EFFECT OF AQUEOUS AND ETHANOLIC EXTRACT OF *Alafia barteri* LEAF ON OXIDATIVE STRESS PARAMETERS IN FORMALIN INDUCED PAW INFLAMMATION IN WISTAR RATS

BY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES COLLEGE OF BASIC AND APPLIED SCIENCES ,MOUNTAIN TOP UNIVERSITY IN PARTIAL FULFILMENT FOR THE REQUIREMENT FOR AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc. HONS) IN BIOCHEMISTRY

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CERTIFICATION

This is to certify that this project research titled **EFFECT OF AQUEOUS AND ETHANOLIC EXTRACT OF *Alafia barteri* LEAF ON OXIDATIVE STRESS PARAMETERS IN FORMALIN INDUCED PAW INFLAMMATION IN WISTAR RATS** was carried out, compiled and written by **ACHEBUMERE KINDNESS** with Matriculation number **15010102013** and has been approved for the partial fulfillment of the award of Bachelor of Sciences, B.Sc(BIOCHEMISTRY) of Mountain Top University.

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DECLARATION

I hereby declare that this project report written under the supervision of Mrs.I.O.Adefisan, is a product of my own research work.Information derived from various sources have been duly acknowledge in the text and a list of references provided.This research project report has not be previously presented anywhere for the award of any degree or certificate.

……..... ……..

ACHEBUMERE KINDNESS DATE

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ABSTRACTS

This research analyzes the anti-oxidant impact of the aqueous and ethanolic leaf extract of *Alafia barteri* on the basis of their medicinal use in the therapy of inflammation,toothaches and fevers. The findings of the phytochemical testing showed that the extract contained alkaloids, steroids, saponins, flavonoids, phenolics and terpenoids which have been connected with anti-inflammatory actions.Ethanolic extract of *A.barteri* at the dose of 200 mg/kg and 400 mg/kg showed a substantial decrease in paw oedema rats volume when compared to the standard drug(diclofenac sodium 10mg/kg).The results support the traditional use of *A. barteri* in the therapy of mutiple pain and inflammation-related illnesses. *A. barteri* caused a major shift in the growth of paw oedema in the formalin induced.This study aimed at evaluating oxidative stress in the liver of formaldehyde-induced rats to cause paw inflammation. SOD, Catalase levels were calculated in the total liver homogenate and in addition to the effect on paw oedema.

Arthritic rats obtained elevated levels of ROS than controls in the total homogenate (46% higher) and in all subcellular fractions (51, 38, and 55% higher for mitochondria, peroxisome, and cytosol, respectively). Arthritic rats also had a greater amount of carbonyl protein in complete homogeneity(75%) and in all subcellular fractions (189, 227, and 260%, respectively, for mitochondria, peroxisomes, and cytosol). The TBARS levels of arthritic rats were more elevated in the total homogenate (36%), mitochondria (20%), and peroxisomes (16%). Arthritic rats also had a greater amount of NO markers in the peroxisomes (112%) and in the cytosol (35%). The catalase action of all cell compartments was stoutly reduced (between 77 and 87%) by arthritis, and glutathione peroxidase actions were reduced in the cytosol (41%) and mitochondria (33.7%) .The cytosolic glucose-6-phosphate dehydrogenase action, on the other hand, was increased (62.9%), the same occurrence with inducible peroxisomal NO synthase (119.3%). The

superoxide dismutase and glutathione reductase actions were not affected. The GSH content was reduced by arthritis in all cellular compartments (50 to 59% diminution). The results showed that the liver of rats with adjuvant-induced arthritis presents a distinct oxidative stress and that, in result, injury to lipids and proteins is highly significant. The increased in ROS content of the liver of arthritic rats seems to be the result of both a stimulated pro-oxidant system and a deficient antioxidant defense with a prevalence of the final as indicated by the strongly diminished actions of catalase and glutathione peroxidase.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Inflammation is a method that results from certain disturbance or disease and it is a process familiar to everyone. It occurs in response to allergen, wounds, infection and auto-immune conditions (Snehal *et al*, 2015). It is caused by the release of chemical mediators from wounded tissue and migrating cells (Godhandaraman *et al***,** 2016). Inflammation signs are elevated heat, loss of function, redness, swelling, pain (Vashishtha *et al, 2014).* Inflammation method plays a guilding role in our body and has an adverse impact such as inflammatory disorders, rheumatoid arthritis, inflammatory bowel diseases, retinitis, multiple sclerosis, osteoarthritis, psoriasis and atherosclerosis. Inflammation may be grouped as either acute or chronic.Increased vascular permeability, capillary infiltration, and emigration of leukocytes is connected with acute inflammation.Infiltration of mononuclear immune cells, macrophages, proliferation (angiogenesis), monocytes, fibrosis, neutrophils, fibroblast activation is connected with chronic inflammation. Inflammation is a common clinical conditions and rheumatoid arthritis (RA) is a persistent debilitating autoimmune disorder.

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Godhandaraman *et al***,** 2016). To overcome this problem, searching for new medicines is very essential, and many plants have phytoconstituents that play a very significant role in inflammation therapy.

Alafia barteri (Apocynaceae) is a high-climbing small white or rose flower arbust. It is used for the therapy of sickle cell anaemia, febrifuges, eye infections, rheumatism, as chewsticks and

toothache in ethnomedicine. The stem of *A.barteri* is used for the therapy of inflammation, fever and root binding materials (Johnson *et al,* 2015).In Nigeria and other African nations, leaf infusion and root deflections are used to treat malaria (Joseph *et al,* 2018). The root decoction is used in Nigeria in the therapy of rheumatic pains (Ishola *et al,* 2014). The leaf infusion is used for malaria treatment in Coˆte d'Ivoire,

1.3 AIM

To determine the effect of aqueous and ethanolic leaf extracts of *Alafia barteri* on oxidative stress parameters in formalin induced inflammation in albino rats.

1.4 OBJECTIVES OF THE STUDY

1. To determine the phytochemical components of *Alafia_barteri* leaf.
2. To evaluate the effect of oral administration of the extract on body weight and paw diameter in formalin induced wistar rats.
3. To evaluate the effect of oral administration of the extract on oxidative stress parameters in wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 MEDICINAL PLANTS

Medicinal plants are plants that used to treat or cure infection or diseases. They are an efficient source of traditional and modern medicine.These plants have been shown to be of real use and as primary health care, about 80% of the rural population rely on them.

According to the World Health Organization (WHO), the definition of traditional medicine can be summarized as the sum total of all the information and practices used in the diagnosis,elimination and prevention of mental, physical or social imbalance.

It has been estimated that in plant medicines represent as much as 25% of complete medicines in advanced nations such as the united state while in developing countries such as China and India, the contribution is as much as 80%. Thus, the financial significance of medicinal plants is much greater for nation such as india than for remainder of the globe(Monier *et al* , 2016).

As a liana, *A.barteri* grows up to 35 metres (115 ft) long, with a stem diameter of up to 3 centimetres (1.2 in).A white corolla.Fruit is dark brown with paired cylindrical follicles each up 50 centimetres (20 in) in diameter featuring fragnant flowers. Habitat is lowland forest from sea-level to 200 metres (660 ft) altitude.

A. barteri is native to Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon and Gabon.

2.1.1 Botanical Classification of Alafia barteri

Scientific classification

Kingdom : plantae

Order : Gentianales

Family : Apocynaceae

Subfamily : Apocynoideae

Tribe : Malouetieae

Genus: Alafia

Species: A.Barteri

Alafia barteri occurs in the forests of West and Central Africa, from Guinea Bissau east to Cameroon and south to Congo. The stems fiber is used as binding material for roofs.In the traditional medicine scheme in Nigeria and other African nations,latex has been used to taint stronger latex. It is an anti-inflammatory and fever remedy.The infusion of the leaves and stem are used to treat fever and inflammation, the decoction of plant root and leaves is also used internally or externally to treat eye diseases, rheumatic pain and toothache. The extracts of the leaves were found to have antibacterial and antifungal activities,anti-inflammatory. The aqueous leaf extract was reported to display potent antiplasmodial activity (Shofidiya *et al*, 2014***).***

2.2 INFLAMMATION

Inflammation is described as the local reaction to injury caused by any agent of living mammalia n tissue. Inflammation comes from the Latin term "inflammare ;" implies burning

The agents causing inflammation may be;

1. Physical agents - Heat, Cold, Radiation.
2. Chemical agents - Inorganic and Organic poisons.
3. Infective agents - Bacteria,Virus and their toxins.
4. Immunological agents - cell-mediated and antigen antibody.

Earlier,Inflammation was thought to be a single disease induced by body fluid disturbances. Infla mmation is a good method that results from some disruption or disease, according to the contemp orary idea.Any form of injury to the human body can elicit a series of chemical changes in the injuredarea.Inflammation generally includes a series of occurrences that can be classified as thre e stages: an acute transient phase, a delayed sub-acute phase and a chronic phase of propagation.

It is characterized in the acute form by the cardinal signs(mohan) such as- Pain(dolor), Heat (calor), Redness(rubor), Swelling(tumor), Loss of function (function laesa). Inflammation is component of the vascular tissue's complicated biological reaction to damaging stimuli like pathogens, damaged cells, or irritants.It is a localized response resulting in redness, warmth, inflammation, and pain caused by infection, irritation, or injury.External or internal infla mmation may occur.(Christian, 2017).There are two major groups of medications used in controlling inflammation: steroidal and non-steroidal ant-iinflammatory agents.Most of the antiinflammatory medicines available now are prospective inhibitors of the ar achidonic acid metabolism cyclooxygenase (COX) pathway that generates prostaglandins. Prostaglandins are hyperalgesic, powerful vasodialers and lead to erythema, edema and pain as w ell. Therefore, analgesic and anti-inflammatory agents are needed to treat inflammatory illnesses.

NSAIDs have three significant activities, all owing to inhibition of arachidonic acid cyclooxygen ase (COX-2 isoenzyme) in inflammatory cells and the resultant decrease in prostanoid synthesis (Satya *et al*, 2013).

All these harmful properties contradict prolonged therapy with glucocorticoids.Recently, some li ght has been thrown on steroids like compounds current in a number of medicinal plants.Medicin al plants contain chemical constituents that chemically resemble steroids in composition and cont emporary clinical studies have endorsed their function as anti-inflammatory agents.Inflammatory response is associated with many acute and chronic inflammatory diseases, including asthma, rheumatoid arthritis, rhinitis, conjunctivitis, and multiple sclerosis. Glucocorticoids have been

widely and successfully used in the treatment of inflammatory diseases.(Snehal et al, 2015.). It is caused by releasing chemical mediators from wounded tissue and migrating cells.Inflammation is a complicated method that is often associated with pain and includes events such as: increasing vascular permeability, increasing protein denaturation, and altering the membrane.Denaturation of proteins is a method where proteins lose their tertiary structure and secondary structure by applying internal stress or compounds like strong acid or base, concentrated inorganic salt, organic solvent or heat.When denatured, most biological proteins lose their biological function.Protein denaturation is a well-documented cause of inflammation(Godhandaraman *et al*, 2016)

Inflammation comes with its initiation, regulation and resolution involving many distinct procedures.A variety of inflammations have been recognized nowadays, 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.

In latest years, many scientists have concentrated on medicinal crops obtained from natural goods such as flavonoids, steroids, polyphenols, coumarins, terpenes and alkaloids because of their wide spectrum of pharmacological importance, including antiinflammatory, analgesic and antipyretic operations with minor side impacts. (Shankar 2017).

2.2.1 THE ROLE OF INFLAMMATION AND ITS EFFECTS

Inflammation plays an significant role in protecting the body from pathogens such as viruses, bacteria, fungi, and other parasites as part of the immune response. Acute inflammation in the impacted tissue releases leukocytes, erythrocytes and plasma elements.It can lead to chronic inflammation if the inflammation is not fixed. Biochemical impacts, such as nutritional fat imbalance, lack

of particular drugs adverse to manufacturing.Chronic inflammation results from antiinflammatory cells as well as particular nutrient issues. Inflammation may have local and systemic impacts.Fever, malaise, and leukocytosis are the systemic impacts of acute inflammation. Local impacts are generally obviously useful, such as the destruction of invading microorganisms, but they do not seem to serve any apparent purpose at other moments, or may even be detrimental.

2.2.2 TYPES OF INFLAMMATION

1. Acute Inflammation

Acute inflammation is characterized by the exudation of fluids and plasma proteins and the migration of leukocytes, most significantly neutrophils into the wounded area.Acute inflammatory response is helpful to the resistance method aimed at killing of virus ,bacteria and parasites. (Shankar, 2017). An acute inflammation is one that starts rapidly and becomes severe in a short space of time. Signs and symptoms are normally only present for a few days but may persist for a few weeks in some cases.(Christian, 2017).

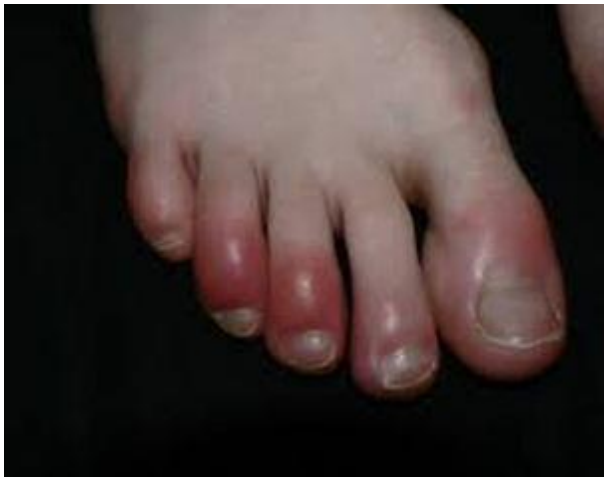
2. Chronic Inflammation

Chronic inflammation refers to long-term inflammation and can last for several months and even years(Christian,2017).Due to the existence of lymphocytes and macrophages, it is of a longer length and histologically, leading in fibrosis and tissue necrosis.Chronic inflammation improves the growth of degenerative illnesses such as rheumatoid arthritis, atherosclerosis, heart illness, Alzheimer's illness, asthma, acquired immunodeficiency disorder (AIDS), cancer, congestive heart failure, various sclerosis, diabetes, infections, gout, IBD inflammatory intestinal illness, aging and ot

her neurodegenerative depression,Chronic inflammation was also involved as part of the muscle loss caused by aging (Shankar, 2017). It can result from

- An autoimmune disorder that attacks ordinary healthy tissue and is mistaken for a disease-causing pathogen.
- Long-term exposure to a small amount of an irritant, such as an industrial chemical.

Examples of diseases and conditions that include chronic inflammation include asthma, chronic peptic ulcer, tuberculosis, rheumatoid arthritis, periodontitis, ulcerative colitis and crohn's disease, sinusitis, active hepatitis.



Foot inflammation

Joint inflammation

2.4 OXIDATIVE STRESS

Oxidative stress is refers to the imbalance between free radicals and their stabilizing agent's antioxidant enzymes in the body(Manisha, 2017).It is caused by an imbalance between excessive reactive oxygen species (ROS) production and antioxidant mechanisms.Increased concentrations of ROS cause oxidative damage to proteins, lipids and DN A nucleic acid bases, which contribute to inflammatory processes.(Berar *et al* , 2015).

Oxidative stress has been involved in several illnesses including cancer, atherosclerosis, malaria, syndrome of acute exhaustion,neurodegenerative, Alzheimer's disease, and Huntington's disease such as Parkinson's illness.Indirect proof through surveillance of biomarkers such as reac tive oxygen species and the manufacturing of reactive nitrogen species shows that the pathogene sis of these illnesses may involve oxidative damage(Rahman,2012).The increasing interest in the role of free radicals in pathogenesis of human disease has led in comprehensive attempts to devel op techniques for evaluating free radicals and their responses in vivo.(Palmieri *et* al,2017).

In order to cope with the oxidative stress of aerobic metabolism, animal and human cells develop ed an omnipresent antioxidant defense system consisting of superoxide dismutase (SOD), catalas e (CAT), glutathione peroxidase (GPx) and glutathione reductase.In combination with a number of low molecular antioxidants such as ascorbate, αtocopherol and glutathione, cystein, thioredoxi n, vitamins, etc. (T. Rahman et al,2012).

In many chronic and degenerative diseases, including atherosclerosis, ischaemic heart disease, ag eing, diabetes mellitus, cancer, immunosuppression, oxidative stress has been created.

Disorders in the ordinary cell redox state can trigger toxic impacts by producing peroxides and fr ee radicals that damage all cell components, including proteins, lipids, and DNA.(Ilechukwu 2014).The damaging element of oxidative stress is the production of reactive oxygen species. Th

ere are free radicals and peroxides in these species.Much attention has been concentrated on the r ole of oxidative stress in latest years, and it has been reported that oxidative stress can be the mai n and common occurrence in secondary diabetic complications pathogenesis.

Chemically oxidative stress is associated with increased production of oxidizing species or a decrease in the usefulness of antioxidant defenses such as glutathione, catalase, superoxide dismutase, gamma- glutamyl transferase, vitamins A, C and E_8. (Ilechukwu, 2014).

2.4.2 Antioxidants

Antioxidants are divided into enzymatic and non-enzymatic antioxidants groups.

Enzymatic antioxidants

2.4.3 Superoxide Dismutase (SOD)

Superoxide dismutase eliminates free radicals and Reactive oxygen species, therefore, it is called a primary enzyme. SOD is an enzyme that converts superoxide to H_2O_2. There are several types of SOD in the cell.

Mn-SOD is found in the mitochondria. It dismutates oxygen ion which is created by oxidative phosphorylation. Cu, Zn-SOD is found mostly in cytosol and dismutates superoxide to H_2O_2 .SOD is also a metallo-enzyme requiring zinc for structural stability and copper for enzymatic activity.Metalloenzyme inhibition induced by cadmium was revealed to result from these metals being displaced from the active site of these enzymes.The decrease in SOD activity in animal tiss ues treated with cadmium alone may therefore be due to the displacement of these metals from th e enzymes.

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2.4.4 Catalase (CAT)

Catalase is an enzyme that breaks down to water the peroxide of hydrogen (H_2O_2). H_2O_2 forms h ydroxyl radical in the presence of transition metals but does not interact with proteins and lipids. Catalase is primarily found in peroxisomes or microperoxisomes.A research showed a reduction in catalase activity in diabetic rabbits ' liver and endothelial cells. This enzyme's activity is also r educed in diabetic patients(Robab 2013). Host cells discharge many enzymes that can debase these oxidizing agents to avoid excessive tissue destruction. One of them is catalase, which is an enzyme directly involved in active oxygen scavenging. Catalase is considered to be a defensive enzyme against the deleterious effects of hydrogen peroxide due to its ability to break it down.(Topcu *et al*, 2016).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Reagents Used For Analysis

- Formaldehyde
- Ethanol
- Sample (liver homogenate)
- Phosphate buffer
- Hydrogen peroxide
- Sodium carbonate
- Distilled water

3.1.2 APPARATUS

- Test tubes
- Test tube rack
- Conical flask
- Beaker
- Burette
- Pipette
- Conical flask
- Sample bottle
- Oral canula

- Weighing balance
- Dissecting Set
- Spectrophotomer
- Water bath
- Ice cube machine
- Centrifuge
- Beaker
- syringe

3.2 METHODS

COLLECTION AND IDENTIFICATION OF THE PLANT MATERIAL

The whole plant material of *Alafia barteri* was collected from Osun State, Nigeria, in the month of March 2019. Botanical identification and authentication was done at University of Lagos, where a voucher specimen was deposited with the herbarium file number LUH5517.

3.2.1 Aqueous Extract Preparation

The Leaves were air-dried in the laboratory for three days and were alienated from the stem. They were then pulverized with means of blender. Hundred grams (100 g) of the powdered leaves was weighed and was macerated in 700mls of distilled water with occasional shaking at room temperature for 72hours. Filtration was done using muslin fabric. The filtrate was concentrated in the oven to obtain the crude extracts.

3.2.2 Ethanolic extract preparation

The Leaves were alienated from the stem and air-dried for three days. They were then pulverized with means of blender. Hundred grams (100 g) of the powdered leaves was weighed and was macerated in 700mls of ethanol with occasional shaking at room temperature for 72hours. Filtration was done using muslin cloth.The filtrate was concentrated to obtain the crude extracts.

3.2.3 Qualitative Phytochemical Analysis

Ethanolic extract was tested for the presence of phytoconstituents using standard methods as described by Sofowora (1993), Harbone (1973) and Trease and Evans (1989), with slight modifications.

- Test For Alkaloids(Meyer's Test)

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

- 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.

- Test for Glycosides

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

- 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.

- 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 indicate the presence of saponins.

- Test for tannins

  2 drops of 5% fecl3 was added to 2mls of plant extract. Appearance of green precipitate on dilution confirms the presences of tannins.

- 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 color that disappears on standing confirms the presence of flavonoids.

- Test for phenol

  3 drops of ferric chloride was added to diluted extract. development of bluish black color signifies 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 confirms the presence of polyphenol.

3.2.4 Quantitative Determination of Phytochemical Constituents

- Test for flavonoids

10g of plant sample is repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. The 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 was obtained.

- Test for alkaloids

Using harbone method the quantity of alkaloids in the plant was determined. 5g of the plant sample is prepared in a 250mls beaker and 200mls 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 4hr. 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.

3.2.5 Experimental Animals

Thirty (30) adult Wistar rats weighing 180-250g were obtained and kept at the animal house, Department of Biological sciences, College of Basic and Applied Sciences, Mountain Top University, Nigeria. The animals were housed in a well-ventilated experimental section of the animal house at room temperature and were allowed free access to feed and water. They were allowed to acclimatize for 7 days before the commencement of the experiment.

3.2.6 Experimental Induction of Inflammation and oral administration

Thirty rats were randomly distributed into six (6) groups (I-V1) of 5 rats per group. Inflammation was induced by sub-planter administration of 0.1ml of 1% formalin into their right hind paw. The paw diameter was measured using digital caliper and anti inflammatory activities was calculated by percentage inhibition of the oedema relative to the control group. The extract was given to the rats for 12days at different doses respectively. Physical parameters (body weight and paw diameter) and oxidative stress parameters(SOD and catalase) were checked respectively.

Experimental design

Group	**Treatment**
I	1% Formalin only
II	1% Formalin + 10 mg/kg Diclofenac
III	1% Formalin + 200mg/kg aqueous extract of *Alafia barteri*
IV	1% Formalin + 400mg/kg aqueous extract of *Alafia barteri*
V	1% Formalin + 200 mg/kg ethanolic extract of *Alafia barteri*
VI	1% Formalin + 200 mg/kg ethanolicextract of *Alafia barteri*

The rats were anesthesized with choloroform and sacrificed by cervical dislocation, a longitudinal abdominal insertion was made ,and the liver was identified, carefully removed and taken for determination of in vivo antioxidant(SOD,CAT).

3.4 Determination of Oxidative Stress parameters

0.2g of liver was homogenized with 1.3mls phosphate buffer and centrifuged at 4000rpm for 15minutes.The cell free supernatant was used for the estimation of superoxide dismutase(SOD) and catalase(CAT).

3.4.1 Catalase assay (CAT)

CAT activity was assayed by the method of Claiborne(1985).The assay mixture comprises of 1.95ml phosphate buffer(0.05M, pH 7.0, 1.0ml H_20_2(0.019M), 0.05ml of sample. Changes in absorbance were recorded at 240nm.Catalase activity was calculated in terms of nmol H_20_2 consumed/mi/mg of protein.

3.4.2 Superoxide Dismutase Assay

The level of superoxide dismutase (SOD) activity was determined following the method described by Misra and Fridovich (1972).

Reagents

1. Carbonate buffer (0.05 M, pH 10.2): Na2CO3.10H2O (14.3 g) and 4.2 g of NaHCO3 were dissolved in 900 ml of distilled water. The pH was adjusted to 10.2 and then made up to 1 litre.

2. Adrenaline (0.3 mM): Adrenaline (0.0137 g) was dissolved in 200 ml distilled water and then made up to 250 ml. This solution was prepared just before the experiment.

Procedure: 1 ml of sample was diluted in 9 mls of distilled water to make a 1 in 10 dilution. 0.2mls of the diluted sample was added to 2.5 mls of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The blank contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance was read at 480 nm every 30 seconds for 150 seconds.

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 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 of the original plant material and the color is brown.

Ethanolic extract yielded 69.41% w/w of the original plant material and the color is dark green.

Table 1. Phytochemical constituents of the aqueous and ethanolic leaf extract of *Alafia barteri* leaves

Phytochemicals	AQUEOUS EXTRACT	ETHANOLIC EXTRACT
Flavonoid	+	+
Glycosides	+	+
Tannin	+	+
Steroids	+	+
Terpenoids	+	+
Anthraquinone	-	-
Saponins	+	+
Alkaloids	+	+
Phenol	+	+
Poly phenol	+	+

+=Present, - =Absent

The Phytochemical screening of the aqueous and ethanolic extracts of *A .barteri* leaf revealed the presence of glycosides, flavonoids, tannins, steroids, terpenoids, saponins, alkaloids, phenol, poly phenols and absence of anthraquinones.

Table 2. Quantitative Phytochemical Analysis On The Leaves Of *A.Barteri*

	Results
Alkaloids	0.7874g
Flavonoid	2.56g

Values are means of duplicate determinations

Quantitative determination of showed that flavonoids was more present in the leaves of the plant than alkaloids.

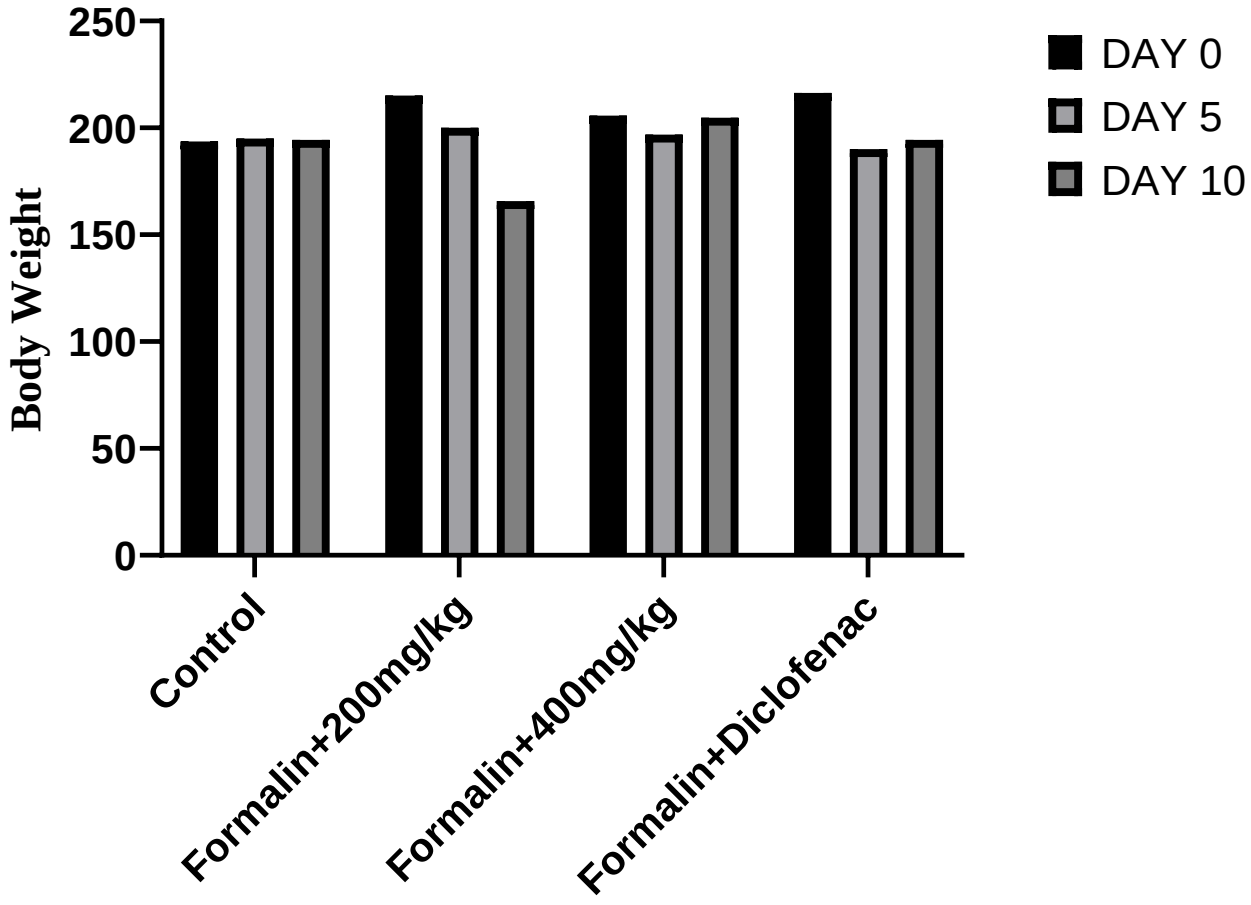

Figure 1. The effect of aqueous *A. barteri* leaf extract on the body weight of control and formalin induced rats

Oral administration of the aqueous leaves extract and the standard drug had no significant effect on the body weight as shown above.

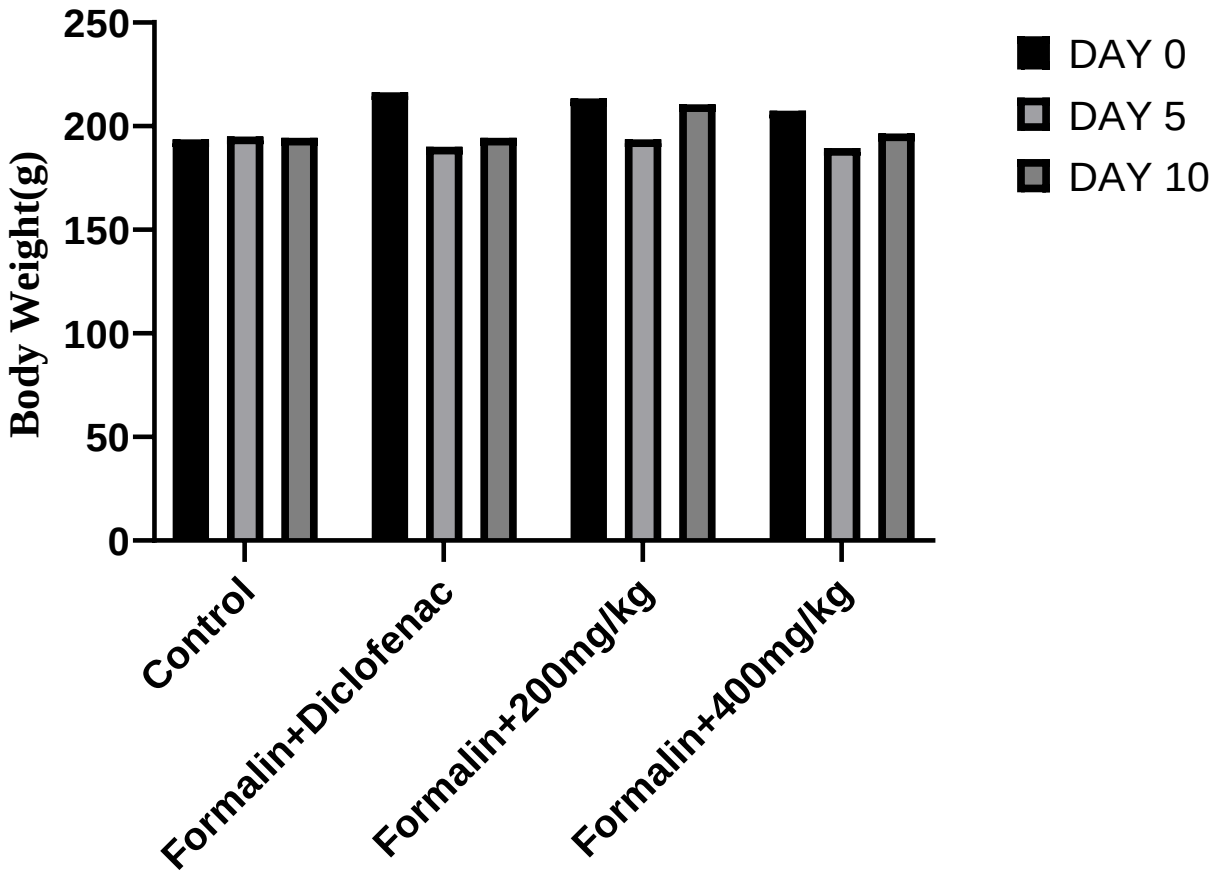

Figure 2. The effect of ethanolic *A.barteri* leaf extract on the body weight.

The effect of oral administration of the aqueous leaves extract on the body weight was insignificant compared to the standard drug (Diclofenac sodium).

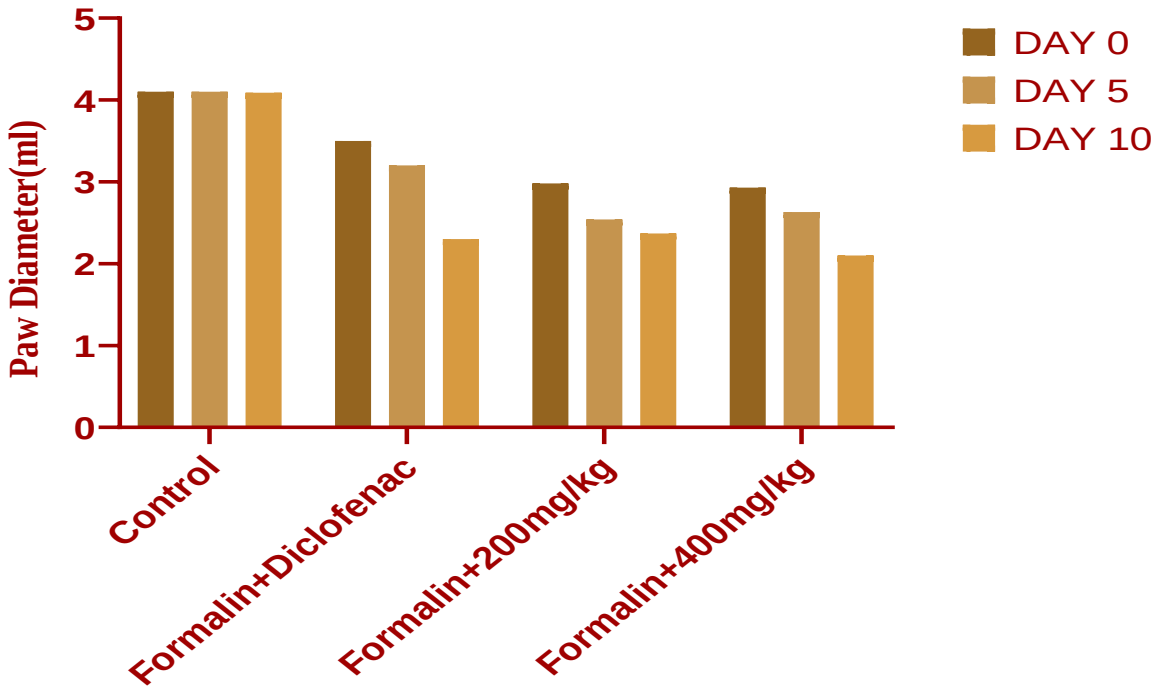

Figure3.Effect of aqueous *A.barteri* extract on paw diameter.

Oral administration of the aqueous leaf extract of *A.barteri* from day 5 to day 10 caused a significant decrease in the paw diameter of the rats.

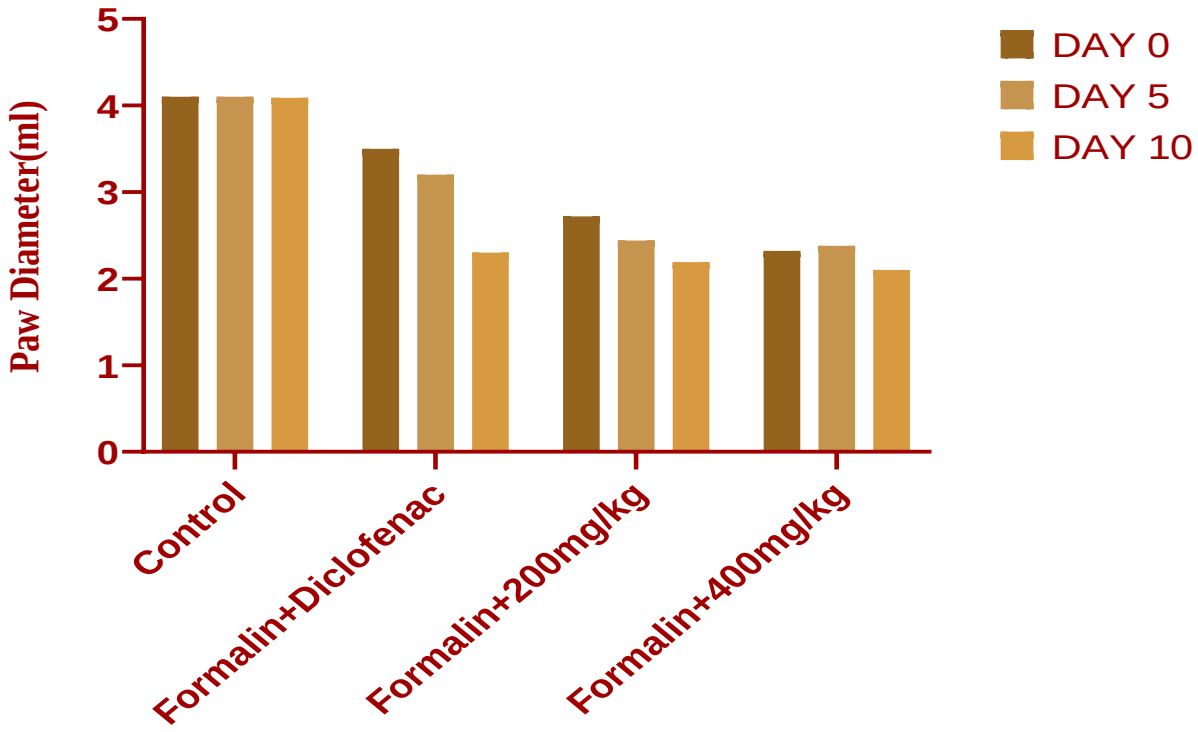

Figure4. Effect of Ethanolic *A.barteri* extract on paw diameter.

Oral administration of the ethanolic leaf extract of A.barteri from day 5 to day 10 caused a significant change in the paw diameter of the rats. In addition, the standard drug also caused a decrease but not as significant as the plant extract.

Table 3. **Effect of *Alafia Barteri* Leaf Extract on catalase activity** in control and formalin induced Rats

GROUPS	AQUEOUS	ETHANOLIC
Negative Control	69.28±7.94	69.28±7.94
Formalin +Diclofenac	78.63±7.77	78.63±7.77
Formalin+200mg/kg	61.23±8.76	60.56±11.54
Formalin+400mg/kg	50.08±6.77	51.46±7.18

Values are expressed as mean ± S.E.M (n=5). $P<0.05$*.

There was no significant difference in the catalase activity of liver tissue of rats in all the groups though group 2 had higher activity.

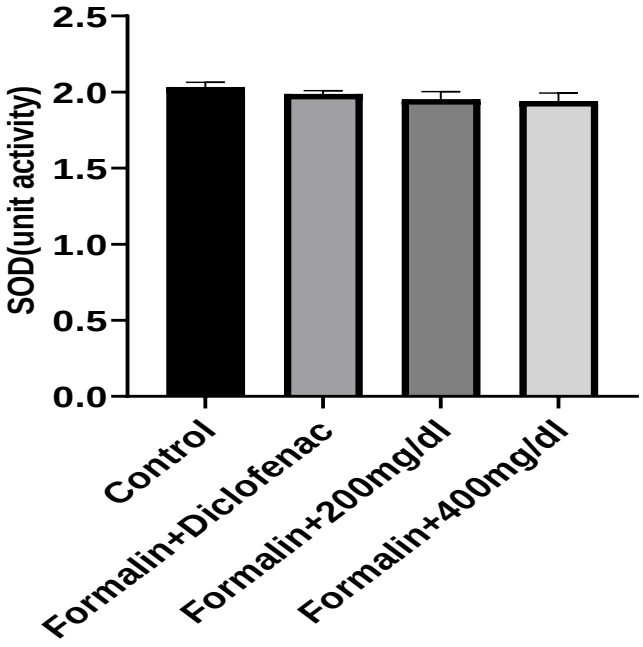

Figure 5. Effect of Aqueous *Alafia Barteri* Leaf Extract on Superoxide dismutase Activity.

There was no significant difference in the SOD activity of liver tissue of rats in all the groups.

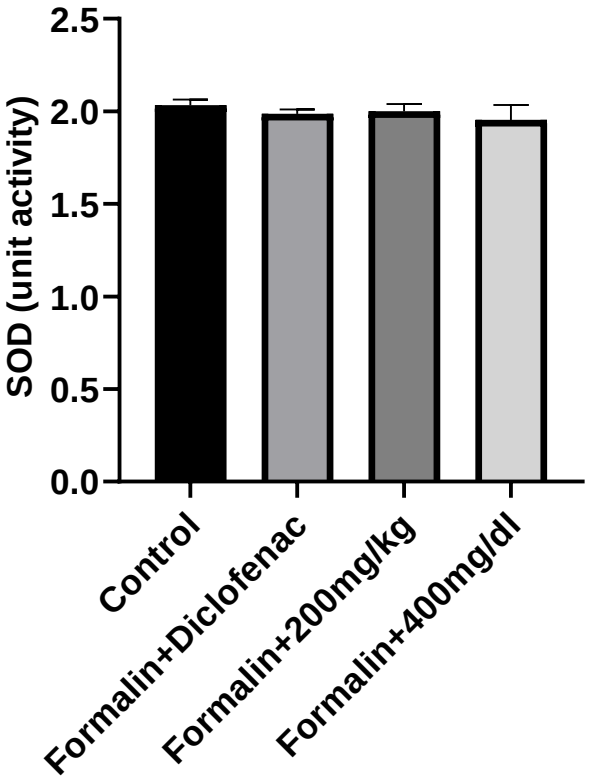

Figure 6. Effect of Ethanolic *Alafia Barteri* Leaf Extract on Superoxide dismutase Activity.

There was no significant difference in the SOD activity of liver tissue of rats in all the groups

CHAPTER FIVE

5.0 DISCUSSION

Inflammation is a response of a living vascularised tissue to an injury. Conventional or synthetic drugs used in the management of inflammatory diseases are inadequate, it sometimes have serious side effects(Agnel,et al,2012). Inflammation comes with many different processes involved in its initiation, regulation and resolution.(Shankar,2017).

Formalin induced paw edema in rats is one of the most suitable test procedure used to screen acute inflammation in experimental animals. Formalin induction causes the changes in connective tissue metabolism,it 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 tissues(Agnel,et al,2012)

Flavonoids are reported for significant antioxidant, vasculoprotector, anti-hepatotoxic, anti allergic, anti inflammatory and anti tumor activity. Its presence in this plant contributes to its ability to reduce paw oedema.

Ethanolic extract of *A.bateri* at the dose of 400 mg/kg and aqueous extract of *A.bateri* at the dose of 200 mg/kg showed a significant reduction in rats paw edema volume when compared with the standard. Although all extract-treated groups showed a decrease in paw thickness as compared to the control, the difference was significant on all observation days only in Group 2(diclofenac sodium), Group 5 (200 mg/kg of ethanolic extract of A.barteri), and Group 3(200 mg kg of aqueous extract of A.barteri).

The enzymatic antioxidant systems such as catalase and superoxide dismutase play a corresponding role in the avoidance of oxidative damage by reactive oxygen species. SOD is one of the chief cellular defence enzymes that dismutate superoxide radicals to water and oxygen. Catalases on the other hand are heme-containing proteins that defend the cells from toxic effects of reactive oxygen species by converting hydrogen peroxide to water and molecular oxygen. The increase in the activities of antioxidant enzymes by the aqueous extract of *A.barteri* leaves is comparable to that reported by Karunna *et al*.

5.1 CONCLUSION

This study revealed that the aqueous and ethanolic extract of *A. barteri* leaves can help fight oxidative stress induced by inflammation in rats by increasing the level of antioxidant enzymes in the liver of control and treated rats.

REFERENCES

Afsar U. Ahmed(2011).An Overview Of Inflammation: Mechanism And Consequences. Front. Biol., 6(4): 274–281.

Agnel.N,Arul John And G. Shobana(2012).Anti-Inflammatory Activity of Talinum Fruticosum

Antonela M. Berar,Dragomir C. David, Liana Lascu, Luminita Matros,Radu S.Campian(2015). Analysis of Hematological and Oxidative Stress Parameters In The Evaluation of Experimentally Induced Periapical Lesions. Human & Veterinary Medicine International Journal of The Bioflux Society. Volume 7 | Issue 3.

B. Palmieri, V. Sblendorio(2016).Oxidative Stress Tests: Overview On Reliability and Use. European Review For Medical And Pharmacological Sciences; 11: 309-342.

Gitahi S. Maina, Juma K. Kelvin, Mwangi B. Maina, Njagi J. Muriithi, Mworia J. Kiambi, Aliyu Umar, Mwonjoria K. John, Njoroge W. Ann, Mburu N. David, Ngugi M. Piero(2015). Antinociceptive Properties of Dichloromethane: Methanolic Leaf and Root Bark Extracts of Carissa Edulis In Rats. The Journal of Phytopharmacology; 4(2): 106-112.

Godhandaraman Sangeetha, Ramalingam Vidhya(2016). In Vitro Anti-Inflammatory Activity of Different Parts Of Pedalium Murex (L.) International Journal of Herbal Medicine; 4(3): 31-36.

Hellen Nyambura Kariuki1, Titus Ikusya Kanui, Abiy Yenesew, Nilesh Patel, Paul Mungai Mbugua(2013). Antinocieptive And Anti-Inflammatory Effects of Toddalia Asiatica Lam. (Rutaceae) Root Extract In Swiss Albino Mice. Pan African Medical Journal.

In Vitro and In Vivo Anti-Inflammatory Activities of Coptosapelta Flavescens Korth Root' S Methanol Extract.Journal of Applied Pharmaceutical Science Vol. 8(09).

Ismail O. Ishola, Esther O. Agbaje, Olufunmilayo O. Adeyemi & Rakesh Shukla(2014). Analgesic and Anti-Inflammatory Effects Of The Methanol Root Extracts of Some Selected Nigerian Medicinal Plantspharmarceutical Biology; 52(9): 1208–1216.

Karuna R, Reddy SS, Baskar R, Saralakumari K(2009).Antioxidant potential of aqueous extract of *Phyllanthus armarus* in rat. Indian J Pharmacol. ;14:64–7.

Khemasili Kosala1, Moch. Aris Widodo, Sanarto Santoso, Setyawati Karyono(2018)

L. On Formalin Induced Paw Edema In Albino Rats. Journal of Applied Pharmaceutical Science 02 (01): 123-127.

Margaret O. Sofidiyaa,, Essien Imeha, Chidebelu Ezeania, Flora R. Aigbeb,Abidemi J. Akindele(2014). Antinociceptive and Anti-Inflammatory Activities of Ethanolic Extract of Alafia Barteri.Nigeria Journal of Pharmacognosy24: 348-354.

Mohiuddin.Md, Syed Masudur Rahman Dewan, Abhijit Das and Md. Shahid Sarwar(2018). Antinociceptive, Anti-Inflammatory And Antipyretic Activities of Ethanolic Extract of Atylosia.

Monier M. Abd El-Ghani(2016).Traditional Medicinal Plants of Nigeria: An Overview Agriculture And Biology Journal of North America .7.5.220.247.

Nishat Fatima, Syeda Jabeen Fatima(2016). Pharmacological Screening For Anti-Arthritic Activity of Moringa Oleifera . Asian J Pharm Clin Res, Vol 9, Issue 3,106-111.

Pushpangadan, T.P. Ijinu And V. George(2015).Plant Based Anti-Inflammatory Secondary Metabolites. Annals of Phytomedicine 4(1): 17-36 .

Review On Some Plants Having Anti-Inflammatory Activity, The Journal Of Phytopharmacology; 3(3): 214-221.

Ruijter, A.De,.Alafia Barteri Oliv. [Internet] Record From Prota4u. Schmelzer, G.H. & Gurib-Fakim, A. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources Végétales De l'Afrique Tropicale), Wageningen, Netherlands.2019.

S. O. Malomo, A. Ore, And M. T. Yakubu(2011).*In Vitro* And *In Vivo* Antioxidant Activities of The Aqueous Extract Of *Celosia Argentea* Leavesindian J Pharmacol 43(3): 278–285.

Scarabaeoides (L.)Benth (Family: Fabaceae) Leaves In Experimental Animal. Journal of Applied Life Sciences International 17(4): 2394-1103.

Sowjanya R., M. Shankar, B. Sireesha, E. Ashok Naik, P. Yudharaj,R. Jasmine Priyadarshini Taibur Rahman, Ismail Hosen, M. M. Towhidul Islam, Hossain Uddin Shekhar(2012). Advances In Bioscience and Biotechnology, 3, 997-1019 .

Vashishtha Vishal, Sharma Ganesh N., Gaur Mukesh, Bairwa Ranjan(2014).

Manisha, Whidul Hasan, Richa Rajak And Deepali Jat(2017). Oxidative Stress And Antioxidants: An Overview. International Journal of Advanced Research And Review. Ijarr, 2(9); 110-119

APPENDIX

Table 4. Effect of aqueous *A.barteri* leaf extract on the body weight.

Groups	Day 0	Day 5	Day 10
Control	195.0±7.99	200.2±7.28	200.5±2.83
Formalin+diclofenac	216.3±3.90	195±2.66	194.3±13.50
Formalin+extract(200mg/kg)	215.4±4.85	215.0±8.40	205.0±10.31
Formalin+extract(400mg/kg)	208.4±9.17	208.8±5.68	204.2±7.75

*represent significant increases at $p<0.05$

Table 5. Effect of ethanolic A.barteri leaf extract on the body weight.

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

*represent significant increases at $p<0.05$

Table 6. Effect of ethanolic *A.barteri* leaf extract on the paw diameter.

Groups	Day 0	Day 5	Day 10
Control	3.44±0.22	2.83±0.20	2.23±0.12
Standard	3.20±0.12	2.84±0.20	2.30±0.05*
Formalin+extract(200mg/kg)	2.92±0.27	2.54±0.14*	2.15±0.06*
Formalin+extract(400mg/kg)	2.59±0.09	2.28±0.11*	2.12±0.09*

*represent significant increases at $p<0.05$ when compared to control value on day 5 to day 10.

Table 7. Effect of aqueous *A.barteri* leaf extract on the paw diameter.

Groups	Day 0	Day 5	Day 10	%inhibition
Control	4.10±0.22	4.10±0.20	4.09±0.12	
Standard	3.20±0.12	2.84±0.20	2.30±0.05*	6%
Formalin+extract(200mg/kg)	2.92+0.05	2.38±0.16*	2.25±0.12	52%
Formalin+extract(400mg/kg)	2.59+0.19	2.36±0.05*	2.08±0.08*	33%

*represent significant increases at $p<0.05$ when compared to control value on day 5 to day 10.

SOD CALCULATION

Increase in absorbance per minute=$\frac{A_3-A_O}{2.5}$

Where,

A_O→Absorbance after 30seconds

A_3→Absorbance after 150seconds

$\%Inhibition=100\left[\frac{\text{Increase in absorbance of substrate}}{\text{Increase in absorbance of blank}}\right]$

1 unit of activity was known as the total of SOD required to source 50% inhibition of the oxidation of adrenaline to adenochrome during 1 minute

Thus,

Unit of activity= $\frac{\%\ Inhibition}{50}$