PREVENTIVE EFFECT OF INDUCED INFLAMMATIONS ON SWISS ALBINO MICE AFTER THE ADMINISTRATION OF ETHANOL EXTRACT OF *Dioscorea alata* (WATER YAM)

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A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY AND SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELORS (B.Sc.) DEGREE IN THE UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

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CERTIFICATION

We certify that this research was carried out by **Miss Glory Kehinde OFORUMEH** with Matriculation Number- **LSC2009914** in the Department of Science Laboratory Technology (Biological Science Techniques), Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

I dedicate this success story to God Almighty, for his grace upon my life.

ACKNOWLEDGEMENTS

I am very grateful to God Almighty for His protection and favour throughout this programme.

My profound gratitude goes to my wonderful supervisor Dr. (Mrs.). O. E. Obaro-Onezeyi and co-supervisor Dr. P. O. Obaro for their love, support and parental criticisms.

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ABSTRACT

Inflammation is a fundamental biological response of the immune system that protects the body against harmful stimuli but can lead to pathological conditions when prolonged or uncontrolled. Current anti-inflammatory therapies, such as non-steroidal anti-inflammatory drugs (NSAIDs), are effective but associated with side effects, prompting the need for alternative remedies from natural sources. This study evaluated the anti-inflammatory effects of the ethanol extract of *Dioscorea alata* (water yam) on experimentally induced paw edema in albino mice. Fresh tubers of *Dioscorea alata* were collected, processed, and extracted with ethanol. Sixty albino mice were used and divided into three (3) groups for acute and chronic inflammation models induced by carrageenan, egg albumin, and formaldehyde. Test groups received oral doses of *Dioscorea alata* extract (20 mg/kg and 250 mg/kg), while negative and positive controls received normal saline and indomethacin (10 mg/kg) as a standard drug respectively. Paw volume was measured at specified time intervals, and data were analyzed using one-way ANOVA with significance set at $p \leq 0.05$. The results revealed that *Dioscorea alata* extract significantly reduced paw edema across all models in a dose-dependent manner, with higher doses exhibiting comparable effects to indomethacin. Both acute and chronic inflammation models demonstrated notable anti-inflammatory activity, suggesting the presence of bioactive phytochemicals such as saponins, phenolic compounds, and diosgenin. Importantly, no mortality was observed among the treated animals. This study concludes that ethanol extract of *Dioscorea alata* possesses significant anti-inflammatory properties, supporting its traditional use in the management of inflammatory conditions. It is recommended that further studies, including isolation of active compounds and clinical evaluations, be conducted to validate its therapeutic potential.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Inflammation is a key aspect of the body's immune system. It is a process by which the immune system recognizes and removes harmful and foreign stimuli and begins the healing process. In inflammation the body's white blood cells and things they make protect the body from outside invaders, such as bacteria and viruses (Pahwa *et al*., 2023). In some diseases, like arthritis. Your body's immune system triggers inflammation when there are no invaders to fight off. In these autoimmune diseases, your immune system acts as if regular tissues are infected or somehow unusual, causing damage (Zelman, 2022).

1.2 Pathophysiology of Inflammation

Inflammation occurs when tissue injury and inflammation-inducing factors such as histamine, cytokines, and others lead to venular dilation, increased vascular permeability, and entrance of inflammatory components. The stress responses promote inflammation, which is an essential component of it (Pahwa *et al*., 2023).

The cardinal signs of inflammation includes; pain, heat, swelling, redness, and loss of function. Inflammation is a generic response, and can be classified as innate immunity, as against acquired immunity, which is directed at a specific pathogen (Zigterman and Dubois, 2022).

1.3 Causes of Inflammation

1.3.1 Physical causes includes;

1. Burns
2. Frostbite
3. Trauma

4. Physical injury
5. Ionizing radiation

1.3.2 Biological sources includes;

1. Stress
2. Infections from pathogen
3. Immune reactions due to hypersensitivity

1.3.3 Chemical sources includes;

1. Alcohol
2. Chemical irritants
3. Toxins

1.3.4 Psychological causes includes;

Emotions such as anger, fear and excitement.

1.4 Types of Inflammation

There are two types of Inflammation which are; acute and chronic inflammation

1.4.1 Acute inflammation

This type of Inflammation is more common. It is the body's response to sudden body damage or stimuli, to which the body produces and sends inflammatory cell to the site of injury. Acute Inflammation has a duration ranging from few hours to few days, depending on the nature and severity of the condition. Acute Inflammation may start rapidly and quickly become severe in a short period of time with symptoms which may last for a few days. Sub-acute is the period between acute and chronic Inflammation may last 2-6 weeks.

1.4.1.1 Symptoms of acute inflammation

1. Redness: This is due increased movement of blood through dilated vessels and additional number of erythrocytes passing through the area.

2. Swelling (edema): This is as result of increased passage of fluid from the dilated and permeable blood vessels into the tissue and infiltration of cells into the damaged area.

3. Heat: The sensation of heat is caused by the increased movement of blood into the cooled extremities.

4. Pain: Pain is due to the direct effects of mediators, either from initial damage or that resulting from the inflammation response itself, and the stretching of nerves due to oedema.

5. Loss of function: This refers to either a simple loss of mobility in a joint, due to edema and pain, or to the replacement of functional cells with scar tissue.

These signs may not always appear. An acute inflammation maybe without symptoms.

The person could feel tired, generally unwell with a fever.

1.4.1.2 Causes of acute inflammation

An acute inflammation may develop from:

An exposure to certain stimuli like a bee sting or substances such as dust or pollen grains.

An infection

• An injury

1.4.1.3 Process of an acute inflammation

The immune system triggers a number of reactions when the body detects damage or the influx of pathogens. These reactions include:

Plasma proteins begin to accumulate in tissues, resulting in the buildup of fluids leading to swelling.

Neutrophil, a type of white blood cell, which migrate towards the affected area.

Certain molecules in the leucocyte assists in fighting pathogens.

Small blood vessels begin to enlarge to allow leucocyte and plasma proteins to reach the site of injury more easily.

Examples of acute inflammation includes:

Acute bronchitis

Acute appendicitis

An ingrown toenail

Sore throat from a flu or cold

• Physical trauma or wound

1.4.2 Chronic inflammation

Chronic inflammation is generally referred to as a slow, long-term inflammation lasting from a periods ranging from several months to years. The degree of damage by chronic inflammation vary with injury and the individual (Pahwa *et al*., 2023)**.**

1.4.2.1 Causes of chronic inflammation

Chronic inflammation generally results from:

• Inability to eliminate the causative agents of an acute inflammation such as an infectious organism like Mycobacterium tuberculosis, other pathogens as well as parasites that resists the host body defenses and remain within for an extended period of time.

Inability to eliminate a low level of irritant or foreign substance by the body or slow, steady absorption of industrial chemicals by the body.

Chronic inflammation can result from an autoimmune disorder, where in the normal body components are recognized as foreign substances by the body as in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE).

Cellular defect leading to persistent or recurrent inflammation, causing auto-inflammatory disorders such as familial Mediterranean fever.

• Oxidative stress and mitochondrial dysfunction such as formation of free radicals, advanced glycation end-products (AGEs), uric acid crystals, oxidized lipoprotein and homocystein caused by inflammatory and biochemical inducers.

• Reoccurring spurts of acute inflammation. Chronic inflammation can also result from independent responses by the body (Pahwa *et al*., 2023).

Chronic inflammation can also be linked to various diseases like:

Diabetes

Cardiovascular diseases

• Arthritis

• Allergies

• Chronic obstructive pulmonary disease

Psoriasis

• Rheumatoid arthritis

1.4.2.2 Symptoms of chronic inflammation

- Abdominal pain
- Chest pain
- Fatigue
- Fever

- Skin rash
- Weight gain
- Weight loss
- Frequent infection

1.5 Management and treatment of inflammation.

The management of inflammation involves a multi-faceted approach that incorporates pharmacological, non-pharmacological, and lifestyle interventions.

1.5.1 Pharmacological Interventions:

Non-steroidal anti-inflammatory drugs (NSAIDs): NSAIDs, such as ibuprofen and naproxen, are commonly used to manage inflammation and pain. (Kumar *et al*., 2020)

Corticosteroids: Corticosteroids, such as prednisone, are potent anti-inflammatory agents used to manage severe inflammation. (Guyton & Hall, 2016)

Biologic Agents: Biologic agents, such as tumor necros is factor-alpha (TNF-alpha) inhibitors, are used to manage chronic inflammatory conditions, such as rheumatoid arthritis. (Harris *et al*., 2016).

1.5.2 Non-Pharmacological Interventions:

Rest and Ice: Rest and ice are commonly used to manage acute inflammation and pain. (Kumar *et al*., 2020)

Physical Therapy: Physical therapy, such as exercise and stretching, can help manage chronic inflammation and improve function. (American College of Rheumatology, 2020)

Stress Management: Stress management techniques, such as meditation and yoga, can help reduce inflammation and improve overall health. (Kabat-Zinn, 2003)

1.5.2.1 Lifestyle Interventions:

Dietary Changes: A healthy diet rich in fruits, vegetables, and whole grains can help reduce inflammation. (Harvard School of Public Health, 2020)

Exercise: Regular exercise can help reduce inflammation and improve overall health. (Centers for Disease Control and Prevention, 2020)

Sleep: Getting adequate sleep can help reduce inflammation and improve overall health. (National Sleep Foundation, 2020).

1.6 Treatment of inflammation

For the treatment of inflammation, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are commonly really used. Most commonly used drugs are:

•Salicylates: Aspirin.

•Propionic acid derivatives: okIbuprofen, Ibuprofen+Paracetamol Combination, Flurbiprofen, Ketoprofen, Naproxen, Fenamates, and Mefenamic acid.

•Pyrazolones: Phenyl butazone and Oxyphen butazone.

•Indole derivative: Ibuprofen.

•Arylacetic acid derivatives: Diclofenac sodium, Diclofenac potassium, Diclofenac+Paracetamol Combination, Combination preparation of diclofenac and Serration peptidase.

•Oxicam derivatives: Piroxicam, Tenoxicam and Meloxicam.

•Pyrrole Derivatives: Ketorolac.

•Para-amino phenol derivative: Paracetamol.

•Others: CCelecoxib Rofecoxib, Valdecoxib and Nimesulide, Combination preparation of Nimesulide, Nabumetone.

1.7 Drugs used in management of Inflammation (Diclofenac)

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID). This medicine works by reducing substances in the body that cause pain and inflammation.

Diclofenac is used to treat mild to moderate pain, or signs and symptoms of osteoarthritis or rheumatoid arthritis.

1.8 Uses of Diclofenac

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) with a wide range of uses. Here are some of the most common uses of diclofenac:

Pain Relief

•Osteoarthritis: Diclofenac is used to relieve pain and inflammation associated with osteoarthritis. (Hochberg *et al*., 2015)

•Rheumatoid Arthritis: Diclofenac is used to relieve pain and inflammation associated with rheumatoid arthritis. (Smolen *et al*., 2017)

•Menstrual Cramps: Diclofenac is used to relieve pain associated with menstrual cramps. (Zhang *et al*., 2018)

1.8.1 Inflammatory Conditions

•Ankylosing Spondylitis: Diclofenac is used to relieve pain and inflammation associated with ankylosng spondylitis. (Braun *et al*., 2011)

•Psoriatic Arthritis: Diclofenac is used to relieve pain and inflammation associated with psoriatic arthritis. (Gladman *et al*., 2017)

•Gout: Diclofenac is used to relieve pain and inflammation associated with gout. (Zhang eta l., 2016)

Other uses

Post-operative Pain: Diclofenac is used to relieve pain after surgery. (White *et al*. 2017)

•Migraine: Diclofenac is used to relieve pain associated with migraine. (Langeveld *et al*., 2018)

•Dysmenorrhea: Diclofenac is used to relieve pain associated with dysmenorrhea. (Zhang *et al*., 2018)

1.9 Mechanism of action

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) that works by inhibiting the enzyme cyclo oxygenase (COX), which is involved in the production of prostaglandins. (Kumar *et al*., 2020).

Inhibition of COX Enzymes

Diclofenac inhibits both COX-1 and COX-2 enzymes, which are involved in the conversion of a rachidonic acid to prostaglandins. (Vane, 1971)

COX-1inhibition: Diclofenac inhibits COX-1, which is involved in the production of prostaglandins that cause pin, inflammation, and fever. (Kumar *et al*., 2020)

COX-2 inhibition: Diclofenac also inhibits COX-2, which is involved in the production of prostagland in that cause inflammation and pin. (Fitz Gerald & Patrono, 2001).

Reduction of Prostaglandin Production

By inhibiting COX enzymes, diclofenac reduces the production of prostaglandins, which are hormone-like substances that cause pain, inflammation, and fever. (Kumar *et al*., 2020)

Anti-Inflammatory Effects

The reduction in prostaglandin production leads to anti-inflammatory effects, including:

Reduced inflammation: Decreased production of prostaglandins reduces inflammation and swelling. (Vane, 1971)

Pain relief: Reduced production of prostaglandins leads to pain relief. (Kumar *et al*., 2020)

Fever reduction: Decreased production of prostaglandins reduces fever. (Vane, 1971)

1.10 Aim of the study

The aim of this study was to evaluate the preventive effect of induced inflammations in Swiss albino mice after the administration of ethanol extract of *Dioscorea alata* (water yam).

1.11 Objectives of the Study

1. To prepare the ethanol extract of *Dioscorea alata* (water yam)
2. To investigate the anti-inflammatory effect of *Dioscorea alata* extract on acute inflammation using carrageenan- and egg albumin-induced paw edema in albino mice.
3. To assess the effect of the extract on chronic inflammation using the formaldehyde-induced paw edema model.
4. To compare the anti-inflammatory effects of *Dioscorea alata* extract with a standard anti-inflammatory drug (indomethacin).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Water yam (*Dioscorea alata*)

Water yam (*Dioscorea alata*) is an extensively cultivated species worldwide. Water yam possesses several valuable attributes for cultivation and consumption. These include high multiplication ratio, early vigor for weed smothering, the higher genetic potential for yield (especially under low to average soil fertility), low post-harvest losses, good processing quality, and high nutritional value, including its possession of low glycemic index (Asiedu *et al*., 2017). Here are some key details:

2.1.1 Botanical Characteristics

- Family: Dioscoreaceae
- Common Names: Water yam, purple yam, winged yam, ube (in the Philippines).
- Vine: Twining, climbing vine with a tuberous root.
- Tubers: Large, starchy, and often purple or white-fleshed.
- Leaves: Opposite, heart-shaped with prominent veins.
- Propagation: Mainly through tuber pieces or bulbils.

Here's the taxonomical classification of water yam (*Dioscorea alata*):

- Kingdom: Plantae
- Phylum: Tracheophyta (vascular plants)
- Class: Liliopsida (monocots)
- Order: Dioscoreales
- Family: Dioscoreaceae
- Genus: Dioscorea
- Species: *Dioscorea alata(* Water yam).

2.2 *Dioscorea alata* As an African Diet

2.2.1 FOOD VALUES

Worldwide, *Dioscorea* has been cultivated for its tubers, which are considered a staple food in many regions. Tubers are primarily consumed for their carbohydrates in the form of dry matter and secondarily for protein, vitamins and other minerals. So, it is a crucial tuber crop containing essential nutrition as an alternative food source. *D. alata* is a major component of the diet in Africa, America and South-East Asia. The tuber is eaten by baked, as a sweetened dessert, or as a vegetable. The fresh-cut tuber is also edible and is used as a staple food in Taiwan. In Philippines, it is most common and used as an ingredient in many desserts as well as to flavor some items. These tubers are used to cook creamy soup in Vietnam. In many parts of India, tubers are fried to make chips.

Water and fat content change, especially in chips and flours of *D. alata*. In chips, fat increases by up to 2.314%, and water goes down by 56.61%. In flour, there was a decrease in water up to 88.93%. The highest phenolic compound in steamed *D. alata* is 265.49%, but there is a real difference between processing within phenolic content in *D. alata* (Patel *et al*., 2019).

Plate 2: 1 Water yam as food

2.2.2 MEDICINAL VALUES

Dioscorea species are characterized by the presence of diosgenin, the basis for anti-infertility drugs such as contraceptive pills and sex hormones, such as testosterone, which are consumed by bodybuilders as supplements to increase their testosterone levels and build muscle strength. The tuber of *D. alata* has the potential to treat a wide range of fatal diseases and disorders. Tubers are used in various skin diseases, wounds, burns. 2-3 g of paste of the tuber is tied to the infected part of the body to treat cancerous wounds, leprosy, gonorrhea, blood pressure. Tubers are also used in gastrointestinal disorders, cardiovascular system disorders, central nervous system disorders, disease of bones and joints metabolic disorders, immune deficiency and autoimmune diseases, dysfunctional changes in the female reproductive system, diarrhea, irritability, abdominal pain, and anemia. Besides it, tubers are extensively used in the treatment of dysentery, piles, and chronic liver pain disease (Adesokun *et al*., 2024).

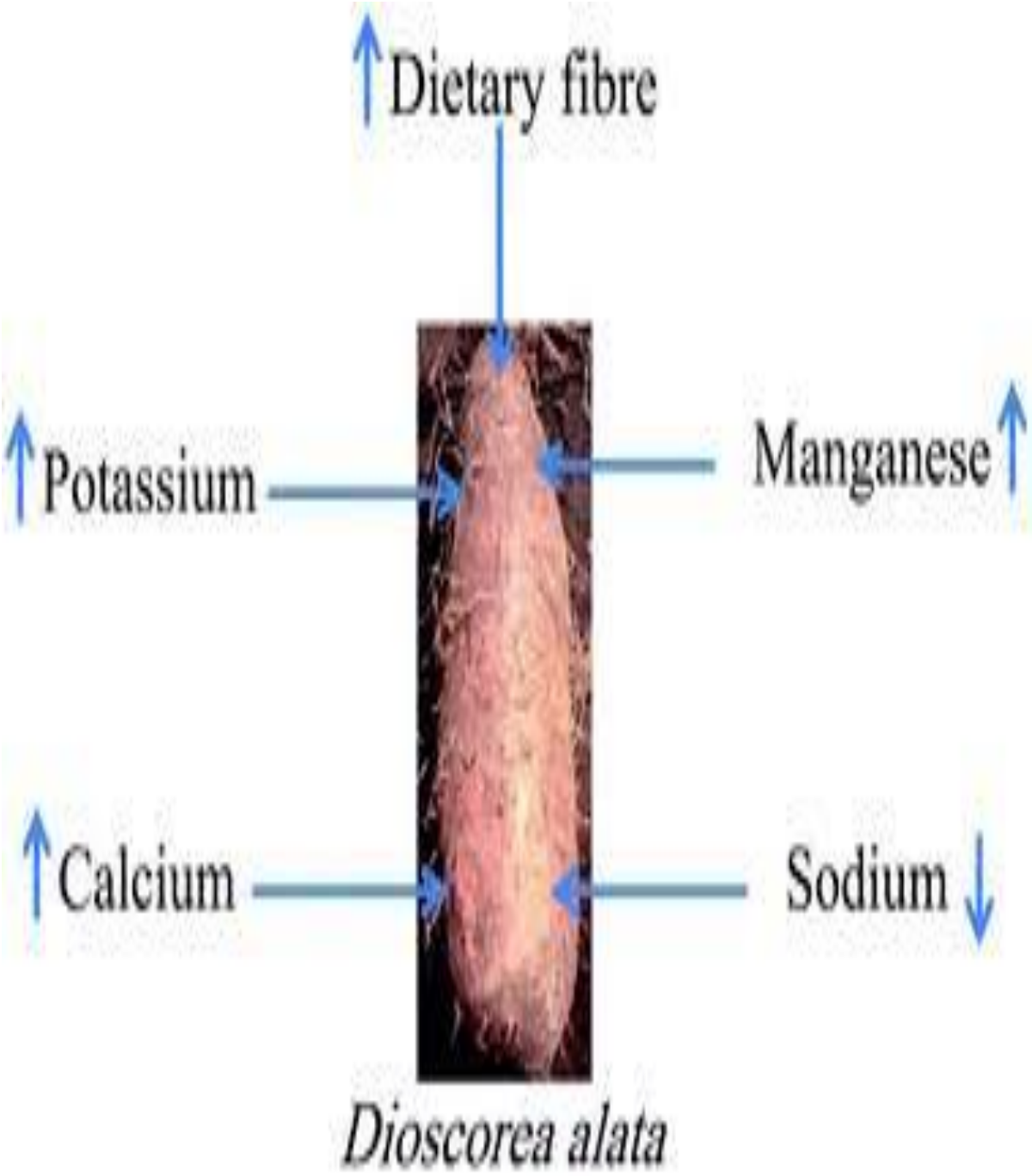

Plate 2.2: Nutrients in *Dioscorea alata*

2.3 PHYTOCHEMISTRY OF *Dioscorea alata*

The phytochemistry of *Dioscorea alata* reveals a rich diversity of bioactive compounds, including anthocyanins, phenolic acids, saponins, and dioscorin, all contributing to its potential health benefits. These compounds exhibit antioxidant, anti-inflammatory, anti-hypertensive, and immune-modulatory activities, making the tuber a functional food with therapeutic potential.

2.3.1 KEY PHYTOCHEMICALS IN *Dioscorea alata*

1. PHENOLIC COMPOUNDS

   The tubers contain various phenolic compounds, such as chlorogenic acid and caffeic acid. These compounds exhibit potent antioxidant activities and contribute to the overall health benefits associated with the consumption of *Dioscorea alata* (Mohammad *et al*., 2022).

2. SAPONINS

   Saponins are present in *Dioscorea alata* and have been reported to possess a wide range of biological activities, including anti-inflammatory, antifungal, and cholesterol-lowering effects. They contribute to the plant's traditional use in herbal medicine (Tetteh *et al*., 2018).

3. VITAMINS AND MINERALS

   Dioscorea alata is a good source of vitamins such as vitamin C, and it also contains minerals like potassium, calcium, and magnesium, which contribute to its nutritional and medicinal properties. (Asiedu *et al*., 2010).

4. ALKALOIDS AND TANNINS

Moderate amounts of alkaloids and tannins are present in the tuber, contributing to antimicrobial and anti-inflammatory properties (Udensi *et al*., 2008).

5. STARCH AND POLYSACCHARIDES

D. alata is a rich source of resistant starch, which has prebiotic effects and contributes to glycemic control. Polysaccharides also contribute to immune modulation (Zhou *et al*., 2020).

6. OTHER VITAMINS AND MINERALS

Besides phytochemicals, *D. alata* provides essential nutrients including vitamin C, vitamin B6, potassium, and manganese, which synergize with phytochemicals in promoting health (Shih *et al*., 2010).

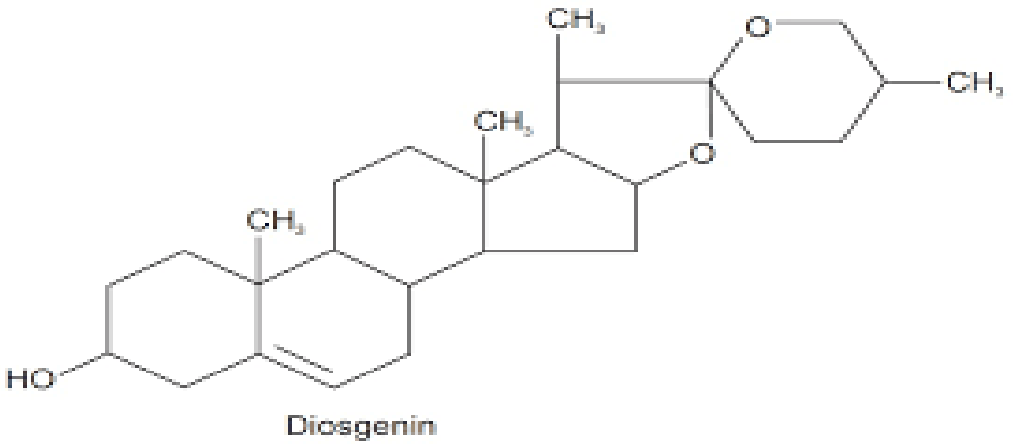

Plate 2. 3: Structure of diogenin

2.4 PRESENCE OF PHYTOESTROGEN IN *Dioscorea alata*

Dioscorea alata, also known as water yam, is a species of yam that has been found to contain phytoestrogens, which are plant-derived compounds with estrogenic activity. Phytoestrogens can mimic or modulate the action of endogenous estrogens in the body by binding to estrogen receptors.Studies on *Dioscorea alata* and other species of yam suggest the presence of compounds like diosgenin, which is a steroidal saponin known for its phytoestrogenic properties. Diosgenin has been researched for its potential to modulate estrogenic effects, although it is not a direct estrogen but rather a precursor that can be converted into steroid hormones in laboratory settings.

2.4.1 KEY STUDIES AND FINDINGS

1. DIOSGENIN IN *Dioscorea* SPICIES

   Diosgenin, present in various species of Dioscorea, including *Dioscorea alata*, has been found to exhibit estrogenic-like activity by interacting with estrogen receptors. Research indicates potential therapeutic uses of diosgenin for hormone-related conditions (Patel *et al*., 2019).

2. PHYTOESTROGENIC PROPERTIES

   Dioscorea alata has been studied for its potential role in alleviating menopausal symptoms due to its phytoestrogen content. Phytoestrogens are believed to help balance estrogen levels in women, particularly during menopause, by binding to estrogen receptors. (Chiang *et al*., 2007).

3. IN VIVO EFFECTS ON HORMONAL REGULATION

Consumption of *Dioscorea alata* has been associated with changes in hormone levels in humans. Some studies suggest it may have beneficial effects on estrogen metabolism in postmenopausal women.

3. DIOSGENIN CONTENT

Dioscorea species, including D. alata, are known for containing diosgenin, a steroidal sapogenin considered a precursor to synthetic hormones such as progesterone and estrogen. Diosgenin has been shown to exhibit phytoestrogenic activity by binding to estrogen receptors.

Plate 2.4: Water yam

2.5 ROLES OF PHYTOESTROGEN IN PRODUCTION OF MULTIPLE BABIES

1. INFLUENCE ON OVULATION

Phytoestrogen, are believed to influence ovulation. The theory is that they can affect hormonal regulation by either mimicking estrogen or by blocking estrogen receptors. This could lead to increased or altered ovulatory cycles. In some cases, it is speculated that such hormonal modulation could result in the release of more than one egg during ovulation, thus increasing the chances of having multiple babies (Cassidy *et al*., 2015).

2. DIETRY IMPACT ON TWINNING

Some epidemiological studies suggest that population with diets high in phytoestrogen-rich foods (e.g water yam, legumes) may have higher rates of twin births. The hypothesis is that phytoestrogens may enhance follicle development, leading to the simultaneous maturation and release of multiple ova (eggs). (Hoekstra *et al*., 2008).

3. ASSISTED REPRODUCTIVE TECHNIQUES (ART)

Some researchers have investigated whether phytoestrogens can enhance the success rate of fertility treatment like in vitro fertilization (IVF). While there is no direct link between phytoestrogens and increased chances of multiple births through ART, their impact on hormone levels and ovarian function is still under investigation. (Chavarro *et al*., 2008).

2.6 TRADITIONAL USE OF *Dioscorea alata*

1. FOOD SOURCE

Dioscorea alata is primarily used as a food crop in Nigeria. It is rich in carbohydrates and provides a significant portion of daily caloric intake in many rural areas. The tuber is

commonly boiled, pounded, or fried and served in various dishes. "Pounded yam" is a popular traditional dish made from boiled and pounded yams, often eaten with soups like egusi or vegetable soup (Asiedu *et al*., 2010).

2. MEDICINAL USES

In traditional Nigerian medicine, *Dioscorea alata* is believed to have health benefits. It is used in treating ailments like diarrhea, dysentery, and some skin conditions. The tuber is often boiled or grated and applied to wounds or consumed to manage gastrointestinal issues. (Okigbo *et al*., 2006).

Digestive Aid: Traditional medicine systems in Asia have used *Dioscorea alata* to aid digestion and relieve stomach discomfort.

Anti-inflammatory & Antioxidant: The anthocyanins in purple yam are believed to help reduce inflammation and oxidative stress.

3. REPRODUCTIVE AND HORMONAL HEALTH

In traditional Chinese medicine, yams (including *D. alata*) are believed to tonify the spleen and kidneys and are used for female reproductive health, particularly to balance hormones.

FOODER AND OTHER USES: In rural areas, the leaves and peels of *Dioscorea alata* are sometimes used as fodder for livestock. Additionally, certain parts of the plant are used in indigenous pest control practices or as compost m

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Plant sample

3.1.1 Sample collection

Disease free samples *Dioscorea alata* was purchased in March 2025 from Ovbiogie market in Ovia South, Benin City, Edo state, Nigeria. The tubers were 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 ethanol solvent using cold extraction method. The ground powdered leave was weighed and placed in a glass jar. 2.5 litres of ethanol solvent 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 extract was concentrated to dryness using an oven at 40^0C. The percentage yield was calculated with reference to the dried powder used.

3.1.3 Preparation of stock solution

Twenty grams (20 g) obtained from the extract were dissolved in 100 ml of water from a distilled source daily to obtain a stock solution (200 mg/ml) from which dilutions were made and calculated doses administered to the animals during the experimental procedures (Oshomoh and Obaro, 2019).

3.2 Solvents/chemicals

Ethanol solvent, chloroform (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 Phytomedicine unit in the Department of Plant Biology and Biotechnology, University of Benin, provided 60 male and female adult albino mice, with an average weight of 30-35 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. In the three (3) models (egg albumin, carrageenan, and formaldehyde) the mice were randomly allotted into four (4) groups of five (5) mice each. At time t = 0, the animal's right hind paw is measured for size. This study was approved by the Faculty of Science Laboratory Technology Research Ethical Committee with Reference Number UNIBEN/FSLT/00008

3.3 Systemic acute inflammation of the mice paw

The systemic acute inflammation was carried out by paw edema model (Obaro-Onezeyi *et al*., 2025). The sub-plantar area of the right hind paw of mice were injected with 1 percent solution of egg albumen and carrageenan in normal saline respectively, to cause acute inflammatory responses in rat paws. The size of the paw was measured in terms of the volume at various intervals, using a

vernier caliper. Single doses of the test substance were administered orally for seven (7) days before i.p. injection of phlogistic agents. At various times (0.5, 1, 2, and 3h) after carrageenan and egg albumin injection respectively, the paw size was measured again. For each group, the paw volume was calculated based on the inhibition level (percent) of edema using the formulae:

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

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

Vc = average paw volume of the control group.

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 rats through formaldehyde, adjuvant induced arthritis, collagen adjuvant-induced arthritis, air pouch inflammation and cotton pellet granuloma tests.

Arthritis - induced by formaldehyde in mice

Twenty (20) adult mice of either sex were divided into four (4) groups of five (5) mice each and administered single doses of the test substances orally. Twenty-four (24) hours after the last administration, a Veiner caliper was used to measure the animal's right hind paw and 0.1 ml of 2 % v/v formaldehyde solution was injected into the sub-plantar region of the right hind paw. After 4 hours, the paw's size is once more measured. On day 2, the mice were treated once and the sizes

of the arthritics feet were measured. On day 2, after treatment and measurement of the arthritic feet size, arthritis is re induced by formaldehyde injection. On day 3, 5 and 7, the animals were treated daily and the sizes of the arthritic feet were measured. Changes in the size of the arthritics feet were 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 was determined using the relation, and the AUC was 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 20 mg/kg of the extract

Group IV 250 mg/kg of the extract

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

4.0 RESULT

4.1 Carrageenan induced for paw edema

Mice treated with the two doses of *ethanol* extract of *Dioscorea alata* (EEDA) exhibited significant decreases in paw size, which when compared to the negative control, were significant (Figure 4.1).

4.1.1 Egg albimen induced for paw edema

Mice treated with the two doses of *ethanol* extract of EEDA exhibited significant decreases in paw size, which when compared to the negative control, were significant (Figure 4.2).

4.1.2 Formaldehyde induced for paw edema

Mice treated with the two doses of ethanol extract of EEDA plant exhibited significant decreases in paw size, which when compared to the negative control, were significant (Figure 4.3).

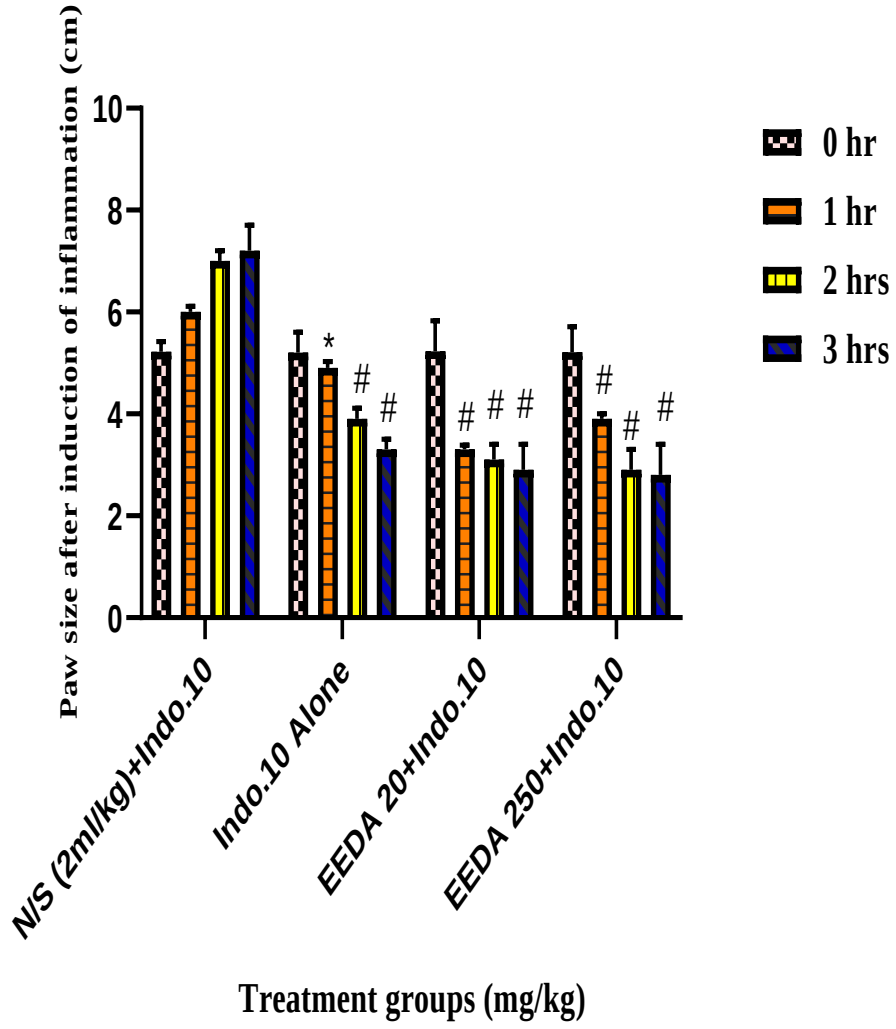

Figure 4.1: Effects of ethanol extract of *Dioscorea alata* (EEDA) (20 and 250 mg/kg) and indomethacin (10mg/kg) on carrageenan induced for paw edema. Results are Expressed as mean ± S.E.M (n=5). **** =P≤0.0001 as compared to control group

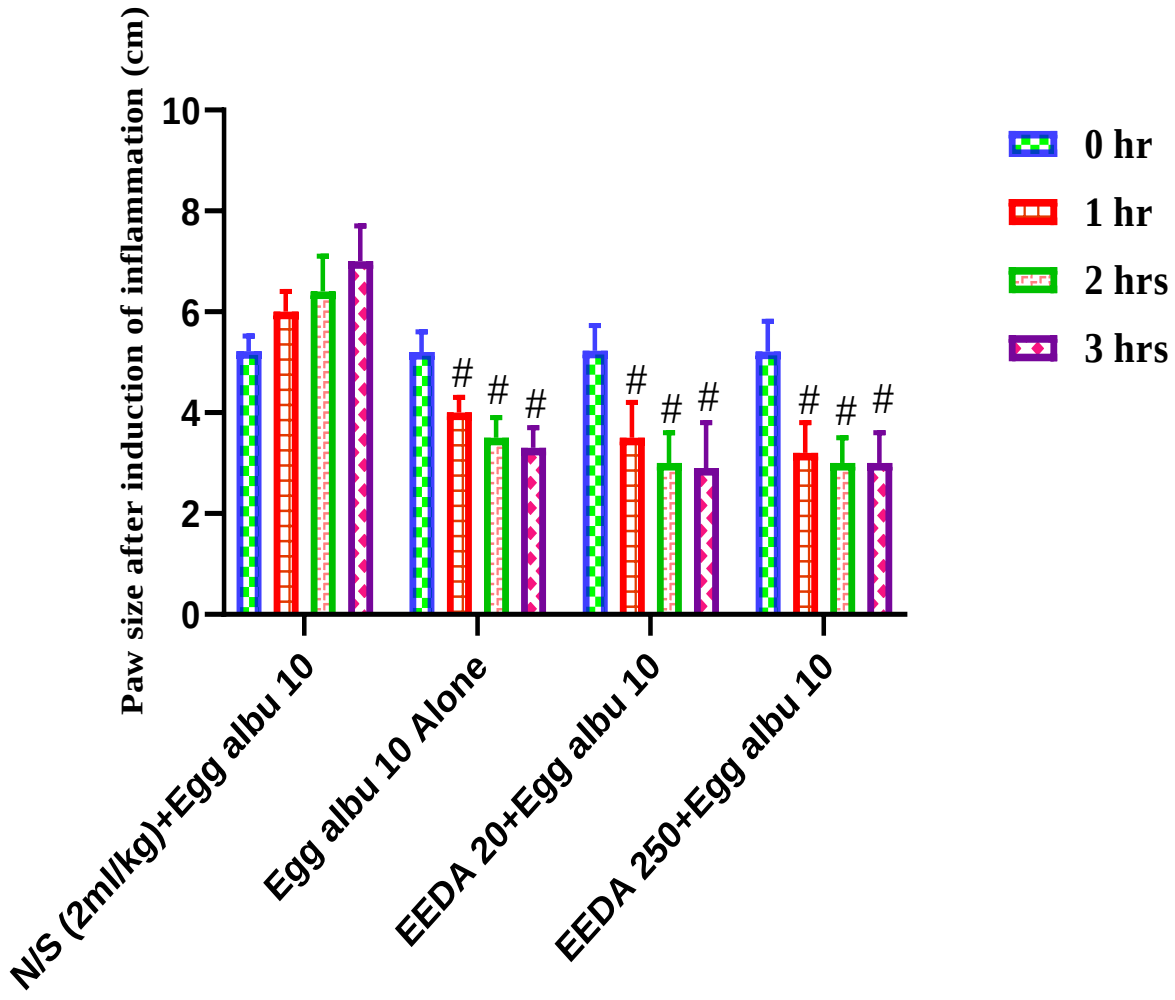

Figure 4.2: Effects of ethanol extract of *Dioscorea alata* (EEDA) (20 and 250 mg/kg) and indomethacin (10mg/kg) on egg albumen induced for paw edema. Results are Expressed as mean ± S.E.M (n=5). **** =P≤0.0001 as compared to control group.

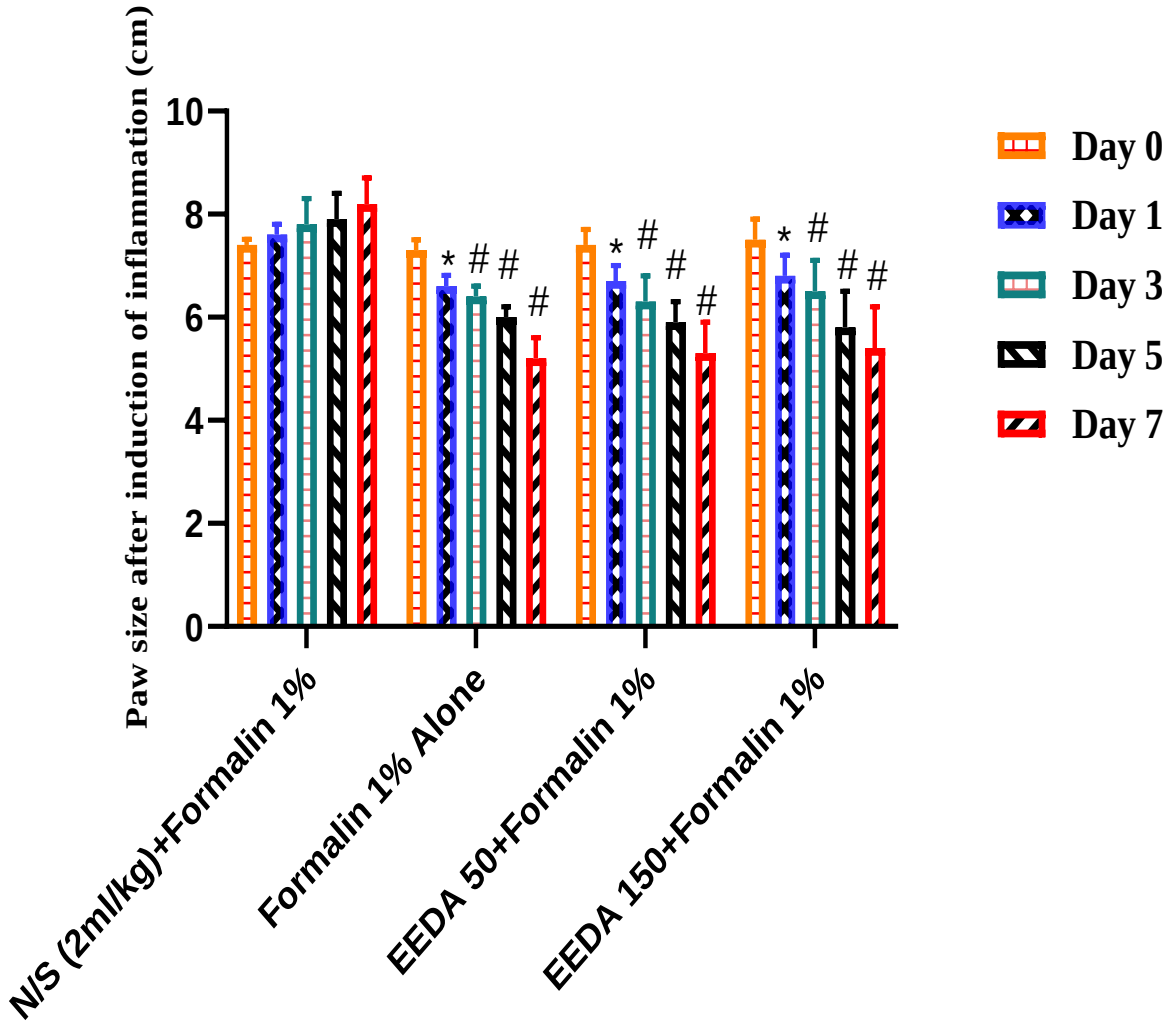

Figure 4.3: Effects of ethanol extract of *Dioscorea alata* (EEDA) (20 and 250 mg/kg) and indomethacin (10mg/kg) on formaldehyde induced for paw edema. Results are Expressed as mean ± S.E.M (n=5). **** =P≤0.0001 as compared to control group.

CHAPTER FIVE

DISCUSSION

5.1 Carrageen an Induced Paw Edema

The development of edema brought about by carrageenan has been routinely utilized to evaluate the anti-edematous qualities of items which are naturally derived (Mansouri *et al*., 2015). Despite having a wide spectrum of inflammatory mediators, it is commonly known that neutrophil infiltration plays a significant role in the inflammation brought on by carrageenan in the hind paw (Gilligan *et al*., 1994). Each

5.1.1 Extract (20 mg/kg)

Treatment group was compared to the standard which was composed of 10 mg/kg of ihuprofen and the negative control which was made up of distilled at various intervals

of 0 hour, 0.5 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 time intervals when compared with the control groups. The onset of action was slow at 2.56% inhibition after 0.5 hour when compared to the standard at 14.63% after 0.5 hour. It attains peak inhibitory action after an hour with the percentage inhibition after 3 hours being 30.77% as against the standard at 31.70%. This shows that the extract is very effective at reducing inflammations even at this dose.

5.1.2 Extract (250 mg/kg)

There is significant decrease in the in the paw size which is steady at the time intervals whhen compared to the control groups. The onset of action was relatively rapid at 11.90% inhibition at t=0.5 hour when compared to the standard drug at 14.63%. It attains peak anti-inflammatory action at 0.5 hour with the percentage inhibition after 3 hours being 35.71% as against the standard drug

at 31.70%. This show that this treatment was more effective in reducing paw edema than the standard drug ibuprofen.

5.2 Egg Albumin Induced Paw Edema

Anti-inflammatory efficacy of the various treatments were tested in te albumin induced paw edema. Further research is needed to identify the protein faction that acts as an inducer of edema because egg white proteins exhibited unique protein properties and nullified inflammatory qualities. Mice were subjected to an egg albumin test to induce paw edema (Anderson, 2015).

Each treatment group was compared to the standard which was composed of 10 mgkg of ibuprofen and the negative control which was made up of distilled at various intervals 0 hours, 0.5 hour, 1 hour, 2 hours and 3 hours. The following was deducted in each treatment group.

5.2.1 Extract (20 mg/kg)

There is significant decrease in the paw size which is steady at the time intervals when compared with the control groups. The onset of action was moderate at 10.00% inhibition after 0.5 hour when compared to the standard at 14.63% after 0.5 hour. It attains peak inhibitory action after an hour with the percentage inhibition after 3 hours being 37.50% as against the standard at 41.46%. This shows that the extract is effective at reducing inflammations even at this dose.

5.2.2 Extract only (250 mg/kg)

There is a significant decrease in the paw size which is steady at the time intervals when compared with the control groups. The onset of action was rapid at 19.05% inhibition at t=0.5 hour when compared to the standard at 14.63% at t=0.5 hour. It attains peak inhibitory action after an hour with the percentage inhibition after 3 hours being 40.48% as against the standard drug at 41.46%. This shows that the extract is effective at reducing inflammations as the standard drug ibuprofen.

5.3 Formaldehyde Induced Paw Edema

The main purpose of Formaldehyde test is to determine the ability of a drug to neutralize inflammatory responses. Formalin irritates the skin which results in pain and localized inflammatory reaction. Since an injection of formalin causes edema and increased vascular permeability, mice were used in the study's formaldehyde test to stimulate paw edema (Hong and Abbott, 1995).

Each treatment group was compared to the standard which was compared to the standard which was composed of 10mg/kg of ibuprofen and the negative control which was made up of distilled at various intervals of day 0, day 3, day 5 and day 7. The following was deduced in each treatment group.

5.3.1 Extract alone (20 mg/kg)

There is a significant decrease in the paw size which is steady at the time intervals when compared with the control groups. The percentage inhibition is 20.00% at t=3 days when compared to the standard drug at 22.54% at t=3 days. The percentage inhibition after at t= being 45.71% ad against the standard at 49.30%. This shows that the extract is effective at reducing subacute inflammations even at this dose.

5.3.2 Extract (250mg/kg)

There is significant decrease in the paw size which is steady at the time intervals when compared with the control groups. The percentage inhibition is 23.94% at t=days when compared to the standard drug at 22.54% at t=3 days. The percentage inhibition after at t= being 47.89% as against the standard at 49.30%. This shows that the extract is effective at reducing subacute inflammations at this dose.

CONCLUSION

The extract EEDA exhibited significant decrease paw size. There was no fatalities among the animals. The findings of this experiment indicates that *Dioscrea alata* maybe use in the treatment and management of inflammation.

RECOMMENDATION

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

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APENDIXES

Carrageenan Induced Inflammation

Control Group

Inducing inflammation on the paw

	0hrs	30mins	1hour
2 tail mark	2.2	2.2	2.4
2 tail mark	2.2	2.2	2.4
4 tail + head mark	2.1	2.3	2.5
Ear mark	2.1	2.1	2.4
Back mark:	2.0	2.2	2.6

20 mg/kg of EEDA Group

Inducing inflammation on the paw

	0hrs	30mins	1hour
2 tail mark	2.2	2.1	2.0
2 tail mark	2.2	2.0	2.1
4 tail + head mark	2.1	2.2	2.0
Ear mark	2.1	2.0	1.9
Back mark:	2.0	1.9	1.8

250 mg/kg of EEDA Group

Inducing inflammation on the paw

	0hrs	30mins	1hour
2 tail mark	2.2	2.2	2.4
2 tail mark	2.2	2.2	2.4
4 tail + head mark	2.1	2.3	2.5
Ear mark	2.1	2.1	2.4
Back mark:	2.0	2.2	2.6