ANTI-INFLAMMATORY EFFECTS OF *Ocimum gratissimum* ESSENTIAL OIL ON SWISS ALBINO MICE

BY

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LSC2009895

(BIOLOGICAL SCIENCE TECHNIQUES)

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

FACULTY OF LIFE SCIENCES

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CERTIFICATION

This is to certify that this project titled **"ANTI-INFLAMMATORY EFFECTS OF *Ocimum gratissimum* ESSENTIAL OIL ON SWISS ALBINO MICE"** was carried out and submitted by **Miss Osarumwense Gift IDUBOR** with matriculation number **LSC2009895** in the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

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DEDICATION

I dedicate this project to God Almighty, who has blessed and sustained me throughout this work and has been my source of inspiration and strength. Without his grace, none of this would have been possible.

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First and foremost, I sincerely thank Almighty God for his unending faithfulness, grace, and mercy throughout the course of my study. His divine guidance, strength and provision have been my anchor in every challenge and success. I also want to appreciate my project supervisor, Dr. P. O. Obaro and his wife, Dr. (Mrs.) O. E. Obaro-Onezeyi, The Dean of Science Laboratory Technology and lecturers of Science Laboratory Technology. I also wish to express my deep gratitude to my dear friend Destiny Aghedo, for being such an amazing friend and a source of strength. Your genuine friendship, support and belief in me meant more than words can express. My heartfelt appreciation goes to my lovely sisters Mrs. Idubor Amenze, Mrs. Idubor Isoken, Miss Idubor Imade, and Miss Idubor Esohe. Your genuine care, affection and support has anchored me in challenges and in success.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Ocimum gratissimum essential oil comes from the plant, commonly called African basil or Clove basil. It is indigenous to mainly Africa. However, they have been found in other tropical and subtropical parts of the world, like Southern Asia and America (Ezeorba *et al*., 2024).

The African basil is a perennial herb. It is woody at the base with an average height of 1–3 m, many branches, broad leaves and narrow ovate. It is a plant propagated through seed planting and stem cutting and is well-known for its aromatic nature, hence its name, “scent leaf” in Nigeria and some West African countries (Ezeorba *et al*., 2024).

Ocimum gratissimum is known by various names in different parts of the world. In India it is known by its several vernacular names, the most commonly used ones being Vriddhutulsi (Sanskrit), Ram tulsi (Hindi), Nimma tulasi (Kannada). In the southern part of Nigeria, the plant is called “effinrin-nla” by the Yoruba speaking tribe. It is called “Ahuji” by the Igbos, while in the Northern part of Nigeria, the Hausas call it “Daidoya” (Prabhu *et al*., 2009).

The flowers and the leaves of this plant are rich in essential oils which been used extensively in the traditional system of medicine in many countries. The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, epilepsy, high fever, skin diseases and diarrhoea. It is also used in the treatment of microbial and fungal infections (Prabhu *et al*., 2009).

1.2 TAXONOMY OF *Ocimum gratissimum*

1. Kingdom: Plantae

2. Class: Magnoliopsida

3. Order: Lamiales

4. Family: Lamiaceae

5. Genus: *Ocimum*

6. Species: *O. gratissimum*

Ocimum gratissimum is a fragrant herbaceous plant in the *Ocimum* genus, a species of the Lamiaceae family. It grows in disturbed regions surrounding settlements, coastal scrubland, lakeshores, savannas, submontane forest, and along roadside and stream margins. In domestic gardens, it is also grown as an ornamental and hedge plant (Akolkar *et al*., 2023).

1.3 PREPARATION OF ESSENTIAL OIL FROM AFRICAN BASIL

Essential oils are highly concentrated plant extracts valued for their medicinal and therapeutic properties. They are called “essential” because they contain unique characteristics of the plant (Ezeorba *et al*., 2024).

They are complex and multifunctional compounds present in plants and used by plants for various roles including defence against herbivores, insects and microorganisms (Lesgard *et al*., 2014).

Plate 1.1: Leaves of *Ocimum gratissimum* plant **Photo Credit:** Osarumwense Idubor

1.3.1 Steam Distillation:

Steam Distillation involves heating water to produce steam that passes through the plant material in a distillation flask. The steam encourages the opening of the glands enclosing the essential oil which is then collected and separated from the condensed steam based on density. Steam distillation is considered a more efficient method, providing a better yield compared to hydrodistillation (Ibeh *et al*., 2017).

The preparation of *Ocimum gratissimum* essential oil extract involves the leaves of the African Basil plant. The most widely (commonly) used methods for preparing these essential oil are: steam distillation and hydrodistillation (Ezeorba *et al*., 2024).

1.3.2 Hydrodistillation:

Hydrodistillation is a common method for essential oil extraction. It involves heating the plant material and water in a Clevenger apparatus, vaporizing the essential oils, and then condensing and collecting them. Hydrodistillation is a traditional method that has been widely used, but it may not be as efficient as steam distillation in terms of yield (Ikeotuonye *et al*., 2023).

1.3.3 Differences Between Steam Distillation and Hydrodistillation

1. Yield: Steam distillation generally provides a higher yield of essential oil compared to hydrodistillation (Ibeh *et al*., 2017).

2. Efficiency: Steam distillation is considered a more efficient method, requiring less time and labor compared to hydrodistillation (Ibeh *et al*., 2017).

3. Quality: Both methods can produce high-quality essential oil, but the quality may vary depending on factors like plant material quality, equipment, and technique (Ikeotuonye *et al*., 2023).

While both steam distillation and hydrodistillation can produce high-quality essential oil, steam distillation may be preferred for its efficiency and higher yield. However, the choice of extraction method ultimately depends on the specific requirements and goals of the extraction process (Ikeotuonye *et al*., 2023).

1.4 BIOACTIVE COMPONENTS

The essential oil extract of the *Ocimum gratissimum* plant is rich in bioactive compounds. Some of these notable components include eugenol, caryophyllene, thymol, cineole, linalool, and geraniol (Ikeotuonye *et al*., 2023).

- **Eugenol:** This phenolic compound is renowned for its antiseptic and analgesic properties
- **Caryophyllene:** This bicyclic sesquiterpene is notable for its anti-inflammatory and analgesic properties.
- **Thymol**: A monoterpenoid phenol with antiseptic attributes.
- **Cineole:** A monoterpene ether recognized for its anti-inflammatory and decongestant effects.
- **Linalool:** A naturally occurring terpene alcohol with calming and sedative properties.
- **Geraniol:** A monoterpenoid and alcohol that contributes to the oil's sweet fragrance.

1.5 NUTRITIONAL BENEFITS OF *Ocimum gratissimum* OIL

Ocimum gratissimum essential oil is not typically considered a source of traditional nutrients such as vitamins, minerals, proteins, or carbohydrates. The essential oil extracted from its leaves is rich in bioactive compounds contributing to its therapeutic properties (Prabhu *et al*., 2009).

1.5.1 Antioxidant Properties

The essential oil of *Ocimum gratissimum* contains eugenol, which exhibits antioxidant properties. These compounds can neutralize free radicals, thereby reducing oxidative stress and potentially mitigating the risk of chronic diseases associated with oxidative damage (Prabhu *et al*., 2009).

1.5.2. Anti-inflammatory Effects

Traditionally, *Ocimum gratissimum* has been used to treat various inflammatory conditions. Experimental studies have shown that the essential oil can inhibit the production of pro-inflammatory mediators, suggesting its potential in managing inflammatory responses (Ezeorba *et al*., 2024).

1.5.3. Antimicrobial Properties

The essential oil exhibits significant antimicrobial activity against a variety of pathogens. Research indicates that it possesses antibacterial properties effective against both Gram-positive and Gram-negative bacteria. Additionally, the oil has demonstrated antifungal activity, making it a potential candidate for combating fungal infections (Ezeorba *et al*., 2024).

1.6 BIOLOGICAL EFFECTS OF *Ocimum gratissimum* OIL

Ocimum gratissimum, commonly known as African basil or clove basil, is a plant whose essential oil has been extensively studied for its diverse biological activities. Below is an overview of its pharmacological effects, toxicological profile, and cytotoxicity (Akolkar *et al*., 2023).

1.6.1 Pharmacological Effects

1. Analgesic Properties: The essential oil of *Ocimum gratissimum* has demonstrated significant analgesic effects. Studies indicate that its administration can alleviate pain sensations, supporting its traditional use in pain management (Ezeorba *et al*., 2024).

2. Antipyretic Properties: Research has shown that the essential oil exhibits antipyretic effects, effectively reducing fever in experimental models. This supports its traditional use in managing febrile conditions (Prabhu *et al*., 2009).

3. Anti-diabetic Properties: The essential oil has been observed to possess anti-diabetic properties, potentially aiding in the management of diabetes. Studies have reported its ability to modulate blood sugar levels and improve insulin sensitivity (Ezeorba *et al*., 2024).

1.6.2 Toxicological Evaluation

Some studies have reported that the essential oil can induce inflammatory responses upon topical application, indicating potential dermal toxicity. Therefore, appropriate dilution with carrier oils (olive oil or coconut oil) is recommended to minimize adverse effects (Akolkar *et al*., 2023).

1.6.3 Cytotoxicity

The essential oil has demonstrated cytotoxic effects against certain cancer cell lines. Specifically, studies have shown that both the essential oil and its major constituent, thymol, exhibit cytotoxic activity against breast and cervical cancer cell lines, suggesting potential anticancer properties (Prabhu *et al*., 2009).

1.7 APPLICATIONS IN MEDICINE

Ocimum gratissimum has been utilized for centuries in both traditional African medicine and in modern therapeutic medicine to treat a variety of ailments and diseases (Prabhu *et al*., 2009).

1. Blood Sugar Modulation: Used to modulate blood sugar levels and improve insulin sensitivity in cases of type 1 diabetes (Ezeorba *et al*., 2024).

2. Gastrointestinal Disorders: The plant has been used to address stomach issues, including diarrhea and stomach pains (Prabhu *et al*., 2009).

3. Respiratory Ailments: The essential oil has been applied to alleviate throat inflammations and other respiratory ailments (Prabhu *et al*., 2009).

4. Antiseptic Use: Due to its antimicrobial properties, it has been utilized as a local antiseptic and as a mouth freshener (Akolkar *et al*., 2023)

5. Antifungal Applications: Traditional practices include using the plant to combat various fungal infections (Akolkar *et al*., 2023).

6. Respiratory Health: The essential oil is utilized in aromatherapy and traditional remedies to relieve symptoms of respiratory tract infections, such as coughs and bronchitis (Prabhu *et al*., 2009).

7. Aromatherapy: Used in aromatherapy due to its calming and sedative effects (Ikeotuonye *et al*., 2023).

8. Wound Healing and Skin Care: The oil's antimicrobial and anti-inflammatory properties make it suitable for healing wounds and managing skin conditions like eczema or acne (Akolkar *et al*., 2023).

9. Cardio-protective Effects: Offer cardio-protective benefits due to its potential antioxidant, anti-inflammatory, and antihypertensive properties, which help protect against cardiovascular disease (Ezeorba *et al*., 2024).

10. Pain Management: Alleviates pain sensations, supporting its use in pain management (Prabhu *et al*., 2009).

11. Hair Loss Prevention: Vasodilation properties may help improve blood flow to the scalp, promoting healthy hair growth. (Prabhu *et al*., 2009).

12. Anti-cancer: Exhibit cytotoxic activity against breast and cervical cancer cell lines (Prabhu *et al*., 2009).

1.7.1 CHALLENGES AND POSSIBLE SOLUTIONS

Challenges

1. **Standardization:** The chemical composition of *Ocimum gratissimum* essential oil varies due to factors like geographical location, cultivation conditions, and extraction methods. This variability complicates the standardization of the oil for therapeutic use.
2. **Quality Control:** Ensuring consistent quality is challenging due to the oil's chemical diversity. Advanced extraction and purification techniques are essential to maintain efficacy and safety.
3. **Regulatory Issues:** The lack of standardized protocols and comprehensive toxicological data impedes regulatory approval for therapeutic applications.

Solutions

1. **Develop a standardized extraction protocol:** This includes parameters such as plant material quality and quantity, extraction method (e.g., steam distillation, solvent extraction), extraction conditions (e.g., temperature, pressure, time), and solvent quality and quantity if applicable.
2. **Clinical Trials:** Conducting rigorous clinical trials is crucial to validate the therapeutic efficacy of *Ocimum gratissimum* essential oil and establish appropriate dosages.

3. **Toxicity Studies:** Comprehensive toxicity evaluations are necessary to determine safe usage parameters. Preliminary studies have shown varying toxicity levels, highlighting the need for further research.

Addressing these challenges through targeted research will facilitate the safe and effective integration of *Ocimum gratissimum* essential oil into modern therapeutics (Gurav *et al*., 2021).

1.8 AIM OF THE STUDY

This study was aimed to investigate the Anti-inflammatory effect of *Ocimum gratissimum* essential oil on Swiss albino mice.

1.9 OBJECTIVE OF THE STUDY

The specific objectives were to:

1. prepare the coconut oil extract of *Ocimum gratissimum* (scent leaf).

2. investigate the anti-inflammatory effect of *Ocimum gratissimum* essential oil on

acute inflammation using carrageenan and egg albumin induced paw edema in albino mice.

3. assess the effect of the extract on chronic inflammation using the formaldehyde induced paw edema model.

4. compare the anti-inflammatory effects of *Ocimum gratissimum* essential oil with a standard anti-inflammatory drug (indomethacin).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ANTI-INFLAMMATION

Anti-inflammation can be referred to as a set of biological activities–both natural and therapeutically induced that prevent, suppress, or resolve inflammation. It can also be simply defined as the process of reducing or preventing inflammation in the body (Medzhitov, 2008). Inflammation is a natural response of the immune system to injury, infection, or damage, characterized by redness, swelling, pain, and heat. It is a cornerstone of the body's immune defense and repair mechanisms (Nathan and Ding, 2010).

2.2 CAUSES OF INFLAMMATION

1. Biological Agents:

a) Pathogenic infections caused by bacterial, viral, fungal, and parasitic invasions can trigger acute inflammation (Serhan and Savill, 2005; Nathan and Ding, 2010).

b) Tissue death, as occurs in myocardial infarctions, also elicits inflammatory response in the body (Medzhitov, 2008).

2. Physical Agents and Environmental Injury:

a) Physical trauma of which includes cuts, burns, fractures, frostbite, radiation exposure, and extreme temperatures (Nathan and Ding, 2010).

b) Caustic substances like acids, alkalis, oxidizing agents, environmental irritants, and toxins which cause chemical irritation can provoke inflammation (Martin, 2015).

c) Airborne particles (pollutants) and environmental toxins can cause lung and systemic inflammation by inducing oxidative stress and inflaming the airways (Acciani *et al.,* 2013).

3. Immunological Responses:

a) Immune over-reactions to harmless substances (e.g. hypersensitivity and allergy to pollen) lead to inflammatory responses (Abbas *et al.,* 2018).

b) Autoimmune diseases like rheumatoid arthritis, lupus, and multiple sclerosis are driven by immune attacks on one's own tissues (Davidson and Diamond, 2001).

4. Lifestyle and Metabolic Factors:

a) High consumption of processed foods, sugars, saturated fats, and ultra-processed products promotes inflammation (Hotamisligil, 2006). Obesity itself leads to a pro-inflammatory state via cytokine release from adipose tissue and adipose-associated immune cell shifts (Gregor and Hotamisligil, 2011).

b) Lifestyle factors like inactivity, stress, and sleep deprivation contribute to inflammation by dysregulating immune and hormonal responses (Irwin, 2009).

5. Chronic Infections and Persistent Irritants:

When certain infections (e.g., tuberculosis) or irritants (e.g., silica dust) cannot be cleared, inflammation becomes chronic (Nathan and Ding, 2010).

6. Cellular aging:

Accumulation of reactive oxygen species (ROS), senescent cells (SASP phenotype), mitochondrial dysfunction, and impaired autophagy lead to low-grade, persistent inflammation in aging or inflammaging (Franceschi and Campisi, 2014).

2.3 TYPES OF INFLAMMATION

2.3.1 Acute Inflammation:

Acute inflammation is a short-term response to injury or infection, characterized by increased blood flow, swelling, pain, and heat (Medzhitov, 2008). This type of inflammation is a protective

mechanism that aims to eliminate the source of injury, promote healing, and restore tissue function. Acute inflammation is typically resolved through the production of anti-inflammatory cytokines and the activation of repair mechanisms (Serhan and Savill, 2005).

2.3.2 Chronic Inflammation:

Chronic inflammation, on the other hand, is a prolonged and persistent response to ongoing tissue damage or infection. This type of inflammation can lead to tissue destruction, organ dysfunction, and increased risk of chronic diseases such as cardiovascular disease, cancer, and neurodegenerative disorders. Chronic inflammation can be caused by persistent infection, autoimmune disorders, chronic stress, and lifestyle factors such as poor diet and lack of exercise (Nathan and Ding, 2010).

2.3.3 Neuroinflammation:

Neuroinflammation is a specific type of inflammation that occurs in the central nervous system (CNS). This type of inflammation is characterized by the activation of microglia, the resident immune cells of the CNS, and the release of pro-inflammatory mediators. Neuroinflammation can be caused by infection, traumatic brain injury, and neurodegenerative diseases such as Alzheimer's and Parkinson's. While neuroinflammation is a necessary response to CNS injury, chronic or excessive inflammation can lead to neuronal damage and cognitive impairment (Glass *et al.,* 2010).

2.3.4 Systemic Inflammation:

Systemic inflammation is a systemic response to infection or injury, characterized by the release of pro-inflammatory cytokines into the bloodstream. This type of inflammation can lead to organ dysfunction, multi-organ failure, and increased mortality. Systemic inflammation can be caused by sepsis, trauma, burns, and infections (Hotchkiss and Karl, 2003).

2.4 TREATMENT OF INFLAMMATION

Effective management or treatment of inflammation is dependent on the type, severity, and underlying cause of the condition (Medzhitov, 2008). Some treatments include:

2.4.1 Medical Treatments

a) Nonsteroidal Anti-Inflammatory Drugs (NSAIDs): NSAIDs, such as ibuprofen and naproxen, are commonly used to reduce pain and inflammation. They work by blocking enzymes called cyclooxygenases (COX), which are involved in the production of inflammatory chemicals (Nathan and Ding, 2010).

b) Corticosteroids: Corticosteroids, such as prednisone, are potent anti-inflammatory agents that mimic hormones produced by the adrenal glands. They are often used to treat severe asthma, autoimmune disorders, and other inflammatory conditions (Medzhitov, 2008).

c) Biologics: Biologics are a class of medications derived from living organisms that target specific components of the immune system contributing to inflammation. They are designed to interfere with specific signaling molecules, like cytokines, and are used to treat autoimmune diseases such as rheumatoid arthritis (Feldmann and Maini, 2003).

d) Disease-Modifying Antirheumatic Drugs (DMARDs): DMARDs, such as methotrexate and azathioprine, are used to treat chronic inflammatory diseases like rheumatoid arthritis (Kremer, 2004)

2.4.2 Lifestyle Modifications

a) Diet and Nutrition: A diet rich in anti-inflammatory foods, such as fruits, vegetables, whole grains, and omega-3 fatty acids, can help mitigate inflammation. The Mediterranean diet, which emphasizes whole foods and healthy fats, is often recommended for its beneficial effects on inflammation (Giugliano *et al.,* 2006)

b) Exercise and Physical Therapy: Regular physical activity, such as walking, swimming, and yoga, can help reduce inflammatory markers and improve overall health. Physical therapies like heat and cold treatments, massage, and acupuncture provide additional relief (Kasama *et al.,* 2005)

c) Stress Management: Psychological stress can contribute to inflammation. Practices like mindfulness, meditation, and yoga can help manage stress and reduce inflammation (Irwin and Cole, 2011).

2.4.3 Natural Remedies

a) Turmeric and Ginger: Turmeric contains curcumin, a powerful anti-inflammatory compound (Chainani-Wu, 2003), while ginger has anti-inflammatory properties that can ease pain and inflammation (Grzanna *et al.,* 2005).

b) Omega-3 Fatty Acids: Omega-3 fatty acids found in fish oil and fatty fish like salmon have potent anti-inflammatory effects (Calder, 2010).

2.5 PLANTS FOR MANAGING INFLAMMATION

While conventional treatments are available, plants have been used for centuries to manage inflammation naturally. Some of the most potent anti-inflammatory plants include:

1. Turmeric: Contains curcumin, a powerful compound that inhibits inflammatory enzymes like COX-2 and reduces cytokine production. Turmeric has been shown to be as effective as some anti-inflammatory drugs in reducing inflammation (Chainani-Wu, 2003).

2. Chamomile: Known for its calming effects, chamomile can reduce inflammation related to the digestive system and skin (Srivastava *et al.,* 2010).

3. Rosemary: Contains rosmarinic acid, which suppresses free radicals and modulates immune responses, reducing inflammation (Bakirel, 2008).

4. Ginger: Rich in gingerols, which block prostaglandin synthesis, reducing pain and inflammation in conditions like arthritis. Ginger also increases antioxidant genes and stimulates the body's natural defenses against inflammatory processes (Grzanna *et al.,* 2005).

5. Cayenne Pepper: Capsaicin in cayenne pepper lowers substance P, a chemical linked to inflammation and pain (Tominaga *et al.,* 1998).

6. Purslane: Rich in omega-3 fatty acids, which help reduce inflammation and promote overall health (Simopoulos *et al.,* 1992).

7. Garlic: Has anti-inflammatory and antioxidant properties, which can help reduce inflammation and promote overall health. Garlic should be eaten raw and crushed to maximize its medicinal benefits (Amagase, 2006).

8. Nettle: Packed with minerals like magnesium and calcium, nettle can help reduce inflammation and support overall well-being (Chrubasik *et al.,* 2007).

9. African basil: Contains eugenol which helps reduce inflammation, making it useful for managing conditions like arthritis and respiratory issues (Tanko *et al.,* 2008).

2.6 STANDARD DRUGS FOR THE TREATMENT OF INFLAMMATION

2.6.1 NSAIDs

NSAIDs are commonly used to reduce inflammation, pain, and fever. They work by inhibiting cyclooxygenase (COX) enzymes, which are involved in the production of prostaglandins, compounds that mediate inflammation and pain (Hulatt and Freitas, 2024). Examples of NSAIDs include:

Over-the-counter NSAIDs:

1. Ibuprofen: 200-400 mg every 4-6 hours as needed for pain and inflammation
2. Aspirin: 325-650 mg every 4-6 hours as needed for pain and inflammation

3. Naproxen: 220 mg every 8-12 hours as needed for pain and inflammation
4. Diclofenac: 2-4 g applied to the affected area 4 times daily

Prescription NSAIDs:

1. Celecoxib: specifically inhibits COX-2, reducing gastrointestinal side effects
2. Diclofenac: available in higher strengths with a prescription
3. Indomethacin: used to treat moderate to severe rheumatoid arthritis
4. Meloxicam: used to treat osteoarthritis and rheumatoid arthritis

2.6.2 Corticosteroids

Corticosteroids are potent anti-inflammatory medications that mimic the effects of hormones produced by the adrenal glands. They work by suppressing the immune system, reducing inflammation and symptoms associated with it (Barnes, 2006).

Examples of corticosteroids include:

1. Prednisone: often prescribed for severe inflammation, allergic reactions, and autoimmune disorders (Franchimont, 2004).
2. Dexamethasone: used to treat various conditions, including inflammation and immune system disorders (Buttgereit *et al.,* 2002).
3. Hydrocortisone: used to treat skin conditions, allergies, and inflammatory bowel disease (Czock *et al.,* 2005).

2.6.3 Biologics

Biologics are a newer class of medications designed to target specific components of the immune system involved in inflammation. They are used to treat chronic inflammatory conditions, such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease, by blocking specific molecules or cellular pathways (Feldmann and Maini, 2001). Examples of biologics include

Etanercept, used to treat rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. By blocking TNF-alpha, Etanercept helps reduce inflammation and slow disease progression (Sumiyoshi *et al.,* 2025).

2.7 MECHANISM OF ACTION: DICLOFENAC, ASPIRIN AND IBUPROFEN

Ibuprofen, aspirin, and diclofenac are nonsteroidal anti-inflammatory drugs (NSAIDs) that work by inhibiting the production of prostaglandins, which are hormone-like substances that mediate inflammation and pain (Rainsford, 2009).

2.7.1 Diclofenac

Diclofenac works by inhibiting COX enzymes, similar to ibuprofen and aspirin. However, diclofenac has a slightly different mechanism of action, as it also inhibits the production of leukotrienes, which are involved in the inflammatory response. Diclofenac is a potent inhibitor of COX-2, which makes it effective in reducing inflammation and pain. Diclofenac is available in various formulations, including topical gels and patches, which allow for localized delivery of the medication (Gan, 2010)

2.7.2 Aspirin

Aspirin works by irreversibly inhibiting the COX enzyme, thereby reducing the production of prostaglandins and thromboxanes. Aspirin's mechanism of action is similar to ibuprofen's, but aspirin's effect on COX enzymes is longer-lasting due to its irreversible inhibition. Aspirin's anti-inflammatory and analgesic effects are primarily due to its inhibition of COX-2, while its antiplatelet effect is due to its inhibition of COX-1 in platelets (Vane and Botting, 2003).

2.7.3 Ibuprofen

Ibuprofen works by inhibiting the enzyme cyclooxygenase (COX), which is responsible for converting arachidonic acid into prostaglandins. By blocking COX, ibuprofen reduces the

production of prostaglandins, thereby decreasing inflammation and pain. Ibuprofen is a non-selective COX inhibitor, meaning it blocks both COX-1 and COX-2 enzymes. COX-1 is involved in maintaining the health of the stomach lining, while COX-2 is involved in the production of prostaglandins that mediate inflammation (Rainsford, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Plant sample

3.1.1 Sample collection

Disease free samples of rhizomes of *Ocimium gratissimum* were collected in March 2025 from Ovbiogie village in Ovia South, Benin City, Edo state, Nigeria. The leaves were first washed, cut into smaller pieces and air-dried at room temperature after collection for 2 weeks then dried in an oven at 40^0C for 1 hour. The dried sample was then reduced to fine powder using an electric milling machine and stored in air tight containers for future use.

3.1.2 Preparation of extract

Five hundred gram (500 g) of the powder was extracted with coconut oil using cold extraction method. The ground powdered leave was weighed and placed in a glass jar. 2.5 litres of coconut oil was poured into the glass jar to make ¾. The solution was macerated and then shake vigorously as often as possible. After 72 hours the solution was macerated and filter using a cheese-cloth. The resulting essential oil was then turn into an air tight container.

3.2 Solvents/chemicals

Coconut oil, Formalin solvent (supplied by Fharmatrends Nigeria Ltd), Sodium Chloride all of analytical standards.

3.2.1 Drugs

Indomethacin, were of pure samples and pharmaceutical standards.

3.2.2 Experimental Design

3.2.3 Experimental animals

The animal house of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin. Sixty (60) male and female adult albino mice, with an average weight of 20-40 g. The animals were housed in wooden cages at room temperature and kept in standard laboratory environment with 12-hour cycles of light and darkness. Prior to the experimental study, the mice were given clean water and standard pelletized layers mash to acclimate for two weeks.

3.3 Systemic acute inflammation of the mouse paw

The mouse paw edema model (Winter *et al*., 1962) involves experimentally inducing acute edema in the right hind paw of the mice in order to assess the systemic effects of test substances on acute inflammation. By injecting phlogistic agents (such as fresh egg albumen or a 1 percent solution of carrageenan in normal saline) into the sub-plantar area of the paw, it is possible to cause an acute inflammatory response in mouse paw records. The mouse paw develops edema, which is seen as an intense pink swelling, as a result of the acute inflammatory response. The size of the paw is measured in terms of the volume at various intervals, using a vernier caliper. Other methods of measurement paw size include volume displacement from a measuring cylinder containing water, measurement of linear circumference of the paw for using a tape and measurement of paw thickness with a micrometer screw gauge or caliper. In some instance, the animal is sacrificed and the inflamed paw amputated and weighed. However, the latter should be relegated to extremely necessary experimental needs. Anti-inflammatory substance abolished or reduce the extent of the swelling or edema when compared to the negative control. Adult mice of either sex are used usually in this experiment. The animals are randomly allotted into groups

based on the dose levels of the test substance and appropriate controls (NSAIDs such as acetylsalicylic acid and indomethacin for positive control; the vehicle or suspending agents served as negative control). At time t = 0, the animal's right hind paw is measured for size. The test substance is administered. One hour after oral administration or 30 minutes after i.p. injection of test substance, the mice right hind paw's subplanter region receives an injection of phlogistic agent measured at 0.1 milliliter.

At various times (0.5, 1,2,3,4 and 5h or 1 and 3h, etc) after carrageenan injection, the paw size is measured again. Edema is measured as a rise in size of the paw size at 0 hours and at various intervals following injection of a phlogistic agent. For each group, the relation (where paw volume is used) is used to calculate the inhibition level (percent) of edema.

Inhibition (%) = [1-(Vt/Vc)] 100

Where, Vt = average paw volume of the treated group,

Vc = average paw volume of the control group.

3.4 Chronic Inflammation

Evaluation of the effect of substances on chronic inflammation involves models using repeated administration of the test agent as well as inflammation sustained beyond 24 h. Inflammation becomes chronic when the assault on the body is not contained within the acute phase. Models that mimic pathological chronic inflammation have been developed and used in screening suitable substances for its management. These models include induction of arthritis in mice through formaldehyde, adjuvant induced arthritis, collagen adjuvant-induced arthritis, air pouch inflammation and cotton pellet granuloma tests.

3.4.1 Arthritis - induced by formaldehyde in mice

Formaldehyde-induced arthritis in mice (Seyle, 1949) can be used to assess how test substances affect the chronic phase of inflammation. Formaldehyde edema has been shown to be mediated by histamine, 5-HT, substance P, and bradykinin and prostaglandins which are involved in the acute phase of inflammatory response. Sustenance of the edema beyond the initial 24 h may involve induction of other humoral and cell- mediated responses such as lymphocyte accumulation and proliferation, indicative of chronic inflammation. Due to the ability of formaldehyde to cause necrosis of the tissues of the paw, it is also thought that necrosis amplifies the later stages of the formaldehyde edema through activation of kinin, coagulation, complement and fibrinolytic systems and the mediator release from passenger leukocytes that are dying or dead. The arthritic foot appears swollen and painful initially but wanes within 1-3 days. To prolong and further sustain the arthritis, the paw is re-inflamed on day 3 with formaldehyde injection. The experiment typically lasts for 10 days.

The experiment uses adult mice of either sex. The animals are divided into groups at random, depending on the doses of the test substance, and appropriate controls (NSAIDs such as acetylsalicylic acids and indomethacin or steroidal anti-inflammatory drugs for positive control; the vehicle or suspending agent as negative control). On day 1 of the experiment, we use a Vernier caliper to measure the animal's right hind paw or volume (by displacement) and the test substance is administered (orally or intraperitoneally). One hour after oral administration or 30 min after i.p. injection of test substance, arthritis is induced through an injection administered sub-plantar at 0.1ml of 2 % v/v formaldehyde solution. After 4 hours, the paw's volume or size is once more measured. On day 2, the animal is treated once again and the size (or volume) of the arthritic foot is measured. On day 3, after treatment and measurement of the arthritic foot size (or

volume), arthritis is re induced by formaldehyde injection. From day 4- 10, the animal is treated daily and the size (or volume) of the arthritic foot measured. Changes in the size or volume of the arthritics foot is used as a measure of arthritics. The overall result of the anti-arthritic treatment is quantified by the area under the curve (AUC) of the time-course of the arthritic event, which represents the global response of edematous to arthritis from formaldehyde. The inhibition level in percentage of arthritis is determined using the relation, and the AUC is determined using the trapezoidal rule:

Inhibiton (%) = [1 – (AUCt / AUCc)] 100

Where, AUCt = AUC of the treated group

AUCc = AUC of the control group.

3.5 Administration of extract

Extract was freshly prepared every morning and administered orally to mice by carefully inserting an orogastric tube into the oral cavity of the mice. The animals were grouped into three categories groups; carrageenan induced for paw edema ([a]), egg albumen induced for paw edema ([b]) and formaldehyde induced for paw edema ([c]), consisting of 5 animals each.

Group I (Negative control) – Normal saline (2 ml/kg)

Group II- Positive control (10 mg/kg indomethacin)

Group III and IV (20 and 50 ml/kg of the extract respectively after acute toxicity study).

Throughout the period of administration, food and water were given to the mice.

3.6 Statistical analysis

Every value is presented as Mean ± Standard Error of Mean (SEM). Using the UK's Graph Pad Prism 8.2 software, one-way ANOVA was used to analyze the data. $P \leq 0.05$ was used to define significance for differences.

CHAPTER FOUR

5.0 DISCUSSION

5.1 Carrageenan Induced Paw Edema

Carrageenan model induces acute inflammation, and assessment of anti-inflammatory activity of extracts is carried out by measuring changes in paw edema or inflammation markers. Carrageenan, a polysaccharide derived from seaweed, triggers the release of histamine, serotonin, and prostaglandins, which contribute to the development of acute inflammation when injected into the paw or other tissues (Morris, 2003; Winter *et al*., 1962).

5.1.1 Extract only (20 ml/kg)

Treatment group was compared to the standard which was composed of 10 mg/kg of indomethacin and the negative control which was made up of 2 ml/kg of Normal saline at various intervals of 0 hour, 1 hour, 2 hours and 3 hours. The following was deduced in each treatment group. There is a significant decrease in the paw size which is steady at the different time intervals when compared with the control groups. This shows that the extract is very effective at reducing inflammations even at this dose.

5.1.2 Extract only (50 ml/kg)

Treatment group was compared to the standard which was composed of 10 mg/kg of indomethacin and the negative control which was made up of 2 ml/kg of Normal saline at various intervals of 0 hour, 1 hour, 2 hours and 3 hours. The following was deduced. There is significant decrease in the in the paw size at steady time intervals when compared to the control groups. This shows that the extract is very effective at reducing inflammations at this increased dose.

5.2 Egg Albumen Induced Paw Edema

Egg albumin, a protein found in egg whites is another model used to study acute inflammation. It induces an immune response and inflammation when injected into experimental animals triggering an immune response, leading to the activation of immune cells and the release of inflammatory mediators (Choi and Hwang, 2004; Olajide *et al*., 2003).

5.2.1 Extract only (20 ml/kg)

Each treatment group was compared to the standard which was composed of 10 mg/kg of indomethacin and the negative control which was made up of 2 ml/kg of Normal saline at various intervals of 0 hour, 1 hour, 2 hours and 3 hours. The following was deducted in each treatment group. There is significant decrease in the paw size which is steady at the time intervals when compared with the control groups. This shows that the extract is very effective at reducing inflammations even at this dose.

5.2.2 Extract only (50 ml/kg)

Treatment group was compared to the standard which was composed of 10 mg/kg of indomethacin and the negative control which was made up of 2 ml/kg of Normal saline at various intervals of 0 hour, 1 hour, 2 hours and 3 hours. The following was deducted in each treatment group. There is significant decrease in the paw size which is steady at the time intervals when compared with the control groups. This shows that the extract is very effective at reducing inflammations at this increased dose.

5.3 Formaldehyde Induced Paw Edema

Formaldehyde mode lnduce chronic inflammation. This chemical irritant, induces inflammation and tissue damage when applied to the skin or injected into experimental animals, to leading to

the release of inflammatory mediators. This inflammation can persist for an extended period of time (Olajide *et al*., 2004).

5.3.1 Extract only (20 ml/kg)

Each treatment group was compared to the standard which was compared to the standard which was composed of 10 mg/kg of indomethacin and the negative control which was made up of 2 ml/kg of Normal saline at various intervals of day 0, day 1, day 3, day 5 and day 7. The following was deduced in each treatment group. There is a significant decrease in the paw size which is steady at the time intervals when compared with the control groups. This shows that the extract is effective at reducing chronic inflammations even at this dose.

5.3.2 Extract only (50 ml/kg)

treatment group was compared to the standard which was compared to the standard which was composed of 10 mg/kg of indomethacin and the negative control which was made up of 2 ml/kg of Normal saline at various intervals of day 0, day 1, day 3, day 5 and day 7. The following was deduced in each treatment group. There is a significant decrease in the paw size which is steady at the time intervals when compared with the control groups. This shows that the extract is effective at reducing chronic inflammations at this increased dose.

CONCLUSION

The extract EOOG exhibited significant effectiveness in the decrease of paw size. There was no fatalities among the animals. The findings of this experiment indicate that *Ocimum gratissimum* maybe use in the treatment and management of inflammation.

RECOMMENDATION

I recommend that this extract EOOG can be used, in the management and treatment of inflammation and its relative conditions.

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