the development of metabolic disorders such as type 2 diabetes, cardiovascular diseases, and insulin resistance (Cao *et al.,* 2016). Hyperglycemia, another metabolic disturbance, leads to the formation of advanced glycation end products (AGEs), which activate inflammatory pathways, especially in the vasculature. This activation can damage blood vessels, increase oxidative stress, and exacerbate the progression of vascular diseases, including atherosclerosis (Hotamisligil, 2006). Thus, metabolic disturbances not only promote inflammation but also create a vicious cycle that exacerbates the underlying metabolic disorders.

2.4 SYMPTOMS AND SIGNS OF INFLAMMATION

Inflammation, whether acute or chronic, manifests through a variety of characteristic symptoms and signs that result from the underlying physiological processes. These signs are crucial for recognizing the inflammatory response and can vary based on the severity, duration, and location of the inflammation. The classical signs of acute inflammation were first described by Celsus in the first century AD and have since been used as the foundation for understanding the inflammatory response.

2.4.1 CLASSICAL SIGNS OF ACUTE INFLAMMATION

- Redness (Rubor): One of the most noticeable signs of acute inflammation is redness, which occurs due to vasodilation, or the widening of blood vessels. Increased blood flow to the affected area causes erythema, or redness, as erythrocytes (red blood cells) accumulate in the dilated vessels near the site of injury or infection (Ferrero-Miliani*et al.,* 2007). This increased blood flow is part of the body's natural response to bring immune cells and nutrients to the affected tissue, aiding in the immune defense and tissue repair.
- Heat (Calor): Heat is another hallmark of acute inflammation, arising from the increased blood flow to the inflamed area. Blood carries heat from the core of the body, and as blood vessels dilate in the inflamed area, it causes an increase

in local temperature. This response is intended to facilitate the immune response and metabolic activity required to combat infection or repair tissue damage (Ferrero-Miliani*et al.,* 2007). Heat can be particularly noticeable in areas like the skin and joints.

- Swelling (Tumor): Swelling is due to the accumulation of fluid (exudate) in the interstitial tissues, a consequence of increased vascular permeability during inflammation. The endothelial cells of blood vessels become more permeable, allowing plasma proteins, leukocytes, and other immune cells to migrate into the surrounding tissues. This leads to the accumulation of interstitial fluid, which causes the tissue to swell (Ferrero-Miliani*et al.,* 2007). Swelling is often accompanied by an increase in tissue volume and can lead to the formation of edema in both superficial and deep tissues.
- Pain (Dolor): Pain in inflammation is the result of various biochemical mediators, including prostaglandins, bradykinin, and histamine, which are released by immune cells in response to tissue damage. These mediators increase the sensitivity of nociceptors (pain receptors) located in the affected tissue, leading to the sensation of pain (Ferrero-Miliani*et al.,* 2007). Inflammatory pain is often sharp, throbbing, and worsens with movement or pressure on the inflamed area. Additionally, swelling can exert pressure on surrounding nerves, further contributing to pain.
- Loss of Function (FunctioLaesa): The final classical sign of inflammation is loss of function, which occurs due to a combination of pain, swelling, and

tissue damage. The accumulation of fluid and the pain associated with inflammation interfere with the ability of tissues or organs to perform their normal function. For instance, in joint inflammation, swelling and pain limit the range of motion, while in the case of respiratory inflammation, swelling in the airways can impair breathing (Ferrero-Miliani*et al.,* 2007). Function loss may vary depending on the organ involved and the extent of the inflammatory response.

2.4.2 SYMPTOMS OF CHRONIC INFLAMMATION

Chronic inflammation is characterized by a more insidious and prolonged immune response. Unlike acute inflammation, which often presents with overt and obvious symptoms, chronic inflammation may be less visually striking but can have significant effects on the body over time. The symptoms of chronic inflammation include:

- Fatigue: One of the most common symptoms of chronic inflammation is fatigue, which is often due to the persistent activation of the immune system. The continuous release of proinflammatory cytokines, such as TNF-α and IL-6, can disrupt normal metabolic processes and cause tiredness or exhaustion. Prolonged inflammation can also lead to disrupted sleep patterns, contributing further to fatigue (Cao *et al.,* 2016).
- Fever: Low-grade fever is frequently observed in conditions of chronic inflammation, particularly in diseases such as autoimmune disorders and

chronic infections. The release of pyrogens, particularly those from immune cells, triggers the hypothalamus in the brain to increase body temperature, which is believed to help fight off infections and encourage immune cell activity (Cao *et al.,* 2016).

- Mouth Sores and Rashes: Chronic inflammatory conditions, such as lupus or inflammatory bowel disease (IBD), often present with mouth sores or rashes as external manifestations of ongoing inflammation. In lupus, skin rashes like the butterfly rash are characteristic, while IBD can lead to painful sores in the mouth due to prolonged immune activation (Cao *et al.,* 2016).
- Abdominal Pain: Inflammatory conditions affecting the gastrointestinal tract, such as Crohn's disease or ulcerative colitis, often present with abdominal pain. The inflammation of the intestines can cause cramping, bloating, and discomfort, further compounded by the body's immune response to infection or injury (Schneider *et al.,* 2014).
- Chest Pain: Chronic inflammation can also affect the cardiovascular system, leading to chest pain. In conditions such as pericarditis (inflammation of the heart lining) or rheumatoid arthritis, the inflammatory mediators can affect the heart or blood vessels, resulting in pain and discomfort in the chest area (Nathan and Ding, 2010).

2.4.3 THE ROLE OF CYTOKINES IN INFLAMMATION SYMPTOMS

The symptoms of inflammation are largely governed by the action of cytokines, small proteins that play a central role in the regulation of the immune response. Cytokines such as TNF-α, IL-1, and IL-6 are produced during inflammation and are responsible for initiating various changes in tissue, including vasodilation, increased vascular permeability, and the recruitment of immune cells. These cytokines also affect the central nervous system and contribute to the systemic symptoms of inflammation, such as fever, fatigue, and anorexia (Hotamisligil, 2006). Chronic activation of cytokine pathways can lead to long-term symptoms and contribute to the progression of inflammatory diseases.

2.5 INFLAMMATORY MEDIATORS

Inflammation is a complex and tightly regulated process that involves the coordinated action of a variety of molecular mediators. These mediators, produced by immune cells and other tissues, play essential roles in the initiation, progression, and resolution of inflammation. The key inflammatory mediators include cytokines, prostaglandins, leukotrienes, histamine, bradykinin, and nitric oxide (NO). They function through distinct yet interconnected mechanisms, amplifying one another’s effects to orchestrate the body’s response to injury or infection.

2.5.1 CYTOKINES

Cytokines are small proteins that act as critical signaling molecules in the immune

system. They are released by various cell types, including macrophages, lymphocytes, and endothelial cells, to regulate immune responses during inflammation. Among the most important pro-inflammatory cytokines are interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) (Liu *et al.,* 2017). These cytokines coordinate various aspects of the inflammatory response.

IL-1 plays a central role in the initiation of inflammation by promoting the activation of endothelial cells, which enhances the migration of immune cells to the site of injury or infection. It also stimulates the liver to produce acute-phase proteins, which help in pathogen defense (Liu *et al.,* 2017). IL-6 not only aids in immune cell activation but also triggers the systemic response to inflammation, such as fever. IL-6 is critical in the acute-phase response, where it enhances the production of C-reactive protein (CRP), a marker of inflammation (Gabay, 2021). TNF-α is known for its role in promoting apoptosis (programmed cell death). TNF-α is pivotal in driving inflammation by activating immune cells and inducing the production of other cytokines. It also increases the expression of adhesion molecules on endothelial cells, which facilitates the migration of leukocytes to the inflamed tissue (Gabay, 2021). These cytokines work synergistically to amplify the inflammatory response, recruit additional immune cells, and increase the expression of various pro-inflammatory molecules at the site of injury.

2.5.2 PROSTAGLANDINS

Prostaglandins are lipid mediators derived from arachidonic acid through the action of

the enzyme cyclooxygenase (COX). They are involved in various physiological processes, including vasodilation, fever, and the induction of pain. Prostaglandins can enhance the effects of other mediators like cytokines, and they play a critical role in the development of the pain and fever associated with inflammation (Vane and Botting, 2020).

PGE2, a specific type of prostaglandin, is particularly important in the inflammatory response. It induces vasodilation (increasing blood flow to the affected area), causes pain by sensitizing nociceptors (pain receptors), and contributes to fever by acting on the hypothalamus to raise body temperature. The inhibition of prostaglandin production by drugs like aspirin and ibuprofen is one of the mechanisms by which these medications reduce pain and fever in inflammatory conditions (Vane and Botting, 2020).

2.5.3 LEUKOTRIENES

Leukotrienes are another class of lipid mediators derived from arachidonic acid, but they are synthesized through the 5-lipoxygenase pathway. They play crucial roles in inflammation, particularly in the lung, where they mediate bronchoconstriction (narrowing of the airways) and promote the recruitment of neutrophils and other leukocytes to the site of inflammation (Morrow *et al.,* 2018).

Leukotriene B4 (LTB4) is known for its potent chemotactic effect, meaning it attracts neutrophils and other immune cells to the site of injury or infection. This process is vital for combating bacterial infections, but it can also contribute to chronic

inflammatory diseases such as asthma and chronic obstructive pulmonary disease (COPD), where excessive leukotriene production leads to sustained inflammation in the airways (Li *et al.,* 2020).

2.5.4 HISTAMINE

Histamine is a biogenic amine that is primarily stored in mast cells, basophils, and platelets. Upon release, histamine binds to specific receptors (H1, H2, H3, and H4) to mediate a variety of physiological responses. In inflammation, histamine is primarily involved in increasing vascular permeability and inducing vasodilation, which allows immune cells and proteins to move out of the bloodstream and into the tissue to combat the underlying cause of inflammation (Seshadri*et al.,* 2020).

The release of histamine contributes to the swelling and redness often seen in inflamed tissues, as it causes capillaries to become more permeable, leading to an influx of fluid and white blood cells into the affected area. Additionally, histamine's effects on the nervous system contribute to the pain and itching sensations associated with allergic reactions and certain types of inflammation (Maguire *et al.,* 2021).

2.5.5 BRADYKININ

Bradykinin is a peptide that acts as a potent vasodilator and plays a crucial role in the inflammatory response. It is generated by the kallikrein-kinin system in response to tissue damage. Bradykinin's primary functions include increasing vascular

permeability, contributing to pain by sensitizing nociceptors, and promoting vasodilation (Saklatvala, 2020).

Bradykinin enhances the pain response during inflammation by binding to its receptors on pain-sensing neurons, thereby amplifying the sensation of pain. It also contributes to the swelling seen in inflamed tissues by promoting the leakage of fluid from blood vessels into the interstitial space (Knezevic*et al.,* 2019).

2.5.6 NITRIC OXIDE (NO)

Nitric Oxide (NO) is a gaseous molecule that plays a dual role in inflammation. It is synthesized by nitric oxide synthases (NOS) in response to inflammatory stimuli. NO acts as a vasodilator, helping to increase blood flow to the affected area, which is essential for the delivery of immune cells and nutrients to sites of injury (Medzhitov*et al.,* 2019).

Additionally, NO helps regulate platelet aggregation and inhibits the formation of thrombi (blood clots), which is crucial for preventing inappropriate clotting in inflamed tissues. However, excess production of NO can contribute to tissue damage, especially in chronic inflammatory diseases, where it can lead to oxidative stress and further exacerbate inflammation (Medzhitov*et al.,* 2019).

2.5.7 THE CASCADE OF INFLAMMATORY MEDIATORS

The inflammatory mediators mentioned above do not act in isolation; rather, they work in a complex cascade that amplifies and sustains the inflammatory response. For

instance, the activation of cytokines like TNF-α can increase the production of prostaglandins, which then sensitize pain receptors and promote further cytokine release. Similarly, the action of histamine and bradykinin in increasing vascular permeability is crucial for the recruitment of immune cells, which are necessary for tissue repair and infection control.

This intricate network of mediators ensures that the inflammation process is not only effective in resolving the initial cause but also tightly regulated to avoid excessive damage to surrounding tissues. Excessive or prolonged activation of these mediators can lead to chronic inflammation, which is associated with a wide range of diseases, including autoimmune disorders, cardiovascular diseases, and cancer (Murray *et al.,* 2021).

2.6 STANDARD DRUGS USED FOR INFLAMMATION

- Ibuprofen: Ibuprofen is one of the most commonly used non-steroidal anti-inflammatory drugs (NSAIDs) worldwide. It is primarily used to relieve pain, reduce fever, and treat inflammation in conditions such as osteoarthritis, rheumatoid arthritis, menstrual cramps, and muscle strains. It works by inhibiting cyclooxygenase (COX) enzymes, which are involved in the production of prostaglandins—molecules that mediate inflammation, pain, and fever. Specifically, ibuprofen inhibits both COX-1 and COX-2 enzymes, which helps decrease inflammation and associated symptoms (Vane and Botting, 2003).

Ibuprofen is considered to have fewer gastrointestinal side effects compared to older NSAIDs like aspirin or indomethacin, making it a safer choice for short-term use. However, long-term use can still pose risks, such as gastrointestinal bleeding, renal impairment, and cardiovascular issues, especially when taken at higher doses (Bhala*et al.,* 2013). Ibuprofen is available over-the-counter in lower doses (e.g., 200 mg), with higher doses requiring a prescription. Its rapid absorption and relatively short half-life make it suitable for treating acute pain, though its effects typically last for only 4-6 hours.

- Aspirin: Aspirin, also known as acetylsalicylic acid, is a well-established NSAID with multiple therapeutic effects, including anti-inflammatory, analgesic, antipyretic, and antiplatelet properties. It is commonly used to manage pain and inflammation associated with conditions such as rheumatoid arthritis, osteoarthritis, and acute injury. Aspirin exerts its anti-inflammatory effects primarily by irreversibly inhibiting COX enzymes, specifically COX-1, which plays a key role in the production of thromboxane A2, a molecule involved in platelet aggregation and vasoconstriction (Vane and Botting, 2003).

In addition to its role in treating inflammation, aspirin is widely used in cardiovascular medicine due to its antiplatelet effects. It is commonly prescribed to prevent heart attacks and strokes, especially in individuals with a history of cardiovascular events or those at high risk. Aspirin's ability to inhibit platelet aggregation helps prevent the formation of blood clots, which can obstruct blood flow and lead to ischemic events. For cardiovascular prevention, low-dose aspirin (usually

81 mg) is often used as it provides sufficient antiplatelet effects while minimizing gastrointestinal side effects (Patocka*et al.,* 2019).

While aspirin is effective in managing inflammation, it is associated with a higher risk of gastrointestinal side effects compared to ibuprofen, including ulcers, bleeding, and gastric irritation. This is due to its strong inhibition of COX-1, which is crucial for protecting the stomach lining. Aspirin can also have adverse effects on renal function and is contraindicated in certain conditions, such as active peptic ulcer disease and in children with viral infections due to the risk of Reye's syndrome (Patocka*et al.,* 2019).

2.6.1 MECHANISM OF ACTION OF NSAIDS

Both ibuprofen and aspirin belong to a class of drugs called NSAIDs, which work by blocking the action of cyclooxygenase (COX) enzymes, which catalyze the conversion of arachidonic acid into prostaglandins. Prostaglandins play a central role in inflammation, pain, and fever. By inhibiting COX enzymes, NSAIDs reduce the synthesis of these molecules, thereby alleviating symptoms associated with inflammation, pain, and fever (Bhala*et al.,* 2013). The distinction between ibuprofen and aspirin lies in their specific interaction with COX enzymes. Ibuprofen is a reversible inhibitor, meaning its effects on COX enzymes wear off once the drug is eliminated from the body, while aspirin is an irreversible inhibitor, binding covalently to the COX enzymes, leading to longer-lasting effects.

2.6.2 SIDE EFFECTS AND CONSIDERATIONS

While ibuprofen and aspirin are both effective for treating inflammation and pain, they come with distinct safety profiles. Ibuprofen is generally safer for short-term use and is less likely to cause gastrointestinal damage compared to aspirin. However, both drugs can pose risks, especially when used long-term or in high doses. The most common side effects of both ibuprofen and aspirin include gastrointestinal discomfort, ulcers, bleeding, and renal impairment. Aspirin, due to its irreversible inhibition of COX-1, is more likely to cause gastrointestinal issues, particularly when taken at higher doses or for prolonged periods (Patocka*et al.,* 2019).

Another significant consideration is the risk of cardiovascular events. While low-dose aspirin is used for cardiovascular protection, high-dose aspirin or chronic use of NSAIDs in general can increase the risk of heart attack, stroke, and hypertension (Bhala*et al.,* 2013). As such, it is important for patients to use these medications under medical supervision, particularly for those with underlying cardiovascular, gastrointestinal, or renal conditions.

2.7 MECHANISMS OF ACTION OF STANDARD DRUGS

Non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and aspirin, are widely used for their ability to alleviate pain, reduce fever, and decrease inflammation. These drugs primarily exert their therapeutic effects by inhibiting cyclooxygenase (COX) enzymes, which play a central role in the biosynthesis of prostaglandins. Prostaglandins are lipid compounds that mediate inflammation, pain, and fever,

making COX inhibition a key mechanism in the treatment of inflammatory conditions.

- Aspirin: Aspirin, also known as acetylsalicylic acid, is one of the oldest and most commonly used NSAIDs. It irreversibly inhibits both COX-1 and COX-2 enzymes, thereby decreasing the production of prostaglandins. COX-1 is constitutively expressed in most tissues and is involved in maintaining normal physiological functions, such as protecting the stomach lining and supporting platelet aggregation. COX-2, on the other hand, is induced during inflammatory responses and is responsible for producing prostaglandins that contribute to pain, fever, and swelling (Vane and Botting, 2003). By inhibiting both enzymes, aspirin reduces inflammation and pain, making it effective in treating conditions like arthritis, muscle pain, and headaches.

Aspirin's irreversible inhibition of COX-1 is particularly important for its cardiovascular benefits. By reducing the production of thromboxane A2, a prostaglandin that promotes platelet aggregation, aspirin plays a crucial role in preventing blood clot formation. This antiplatelet effect is widely utilized in the prevention of cardiovascular events such as heart attacks and strokes, especially in individuals at high risk (Patocka*et al.,* 2019). However, the inhibition of COX-1 can also lead to gastrointestinal side effects, including ulcer formation and bleeding, making long-term use of aspirin potentially problematic for some individuals (Bhala*et al.,* 2013).

- Ibuprofen: Ibuprofen, unlike aspirin, is a reversible inhibitor of COX enzymes.

It functions by binding to the COX enzymes and blocking the conversion of arachidonic acid to prostaglandins, but the effect is not permanent. Ibuprofen inhibits both COX-1 and COX-2, though it has a slightly higher selectivity for COX-2, which is primarily responsible for prostaglandin production during inflammation. The reduced production of prostaglandins in inflamed tissues helps to alleviate pain and swelling, making ibuprofen effective for conditions such as arthritis, musculoskeletal pain, and minor injuries (Hinz and Brune, 2002).

Ibuprofen's ability to selectively inhibit COX-2 is particularly beneficial in minimizing gastrointestinal side effects, as COX-1 inhibition is mainly responsible for the adverse effects on the gastrointestinal tract, such as ulceration and bleeding. While ibuprofen is generally safer for short-term use compared to older NSAIDs, it still carries risks, including kidney damage, especially with prolonged use or in patients with pre-existing renal issues (Hinz and Brune, 2002).

- Comparison of Aspirin and Ibuprofen: The primary difference between aspirin and ibuprofen lies in their effects on COX enzymes. Aspirin irreversibly inhibits COX-1, providing long-lasting effects and making it beneficial for cardiovascular protection. However, this also increases the risk of gastrointestinal side effects and bleeding. On the other hand, ibuprofen is a reversible inhibitor with a stronger preference for COX-2 inhibition, which helps reduce inflammation and pain with a relatively lower incidence of gastrointestinal issues compared to aspirin. However, ibuprofen still carries

risks when used long-term, particularly concerning kidney function (Bhala et al., 2013).

Both aspirin and ibuprofen are highly effective in treating pain, inflammation, and fever, but they work through slightly different mechanisms. Aspirin's irreversible inhibition of COX-1 provides cardiovascular protection but increases the risk of gastrointestinal side effects. Ibuprofen, with its reversible inhibition and preferential COX-2 inhibition, is generally safer for short-term use with fewer gastrointestinal issues, though long-term use may still present renal and other systemic risks. Understanding these mechanisms helps clinicians choose the appropriate NSAID based on the patient's condition and risk factors.

2.8 MECHANISM OF ACTION OF PINEAPPLE AND WATERMELON IN INHIBITING INFLAMMATION

Pineapple (*Ananascomosus*) and watermelon (*Citrullus lanatus*) are not only refreshing fruits but also possess significant anti-inflammatory properties, owing to the presence of bioactive compounds such as bromelain, lycopene, vitamin C, and citrulline. These compounds interact with various biochemical pathways to modulate inflammation, offering potential therapeutic benefits for conditions associated with chronic inflammation.

2.8.1 PINEAPPLE AND ITS ANTI-INFLAMMATORY EFFECTS

Pineapple contains bromelain, a mixture of proteolytic enzymes that have long been recognized for their ability to reduce inflammation. Bromelain exerts its anti-

inflammatory effects by modulating the immune system and inhibiting the release of pro-inflammatory mediators, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which are typically elevated during inflammation (Maurer, 2001). These cytokines are key players in the inflammatory response and are associated with the development of various inflammatory diseases, including rheumatoid arthritis and inflammatory bowel disease. By reducing the levels of these cytokines, bromelain helps to control the inflammatory response.

Bromelain also plays a role in reducing pain and swelling, two hallmark symptoms of inflammation. It accomplishes this by inhibiting the formation of bradykinin, a peptide that contributes to pain and edema in inflamed tissues (Maurer, 2001). In addition to its effects on inflammatory mediators, bromelain has been shown to influence immune cell signaling, which further aids in regulating inflammation. Studies have suggested that bromelain can enhance the activity of macrophages and other immune cells, promoting tissue repair and modulating the balance between pro-inflammatory and anti-inflammatory signals in the body.

2.8.2 WATERMELON AND ITS ANTI-INFLAMMATORY EFFECTS

Watermelon, a rich source of antioxidants such as lycopene, vitamin C, and citrulline, has also demonstrated considerable anti-inflammatory properties. Lycopene, a carotenoid found abundantly in watermelon, has been shown to inhibit the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a transcription factor that plays a central role in regulating the expression of

inflammatory cytokines (Abu-Reidah et al., 2021). By suppressing NF-κB activity, lycopene effectively reduces the production of pro-inflammatory cytokines, thereby decreasing chronic inflammation and its associated symptoms.

Vitamin C, another key antioxidant found in watermelon, contributes to the reduction of oxidative stress, which is a critical factor in inflammation. Oxidative stress occurs when there is an imbalance between free radicals and antioxidants in the body, leading to cellular damage and triggering inflammatory responses. Vitamin C neutralizes free radicals, thereby protecting cells from oxidative damage and preventing the cascade of inflammation (Perkins-Veazie*et al.,* 2001).

Citrulline, an amino acid present in watermelon, also plays a significant role in modulating inflammation. Citrulline helps improve blood flow by enhancing nitric oxide production, which relaxes blood vessels and promotes better circulation. This improved blood flow not only aids in the delivery of nutrients and oxygen to inflamed tissues but also helps reduce the buildup of pro-inflammatory markers. Additionally, citrulline has been found to reduce oxidative stress, which is known to exacerbate inflammation (Abu-Reidah*et al.,* 2021).

2.8.3 SYNERGISTIC EFFECTS OF PINEAPPLE AND WATERMELON

The combined anti-inflammatory effects of pineapple and watermelon could potentially offer enhanced therapeutic benefits. While bromelain in pineapple works by directly modulating immune cell signaling and reducing the levels of pro-inflammatory cytokines, lycopene and vitamin C from watermelon address

inflammation through their antioxidant actions, reducing oxidative stress and cytokine production. Citrulline further contributes to improved blood circulation and the reduction of inflammatory markers. Together, these fruits provide a multifaceted approach to managing inflammation, addressing both the immune and oxidative stress pathways that are often involved in chronic inflammatory diseases.

CHAPTER THREE

MATERIALS AND METHOD

3.1 COLLECTION OF PLANT SAMPLES

Pineapple and watermelon fruits were purchased from Uselu market in Egor local government area, Edo state, Nigeria. The fruits were cleaned, peeled, cut to smaller pieces and juiced with a food processor (Model No. K35). The produced juice was stored in an air tight container and kept in a refrigerator (Model No. TR-131L) for future study.

3.2 DRUG/SOLVENTS AND CHEMICALS

IBUCAP (Ibuprofen capsule 25 mg), Aspirin, carrageenan (1% solution), egg albumin, and formaldehyde d all of analytical standards, were used for the experiment.

3.3 EXPERIMENTAL ANIMALS

Male and female albino mice were used for this study, weighing between 30 – 35 g and were purchased from the Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria. The mice for the study were kept in cages made of plastic, under natural illumination and temperature settings. They were nourished with standard feed (Top Feeds, Nigeria Plc) and water *ad libitum*.

3.4 EXPERIMENTAL DESIGN

3.4.1 ACUTE TOXICITY STUDY

A study on acute toxicity was conducted using methods of OECD (Organization of economic co-operation development), 2008a guidelines. Six mice, three male and three female, received 100ml/kg of the fruit juice and were monitored for 72 hours for any potential toxicity, mortality, or morbidity symptoms.

3.5. ANTI- INFLAMMATORY STUDIES

3.5.1 FORMALIN INDUCED INFLAMMATION METHOD

The anti-inflammatory activity of ethanol extract was measured by formalin method.

The albino mice weighed between 30- 35 g were used for evaluation of anti-inflammatory activity; in each group five (5) mice were used.

Group-A (negative control) -received distilled water

Group-B (positive control) received Ibuprofen/ Aspirin 10 mg/kg

Group C-D (extract treated) received 10 and 20 ml/kg respectively.

Suspensions of plant extracts and Ibuprofen/ Aspirin were prepared by using distilled water.

Food was withdrawn 12 hours prior to drug administration till completion of experiment. The test and standard drugs were given orally. After 30 minutes, the animals were injected with 0.2 ml of 1 % formalin on the dorsal surface of the right hind paw and the paw sizes were measured and recorded as formalin percentages (%) for the time interval of 30 minutes. Anti- inflammatory activities were measured as the percentage (%) reduction in oedema level when drug was present relative to the control and Aspirin (10 mg/kg).

3.6.2.2 CARRAGEENAN INDUCED RAT PAW OEDEMA

Anti- inflammation was measured using carrageenan- induced rat paw oedema assay (Obaro*et al.,* 2024). Groups of male and female rats were given dose of the extracts. After 1hour, 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The linear paw circumferences were measured at hourly intervals for 4 hours. Three groups of extract treated rats and one control group were used. The mean paw oedema value for the test groups were compared with mean value of the control groups.

Anti- inflammatory activities were measured as the percentage (%) reduction in oedema level when drug was present relative to the control. Ibuprofen (10 mg/kg) was used as reference drug while distilled water was use as the control.

3.6.2.2 EGG ALBUMIN INDUCED RAT PAW OEDEMA

Anti- inflammation was measured using carrageenan- induced rat paw oedema assay (Obaro*et al.,* 2024). Groups of male and female rats were given dose of the extracts. After 1hour, 1% Egg albumin suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The linear paw circumferences were measured at hourly intervals for 4 hours. Three groups of extract treated rats and one control group were used. The mean paw oedema value for the test groups were compared with mean value of the control groups.

Anti- inflammatory activities were measured as the percentage (%) reduction in oedema level when drug was present relative to the control. Ibuprofen (10 mg/ml) was used as reference drug while distilled water was use as the control.

3.8 STATISTICAL ANALYSIS

The results from the studies were expressed as mean ± SEM. Statistical analysis were carried out using graph pad prism 8 version software (UK). Comparisms between the control and treated groups were analysed using one-way ANOVA and, Dunnett's multiple comparisms test a and d = $P \leq 0.05$, and *0.0001* was regarded as indicating significant difference.

CHAPTER FOUR

RESULTS

4.1 ACUTE TOXICOLOGICAL STUDY

The acute toxicity study revealed with a dosage of 50 ml/kg per oral, in all treated animals, the extract was discovered to be devoid of harmful and deadly side effects. Hence the doses for anti-inflammatory studies were selected 10 and 20 ml/kg per oral.

Table 4.1: Acute effect of Pineapple and Water Melon Juice (PWMJ) on Swiss albino mice after 72 hours administration of single-dose (100 ml/kg)

Group(s)	Dose (ml/kg)	Cognition	Agility	Signs of Toxicity such as Grooming, nausea, writhing,	Mortality after 72 hours of administration
Control	2	Normal	Normal	None	0/6
PWMJ	100	Normal	Normal	Watery stool	0/6

4.2 ACUTE INFLAMMATORY TEST

4.2.1 EGG ALBUMIN INFLAMMATORY TEST

Mice treated with the two doses of Pineapple and Water Melon Juice (PWMJ) exhibited decreases in inflammation with increase in times, which were significant when compared with the normal control.

4.2.2 CARRAGEENAN INFLAMMATORY TEST

Animals given the two (2) dosages of Pineapple and Water Melon Juice (PWMJ) demonstrated reductions in their inflammation within the treatment periods that were noteworthy when contrasted to controls.

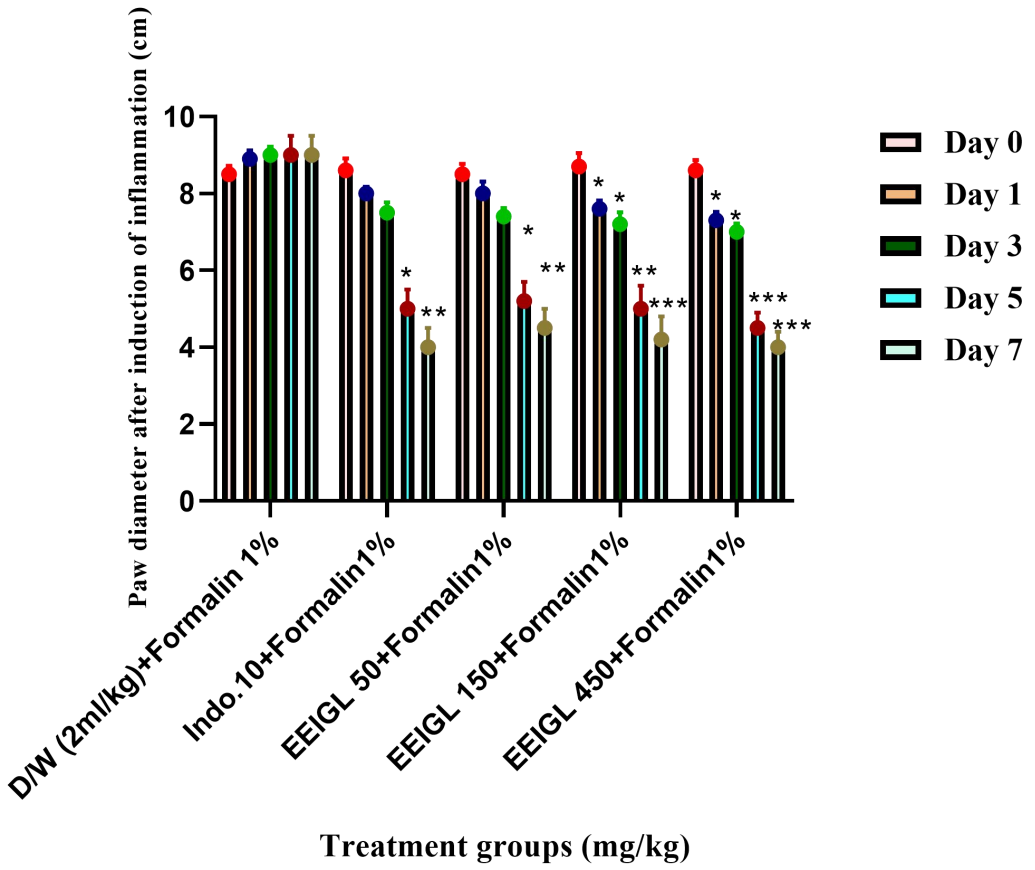

Figure 4.1: Effects of Pineapple and Water Melon Juice (PWMJ) and Aspirin(10 mg/kg) on inflammation in the formalin inflammation. Results are Expressed as mean ± S.E.M (n=5). * =*P≤0.001* as compared to control group

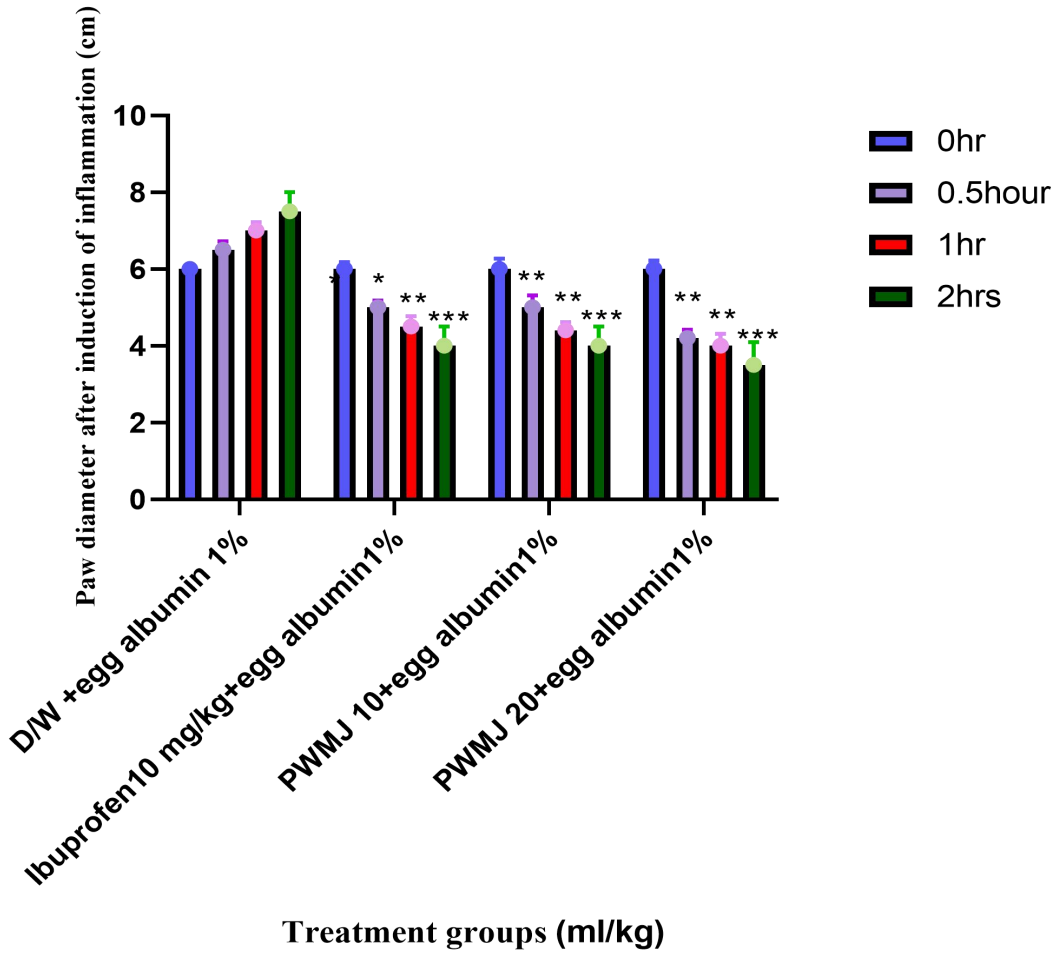

Figure 4.2: Effects of Pineapple and Water Melon Juice (PWMJ) and ibuprofen (10 mg/kg) on inflammation in the Egg Albumin inflammation. Results are Expressed as mean ± S.E.M (n=5). * =*P≤0.001* as compared to control group

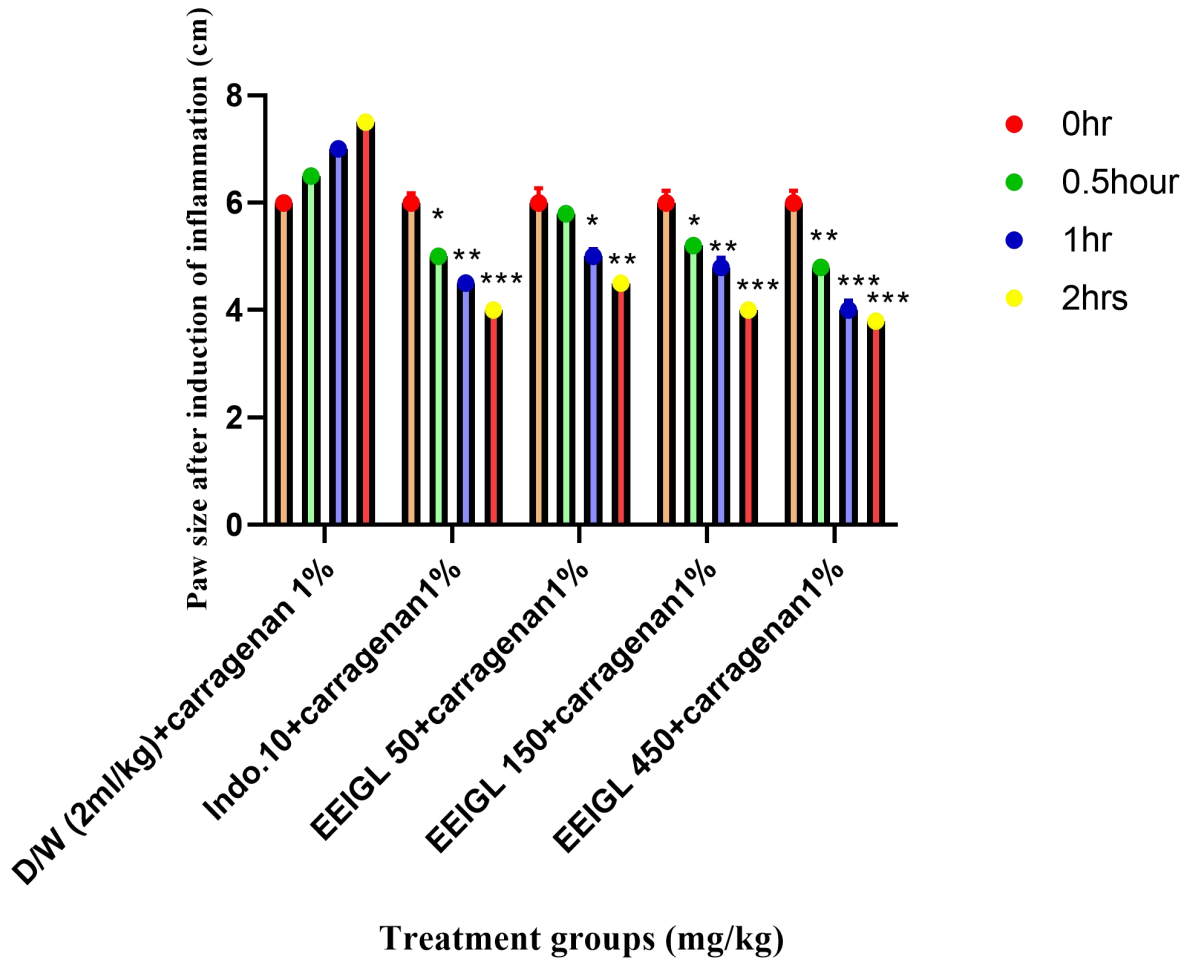

Figure 4.3: Effects of Pineapple and Water Melon Juice (PWMJ) and Ibuprofen(10 mg/kg) on inflammation in the carrageenan inflammation. Results are Expressed as mean ± S.E.M (n=5). * =*P≤0.001* as compared to control group

CHAPTER FIVE

5.1 DISCUSSION OF RESULTS

The present study evaluated the synergistic anti-inflammatory effects of combined pineapple (*Ananascomosus*) and watermelon (*Citrullus lanatus*) juice (PWMJ) using standard animal models of inflammation, including formalin-induced, carrageenan-induced, and egg albumin-induced paw oedema. Acute toxicity testing was also performed to establish the safety of the extract prior to administration.

The acute toxicity study revealed that a single oral dose of 100 ml/kg of PWMJ did not produce mortality or severe toxic manifestations in mice within 72 hours of observation. The only mild response observed was watery stool in some animals, which subsided without further complications. All treated animals maintained normal cognition and agility, and no signs of writhing, nausea, or grooming abnormalities were reported. These results suggest that the juice is relatively safe at high doses, consistent with previous reports on the low toxicity of dietary fruit extracts (Naz*et al.,* 2020; Liu *et al.,* 2022). Therefore, lower doses of 10 and 20 ml/kg were selected for the anti-inflammatory assays.

The safety profile observed here is important for translational research because one of the limitations of synthetic anti-inflammatory drugs is their narrow therapeutic index and potential for gastrointestinal, cardiovascular, or renal toxicity when used

repeatedly (McCarberg andCryer, 2021). By contrast, the lack of significant adverse effects in this study suggests that the consumption of PWMJ may offer a safer alternative for long-term inflammation management.

Formalin injection into the hind paw produced a marked inflammatory response in control mice, characterized by oedema and pain. Treatment with PWMJ at 10 and 20 ml/kg significantly reduced paw swelling compared to untreated controls. The reduction was dose-dependent, with the higher dose exhibiting a greater anti-inflammatory effect. Interestingly, the effect of PWMJ was comparable to that of standard reference drugs such as aspirin and ibuprofen, which were used at 10 mg/kg.

Formalin-induced paw oedema is a well-validated model that involves both neurogenic and inflammatory pain phases. The reduction of paw swelling by PWMJ indicates that the juice has bioactive compounds capable of modulating inflammatory mediators such as histamine, serotonin, prostaglandins, and cytokines (Insuan*et al.,* 2021). Bromelain in pineapple is known to inhibit bradykinin and prostaglandin synthesis, while watermelon-derived lycopene and L-citrulline reduce oxidative stress and nitric oxide imbalance (Jayaprakasha*et al.,* 2019). Thus, the combined extract appears to target multiple inflammatory pathways.

In the egg albumin model, PWMJ significantly reduced oedema compared with the control group, and its effect increased over time. Again, the anti-inflammatory activity at higher doses of PWMJ approached that of ibuprofen. This finding further supports the synergistic role of bromelain, lycopene, and polyphenolic compounds in

mitigating inflammation.

The egg albumin-induced paw oedema model primarily reflects acute inflammatory reactions mediated by histamine and serotonin release. The observed activity of PWMJ therefore suggests that its bioactive compounds can suppress early-phase inflammatory mediators in addition to later-stage cytokines. This aligns with reports that bromelaindownregulates nuclear factor-kappa B (NF-κB) and COX-2 expression, while lycopene blocks reactive oxygen species that perpetuate inflammation (Oguntibeju, 2021; Kansakar*et al.,* 2024).

The carrageenan model is widely used to study both the early and late phases of inflammation. In this study, administration of PWMJ significantly reduced paw oedema at all observation points compared with the control, and the activity increased in a dose-dependent manner. The 20 ml/kg dose of PWMJ showed effects similar to ibuprofen, the reference drug.

Carrageenan-induced inflammation is mediated by an initial release of histamine and serotonin (first phase), followed by prostaglandins, kinins, and leukotrienes in the later phase. The ability of PWMJ to reduce inflammation across these phases suggests broad-spectrum anti-inflammatory action. It is likely that bromelain suppresses cytokine and prostaglandin production, while L-citrulline promotes nitric oxide-mediated vasodilation, reducing oedema formation. The synergy of these mechanisms highlights the therapeutic potential of combining watermelon and pineapple.

Collectively, the findings indicate that PWMJ exerts significant anti-inflammatory effects across different experimental models, demonstrating both safety and efficacy. The activity was dose-dependent and in some cases comparable to standard drugs such as ibuprofen and aspirin. This suggests that combining watermelon and pineapple provides a synergistic effect, possibly due to the complementary actions of bromelain, lycopene, L-citrulline, and polyphenolic compounds.

Importantly, while synthetic NSAIDs act mainly through inhibition of COX enzymes, PWMJ may provide broader modulation of inflammatory pathways, including antioxidant activity, cytokine suppression, and improved vascular regulation. This multimodal action may explain its effectiveness across models and supports its potential role as a functional food-based approach to inflammation management.

CONCLUSION

This study demonstrated that combined pineapple (*Ananascomosus*) and watermelon

(*Citrullus lanatus*) juice (PWMJ) possesses significant anti-inflammatory activity in various experimental models, including formalin-, egg albumin-, and carrageenan-induced paw oedema. The juice was found to be safe in acute toxicity studies, with no mortality or severe adverse effects observed even at high doses. Its anti-inflammatory effects were dose-dependent and in some cases comparable to standard reference drugs such as aspirin and ibuprofen.

The observed activity can be attributed to the synergistic interaction of bromelain from pineapple and bioactive compounds such as lycopene and L-citrulline from watermelon, which collectively modulate cytokine expression, suppress oxidative stress, and regulate nitric oxide pathways. These findings suggest that PWMJ may serve as a safe, affordable, and effective natural alternative or adjunct to conventional anti-inflammatory agents, with potential applications in functional food development and complementary medicine.

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