CERTIFICATION

This is to certify that this project titled IN-VIVO ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS AND ETHANOL *A.barteri* LEAF EXTRACTS IN ALBINO RATS was compiled and written by Akindele Gloria Toluwalope with Matriculation number 15010102004 of the Department of Biological Sciences, College of Basic and Applied Sciences, Mountain Top University, Ogun State, under the supervision of Mrs. I.O Adefisan.

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AKINDELE GLORIA DATE

STUDENT

…… …….

MRS. I.O ADEFISAN DATE

SUPERVISOR

ACKNOWLEDGEMENT

All glory and honor to God Almighty for the grace he has given me to be able to complete this project work.

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DEDICATION

This report is dedicated to God Almighty for his grace, strength and provision throughout my stay in Mountain Top University. Also to my parents for their parental love and support and my supervisor for her endless support during the course of this project.

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 (200 mg/kg) significantly suppressed inflammation with maximal inhibitions of 52% and ethanolic extract (400 mg/kg) with maximal inhibitions 45%. Other doses of the extract did not show significant inhibition. Serum AST and ALT activity 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.

Key words: Anti-inflammation, formaldehyde, *Alafia barteri*

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Inflammation as a vitalreaction to injury has been known for many thousands of years. It is a complex set of interactions among soluble factors (cytokines) and cells that can arise in any tissue in response to traumatic, infectious, post-ischemic, toxic or autoimmune injury. The process normally leads to recovery from infection and to healing, however, if targeted destruction and assisted repair are not properly phased, inflammation can lead to severe tissue damage by leukocytes, lymphocytes or collagen (Nathan, 2002).

Chronic inflammation can contribute to diseases such as arthritis, heart attacks, Alzheimer's disease and also makes individuals susceptible to many forms of cancer. The culprits that drive this process are inflammatory cells and signaling molecules of the innate immune system, which recognizes potential threats without previous exposure to them (Balkwill and Coussens, 2004; Mantovani, 2005). The first extensive description of inflammatory symptoms can be found in De Medicina, written by AulusCelsus (~25 BC–AD 38) who delineated the four symptoms of inflammation as rubor, tumor, color, and dolor (redness, swelling, heat, and pain. The fifth sign of inflammation, functiolaesa (impaired function) was included by Galen of Pergamon some 100 years later.

Non-steroidal anti-inflammatory drugs (NSAID) have been used globally for the treatment of inflammation, pain and fever, as well as for cardiovascular protection. However, it causes severe side-effects, which include gastric ulcer, renal damage,

bronchospasm and cardiac abnormalities, thus restricting their use. (Burke *et al.* 2006).

Examples of these drugs are aspirin, ibuprofen, naproxen, fenoprofen, indomethacin, diclofenac, fenamates, piroxicam, ketorolac, nimesulide, rafecoxib, paracetamol etc. Research has proven the significance of drugs of natural origin as an important source for the treatment of many diseases worldwide (Pandima *et al*., 2003).

Medicinal 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).

Africa flora is very rich in these medicinal plants. The bacteria and fungi infections are also prevalent in our society and this leads to various diseases like inflammation, rheumatism, venereal and skin diseases. The search for new, safer and affordable drugs especially from plants to treat and cure these diseases is on the increase.

1.2 STATEMENT OF RESEARCH PROBLEM

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. It plays an essential role in the problemsrelated with pathological conditions in both advanced and emerging countries, particularly in African countries. For instance, chronic inflammation is known to play a part in the development of obesity-associated diabetes secondary to insulin resistance.(Oguntibeju., 2018).

1.3 JUSTIFICATION

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. The research project is based on evaluating the anti-inflammatory potential of the *Alafia barteri*

1.4 AIM OF STUDY

The research aims at the evaluation of the anti-inflammatory activity of the aqueous and ethanol extract of *Alafia barteri* leaf in Wistar rats.

1.5 OBJECTIVE OF STUDY

- To determine the phytochemical components of *Alafia barteri* leaf
- To evaluate the effect of aqueous and ethanolic leaf extract of *Alafia barteri*on body weight and paw diameter of rats
- To evaluate the effect of the extract on serum biochemical parameters in Wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 WHAT IS INFLAMMATION?

Inflammation is a biochemical response of the body against an assertive agent, defined by vasodilatation, access of fluid and cells to the target tissue (Schmid-Scho¨nbein, 2006, Ismail, *et al*., 2014.). It is a conserving strategy developed in higher organisms in response to prejudicial insults such as microbial infection, tissue injury and other noxious conditions. It is an essential immune response by the host that enables the removal of harmful stimuli as well as the healing of damaged tissue. Inflammation has been considered as a part of innate immunity, the first line of host defense against foreign invaders and danger molecules. Mankind has known the standard symptoms of inflammation for hundreds of years, which include redness, pain, swelling and heat (Medzhitov, 2008). However, the entire course of inflammation comes with many different processes involved in its initiation, regulation and resolution. Presently, a diverse range of inflammations have been identified, with many different forms initiated by numerous stimuli and governed by various regulatory mechanisms.

Due to its extensive and widespread nature, inflammation is believed to have an impact on every aspect of normal human physiology and pathology. (Medzhitoz, 2010).

2.2 STAGES OF INFLAMMATION

Inflammation is the body reaction to injury. Injury can arise from different sources such as physical traumas (strain, sprain or contusion), bacterial or viral infections, heat or chemical injury. Trauma causes direct damage to cells in the immediate area of injury, causing bleeding. (Christiana, 2016.). The bleeding causes series of events in the inflammatory process which enhance healing of the injured tissue. Persistent injury or individual factors such as diabetes, corticosteroid use, blood disorders, can promote the progression of acute inflammation to chronic inflammation. (Christiana, 2016.)

2.2.1 STAGE 1: INFLAMMATORY RESPONSE

The healing of acute injuries starts with acute vascular inflammatory responses. The vascular change is to increase the blood flow to the immediate area and to also transport cells to the area to initiate healing. When the healing is initiated, damaged cells are removed and then new collagen is added by the body to the area of injury. This stage occurs immediately after injury and it lasts for 3-5 daysc During this 3-5 day the area of injury will experience pain, warmth, swelling, palpable tenderness, limitation in joint or muscle range of motion. Pain and swelling can be decreased to prevent chronic inflammation and to maintain mobility and strength in other areas of the body while injured areas are at rest.

2.2.2 STAGE 2: REPAIR AND REGENERATION

The next stage is the formation of new collagen. The new collagen fibers are laid down in a disorganized manner in form of scar with weak links between each fiber. However the new tissue is weak and susceptible to disruption by aggressive activity. This stage usually last from 2 days to 8 weeks. (Christiana, 2016.) The warmth and swelling become lesser and the palpable tenderness decreases. Range of motion exercises, joint mobilization, and scar mobilization should be done to enhance tissue remodel.

2.2.3 STAGE 3: REMODELING AND MATURATION

In this stage there is less formation of new collagen but increased organization of the collagen fibers with stronger bonds in between them. New collagen must align along the lines of stress to accommodate the loads required for function therefore tension is important. The tissue continues to remodel, strengthen and improve its cellular organization as the healing progresses. The end of tissue remodeling and healing is not known. It can take months to years before completion can take place. (Dalgleish et al., 2002)

2.3 TYPES OF INFLAMMATION

The response of inflammation to the body is of two forms:

2.3.1 ACUTE INFLAMMATION

Acute inflammation is the response of the body to injury to mediate the healing of the injury. It usually occurs within minutes or hours after tissue injury and can be defined by the basic symptoms of heat, pain, dysfunction and redness. It is

characterized by the exudation of fluids and plasma proteins, movement of leukocytes (monocytes, macrophages most importantly neutrophils) into the injured area. Acute inflammation response is relevant to the action of defense aimed at eradicating bacteria, viruses, parasites and fungi while still enhancing wound repairs. (Toth., 2004)

2.3.2 CHRONIC INFLAMMATION

Chronic inflammation is characterized histologically by the presence of macrophages and lymphocytes, leading to tissue necrosis and fibrosis. It increases the improvement of degenerative disease 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 neuro-degenerative CNS depression. It is part of the cause of muscle loss which occurs with aging. (Denis et al 2014). It is associated with immune-pathological and infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation proliferation and fibrosis. (Kumar Cet al., 1999)

2.4 CAUSES OF ACUTE INFLAMMATION

Inflammation is a normal reaction of the body to protect the tissues from diseases, injuries and infections. This reaction or response begin with the production and release of chemical agents by cells in the infected, diseased or injured tissue. These agents cause redness, swelling, pain, heat and deranged function. Additional signals that recruit leukocytes to the sites of inflammation is being generated by inflamed tissue. These leukocytes can destroy any injurious or infectious agents; they can also

remove cellular debris from damaged tissue. (Medzhitoz, 2010). Although this inflammatory response usually enhance healing of the tissue but if not controlled may become harmful. The causes of acute inflammation includes

2.4.1 MICROBIAL INFECTION

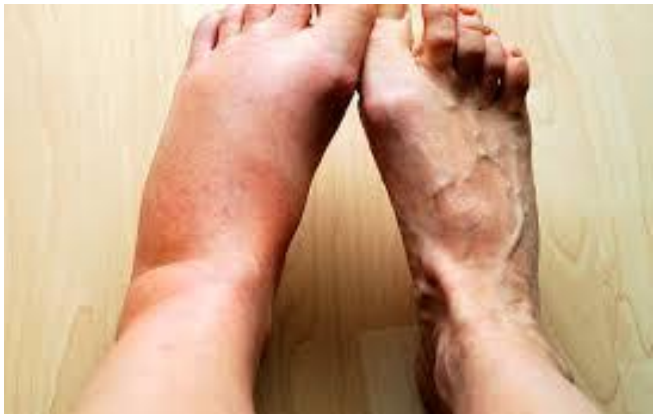
Microbial infection is one of the common causes of inflammation in the body. These microbial infections are caused by the killer activities of microbes. Microbes include bacteria, protozoa, fungi, viruses and several parasites. Bacteria release specific toxins (exotoxins or endotoxins). (Akira *et al*., 2006). Endotoxins are toxins present inside a bacterial cell and released when the cell disintegrates. They are part of the cell wall of Gram negative bacteria and they do terrible things to the body. Exotoxins are toxins released by a bacteria cell into its surroundings. They are produced specifically for export (like anthrax toxins or tetanus toxins). The endotoxins are not as specific in their actions as the exotoxins.

2.4.2 PHYSICAL AGENTS, IRRITANT AND CORROSIVE CHEMICALS

Physical trauma, burns or excessive, ultraviolet or other ionizing radiation can cause the tissue to be damaged leading to inflammation. Corrosive chemicals such as alkalis, acids and oxidizing agents can instigate through direct tissue damage. These corrosive chemical causes the damage of the tissue which immediately provoke inflammation. (Kostandinos, *et al*., 2014.)

2.4.3 TISSUE NECROSIS

Tissue necrosis (death) is a passive process resulting in a breakdown of ordered structure and function following irreversible traumatic damage. (Kostandinos, *et al.*, 2014.) This is a potent inflammatory stimulus. It often shows an acute inflammatory response.



https://www.britannica.com/story/how-is-inflammation-involved-in-swelling

2.5 EFFECTS OF INFLAMMATION

The effects of inflammation can either be local or systemic. The local effects of inflammation are usually beneficial to the body. For instance, the execution of invading microorganisms but sometimes they appear to have no effect or may be harmful. While the systemic effect include fever, leukocytosis and malaise. (Afsar, 2011.)

Table 1. Effect of inflammation

Beneficial Effects of inflammation	Harmful effects of inflammation
Dilution of toxins Entry of antibodies	Persistent cytokines release Destruction of normal tissues

Fibrin formation Delivery of nutrients and oxygen Stimulation of immune response	Swelling Inappropriate inflammatory response

2.6 CELLULAR HOMEOSTASIS AND INFLAMMATORY RESPONSES

One of the main aims of inflammation is to create cellular homeostasis in response to any kind of damage as a conserving strategy for the host. (Akira *et al*., 2006). Cellular homeostasis is tightly connected to the mechanism underlying the initiation of inflammation; therefore, inflammation can be referred to an adaptive response to dangerous effect threatening the stability and integrity of cellular homeostasis. These adaptive responses operate at the expense of normal cellular functions. (Medzhitov, 2010). Therefore, the more this response persists, the more the host will continue to encounter damaging consequences. Apart from its beneficial role as a safeguard for cellular physiology, the inflammatory response is required to be least enduring in order to avoid an increase of unfavorable circumstances. Inflammation is commonly initiated within minutes in any host with functioning innate immune system in response to a proper stimulant such as microbial infection, invaders both external and internal. Just as innate immune system is the major cause to inflammation, immune cells such as dendritic cells, neutrophils, lymphocytes, macrophages, and mast cells also play important roles in inflammatory processes. (Akira *et al*., 2006). Also, non-immune cells such as epithelial cells, fibroblasts and endothelial cells contribute in great deal to inflammatory processes. According to the nature of stimulants, inflammatory pathways and their target tissues differs. When a host has bacterial

infection, the immune cells through specific receptors instantly sense the pathogens. The activation of pathogen-specific receptors there by induces the production of inflammatory mediators such as inflammatory cytokines [e.g. tumor necrosis factor (TNF), interleukin-1 (IL-1) and interleukin-6 (IL-6)] and chemokines. The progression of inflammation through the modification of vascular endothelial permeability as well as the supply of neutrophils and excess plasma which contains antibodies and complement factors into the site of infection is rapidly accelerated by these chemical mediators. (Afsar, 2011.) At the same time, the pathogens are targeted and destroyed by the immune cells. The duration of inflammatory responses differs depending on the level of damage caused by the infection. These responses extend in most cases toward systemic effects through the excessive production of inflammatory cytokines. The secretion of acute phase proteins (i.e. coagulation factors and C - reactive protein) by the liver cells is then mediated by these inflammatory cytokines produced. These proteins then induce brain endothelium and bring about the production of prostaglandins. Prostaglandins are mainly responsible for the symptoms of inflammation through their effects on the central system. Similarly, the viral infection leads to a signaling pathway through the production of another class of cytokines such as type-1 interferon (IFNs) and also involves cytotoxic lymphocytes, Type-1 IFNs which play central roles in antiviral responses. Then again, parasitic infections as well as allergens induce the production of IL-4, IL-5, IL-13 and histamine (Medzhitov, 2010).

2.7 INFLAMMATION AND DISEASES

Inflammatory diseases are a group of medical disorders which are exhibited by chronic inflammation i.e. abnormal inflammatory responses as a distinguish

characteristics. Chronic inflammation and the pathogenesis of inflammatory diseases are both the causes and effect of these diseases. For example, inflammation is caused by obesity, whereas chronic inflammation can lead to obesity-associated diabetes partly because of insulin resistance (Hotamisligil, 2006).

2.7.1 INFLAMMATION AND RHEUMATOID ARTHRITIS

The chronic nature of the inflammation with these diseases can be controlled at least to some extent by the pathological outcome of these diseases there by making them a bit different from those triggered by stubborn infection. Another good example of chronic inflammation-associated disorder is rheumatoid arthritis. Inflammation is a common clinical conditions and rheumatoid arthritis (RA) is a chronic draining autoimmune disorder1 that affects about 1% of the population in developed countries. It is a chronic, systemic inflammatory disorder that affects the small joints in hands and feet. The immune system attacks joint tissues and possibly other parts of the body for unknown reasons. (Miller *et al.*, 2009). Rheumatoid arthritis causes pain, inflammation, joint damage and then malformation. It can cause patient to feel sick, tried, feverish and joint pain at both sides of the body. These symptoms may spread to the knee, ankles, wrists, elbow, hips and shoulders as the disease develops. (Mayo *et al.*, 2016)

The synovium, the lining of the joint which experiences chronic inflammation by the penetration of lymphocytes and macrophages and the activation of synovial cells. The synovial fluid is invaded by billions of neutrophils during rheumatoid arthritis every day. (Akira *et al*., 2006). It has been proposed that neutrophils which have a half-life of about 4 hours make an important contribution to the nature of chronic inflammation in synovial tissue. One of the enzymes of neutrophils, cytosolic peptidyl arginine

deaminase whose activity depends on the level of extracellular Ca+ is being hypothesized to be released from dead neutrophils and then activated. (Afsar, 2011). This enzyme produces citrulline in some proteins by converting the guanidine side chains of L-arginine residues to uredo residues. Surprisingly, citrullinated proteins are found to react with auto anti-bodies associated with rheumatoid arthritis. (Uysal *et al*., 2009).

2.7.2 INFLAMMATION AND CANCER

Over the decade, it has become apparent that inflammation plays an important role in endorsing cancer especially in formation of tumor (tumorigenesis). (Afsar, 2011). Persistent inflammation is linked with DNA damage which in turn lead to cancer making inflammation as the precursor of cancer. Chronic inflammation serves as a precursor to certain cancers. Furthermore, apart from cancer cells several types of immune cells are also found within tumors. Also an inflammatory micro-environment is often found as an essential part of all tumors. (Mantovani *et al*., 2008; de Visser *et al*., 2006). It has been discovered that the responses of inflammation triggered by infection is also linked with increase in cancer risk (de Martel and Franceschi, 2009). Study has revealed that lung tumorigenesis caused by smoking tobacco is actually initiated by IKK-beta and JNK1- mediated chronic inflammation and that the pathway of both inflammation and tumorigenesis is closely associated. (Takahashi *et al*.,2010). Apart from the tumor-promoting inflammatory response, it is believed that an active anti-tumor immunity is also present in most tumor microenvironment. (Smyth et al., 2006; Lin and Karin, 2007). Research has shown that cancer cell growth are not only enhanced by inflammatory micro environments but also increase mutation rates by producing nitrogen intermediates and reactive oxygen species (ROS) which can

lead to DNA damage and genomic instability. (Grivennikov *et al*., 2010). The relationship between cancer and inflammation is not in one direction only just has most studies has revealed that DNA damage can lead to inflammation. Finally, tumor-associated inflammation can be promoted by modern cancer therapy such as radiotherapy and chemotherapy. (Zong and Thompson, 2006).

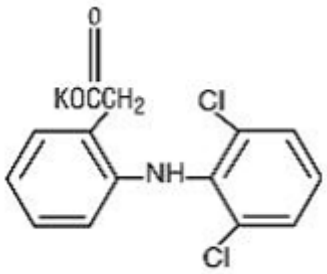
2.7.3 TREATMENT OF INFLAMMATION

Inflammation have been treated with non-steroidal anti-inflammatory drugs for years. (NSAIDs), non-steroidal anti-inflammatory drugs, are one of the most commonly prescribed pain medications. It is an extremely active drug class for pain and inflammation. NSAIDs are one of the most commonly prescribed classes of medication for pain and inflammation. Many nonsteroidal anti-inflammatory drugs are known to reduce pain and inflammation by blocking the metabolism of arachidonic acid by isoform of cyclooxygenase enzyme (COX-1 and/or COX-2), thereby reducing the production of prostaglandin. (Oguntibeju, 2018.) They are responsible for approximately 5-10% of all medications prescribed each year. (Supakanya, *et al.*, 2018) The key therapeutic activities of NSAIDs are principally endorsed by their ability to wedge certain prostaglandins (PGs) synthesis through the cyclooxygenase enzymes (COX-1 and COX-2) inhibition. COX-1 produces prostaglandins and thromboxane A2 which control mucosal barrier in GI-tract, renal homeostasis, platelet aggregation and other physiological functions. COX-2 produces PGs that related to inflammation, pain and fever. COX-1 is articulated in normal cells, while COX-2 is induced in inflammatory cells. (Supakanya, *et al*., 2018) COX-2 inhibition most likely represents the desired effect of NSAIDs' anti-inflammatory, antipyretic and analgesic response; while COX-1 inhibition plays a major role in the

undesired side effects such as GI and renal toxicities. However, research has known that these drugs have side effect on the body such as increased risk for myocardial infarction, stroke, heart failure, and hypertension. (Oguntibeju, 2018.) The risk for these side effects is probable extreme in patients with a previous history of or at high risk for cardiovascular disease. The use of COX-2 inhibitors for pain relief should be limited to patients for whom there are no appropriate alternatives, and then, only in the lowest dose and for the shortest duration necessary. (Elliott, 2007) According to FDA, NSAIDS increases the chance of a heart attack or stroke which can lead to death. The risk of heart attack or stroke can occur as early as the first weeks of using an NSAID and it may increase with longer use of it. Numerous reports have shown that have identified an increased risk of cardiovascular events. (Elliott, 2007) A deliberate worldwide withdrawal of Vioxx (rofecoxib was announced by Merck in September 2004 because of the increased risk of stroke and heart attack. All these side effect has led to the use of medicinal plant for the treatment of inflammation. Plants such as *Alafia barteri* have been used as an anti-inflammatory and fever remedy all over world. *Alafia barteri* is used for the treatment of eyes infections, febrifuges, as chew sticks, sickle cell, anemia, rheumatism and toothache. (Odugbemi, 2008) According to Adeeyo, *et al*., (2018), *Alafia barteri* leaf extract exert reduction in the blood glucose of the diabetic animals and maintained histo-renal architecture. Other anti-inflammatory plants include:*Aeglemarmelos (Rutaceae), Bryophyllumpinnatum (Crassulaceae), Moringaoliefera (Moringaceae), Matricariachamomilla L., Arnica montana L. (Asteraceae), Salix alba (Salicaceae), and Glycyrrhizaglabra (Fabaceae)* etc. Examples of NSAIDs are diclofenac, ibuprofen, aspirin etc.

2.7.4 DICLOFENAC POTASSIUM TABLETS USP, 50 MG

Diclofenac potassium tablets USP are a benzene acetic acid derivative. They are available as orange, film-coated tablets for oral administration. The chemical name is 2[(2, 6-dichlorophenyl) amino] benzene acetic acid, monopotassium salt. The structural formula is CHClKNO and the molecular weight is 334.24. Diclofenac potassium is a slightly yellowish white to light beige, practically odorless, faintly hygroscopic crystalline powder. It is very soluble in methanol, soluble in ethanol and water, and virtually insoluble in chloroform and in dilute acid. The n-octanol/water partition coefficient is 13.4 at pH 7.4 and 1545 at pH 5.2. It has a single dissociation constant (pKa) of 4.0 ± 0.2 at 25°C in water. Each tablet, for oral administration, contains 50 mg of diclofenac potassium. (Carilion material management., 2017.)



Chemical structure of diclofenac . (Carillon material management, 2017.)

Mechanism of Action

Diclofenac has analgesic, anti-inflammatory, and antipyretic properties. The mechanism of action of diclofenac potassium tablets, like that of other NSAIDs, is not totally understood but involves inhibition of cyclooxygenase (COX-1 and COX-2). Diclofenac is aneffective inhibitor of prostaglandin synthesis. Prostaglandins

stimulate afferent nerves and enhance the action of bradykinin in inducing pain in animal models. Prostaglandins are mediators of inflammation and diclofenac is an inhibitor of prostaglandin synthesis, therefore its mode of action is due to the decrease of prostaglandin in peripheral tissues.(Carillon material management, 2017.)

2.8 BIOMARKERS

2.8.1 ASPARTATE AMINOTRANSFERASE (AST)

Aspartate aminotransferase (AST) is a transaminase enzyme that catalyzes the conversion of aspartate and alpha-ketoglutarate to oxaloacetate and glutamate. The AST enzyme was previously known as serum glutamate oxalate transaminase (SGOT) and is present in all tissues except bone, with highest levels in liver and skeletal muscle. Concentration of AST is eminent after bruising, trauma, necrosis, infection, or neoplastic of liver or muscle (Ida, 2012). The AST enzyme is found in cerebrospinal fluid, exudates, and transudates in fraction to the amount of biological damage. Low concentrations of AST are also found in urine but are not useful for diagnosis of renal damage (Evans, 2009).

2.8.2 ALANINE AMINOTRANSFERASE (ALT)

Serum alanine aminotransferase (ALT) is a readily obtainable, inexpensive, and routine biochemical assay used in medical practice. (Zhengtao, 2014). Primarily, many concerns were articulated regarding the clinical implication of ALT activity on viral and toxic hepatitis, muscular dystrophy, and other muscular diseases, which might

cause anextensive increase in the ALT level in a relatively low percentage of the overall population. (Zhengtao, 2014).

2.8.3 TOTAL PROTEIN CONCENTRATION

The total serum protein (TP) concentration is the total amount of specific proteins in plasma with the exclusion of those that are consumed in clot formation, such as fibrinogen and the clotting factors. Plasma protein is about 3–5 g/L greater than serum protein.(Wanda, 2010.) Hydration status of the animal should be considered when interpreting protein changes.

Principle of action

The most commonly used method for analyzing total protein is the biuret reaction, in which an alkaline copper (II) solution reacts with peptide linkage to form a complex that absorbs light at wavelength 546 nm. (William, 2012.)
The sensitivity of the reaction can be increased by the addition of phosphotungstomol ybdic acid (Folin Ciocalteu reagent, phenol reagent) (Lowry method), which, together with other modifications, results in increased absorption for a given amount of protein. (William, 2012.)

2.8.4 CREATININE

Creatinine is produced from the breakdown of creatine and phosphocreatine and can also serve as an indicator of renal function. (Jose, 2019) Creatine is synthesized in the liver, pancreas, and kidneys from the transamination of the amino acids arginine, glycine, and methionine.

2.9 ALAFIA BARTERI

Alafia barteri Olive, Apocyanaceae, commonly known as Alafia chewing stick, is a high climbing, glabrous, scandent shrub with small pure white or pink flowers distributed widely in the tropics. It is a vigorous climbing shrub producing stems up to 35 meters long that scramble over the ground or climb up into trees in the Gabon. Mostly (often), the stem and leaves are medicinal especially in the treatment of malaria and fungal diseases, hence giving focus to the study of the root and leave for anti-inflammatory effect is highly proper and considerable.The plant is harvested from the wild for local use as a tying material and medicine. (Salim et al., 2008). 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

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

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. (Salim et al., 2008).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*

2.9.1 USES OF A*LAFIA BARTERI*

A.barteri is valued for its effectiveness 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 fiber 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). According to Adeeyo, *et al*., (2018), *Alafia barteri* leaf extract exert reduction in the blood glucose of the diabetic animals and maintained histo-renal architecture. Also research as shown thatfundamental and outlying analgesic effect of *A. barteri* through relations with L-arginine-nitric-oxide pathway, alpha(1/2)-adrenoceptors, and/or, opioidergic pathway, while, the anti-inflammatory effect contains marked inhibition of histamine and serotonin release.(Ishola, I.O. et al.,2015).

CHAPTER THREE

3.0 METHODOLOGY

3.1 REAGENTS USED

Formaldehyde

Ethanol

Distilled Water

Aspartate transferases (AST) Kit

Alanine transferases (ALT) Kit

Total protein concentration (TP) Kit

Creatinine kit

(Kits were purchased from Randox Laboratories)

3.2 METHODS

3.2.1 COLLECTION OF PLANTS

Alafia barteri leaves were collected from Ikere, Osun state on March 2019 and identified by Mr. Novsa at Department of Botany University of Lagos and Voucher number LUH5517 was used.

3.2.2AQUEOUS EXTRACT PREPARATION

Leaves were air dried in shade for 3 days and then grinded using grinding machine into powder.

Procedure: 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 acquire the crude extract.

3.2.3 ETHANOLIC EXTRACT PREPARATION

Procedure:100g of dried plant material macerated with 700mls absolute ethanol for 3 days. Filtration was done after 3 days and the filtrate was concentrated to acquire crude extract.

3.2.4 PHYTOCHEMICAL ANALYSIS

Preliminary phytochemical screening was conducted on the aqueous and Ethanolicextract of *A.barteri* 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 was 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 indicated 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 confirmed 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 confirmed the presence of flavonoids. (Trease & Evans 1989)

Test for phenol

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

Test for polyphenol

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

Test for anthraquinone (Clulei, 1975)

2mls of each extract was treated with 5mls of benzene and observed for two layers. The clear colorless upper layer was pipetted and the organic layer was treated with 3mls of 10% NH_3. A change in color from rose pink to red indicated the presences of anthraquinone.

Quantitative Determination of Phytochemical content in plant

Test for alkaloids

Using HARBONE`smethod the quantity of alkaloids in the plant was determined. 5 g of the plant sample is prepared in a 250ml beaker and 200 ml of 10% CH_3CO_2H (acetic acid) in C_2H_5OH (ethanol) is added to the plant sample. The mixture is covered and allowed to stand for 4 h. The mixture then filtered and the extract is allowed to become concentrated in a water bath till it reaches 1/4 of the original volume. Concentrated NH_4OH is added until the precipitation is complete. The whole solution is allowed to settle and the precipitate is collected and washed with dilute NH_4OH and then filtered. The residue is alkaloid, which is then dried and weighed.

Test for flavonoids

10 g of plant sample is repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. The whole solution is then filtered through filter paper and the filtrate is later on transferred into a water bath and solution is evaporated into dryness. The sample is then weighed until a constant weight.

3.3 EXPERIMENTAL ANIMALS

Thirtyfemale adult Wistar rats 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.

3.4 EXPERIMENTAL DESIGN

The Thirty (30) rats were randomly distributed into six (6) groups (I-VI) of five (5) animals per group.

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 0.5ml of aqueous extract (200mg/kg)

Group IV- received 0.5ml of aqueous extract (400mg/kg)

Group V- received 0.5ml of ethanolic extract (200mg/kg)

Group VI- received 0.5ml of ethanolic extract (400mg/kg)

3.5 EXPERIMENTAL INDUCTION OF INFLAMMATION AND ORAL ADMINISTRATION

Inflammation was induced by sub-planar administration of 0.1ml of 1% formaldehyde into their right hind paw.Administration of plant extract and diclofenac started after 24hours of induction of inflammation. The extract and diclofenac was administered for 12 days at different doses respectively. Inflammation in rats was accessed by means of physical and biochemical measurement. The paw diameter, body weight and levels of AST, ALT, TP, and Creatinine was measured. The paw swelling was measured using vernier Caliper.The anti-inflammatory activities was calculated has percentage inhibition of oedma in the extract animals in comparison to the control group.

3.6 COLLECTION AND PREPARATION OF BLOOD SERUM

On the twelfth (12^{th}) day of administration of plant extract and diclofenac, the animals were sacrificed by cervical dislocation under anesthesia. The blood sample were collected ocular puncture. Blood samples were transferred to Lithium heparin (EDTA) bottle and centrifuged at 4000rpm for 15mins to obtain serum.

3.7 ESTIMATION OF SERUM LIVER AND RENAL FUNCTION

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

3.8 STATISTICAL ANALYSIS

The statistical analysis was done using Graph Pad prism 8.2. The results were reported as mean ± SEM (standard error of mean). The data collected were subjected

to one- way analysis of Variance (ANOVA) to test for variations of the different parameters observed in the study. Test of significance was at 0.05% probability ($p<0.05$).

CHAPTER FOUR

4.0 RESULTS

4.1 PERCENTAGE YIELD

Percentage yield = $\frac{\text{weight after extraction}}{\text{Weight before extraction}} \times 100$

Aqueous extract yielded 74.62 %w/w with a brown colour while the ethanol 69.41 %w/w with dark green colour.

4.2a Qualitative phytochemical analysis on the leaves of *A.barteri*

Constituents	**Ethanol extract**	**Aqueous extract**
Flavonoid	+	+
Glycosides	+	+
Tannin	+	+
Steroids	+	+
Terpenoids	+	+
Anthraquinone	-	-
Saponins	+	+
Alkaloids	+	+

Phenol	+	+
Poly phenol	+	+

+ =Present, - = Absent Table 1a

4.2b Quantitative phytochemical analysis on the leaves of *A.barteri*

Phytochemical	Consistituent (g/mg)
Alkaloids	0.7874
Flavonoid	8.56

Table 1b Values are means of duplicate determination

The Phytochemical screening of the aqueous and Ethanolic extract showed the presence of different secondary metabolites such as alkaloids, saponins, flavonoids, steroids, glycosides, phenol, polyphenol, tannins, terpenoids while anthraquinone was absent. Quantitative analysis show that flavonoids was more present in the plant extract than alkaloids.

4.3 EFFECT OF *ALAFIA BARTERI* LEAF EXTRACT ON BODY WEIGHT

The oral administration of aqueous and ethanolic extract had little or no significant differences on the body weight of the rats

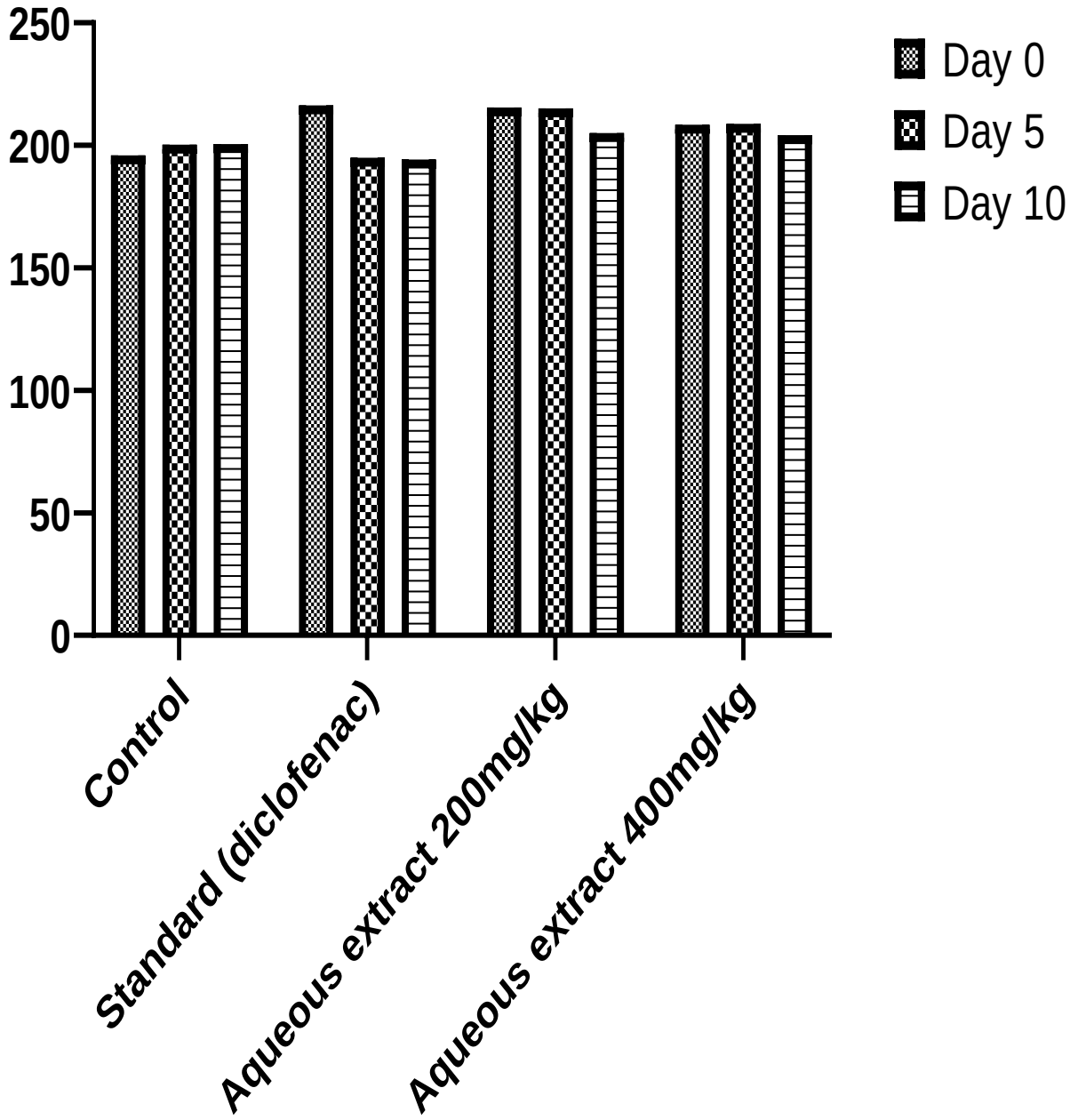


Figure 1. The effect of aqueous A.barteri leaf extract on the body weight of rats

Table 2.Effect of aqueous *A.barteri* leaf extract on the body weight of control and formalin-induced rats

Group	Day 0(g)	Day 5(g)	Day 10 (g)
Control	195.0±7.99	200.2±7.28	200.5±2.83
Standard Drug(Diclofenac)	216.3±3.90	195±2.66	194.3±13.50
Aqueous Leaf Extract(200mg/kg)	215.4±4.85	215.0±8.40	205.0±10.31
Aqueous Leaf Extract (400mg/kg)	208.4±9.17	208.8±5.68	204.2±7.75

Table 3. Effect of ethanolic A.barteri leaf extract on the body weight of control and formalin-induced rats

Groups	Day 0(g)	Day 5(g)	Day 10(g)
Control	195.0±7.99	200.2±7.28	200.5±2.83
StandardDrug(Diclofenac)	216.3±3.90	195±2.66	194.3±13.50
Ethanolic Leaf extract(200mg/kg)	219.3+7.54	204.7±8.45	218.4±6.68
Ethanolic leaf extract(400mg/kg)	207.4+13.52	193.0±5.99	196.2±8.15

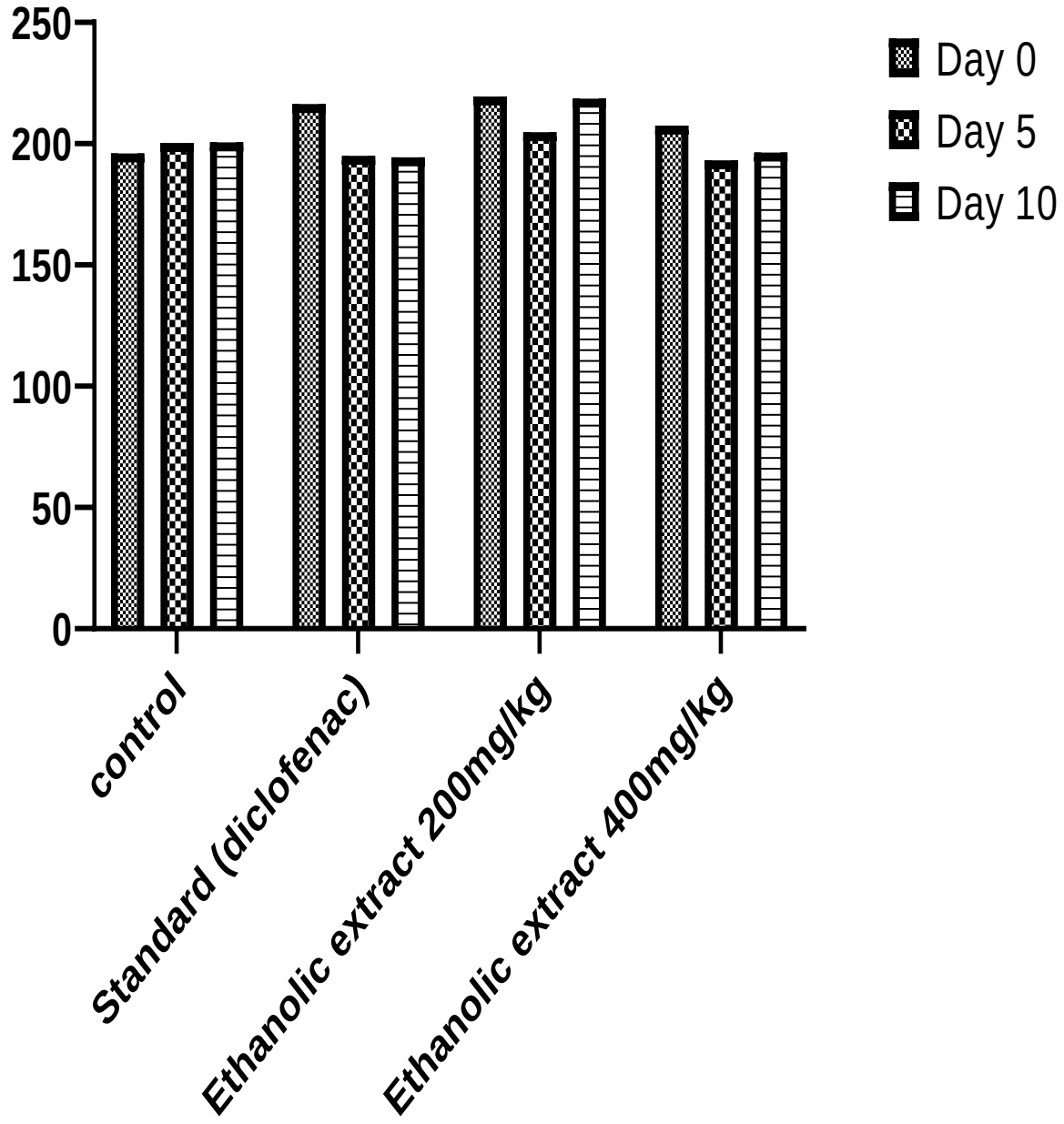


Figure 2. Effect of ethanolic A.barteri leaf extract on the body weight of rats

4.4 EFFECT OF ALAFIA BARTERI LEAF EXTRACT ON PAW DIAMETER

The injection of formalin into the right hind paw triggered an increase in the paw diameter. Oral administration of *A.barteri* leaf extract led to a decrease in paw diameter. The aqueous extract (200mg/kg) caused a decrease in paw diameter on day 5 while aqueous extract (400mg/kg) caused a decrease in paw diameter on both day 5 and 10. Also, the ethanolic extract (200mg/kg and 400mg/kg) caused decrease in paw diameter on day 5 and 10. However, the diclofenac drug also caused a reduction but not as significant as the plant extract.

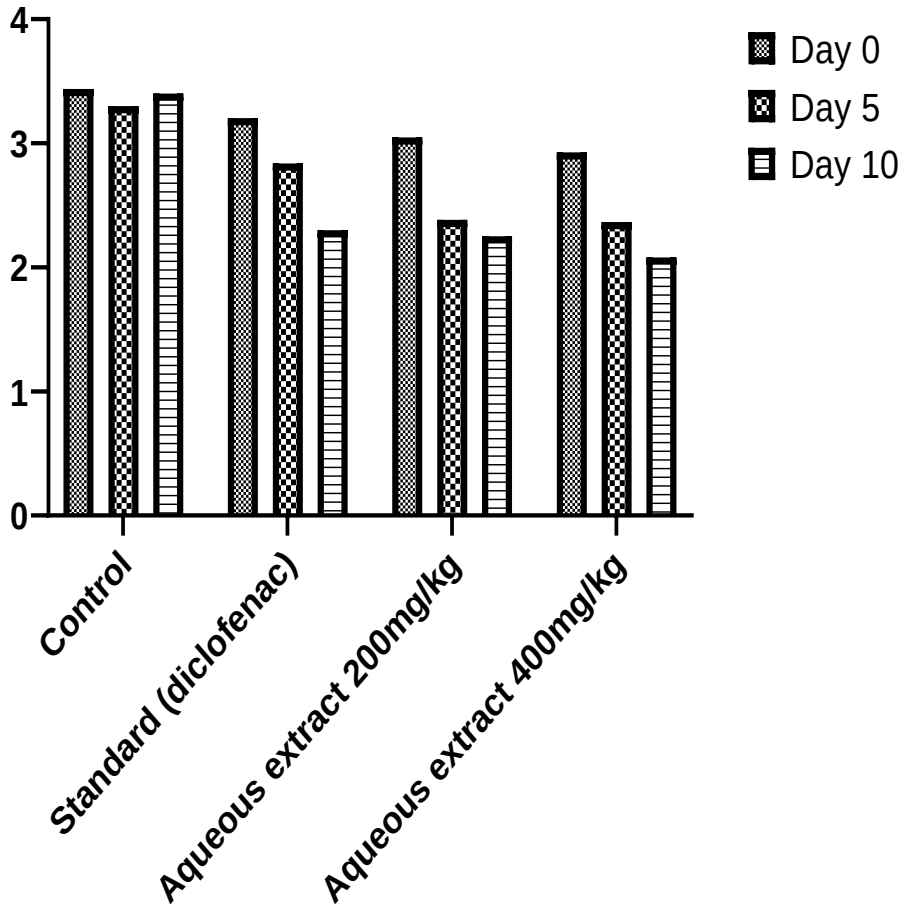


Figure 3. Effect of aqueous A.barteri extract on paw diameter of rats

Table 4. Effect of aqueous A.barteri leaf extract on paw diameter of control and formalin-induced rats

Group	Day0 (mm)	Day 5 (mm)	Day10 (mm)	% inhibition
Control	3.44±0.22	2.85±0.20	2.23±0.12	
Standard	3.20±0.12	2.84±0.20	2.30±0.05*	6%
Aqueous leaf extract(200mg/kg)	2.92+0.05	2.38±0.16*	2.25±0.12	52%
Aqueous leaf extract(400mg/kg)	2.59+0.19	2.36±0.05*	2.08±0.08*	32%

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

Table 5. Effect of ethanolic A.barteri leaf extract on paw diameter of control and formalin-induced rats

Group	Day 0 (mm)	Day 5 (mm)	Day 10 (mm)	% inhibition
Control	3.44±0.22	2.83±0.20	2.23 ±0.12	
Standard (diclofenac)	3.20±0.12	2.84±0.20	2.30±0.05*	6%
Ethanolic leaf extract(200mg/kg)	2.92±0.27	2.54±0.14*	2.15±0.06*	24%
Ethanolic leaf extract(400mg/kg)	2.59±0.09	2.28±0.11*	2.12±0.09*	45%

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

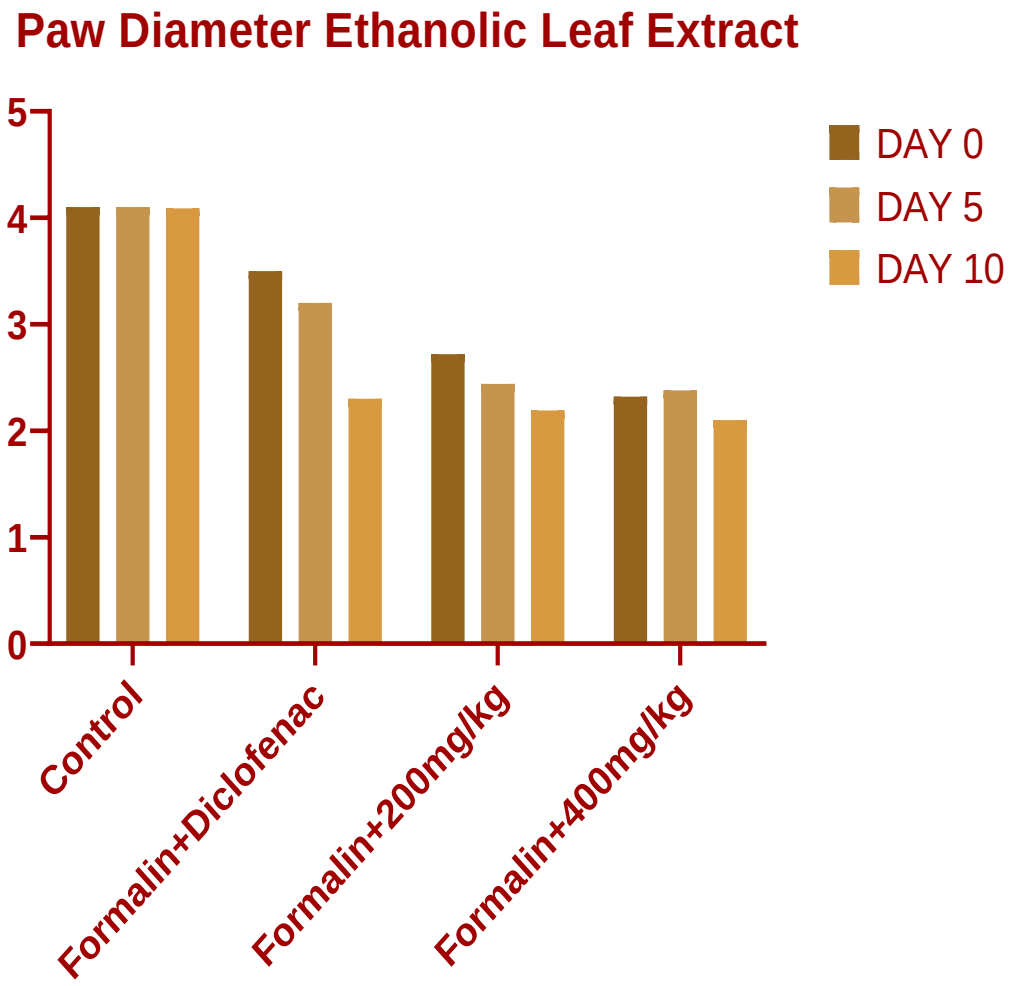


Figure 4. Effect of Ethanolic A.barteri extract on paw diameter of rats

4.5 EFFECT OF AQUEOUS *ALAFIA BARTERI* LEAF EXTRACT ON SERUM BIOCHEMICAL PARAMETERS OF RATS

Groups	AST(mg/dl)	TP(g/l)	ALT(mg/dl)	CREATININE (mg/dl)
Control	10.00±0.00	8.26±0.17	8.00±0.00	0.22±0.01
Standard (diclofenac)	7.00±0.00	7.13±0.17	6.66±0.94	0.32±0.16
Aqueous leaf extract(200mg/kg)	7.00±0.00	9.01±0.53*	24.75±13.22	0.49±0.26
Aqueous leaf extract(400mg/kg)	9.25±2.25	9.45±0.39*	9.25±2.75	0.77±0.22

Table 6a

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.

*represent significant increases at p<0.05 when compared to control value

Alanine aminotransferase showed no significant difference in standard (diclofenac), aqueous leaf extract 200mg/kg, aqueous leaf extract 400mg/kg compared to the control.

Aspartate aminotransferase showed no significant difference in standard (diclofenac), aqueous leaf extract 200mg/kg, aqueous leaf extract 400mg/kg compared to the control.

Total protein showed significant differences in aqueous leaf extract 200mg/kg, aqueous leaf extract 400mg/kg compared to the control while there was no significant differences in standard (diclofenac)compared to control.

Creatinine showed no significant differences in standard (diclofenac), aqueous leaf extract 200mg/kg, aqueous leaf extract 400mg/kg compared to the control.

4.6 EFFECT OF ETHANOLIC ALAFIA BARTERI LEAF EXTRACT ON SERUM BIOCHEMICAL PARAMETERS OF RATS

Group	AST(mg/dl)	TP(g/l)	ALT(mg/dl)	CREATININE (mg/dl)
Control	10.00±0.00	8.26±0.17	8.00±0.00	0.22±0.01
Standard (diclofenac)	7.00±0.00	7.13±0.17	6.66±0.94	0.32±0.16
Ethanolic leaf extract(200mg/kg)	19.00±17.02	8.53±0.31*	8.25±3.06	0.49±0.15
Ethanolic leaf extract(400mg/kg)	7.00±0.00	8.96±0.42*	7.00±0.00	0.35±0.04

Table 6b

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.

*represent significant increases at p<0.05 when compared to control value

Alanine aminotransferase showed no significant difference in standard (diclofenac), ethanolic leaf extract 200mg/kg, aqueous leaf extract 400mg/kg compared to the control.

Aspartate aminotransferase showed no significant difference in standard (diclofenac), ethanolic leaf extract 200mg/kg, aqueous leaf extract 400mg/kg compared to the control.

Total protein showed significant differences in ethanolic leaf extract 200mg/kg, ethanolic leaf extract 400mg/kg compared to the control while there was no significant differences in standard (diclofenac) compared to control.

Creatinine showed no significant differences in standard (diclofenac), ethanolic leaf extract 200mg/kg, aqueous leaf extract 400mg/kg compared to the control.

CHAPTER FIVE

5.0 DISCUSSION

Inflammatory processes encompasses of the release of various mediators and substances that standardize adhesion of molecules and the processes of cell migration, activation and degranulation. (Hellen Kariuki et al., 2013). Medicinal plants are mostly used for the treatment of inflammation due to fact that they exhibit no or little side effect compared to drugs. The study investigated the anti-inflammatory effects of *A.barteri* leaves aqueous and ethanolic extract. Formalin was used to induce inflammation on the right hind paw of the rats. It has been used as a classic model to produce experimental inflammation. It is a modern, scientific, international approved standard experimental procedure. (Fatima, N. and Fatima, S.J., 2016). On the injection of formalin there was formation of inflammation and pain on the rats. Oedema was obtained after 24hours of induction (0.1 of 1.0% formalin) and this is due to the release of chemical mediators such as histamine, leukotriene, kinin, cyclooxygenase. However, on a delayed phase, bradykinin, prostaglandins and neutrophil infiltration are generated. Diclofenac is a non-steroidal anti-inflammatory drug whose mechanism action is by inhibiting prostaglandins which is synthesized by cyclooxygenase-2. The anti-inflammatory effect of *A.barteri* as the result suggests was due to the presence of secondary metabolites such as flavonoids, alkaloids, tannins, glycosides, saponins, steroids terpenoids, phenol, polyphenol which have the ability to inhibit the production of prostaglandins by cyclooxygenase-2. According to Agnel, N., Arul, J. And Shobana, G., (2012) flavonoids have advantageous effects in the inflammatory conditions and that the anti-inflammatory activity is a common property of many terpenoids.

Aqueous and ethanolic extracts of *A. barteri* leaf at doses of 200mg/kg and 400mg reduced formalin induced paw compared with that of the standard drug, diclofenac. According to Sofidiya et al. (2014) the ethanolic extract of Alafia barteri (100mg/kg) caused a significant inhibition against carrageenan induced paw edema test. The result showed that serum AST, ALT, creatinine were not significantly removed or elevated. It can be inferred that the extract have no hepatic toxicity on the liver and no kidney damage after oral administration. However, increase in TP concentration as seen in the results may be attributed to stress which arises during the inflammatory process.

5.1 CONCLUSION

This study showed that the aqueous and ethanolic leaf extracts of *Alafia barteri* exhibits anti-inflammatory effect without altering liver function, hence validating its traditional claims.

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APPENDIX

Paw diameter

Group	Day 0	Day 5	Day 10
Negative control	3.65	2.83	2.23
Formalin+diclofenac	3.20	2.84	2.30
Formalin+ 200mg/kg aqueous extract	3.04	2.38	2.25
Formalin+ 400mg/kg aqueous extract	2.95	2.37	2.08
Formalin +200mg/kg ethanolic extract	2.92	2.54	2.15
Formalin + 400mg/kg ethanolic extract	2.59	2.28	2.12

- Body weight

Groups	Day 0	Day 5	Day 10
Negative control	195.85	200.22	200.47
Formalin+diclofenac	216.30	194.95	194.27
Formalin+ 200mg/kg aqueous extract	215.40	215.03	205.03
Formalin+ 400mg/kg aqueous extract	208.43	208.77	204.20
Formalin +200mg/kg ethanolic extract	219.29	204.65	218.42
Formalin + 400mg/kg ethanolic extract	207.38	193.00	196.24