IN-VIVO ANTI-INFLAMMATORY ACTIVITY OF THE AQUEOUS AND METHANOLE ROOT EXTRACT OF *ALAFIA BARTERI*IN ALBINO RATS

BY

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CERTIFICATION

This is to certify this project work entitled as INVIVO ANTI-INFLAMMATORY ACTIVITY OF THE AQUEOUS AND METHANOLIC ROOT EXTRACT OF *ALAFIA BARTERI*IN ALBINO RATS was carried out by **ESONWUNE CLINTON OGOCHUKWU** with matriculation number **15010102015** department of Biochemistry Mountain Top University in partial fulfillment of the award of Bachelor of Science, B.Sc in Biochemistry under the supervision of MrsI.O. Adefisan

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DEDICATION

This report is dedicated to God Almighty for his grace and strength and provision throughout my stay in Mountain Top University. Also to my family for their love and support and my supervisor for her endless support.

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ABSTRACT

Alafia barteri (Apocyanaceae) is a medicinal plant used traditionally in Ghana and other parts of Africa for the control of various inflammatory and pain conditions. The aim of this study, therefore was to estimate the anti-inflammatory effect of the plant using formaldehyde induced inflammation, a chronic inflammatory model in humans. Inflammation was induced by injection of 0.1 ml of 1% formaldehyde into the right paw of rats and paw diameter was measured by digital Vernier calliper. Diclofenac was used as the reference drug. Physical parameters as well as serum liver function markers were also assessed.

The results obtained showed that oral treatment with the aqueous extract (400 mg/kg) significantly suppressed inflammation with maximal inhibitions of 34.8% and methanolic extract (400 mg/kg) with maximal inhibitions 38.6 %. Other doses of the extract did not show significant inhibition. Serum AST and ALT were unaffected as well as creatinine and total protein levels.

From the results, *A.barteri*exhibits anti-inflammatory effect and the effect revealed is comparable to that of diclofenac. This provides an authentication for the traditional use of the plant in controlling inflammation.

Keywords: Inflammation, serum, formaldehyde, anti-inflammation, total protein concentration

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

The inflammation term is taken from the Latin word “inflammare” (to burn) (*de oliveira*). Inflammation is one of the most central processes required in defense of animal cells against certain injuries or microbial infections [Isailovic*et al*., 2015;Todd *et al*., 2015]. Nevertheless, inflammation regularly progresses to acute or chronic [Serhan*et al*., 2015]. Chronic inflammation is caused due to a variety of diseases including neurodegenerative disorders, cancer, and cardiovascular diseases.

Inflammation is a local response of living mammalian tissues to injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation (Mitchell and Cotron, 2010).Mechanism of inflammation represents a chain of organized, dynamic responses including both cellular and vascular events with specific humoral secretions.

A group of secreted mediators and other signaling molecules (e.g., histamine, prostaglandins, leukotrienes, oxygen- and nitrogen-derived free radicals, and serotonin) are released by immune defense cells principally in the mechanism which can contribute in the event of inflammation [Anwikar*et al*., 2010].

Traditional and folklore medicines play an important role in healthcare. There exist a plethora of knowledge and benefits of herbal drugs in our ancient literature of ayurvedic and unani medicine. According to the World Health Organization (WHO), 80% of the population in developed countries relies on traditional medicine for their primary healthcare. Despite considerable progress in therapies using expensive synthetic drugs, the search for herbal remedies is growing which can be accounted for the effectiveness, minimal side effects in clinical experience, and relatively low cost of the herbal drugs. Herbal drugs or their extracts are prescribed widely, even when their biologically active compounds are unknown. Exploration of the chemical constituents of plants and pharmacological screening would provide the basis for developing new lead molecules in strategic favor of

Natural product Drug discovery. This shows the need for planned activity guided pharmacological evaluation of herbal drugs [Kumar et al., 2013].

Medicinal plants constitute an effective source of both traditional and modern medicine. Plants have been used as sources of remedies for the treatment of many diseases since ancient times and people of all continents especially Africa have this old tradition.A plant is said to be medicinal when one or more of its parts have been claimed or used to cure disorders or ailments.

Despite the remarkable progress in synthetic organic medicinal products of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants (Newman et al., 2000). However, plants used in traditional medicine are still understudied (Kirby, 1996). In developing countries, notably in West Africa, new drugs are not often affordable. Thus, up to 80% of the population uses medicinal plants as remedies (Kirby, 1996; Hostellmann and Marston, 2002). In Africa, traditional healers and remedies made from plants play an important role in the health of millions of people.

The current idea on inflammation has grown significantly over the years because of the huge elaboration of the field in more oblique directions. As a result, we are currently far from being able to fully apprehend the aftermath of inflammation in human health and diseases. Inflammation for years has been the sources and causes of different kinds of disease such as arthritis, heart attacks, Alzheimer's disease, cardiovascular diseases, rheumatoid arthritis, cancer, asthma etc.

Although, therapeutic drugs have been used to treat acute inflammation these drugs have been discovered to cause severe side effects which may lead to death.

1.2 SIGNIFICANCE OF STUDY

The study is carried out to complement the works of various researchers who thirst to solve the problem of inflammation without the use of synthesized drugs such as Diclofenac potassium by carrying out a research on *Alafia barteri* aqueous extract and methanolic root extract.

1.3 AIM OF STUDY

The research aims at evaluating the anti-inflammatory activity of the aqueous and methanolic root extract of *Alafia barteri* (plant) in formaldehyde induced inflammation in Wistar rats.

1.4 OBJECTIVES

1. To determine the phytochemicals present in *Alafia barteri* root
2. To evaluate the effects of the aqueous and methanolic root extract on body weight and paw diameter of rats
3. To determine the effect of the extracts on some serum biochemical parameters

CHAPTER TWO

LITERATURE REVIEW

2.1*ALAFIA BARTERI*PLANT

Alafia barteri Olive, Apocynaceae, frequently referred to as Alafia chewing stick, is a highly climbing, glabrous, scandalous tree with tiny pure white or pink flowers that are widely spread in tropics.The stem and leaves are mostly (often) medicinal, particularly in the therapy of malaria and fungal diseases, so it is extremely appropriate and substantial to concentrate on root research and leave for anti-inflammatory impact.

2.1.1 BOTANICAL DESCRIPTION OF *ALAFIA BARTERI*

A liana up to 35meters (115ft) long, clear sap or sometimes with white latex; stem diameter of up to 3 centimeters (1.2 in), bark pale grey-brown with many lenticels. Leaves opposite, simple and entire; stipules in axil of petiole; petiole 2-5 mm long; blade elliptical to narrowly elliptical, 4-16.5 cm x 2-6.5 cm, base obtuse to cordate, apex rounded to shortly acuminate, leathery, glabrous.

Inflorescence a rather lax terminal dischasial cyme, many flowered; peduncle 5-20 mm long; bracts sepal-like. Flower bisexual, regular, 5-merous, fragrant; pedicel 2-6mm long; sepals free, ovate, 1.5-2 mm long, rounded or obtuse; corolla white, often with greenish tube, 5-8 mm long, 1-2 mm wide above the base, widening near the insertion of the stamens and narrowed towards the throat, glabrous or slightly hairy outside, inside with hairy belt below insertion of stamens, lobes obliquely orbicular to elliptical or obovate, 4.5-8 mm long, apex rounded and often wavy, spreading, glabrous outside, hairy with curled to rather straight hairs at the part of the lobes covered in the bud and hairy inside at the base and in the upper part of the throat; stamens inserted halfway the corolla tube, just included or exserted, anthers sessile, arrow-shaped; ovary superior, ovoid, consisting of 2 separate carpels, style narrowly obconical, 2.5-3 mm long, pistil head consisting of basal ring, cylindrical part and 2-lobed stigmoid apex.

Fruit consisting of 2 separate, cylindrical, linear follicles 15-50 cm x 0.5-1 cm, dehiscent, dark brown, many selected. Seeds narrowly ellipsoid, 20 mm long at the top with a tuft of hairs 2.5 cm long.

2.1.2 TAXONOMY

Alafia barteri is a species in the genus of Alafia which contain 23 species 15 of which occur in continental Africa and 8 in Madagascar. It belongs to the family of Apocynaceae. The taxonomy of *Alafia barteri* for classification includes the following;

Division: Angiosperm

Class: Eudicots

Subclass: Asterids

Order: Gentianales

Family: Apocynaceae

Subfamily: Apocynoideae

Tribe: Malouetieae

Genus: Alafia

Species: *A.barteri*

2.1.3 CULTIVATION

The plant is harvested from the wild for local use as a tying material and medicine. It is said to be poisonous. It other common names are;

Sierra leone: LOKO kpeeng, MENDE gbenge

Ivory Coast: ANYI si-diafua-angbe

Ghana: AKAN-ASANTE momunimo, FANTE edru

Nigeria: IGBO (Obompa) otanza, YORUBA agbarietu

2.1.4 GEOGRAPHIC DISTRIBUTION:

Alafia barteri is a native to Nigeria, Congo, Sierra Leone, Liberia, Togo, Gabon, Cameroon, Benin, and Guinea Bissau.

2.1.5 USES OF *ALAFIA BARTERI*

Alafia barteri is valued for its effectivity in the traditional medicine system in Nigeria and other African countries, as an anti-inflammatory and fever remedy. Also it is used for the treatment of eyes infections, febrifuges, as chew sticks, sickle cell, anemia, rheumatism and toothache. The infusion of the leaves and twining stem are used for the treatment of inflammation and fever. The decoction of root and leaves of the plant is also taken internally or applied externally to treat rheumatic pain, toothache and eye infections (Odugbemi, 2008) and the fibre from the stem of the plant serves as tying material for roofs. The extracts of the leaves were found to have antibacterial and antifungal activities (Adekunle and Okoli, 2002; Hamid and Aiyelaagbe, 2011). The aqueous leaf extract was reported to display potent anti-plasmodia activity i.e. it is use for the treatment of malaria. (Lasisi et al., 2012).

2.1.6 Medicinal plants with anti-inflammatory activities from countries and regions of Africa

EGYPT:

In Egypt, plants have been reported to supply natural anti-inflammatory agents that may display minimal side effects.

Species of the genus *Ipomoea* and *Alstonia*have been documented to possess anti-inflammatory activities and scientists have isolated lipoidal and phenolic compounds from this genus.In a laboratory-controlled experimental animal model conducted in Egypt, Karawya et al used aerial parts of *Ipomoeapalmate*; *Alstoniascholaris*; and the leaves of *Salixsubserrata*, *S. tetrasperma*, and *Phyllostachysnigrar*(500mg/kg rat body weight of each extract) and anti-inflammatory activities of the plant extracts were subsequently assessed in male and female albino mice.12 Different extracts of the aerial parts of *A. scholaris*showed variable anti-inflammatory activity from which the aqueous methanol extract was the most promising, showing significant

anti-inflammatory effect 1 hour after carrageenan injection with 64% inhibition with a maximum inhibition of 91%. (Oluwafemi, 2018).

ALGERIA:

Using female rats, Boubekri et al performed anti-inflammatory activity on *Genistaquadriflora*munby extract. The plants were collected during the flowering season in May 2008 from the area of El Kala in Algeria. The plant is commonly used in Algeria and Morocco in traditional medicine. In the study, anti-inflammatory activity of the plant extract (100 and 200 mg/kg) was assessed by carrageenan-induced rat paw while edema was induced by injecting 1% suspension of carrageenan in 0.09% sterile physiological saline into the right plantar region of the rat. The n-butanol extract of the *G.quadriflora*demonstrated significant reduction in the edema paw volume in a dose-dependent manner and the aspirin standard (100 mg/kg) produced a significant inhibitory effect comparable to the group that took the plant extract. Carrageenan paw is an acceptable approach in the determination of anti-inflammatory activity as it involves various mediators. The development of edema induced by carrageenan injection is a three-phase process; the first 90 minutes involvesthe early phase which involves the release of histamine and serotonin, the next phase which commences from 90 to 150 minutes is mediated by kinin, and the last phase (usually after 180 minutes) is mediated by prostaglandin. It was observed that significant inhibitory activity occurred at the third phase (after 3 hours) of edema development. This observation prompted the authors to opine that anti-inflammatory activities of the extract are possibly related to the inhibition of one or more intracellular signaling pathways involved in the effects of inflammatory mediators. Based on the role of free radicals in inflammatory processes, the authors suggested that the anti-inflammatory effect of the extract may be partly linked to its antioxidant content since the extract is very rich in phenolic compounds.(Oluwafemi, 2018).

CAMEROON:

Medicinal plants have been used in traditional medicine in meeting the health needs of people in Cameroon, a country located in central Africa. In Cameroon, research emphasizing discovery of medicinal plants with antioxidant and anti-inflammatory activities is ongoing and recognition has been given to the application of

medicinal plants in the treatment and management of several diseases, including inflammatory diseases. In a laboratory scientific study in Cameroon, Sagnia et al examined fresh leaves of *Caricapapaya, Eremomastaxspectiosa, Eleusineindica, Cassiaalata,* and *Polysciasfulva*for their anti-inflammatory potentials.The air-dried and powdered leaf specimen from each plant were macerated separately in ethanol for 48 hours at room temperature and shaking at intervals. Following the extraction procedures, the plant extracts were tested for their anti-inflammatory activities in an experiment. In the assessment of anti-inflammatory activity of the plant extracts, alpha tumor necrosis factor (TNF-α) production from monocyte was evaluated after culture with different concentration of plant extracts in the presence of lipopolysaccharide (LPS). The authors reported that the plant extracts inhibited the LPS-induced TNF production by monocyte purification in a dose-dependent manner and that the most inhibition was achieved with 1 μg of plant extracts by *Er. speciosa, P. fulva, and Casiaalata followedby El. indica*and *Carica papaya.* It was noted that the anti-inflammatory activity of each plant extract is directly related to the concentration of the phenolic compounds and other constituents that were present in the extracts. Other studies have reported that compounds such as alkaloids, saponins, phytosterols, tannins, flavonoids present in medicinal plants possess anti-inflammatory activities *Dichaetantheraafricana*is a medicinal plant commonly used in Cameroon and Congo to treat various ailments as well as inflammatory diseases.(Oluwafemi, 2018).

GABON:

Gabon, like many African countries, has a population that increasingly uses herbal medicine to manage multitude of diseases. In recognition of this, a large program of research on medicinal plants has been carried out at the Institute of Pharmacopoeia and Traditional Medicine in Gabon to contribute effectively to the management of various diseases via development of phytomedicines in accordance with government guidelines. The program involves ethnobotanical surveys, experimental laboratory research, and the production of herbal medicines. There are large number of documented plants used for management of diabetes, hypertension, pain, and inflammation. In Gabon, the stem bark of *Pseudospondiaslongifolia*and *Antrocaryonklaineanum*are used in

traditional medicine as analgesic and anti-inflammatory agents. To test the phytochemical contents of these anti-inflammatory and analgesic agents, Mebale et al collected stem barks of two plants (*P. longifolia and A. klaineanum*) in the northern part of Gabon. After extraction, phytochemical analysis was performed. The results showed that the extracts were rich in alkaloids, tannins, saponins, and flavonoids that possibly explain their potential anti-inflammatory and analgesic activities. In an ethnobotanical survey in Gabon, Tchouya et al reported on 52 medicinal plants belonging to 50 genera and 34 families that are traditionally being used in the treatment of HIV/AIDS opportunistic infections.These plants are also being used as anti-inflammatory agents since inflammation is associated with viral and bacterial infections.(Oluwafemi, 2018).

KENYA:

In Kenya, traditional medicine is, broadly, culturally accepted and practiced in various regions where over 400 plant species have been documented to be in use as therapeutic agents and in traditional remedies by traditional healers and by the general public in the treatment and management of many diseases, including inflammatory diseases. Bacterial infections and inflammatory diseases are some of the conditions treated with preparations from medicinal plants in Kenya. It is important to note that many of the inflammatory diseases are associated with synthesis of prostaglandins. In 2003, Matu and van Staden evaluated the anti-inflammatory activities of selected medicinal plants used in traditional medicine in Kenya. Anti-inflammatory activity of each plant extract was assessed by using COX assay.The results showed that the highest inhibition was with organic extracts of *Galinsogaparviflora,Maytenussenegalensis, Mondiawhitei, O. sinuatum, P. barbatus, Xzanthoxylumchalybem*, and *Zanthoxylumusambarense*.

It was reported that the aqueous extracts showed lower anti-inflammatory activity, and it was concluded that the results confirmed the therapeutic potency of the 12 medicinal plant species assessed in the study and provide justification for their applications in traditional medicine in the treatment of inflammatory diseases and other diseases in Kenya.(Oluwafemi, 2018).

TANZANIA:

The people of Tanzania have a rich culture of traditional medicine usage and practices with dynamic interethnic cultural interactions of different people from different parts of the country constituting a rich base of herbal and traditional practices. It was noted that most of the medicinal plants used had more than one therapeutic usage. For instance, *Draceanasteudneri*was used for treating fibroids and asthma. The prevailing conditions treated with medicinal plants include respiratory tract infections, cardiovascular disease, infectious diseases, and skin diseases and joint pains. It is important to note that all these disease conditions elicit inflammatory responses. The leaves from the plants were the most commonly used parts (20 species) followed by the roots (13 species) and that monotherapy prepared from the medicinal plants was common compared to mixed preparations from two or more medicinal plants. Sixteen of the 34 plants used are supported by reports of their usage in literature and there are no reports of their harmful effects in human. The plants whose traditional and therapeutic claims are supported in literature in Tanzania are as follows: *Ageratum conyzoides*, *Bidenspilosa*, *Boerhaviadiffusa*, *Capparistomentosa*, *Casiaalata*, *Clerodendrummyricoides*, *Lantana camara*, *Flueggeavirosa*, and *Veroniaamybdalina*. The above-mentioned plants, which are commonly used in Tanzania, have the possibility of being used in drug development and some of them constitute source of food and nutrition for the local community. (Oluwafemi, 2018).

SWAZILAND:

Medicinal plants form an essential part of Swaziland's biological resources, and it is important to report that many Swazis depend on traditional medicine for their health care needs. This is because traditional medicine is linked to the culture and religious beliefs of the Swazis. The need to preserve the cultural heritage by documenting information on medicinal plants used in traditional medicine in Swaziland motivated Amusan and colleagues to perform ethnobotanical surveys of medicinal plants in the Kingdom of Swaziland.42 Few of the plants used in the region of Manzini, Swaziland include: *Spirostachysafricana, Trichilia emetic, Ximeniaamericana,Peltophorumafricanum*. The investigators reported on traditional medical practitioners in the

Mazini region of Swaziland describing 41 remedies for 25 disease conditions including inflammatory disease. (Oluwafemi, 2018).

2.2 INFLAMMATION IN HUMAN BODY

Inflammation is the body's biochemical reaction to an assertive agent, characterized by vasodilatation, access to target tissue by liquid and cells (Schmid-Scho¨nbein, 2006). It is a conservation approach established in greater organisms in reaction to harmful insults such as microbial infection, tissue injury and other harmful circumstances**.**The host is an important immune response that allows damaging stimuli to be removed as well as damaged tissue to be healed. Inflammation was seen as part of innate immunity, the first line of host defense against foreign invaders and molecules of risk.For hundreds of years, human beings have known the standard inflammation symptoms including redness, pain, swelling, and heat (Medzhitov, 2008). However, there are many distinct procedures engaged in initiating, regulating and resolving the entire course of inflammation. A variety of inflammations have been recognized at the moment, with many distinct types being initiated by numerous stimuli and regulated by distinct regulatory processes.

Inflammation is thought to have an effect on every aspect of ordinary human physiology and pathology due to its comprehensive and widespread nature.

2.2.1 Types / Inflammatory characteristics

The response of inflammation to the body is of two types;

Acute inflammation:Acute inflammation is the body's reaction to injury to mediate the injury's healing. It generally happens within minutes or hours of tissue injury, and the fundamental symptoms of heat, pain, dysfunction, and redness can be described. It is defined by fluid and plasma protein exudation, leukocyte movement (monocytes, macrophages mostly neutrophils) into the wounded region. Acute response to inflammation is relevant to defense action aimed at eradicating bacteria, viruses, parasites and fungi while at the same time enchanting wound repairs.

Chronic Inflammation: The existence of macrophages and lymphocytes, leading to tissue necrosis and fibrosis, characterizes chronic inflammation histologically.It improves degenerative disease enhancement, such as IBD-inflammatory bowel disease, rheumatoid arthritis, atherosclerosis, heart disease, Alzheimer, asthma, acquired immunodeficiency disorder (AIDS), cancer, congestive heart failure, multiple sclerosis, diabetes, infections, gout, aging and other neurodegenerative CNS depression. It is part of the cause of muscle loss which occurs with aging. It is associated with mononuclear immune cell infiltration, macrophages, monocytes, neutrophils, proliferation and fibrosis of fibroblast activation.

2.2.2 Stages of inflammation

Inflammation is the body's injury response. Injury can occur from a variety of sources, including physical trauma (strain, sprain or contusion), bacterial or viral diseases, heat or chemical injury.In the immediate region of injury, trauma causes direct harm to cells, causing bleeding. The bleeding lead s in the inflammatory process sequence of occurrences that enchant the healing of the wounded tissue. Persistent injury or individual factors such as diabetes, use of corticosteroids, blood disorders may encourage progression of acute inflammation to chronic inflammation.

Inflammatory Response

Acute injury healing begins with acute inflammatory vascular reactions. The vascular modifications are intended to boost blood flow to the immediate region and also to bring cells to the region to initiate healing.When the healing begins, damaged cells are separated and the body adds fresh collagen to the injury region. This phase follows the injury instantly and lasts 3-5 days.The injury region will experience pain, warmth, swelling, palpable tenderness, limited joint or muscle variety of movement during these 3-5 days. Pain and swelling can be reduced to avoid chronic inflammation and to retain mobility and strength in other regions of the body while resting in wounded regions.

Repair and Regeneration

The next phase is fresh collagen formation. The fresh collagen fibers are laid down in the shape of a scar with weak links between each fiber in a disorganized way. The fresh tissue, however, is fragile and prone to aggressive activity disturbance.Usually this phase lasts from 2 days to 8 weeks. Warmth and swelling are decreasing and the palpable pain is decreasing. Range of exercises, joint mobilization, and scar mobilization to improve tissue remodeling should be performed.

Re-modeling and Maturation

There is less formation of fresh collagen at this point but enhanced collagen fiber organization with greater bonds between them. Therefore, new collagen must align along the stress lines to accommodate the loads needed for the function.As the healing advances, the tissue continues to remodel, reinforce and enhance its cellular organization. There is no known end to tissue re-modeling and healing. It may take months to years before it can be completed.

2.2.3 Causes of inflammation

Inflammation is a normal reaction of the body to protect the tissues from disease, injury, and infection. This reaction or response starts with the manufacture and release by cells of chemical agents in the infected, diseased or injured tissue. These agents are cause redness, inflammation, pain, heat, and deranged work.These leukocytes can destroy any injurious or infectious agent and can also remove cellular debris from damaged tissue. While this inflammatory response usually increases tissue healing, if not controlled, it may become hazardous.Acute inflammation causes include

Microbial infection:

One of the prevalent causes of inflammation in the body is microbial infection. The killer operations of microbes cause this microbial infection. Bacteria, protozoa, fungi, viruses and several parasites are included in microbes.Specific toxins (exotoxins or endotoxins) released by bacteria. Endotoxins are toxins found in a bacterial cell and released when the cell disintegrates. They are component of Gram's cell wall of adverse

bacteria and they do terrible things to the body. While exotoxins are toxins that are released into the environment by a bacteria cell. They are produced specifically for export (like anthrax toxins or tetanus toxins). The endotoxins in their behavior are not as particular as the exotoxins.

Hypersensitivity reactions

A response to hypersensitivity happens when an altered immunological response status causes an inappropriate or excessive immune reaction that damages the tissues. The reaction types will be discussed later in more detail (In the Immune Mediated Inflammation class).

Physical agents, irritant and corrosive chemicals

Physical trauma, burns or excessive, ultraviolet or other ionizing radiation may cause inflammatory damage to the tissue. Corrosive chemicals like alkalis, acids, and oxidizing agents can cause direct harm to the tissue. These corrosive chemicals cause the tissue damage that causes inflammation immediately.

Tissue necrosis

Infarction i.e. insufficient blood flow leads to absence of oxygen or nutrients and then to tissue death. This is a powerful stimulus for inflammation. It often demonstrates an acute response to inflammation.

2.2.4 Inflammatory Effects

Inflammation may have local or systemic impacts. Inflammation local impacts are generally useful to the body. Execution of invading microorganisms, for example, but they sometimes seem to have no impact or may be dangerous. While fever, leukocytosis, malaise are part of the systemic effect.

Systemic Effects of Inflammation

Inflammation, either acute or chronic, even if well located, can have a huge impact on the body. The body's primary portion that experiences these impacts is:

Leukocytosis

A prevalent feature of inflammatory responses is leukocytosis. It happens when the body's white blood cells are abnormally large in circulation. Increased neutrophils (white blood cells) show a bacterial infection while lymphocyte increases imply a viral infection.

Fever

Fever (heat) is a prevalent systemic reaction to inflammation. It is frequently associated with infectious disease inflammation. The rise in body temperature is aimed at improving the effectiveness of killing leukocytes and also impairing the replication of many invading bacteria. The hypothalamus coordinates fever and includes a broad variety of variables.

2.2.5 Inflammatory linked diseases

Inflammatory diseases are a group of medical illnesses that are displayed as distinctive features by chronic inflammation i.e. abnormal inflammatory reactions. The causes and effects of these illnesses are chronic inflammation and the pathogenesis of inflammatory diseases. For instance, obesity causes inflammation, while chronic inflammation can lead to obesity-associated diabetes due in part to insulin resistance (Hotamisligil, 2006).

2.2.6 Rheumatoid arthritis

By making them a little different from those caused by stubborn infection, the chronic nature of the inflammation with these illnesses can be regulated at least to some extent by the pathological outcome of these illnesses. Another excellent instance of chronic inflammation-associated disease is rheumatoid arthritis. Inflammation is a prevalent clinical condition and rheumatoid arthritis (RA) is a chronic autoimmune draining disorder1, which impacts about 1% of the population in advanced nations. It is a chronic, systemic inflammatory disease that impacts the hands and feet of the tiny joints. For unknown reasons, the immune system attacks joint tissue and potentially other components of the body. (Miller et al., 2009). Pain, inflammation, joint harm and then malformation are caused by rheumatoid arthritis. It can cause patients on both sides of the body to feel sick, attempted, feverish, and joint pain. As the disease develops, these symptoms can spread to the knee, ankles, wrists, elbow, hips, and shoulders. (Mayo et al., 2016).

The synovium, the joint lining experiences chronic inflammation through lymphocyte and macrophage penetration and synovial cell activation, synoviocytes that produce synovial fluid. Billions of neutrophils invade the synovial fluid every day during rheumatoid arthritis. (Akira et al., 2006). Neutrophils with a half-life of about 4 hours have been suggested to create a significant contribution to the nature of synovial tissue chronic inflammation. One of the neutrophil proteins, cytosolic peptidyl arginine deaminase, whose activity depends on the level of extracellular Ca+, is hypothesized to release and then activate from deceased neutrophils. (Afsar.U. Ahmed., 2011). This enzyme produces citrulline in certain proteins by converting L-arginine residue guanidine side chains to uredo residues. Surprisingly, citrullinated proteins respond with rheumatoid arthritis-related auto-anti-bodies. (Uysal et al., 2009).

2.2.7 Asthma

In asthma, the inflammatory response mechanism is more complicated compared to others. Asthma's first cause is more complex. While genetic asthma susceptibility has been reported for years, latest genome-wide studies indicate comparatively tiny inherited contributions to asthma (Rogers et al., 2009Inflammation is triggered by unregulated interactions between the inborn immune cells and the epithelial mucosa .(Afsar.U. Ahmed 2011). In addition to innate immunity, adaptive immune cells like CD4 T helper 2 cells are heavily related to asthma by Th2-associated cytokines. (Robinson et al., 1992). On the other side, environmental factors were evaluated as a significant cause of danger of asthma. For instance, several studies indicate that many early-life viral upper respiratory infections along with family genetic predilection affect a substantial danger of asthma. (Locksley, 2010).

It is connected with many neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and multiple sclerosis due to the effect of inflammatory illnesses on human health that have had a major effect on every single function of the body. (Glass et al., 2010). Other illnesses include inflammatory intestinal disease that impacts the bowel (Garrett et al., 2010) and Inflammation of the glomeruli, tiny blood vessels, in the kidneys causes glomerulonephritis. (Fakhouri et al., 2010).

2.2.8 Cancer

It has become evident over the century that inflammation plays a major role in endorsing cancer, particularly in tumor formation (tumorigenesis). (Afsar.U. Ahmed., 2011). Persistent inflammation is associated with harm to DNA, which in turn leads to cancer as a precursor to cancer. Chronic inflammation for certain cancers serves as a precursor. In addition, several kinds of immune cells are also discovered in tumors apart from cancer cells. As an important aspect of all tumors, an inflammatory micro-environment is also often discovered. (Mantovani et al., 2008; de Visser et al., 2006). It has been found that the responses of inflammation caused by infection are also associated with a rise in the danger of cancer (de Martel and Franceschi, 2009). Studies have shown that lung tumorigenesis induced by smoking tobacco is in fact initiated by chronic inflammation mediated by IKK-beta and JNK1 and that the pathway of inflammation and tumorgenesis is strongly linked.(Takahashi et al.,2010). Besides the inflammatory response to the tumor, it is believed that in most tumor microenvironments an effective anti-tumor immunity is also present. (Smyth et al., 2006; Lin and Karin, 2007). Research has shown that cancer cell growth is enchanted not only by inflammatory micro-environments, but also by the production of intermediate nitrogen and reactive oxygen species (ROS), which can lead to DNA damage and genomic instability. (Grivennikov et al., 2010). In addition to genomic instability, environmental factors including carcinogens, infectious microbes, tobacco smoke and inhaled pollutants were measured to play critical roles in inflammation-induced cancers. (Aggarwal et al., 2009). For instance, rhematoid arthritis is not caused by chronic inflammatory illnesses, while inflammatory intestinal disease and chronic hepatitis can lead to cancer owing to nutritional and environmental exposure. (Afsar.U. Ahmed., 2011). The cancer-inflammatory connection is not only in one direction, but most studies have demonstrated that damage to DNA can lead to inflammation. Finally, contemporary cancer treatment such as radiotherapy and chemotherapy can promote tumor-associated inflammation. (Zong and Thompson, 2006).

2.2.9 **TREATMENT OF INFLAMMATION**

There are a number of treatment options for inflammatory joint diseases including medications, rest, exercise, and surgery to correct jointdamage. The type of treatment prescribed will depend on several factors including the type of disease, the person's age, type of medications he or she is taking, overall health, medical history, and severity of symptoms.

The goals of treatment are to:

1. Treat the underlying inflammatory disease and decrease inflammation
2. Relieve pain by medication, activity modification
3. Maintain joint movement, muscle strength and overall function through physical therapy and exercise
4. Decrease stress on the joints by using braces, splints, or canes as needed

There are many drugs available to decrease joint pain, swelling, and/or inflammation and hopefully prevent or minimize the progression of the inflammatory disease. These medications include:

- Anti-inflammatory pain reliever drugs (NSAIDs -- such as aspirin, ibuprofen, or Celebrex)
- Corticosteroids (such as prednisone)
- Other medications which include chemotherapy drugs, disease modifying treatments, biologic therapy, or narcotic pain relievers.

Also, your diet is especially relevant when managing inflammation and reducing swelling in joints. While some foods can make things worse, there are plenty of tasty anti-inflammatory foods that can ease swollen joints, finger pain, and even symptoms of rheumatoid arthritis (RA). Such foods are;

1. Good oils: It is incomparably rich in oleic acid, an omega-9 fatty acid that helps to minimize inflammation. Ditch the vegetable oil for healthier options like olive, grapeseed, and avocado oils.Using extra virgin olive oil in cooking and on salads can get your food working faster. It’s good for the heart and the brain.

2. Fish: Red meat has earned its bad reputation for a reason. It is higher in cholesterol and salt, which can trigger inflammation. To get protein fish like salmon, snapper, tuna, cod, halibut, and bass that are high in omega-3 fatty acids, which also help to reduce inflammation.

3. Nut: Between meals nuts are also good food factors o treatment for inflammation. Some great choices include: Walnuts, Almonds, and Hazelnuts.

They are also high in omega-3 fatty acids and make a great snack. Sunflower seeds also share some of these nutty benefits.

4. Fruits: Also replacing processed snack foods with an array of fruits like: apples, blueberries, cherries, pineapple, raspberries, strawberries

According to the Arthritis Foundation, the antioxidants in fresh fruits and veggies help your body fight off free radicals that can cause cellular damage.

5. Chocolate: Eating healthy does not mean missing out on the sweet stuff. Chocolate that is at least 70 percent pure cocoa is the way to go.Other desserts that are low in fats and heavy in the fruits and nuts mentioned earlier, are also great ways to keep inflammation down.

6. Tea time: Besides reducing your risk of heart disease and cancer, green tea also stages an anti-inflammatory fight inside your body, according to research.Drink it hot or cold and add some lemon juice to perk up the tea's flavor — and kick up the antioxidants.

7. Beans: Serving of beans about 1 cup has 15 grams of protein. They are not only affordable, they are also packed with fiber and phytonutrients, which help decrease inflammation.They even have folic acid and important minerals, including: Magnesium, iron, zinc, potassium

8. Onions: Onions are full of nutritious antioxidants, and may in fact reduce: Inflammation, risk of heart disease, high cholesterol levels.

Adding them to the base of soups, sauté them in your favorite sauce, eat them raw in a sandwich, or toss them in an easy, nutritious stir-fry to instantly reap the benefits.

9. High fiber foods: Fiber is known to lower C-reactive protein (CRP), a substance found in our blood that suggests the presence of inflammation. Foods high in fiber include: Whole grains, beans, vegetable and fruits

Consuming whole grains made with the entire grain kernel, such as oatmeal, bulgur, brown rice, quinoa, and whole-wheat flour ensure a higher level of nutritious fiber. But if you have a gluten allergy, whole grains made of wheat can inversely contribute drastically to your inflammation.

10. Avocado: The avocado is rich in monounsaturated fat and high in vitamin E, two anti-inflammatory properties linked to a reduced risk of joint damage seen in early osteoarthritis.Eating avocados regularly can also contribute to regulating cholesterol levels.

(Nancy Carteron, MD, FACRon January, 2018)

CHAPTERTHREE

3.0 MATERIALS AND METHODS

3.1MATERIALS

3.1.1 Reagent used

Formaldehyde

Methanol

Sample (Liver Homogenate)

Phosphate Buffer

Hydrogen Peroxide

Sodium Carbonate

Distilled Water

3.1.2 Apparatus

Test Tubes

Test Tube Rack

Conical Flask

Beaker

Burette

Micro-Pipette

Conical Flask

Sample Bottle

Oral Cannula

Weighing Balance

Surgical Blade

Spectrophotometer

Water Bath

Ice Cube Machine

Centrifuge

Beaker

Burette

Syringe

3.2 Methods

3.2.1 Collection of plants

Alafia barteri roots were collected from Osun State in the month of March 2019 and identified at the Department of Botany, University of Lagos where a specimen numberLUH5517 was issued.

3.2.2Aqueous extract preparation

Leaves were air dried in shade for 3 days and then grinded with the milling machine.100g of dried grinded plant was macerated in 700mls of distilled water and left at room temperature with occasional stirring for 3 days. After 3 days, filtration was done and the filtrate was concentrated to obtain the crude extract.

3.2.3 Methanolic extract preparation

100g of dried plant material macerated with 700mls absolute methanol for 3 days. After 3 days, filtration was done and the filtrate was concentrated to obtain the crude extract.

3.2.4 Phytochemical analysis

Phytochemical screening was conducted on the plant root extract using standard methods.

Qualitative analysis on phytochemical constituents

Test for Alkaloids (Meyer's test)

To a few mls of plant sample, two drops of Meyer's reagent was added and 1% HCl along sides of the test tube. A yellow precipitate confirmed the presence of alkaloid.

Test for Glycosides

2mls of each extract was added to 3ml of 3.5% iron (III) chloride, and then 3mls of ethanolic acid was added. A green precipitate and dark colored solution respectively confirmed the presence of glycoside (Sofowora 1984)

Test for Terpenoids

5mls of each plant extract was added to 2mls of chloroform in a tube. 3mls of sulphuric acid was carefully added to the mixture. A reddish-brown interface confirms its presences. (Trease and Evans 1989)

Test for steroids (Sakowski`s test)

2ml of $H_2SO_4$was added to 2mls of the extract. Appearance of effervescence after which a clear reddish-brown color appear indicates the presences of steroids. (Herbone 1973)

Test for Saponins

2g of the plant extract with 20mls of distilled water was placed in the water bath. It is then filtered using a filter paper. 5mls of distilled water is added to 10mls of the filtrate and then shaken vigorously for a stable persistent

foaming. 3 drops of olive oil is then added to the froth and shaken vigorously again. The formation of emulsion indicates the presence of saponins. (Trease& Evans 1989)

Test for Tannins

2 drops of 5% $Fecl_3$ was added to 2mls of plant extract. Appearance of green precipitate on dilution confirms the presences of tannins. (Sofowora, 1984)

Test for Flavonoids

5mls of dilute ammonia solution was added to 2mls of aqueous filtrate of plant extract followed by the addition of 1ml of concentrated H_2SO_4. A yellow coloration that disappears on standing confirms the presence of flavonoids. (Trease& Evans 1989)

Test for Phenols

3 drops of ferric chloride was added to diluted extract. Formation of bluish black color indicates the presence of phenol.

Test for Polyphenols

3 drops of ferric chloride was added to diluted extract followed by 2mls of potassium chloride. A bluish black coloration confirms the presence of polyphenol.

3.3.1 Experimental Animals

Thirty (30) adultwistarrats with weight range of 180g-250g were obtained and kept at the animal house, Department of Biological sciences, College of Basic and Applied Sciences, Mountain top university Ibafo, Ogun state. The rats were allowed to acclimatize to the laboratory conditions for 7 days preceding the experiment. The animals had access to feeds and water and were housed in cleaned cages.

3.3.2 Experimental design

The animals received treatment as follows;

- Group I- normal control, received 1.0 ml of distilled water
- Group II- standard received 1.0 ml of diclofenac
- Group III- received aqueous extract (200mg/kg)
- Group IV- received aqueous extract (400mg/kg)
- Group V- received methanolic extract (200mg/kg)
- Group VI- receivedmethanolic extract (400mg/kg)

3.3.3 Collection and preparation of Blood serum

On day twelveof administration of the plant extract and diclofenac, the animals were sacrificed by cervical dislocation under anesthesia. The blood sample were collected by ocular puncture into Lithium heparin (EDTA) bottles and centrifuged at 4000RPM for 15mins to obtain the serum.

Estimation of Serum Liver and Renal Function Parameters

Aspartate transaminase (AST), Alanine transaminase (ALT) and Total protein concentration (TPC) as liver function parameters and serum creatinine as renal function parameters were assayed for using Commercial test kits obtained from Randox Laboratories, UK

Aspartate Transaminase (AST)
Reagent composition

Contents	Initial concentration of solutions

R1. Buffer	
Phosphate buffer	100 mmol/l, pH 7.4
L-aspartate	100 mmol/l
∝-oxoglutarate	2 mmol/l
R2. 2,4-dinitrophenylhydrazine	2 mmol/l

a. Procedure for reagent blank

250μl of reagent 1 was added to 50μl of distilled water. The solution was mixed and allowed to stand for 30mins at 37℃. Reagent 2 was added and the solution was allowed to stand for 20mins at 25℃. 2500 μl of 0.4mol NaOH was added. The solution was mixed and the absorbance was taken.

b. Procedure for sample

50 μl of sample was added to 250μl of reagent 1. The solution was mixed and allowed to stand for 30mins at 37℃.Reagent 2 was added and the solution was allowed to stand for 20mins at 25℃. 2500 μl of 0.4mol NaOH was added. The solution was mixed and the absorbance of the sample was read at 546nm against the reagent blank after 5min

Alanine Transaminase

Reagent composition

Contents	Initial concentration of solutions
R1. Buffer	
Phosphate buffer	100 mmol/l, pH 7.4
L-alanine	200 mmol/l
∝-oxoglutarate	2 mmol/l
R2. 2,4-dinitrophenylhydrazine	2 mmol/l

a. Procedure for reagent blank

250μl of reagent 1 was added to 50μl of distilled water. The solution was mixed and allowed to stand for 30mins at 37℃. Reagent 2 was added and the solution was allowed to stand for 20mins at 25℃. 2500 μl of 0.4mol NaOH was added. The solution was mixed and the absorbance was taken.

b. Procedure for sample

50 μl of blood plasma was added to 250μl of reagent 1. The solution was mixed and allowed to stand for 30mins at 37℃. Reagent 2 was added and the solution was allowed to stand for 20mins at 25℃. 2500 μl of 0.4mol NaOH was added. The solution was mixed and the absorbance of the sample was read at 546nm against the reagent blank after 5minutes.

Total Protein (TP)

Reagent composition

Contents	Concentration of solutions
R1. Biuret reagent	
Sodium hydroxide	100 mmol/l
Na-K-tartrate	16 mmol/l
Potassium iodide	15 mmol/l
Cupric sulphate	6 mmol/l
R2. Blank reagent	
Sodium hydroxide	100 mmol/l
Na-K-tartrate	16 mmol/l
CAL. Standard	
Protein	
Sodium Azide	<0.1% w/v

R1 was diluted with 400ml of distilled water. The contents of R2 were diluted with 400ml of distilled water.

a. Procedure for reagent blank

20μl of distilled water was added to 1000μl of R1. The solution was mixed and incubated for 30mins in the water bath at 25℃.

b. Procedure for standard

20μl of standard (CAL) was added to 1000μl of R1. The solution was mixed and incubated at 25℃.

c. Procedure for sample

20μl of blood plasma was added to 1000μl of R1. The solution was mixed and incubated at 25℃.The absorbance of the sample and of the standard was measured against the reagent blank at 546nm.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage yield

Percentage yield = Weight after extraction x 100

Weight before extraction

Aqueous extract yielded 80.73% w/w of the original plant material and the color is brown

Methanolic extract yielded 88.96% w/w of the original plant material and the color is light brown

4.2 Qualitative phytochemical analysis of *A.barteri*root extract

Table 1

Constituents	Methanolic extract	Aqueous extract
Flavonoid	+	+
Glycosides	+	+
Tannin	+	+
Steroids	+	+
Terpenoids	+	+
Anthraquinone	-	-
Saponins	+	+
Alkaloids	+	+
Phenol	+	+
Poly phenol	+	+

+ =Present, - = Absent

The Phytochemical screening of the aqueous and methanolic extract showed the presence of different secondary metabolites such as alkaloids, saponins, flavonoids, steroids, glycosides, phenol, polyphenol, tannins, terpenoids while anthraquinone was absent.

4.3 Effect of *Alafia barteri* root extract on body weight

The oral administration of aqueous and ethanolic extract had no significant differenceon the body weight of the rats

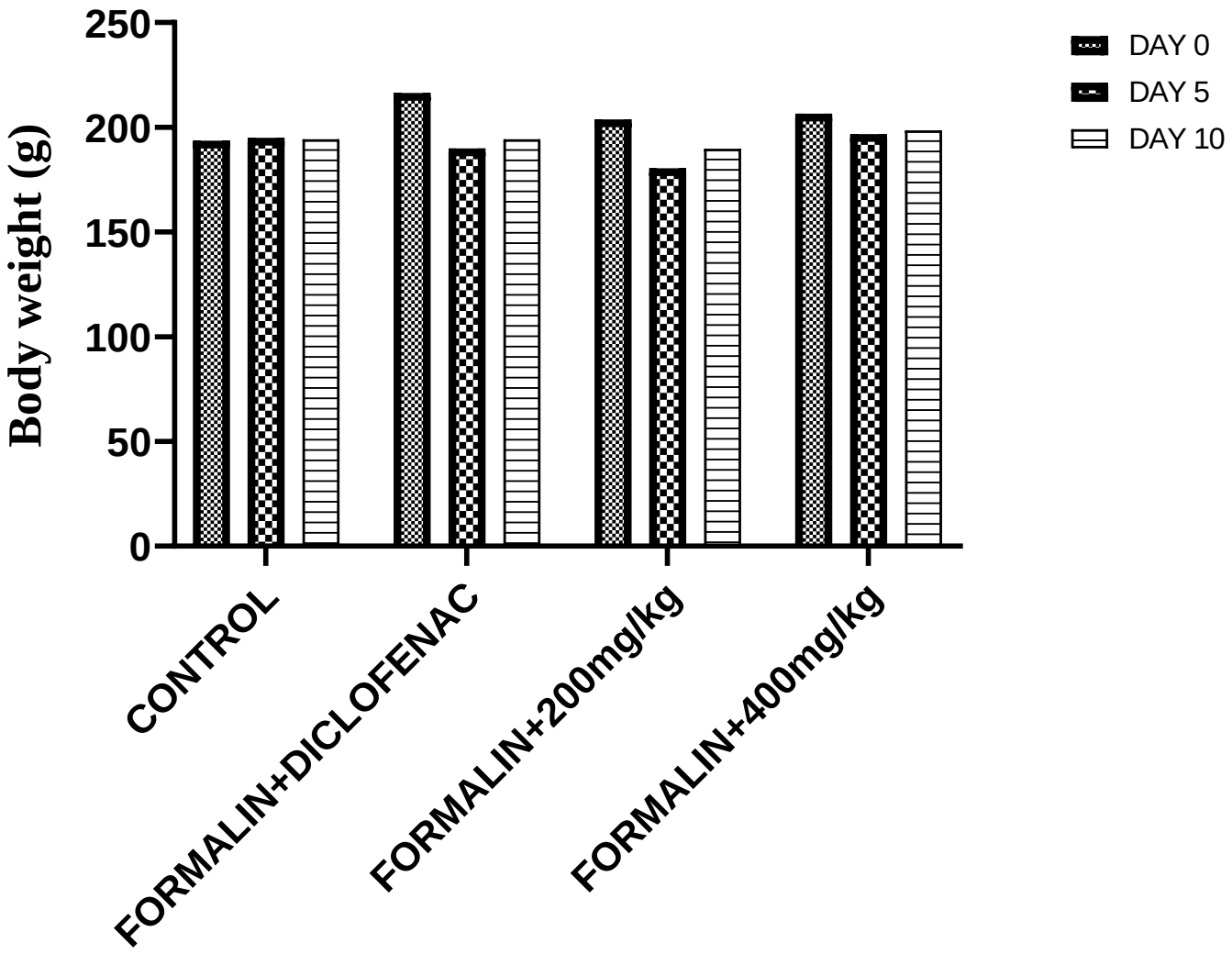

Fig 1:Effect of *Alafia barteri aqueous* root extract on body weight of rats

Table 2: Effect of aqueous *A.barteri* root extract on the body weight

Groups	Day 0	Day 5	Day 10
Control	193.5±11.47	195.0±2.66	194.3±6.66
Formalin+diclofenac	216.3±3.90	189.9±13.35	194.3±13.50
Formalin+extract(200mg/kg)	200.0±23.35	183.4±8.42	195.8±10.54
Formalin+extract(400mg/kg)	208.1±10.43	179.0±6.52	198.9±13.47

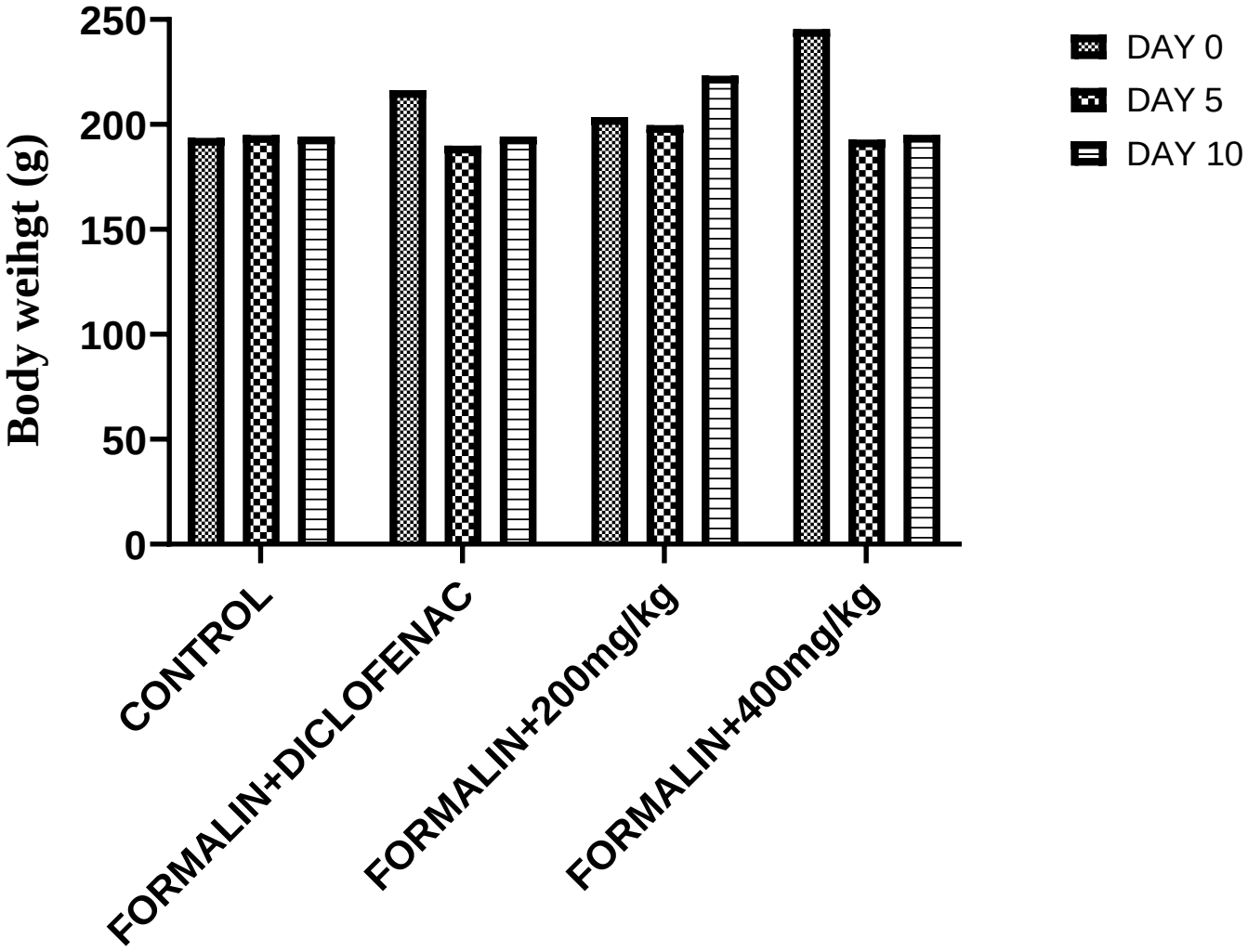

Fig 2:Effect of *Alafia barteri methanole* root extract on body weight of rats

Table 3. Effect of methanolic*A.barteri* root extract on the body weight

Groups	Day 0	Day 5	Day 10
Control	193.5±11.47	195.0±2.66	194.3±6.66
Standard	216.3±3.90	189.9±13.35	194.3±13.50
Formalin+extract(200mg/kg)	198.5±29.68	199.2±21.04	247.6±23.19
Formalin+extract(400mg/kg)	196.0±20.09	184.2±12.86	179.2±6.79

4.4 Effect of *Alafia barteri* root extract on paw diameter

Methanolic and aqueous root extract of *Alafiabarteri*at doses of 200 and 400 mg/kg showed a significant reduction in rats paw edemaon days 5 and 10 when compared with the inflamed rats. However, the standard diclofenac drug also caused a reduction but not as significant as the plant extract.

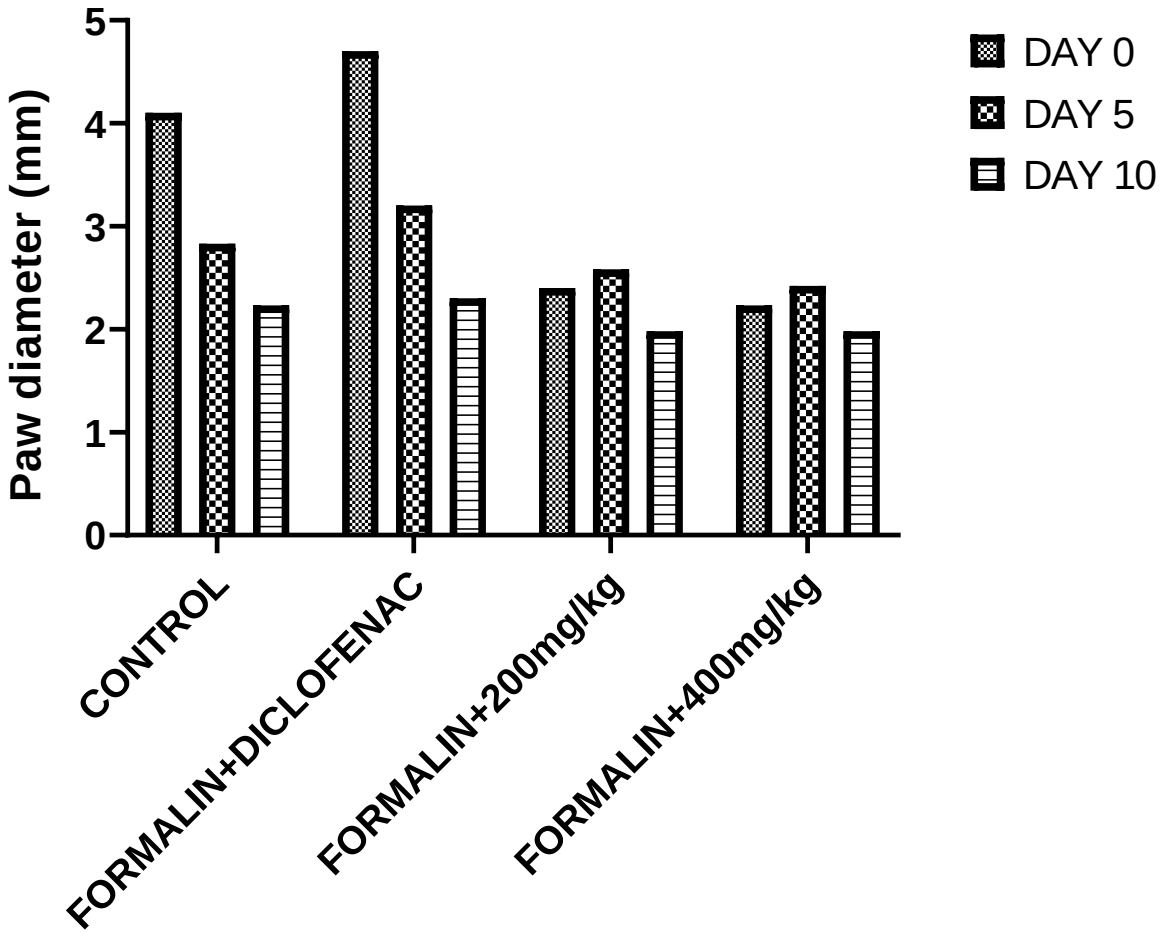

Fig 3:Effect of *Alafia barteri aqueous* root extract on paw diameter of rats

Table 4.Effect of aqueous*A.barteri* root extract on the paw diameter

Groups	Day 0	Day 5	Day 10	%Inhibition
Control	4.10±0.21	2.83±0.20	2.23±0.12	
Standard	4.67±1.49	3.20±0.21	2.30±0.06*	
Formalin+extract(200mg/kg)	2.59±0.45	2.33±0.08*	1.97±0.09*	31.6
Formalin+extract(400mg/kg)	2.10±0.70	2.53±0.30*	2.07±0.03*	34.8

*represent significant increases at $p<0.05$ when compared to control value on 5^{th} and 10^{th} day

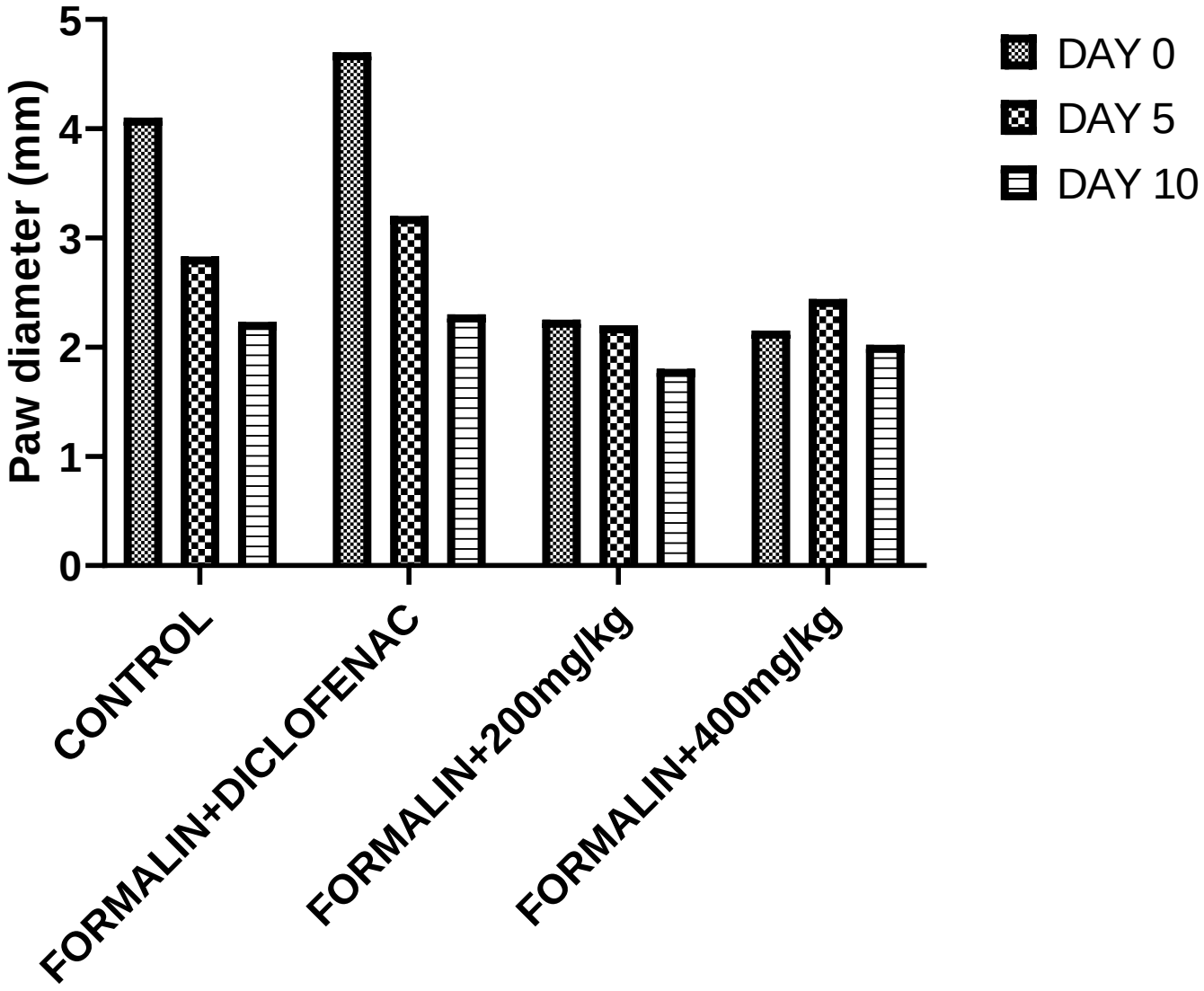

Fig 4:Effect of *Alafia barteri methanole* root extract on paw diameter of rats

Table 5. Effect of methanolic*A.barteri* root extract on the paw diameter

Groups	Day 0	Day 5	Day 10	%Inhibition
Control	4.10±0.21	2.83±0.20	2.23±0.12	
Standard	4.67±1.49	3.20±0.21	2.30±0.06*	
Formalin+extract(200mg/kg)	2.28±0.33	2.13±0.15*	1.73±0.07*	35.1
Formalin+extract(400mg/kg)	2.22±0.25	2.48±0.20*	2.00±0.12*	38.6

*represent significant increases at $p<0.05$ when compared to control value on 5^{th} and 10^{th} day

4.5 Effect of aqueous *Alafia barteri* root extract on serum biochemical parameters

Table 6a

Groups	AST	TP	ALT	CREATININE
Control	10.00±1.73	8.26±0.24	10.67±1.33	0.97±0.75
Formalin+diclofenac	8.00±1.00	4.87±2.33	6.67±1.33	0.33±0.23
Formalin+200mg/kg	9.00±2.00	9.28±0.09	6.67±1.33	0.13±0.04
Formalin+400mg/kg	7.00±0.00	9.78±0.32	14.00±3.00	0.88±0.32

Values are expressed as mean±SEM (n=5). The level of statistical Significance was measured using one way ANOVA followed by multiple comparison test. P<0.05 versus negative control; p<0.05 versus aqueous *A. barteri*, 200mg/kg and 400mg/kg.

1. Aspartate transaminase showed no significant difference in 200mg/kg and 400mg/kg of *Alafia barteri* aqueous root extract when compared to the control and also in the standard (Diclofenac Potassium) when compared to the control.
2. Total Protein showed no significant difference in 200mg/kg and 400mg/kg of *Alafia barteri*aqueous root extract when compared to the control and also in the standard (Diclofenac Potassium) when compared to the control.
3. Alanine transaminaseshowed no significant difference in 200mg/kg and 400mg/kg of *Alafia barteri* aqueous root extract when compared to the control and also in the standard (Diclofenac Potassium) when compared to the control.
4. Creatinine showed no significant difference in 200mg/kg and 400mg/kg of *Alafia barteri* aqueous root extract when compared to the control and also in the standard (Diclofenac Potassium) when compared to the control.

4.6 Effect of methanolic *Alafia barteri* root extract on serum biochemical parameters

Table 6b

Groups	AST	TP	ALT	CREATININE
Control	10.67±1.33	8.26±0.24	10.67±1.33	0.97±0.75
Formalin+diclofenac	6.67±1.33	4.87±2.33	6.67±1.33	0.33±0.23
Formalin+200mg/kg	12.00±2.65	8.92± 0.31	8.00±2.31	1.27±0.17
Formalin+400mg/kg	9.00±1.00	9.26±0.84	29.67±5.21	0.81±0.28

Values are expressed as mean±SEM (n=5). The level of statistical Significance was measured using one way ANOVA followed by multiple comparison test. P<0.05 versus negative control; p<0.05 versus ethanolic*A. barteri*, 200mg/kg and 400mg/kg.

1. Aspartate transaminase showed no significant difference in 200mg/kg and 400mg/kg of *Alafia barteri* methanolic root extract when compared to the control and also in the standard (Diclofenac Potassium) when compared to the control.
2. Total Protein showed no significant difference in 200mg/kg and 400mg/kg of *Alafia barteri* methanolic root extract when compared to the control and also in the standard (Diclofenac Potassium) when compared to the control.
3. Alanine transaminaseshowed no significant difference in 200mg/kg and 400mg/kg of *Alafia barteri* methanolic root extract when compared to the control and also in the standard (Diclofenac Potassium) when compared to the control.
4. Creatinine showed no significant difference in 200mg/kg and 400mg/kg of *Alafia barteri* methanolic root extract when compared with the control and also in the standard (Diclofenac Potassium) when compared with the control.

CHAPTER FIVE

5.0 DISCUSSION and CONCLUSION

5.1 DISCUSSION

Inflammation is the reaction of the body to injury. It operates to cure injuries, but in some chronic diseases it can also play a part. It is the way the body signals the immune system to cure and repair damaged tissue and to protect itself against foreign invaders, such as viruses and bacteriaMedicinal plants have played exceptional and indispensable roles in early times in alternative traditional medicine. The research and analysis of plants employed as pain-relievers and anti-inflammatory agents in traditional medicine is one of the productive and logical strategies in the search for new drugs (Vongtau*et al*., 2004).

The formalin test is a popular chemical assay of injury-produced inflammatory pain. It is regarded as a more satisfactory model of clinical pain and is a useful model for the screening of novel compounds, as it encompasses inflammatory, neurogenic, and central mechanisms of nociception. The advantage of the formalin assay over other models of inflammatory pain is that the injection of a dilute solution of formalin into the surface of a mouse or rat's hind paw allows modeling of both acute and tonic pain using a single chemical in a relatively limited time (Dzoyem*et al*., 2017)

Formalin induced paw edema in rats is one of the mostsuitable test procedure to screen the acute inflammation and it is believed to be a biphasic event. Formalin induction causes the changes in connective tissue metabolism, is one of the major biochemical events during the process of inflammation. These changes are effected in the alteration of relative composition of various constituents of connective tissue such as muco-polysaccharides, glycoprotein,

Hexosamine and hydroxy proline sialic acid (Houck and Jacob 1969).

Phytochemical investigations and review of the literature reveal the presence of alkaloids, tannins, flavonoids, glycosides, reducing sugars, and saponins. These components may exert its anti-inflammatory activity, which is very important in producing and maintaining inflammation. Saponins and alkaloids are known to inhibit articular swelling, decrease index, and regulate down the content in the inflammatory tissue (Nishat.*et al.*,

2015). Among the different phyto-constituents, flavonoids have positive impacts on inflammatory circumstances and the anti-inflammatory activity is prevalent to many terpenoids. Flavonoids are particularly reported for significant antioxidant, vasculo-protector, anti hepato toxic, anti-allergic, anti-inflammatory and anti-tumor activity (Agnel*et al.*,2012)

Diclofenac sodium which is used as a standard drug is an NSAID, which acts by inhibition of PGs synthesis by blocking COX enzymes responsible for inflammation (Yend*et al*., 2010).

Similarly, inhibition of paw edema volume was treatment with methanolic and aqueous root extracts of *A.barteri*200 and 400 mg/kg orally in this model suggests significant improvement in inflammation induced rats and was comparable to the standard drug diclofenac potassium.

It can be proposed that the anti-inflammatory and anti-arthritic activity of *Alafia barteri* root extracts could be due to combined effect of flavonoids, saponins, and alkaloids, which are the major chemical constituents of the methanolic and aqueous root extracts. The presence of these compounds in the extract may explain the possible mechanism for anti-inflammatoryproperties of this plant and its use in traditional alternative and complementary medicine.

5.2 CONCLUSION

The results of this research helped validate the traditional use of the formulation of *Alafia barteri* in inflammation therapy. When compared between the two extracts, aqueous extract was found to be potent than the methanolic extractHowever, it may not be feasible to use animal models to describe the precise pathophysiology and development of this disease.Further research studies in human subjects are therefore warranted to clarify the exact mechanism of this formulation's anti-inflammatory activity.

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