ANTIINFLAMMATORY EFFECTS AND ACUTE TOXICITY STUDIES OF ETHANOL EXTRACT OF THE STEM BARK OF FADOGIA CIENKOWSKII SCHWEINF. IN RODENTS

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ACKNOWLEDGEMENT

Firstly, my acknowledgement goes to God almighty for his Grace and Favour upon my life. Then to my parents Late Engr. Noble Egharevba and Mrs. Chinwe Egharevba for their unwavering support towards my education.

My gratitude goes to my amiable supervisor, Prof Ighodaro Igbe, for granting me the opportunity to work with him and for making it a very seamless process for I and my colleagues of which his guidance was of great value.

I am indeed grateful for all the support from the staff (Academic and non-academic) of the department of Pharmacology and Toxicology) thank you.

To my colleagues Nkechi, precious and victor thank you for your cooperation.

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LIST OF ABBREVIATIONS

COX- cyclooxygenase

DMARDS- disease modifying antirheumatic drugs

NSAIDs- non steroidal anti-inflammatory drug

INDO-indomethacin

CYPRO-cyproheptadine

PAF- Platelet activating factor

PGs- prostaglandins

ABSTRACT

Background: Fadogia cienkowskii is used as a herb for treating inflammation

Aim: To evaluate the acute toxicity profile of Fadogia cienkowskii and determine the antiinflammatory properties of Fadogia cienkowskii.

Method: the acute toxicity profile was evaluated using lorkes model, the antiinflammatory effect of the extract Fadogia cienkowskii (stem bark) was determined using the carrageenan induced paw edema and dextran induced paw edema models.

Results: Oral administration of the extract produced no mortality and acute toxicity and also produced significant ($p<0.05$) antiedematogeni effect with the dose of 100,200 and 400mg/kg in the carrageenan-induced edema model. In the dextran-induced edema model significantly inhibited dextran-induced edema sustained through the experiment.

Conclusion: The result indicates that ethanol extract of Fadogia cienkowskii stem bark has no potential for sure Toxicity and possess acute antiinflammatory activity mediated by either blocking release of histamine and prostaglandins. Thus supporting the usage of the plant in traditional medicine treatment of inflammation.

CHAPTER ONE.

1.0 INTRODUCTION

Natural products from medicinal plants, Because of their unparalleled chemical variety, medicinal plants, whether as pure chemicals or standardised extracts, give limitless prospects for novel therapeutic leads. Interest in edible plants has developed worldwide as a result of an increasing desire for chemical variety in screening programs, as well as the search for therapeutic medications from natural sources. (Duraipandiyan et al, 2006).

The immune system's reaction to an irritant is inflammation. Numerous immune system cells may be implicated when inflammation arises in your body. They emit a variety of chemicals referred to as inflammatory mediators. Among them are the hormones histamine and bradykinin. More blood can reach the damaged tissue as a result of their dilatation, which widens the tissue's tiny blood capillaries. More immune system cells can reach the damaged tissue thanks to the increased blood flow, aiding in the healing process. Furthermore, both of these hormones aggravate nerves, which results in the brain receiving pain signals. (Ahmad and duke, 2008)

1.12 CAUSES OF AN INFLAMMATION

External injuries and damage through foreign objects or effects of chemicals or radiation.

1.1.3 SIGNS OF AN INFLAMMATION

There are five signs of an acute inflammation:

Redness, heat, swelling, pain and loss of function

Some inflammations occur “silently”and don’t cause any symptoms

1.1.4 TYPES OF INFLAMMATION

According to the length of time the body responds to the injury, inflammation can be divided into three types: acute inflammation, which appears right after an injury and usually lasts a few days; chronic inflammation, which can linger for months or even years if acute inflammation doesn't go away; and subacute inflammation, which is a phase that occurs between acute and chronic inflammation and lasts for two to six weeks.

Acute inflammation;

After a particular damage, soluble mediators such cytokines, acute phase proteins, and chemokines are released, which starts acute inflammation. These compounds, which are an essential part of the innate immune response during acute inflammation, encourage neutrophil and macrophage migration to the site of inflammation.

Sub acute inflammation

If the acute inflammation does not quickly resolve, it progresses to subacute inflammation.

Chronic inflammation

The migration of T-lymphocytes and plasma cells to the site of inflammation indicates that the inflammation has changed from subacute to chronic if it lasts longer than six weeks. Long-term inflammation that doesn't go away causes fibrosis and tissue damage.

1.1.5 PATHOPHYSIOLOGY OF INFLAMMATION

The immune system becoming activated in response to a wide variety of stimuli causes inflammation. With both humoral and cellular components, the immune system is an extremely intricate and evolutionary-optimized defence mechanism. The host's immune status, the virulence of an infectious agent, and the fine-tuning of the local tissue reaction—which may be impacted by personal genetic factors—all affect how an inflammatory response develops. Immunity is a balance between excessive (autoimmunity) or insufficient (immunodeficiency) immune responses. (Nuklearmedizin,et al 2016) On the one hand, "checkpoint control" in peripheral lymphatic tissues and strict T- and B-cell selection in the bone marrow and thymus are responsible for the dynamic equilibrium between these two extremes. One of the alleged "hallmarks of cancer" is the ability of many tumours to evade immune system destruction and decrease local immune responses. This local immunosuppression has been successfully reversed in recent years using a variety of techniques. Early clinical trials employing these techniques have produced very encouraging findings, suggesting that the immune system's therapeutic application will prove to be a highly useful tool in the toolbox of cancer therapy agents.(Sahlmann et al, 2016.)

1.1.6 INFLAMMATORY MEDIATORS.

One essential component of the tissues' reactions to harmful inflammogens is the inflammatory response. Leukocytes, also referred to as inflammatory cells, include neutrophils, macrophages, and lymphocytes in this intricate reaction. Vasoactive amines and peptides, eicosanoids, proinflammatory cytokines, and acute-phase proteins are among the specialised substances released by these cells in response to the inflammatory process. These substances mediate the inflammatory process by halting additional tissue damage and ultimately leading to tissue function restoration and healing.

(Abdulkhaleq et al, 2007)

MEDIATORS

The inflammatory response is actively influenced and modulated by a range of chemical mediators from the circulatory system, inflammatory cells, and wounded tissue. Among the chemical mediators that are released are

(1) Vasoactive amines (histamine and serotonin)

(2) Peptide (bradykinin)

(3) Eicosanoids (thromboxanes, leukotrienes, and prostaglandins)

1. VASOACTIVE AMINES AND PEPTIDE

HISTAMINE

Histamine is an organic nitrogenous substance that regulates gastrointestinal physiological processes, communicates local immunological responses, and serves as a neurotransmitter for the brain, spinal cord, and uterus. Since histamine is produced independently of the traditional endocrine glands, it has been regarded as a local hormone (autocoid); but, in recent years, histamine has been identified as a central neurotransmitter. Histamine has a key role in mediating itching and is implicated in the inflammatory response. Basophils and mast cells in surrounding connective tissues generate histamine as part of the immune system's reaction to foreign invaders. Histamine makes capillaries more permeable to white blood cells and certain proteins, which enables them to interact with pathogens in affected tissue. (Arthurfragaso, 2024)

Serotonin

Four serotonin receptors—5-HTl, 5-HT2, 5-HT3, and 5-HT4—have been shown to mediate the biological functions of serotonin, which is produced by decarboxylating tryptophan and stored in granules. In humans, serotonin is found in platelets, while in mice, it is available in basophilic granules.. (Shamim et al, 2023)

2. PEPTIDE.

BRADYKININ

Bradykinin is a plasma Kinin–Kallikrein system-derived nanopeptide. Bradykinins have two or more different receptors, which have been dubbed B1 and B2 [29]. It can raise prostaglandin production and cause discomfort locally, just like histamine and serotonin do (Mohiuddin et al 2003)

3. EICOSANIODS

ARACHIDONIC ACID

The primary constituent of membrane phospholipids in all cells, arachidonic acid is a crucial substrate for the production of eicosanoids, which are physiologically active mediators of inflammation. The latter comprises the byproducts of cyclooxygenases (prostaglandins and thromboxanes), 12-lipoxygenase (12-hydroxyeicosatetraenoic acid), and 5-lipoxygenase (leukotriene and 5-hydroxyeicosatetraenoic acid) (Davies, 2008)

5-LIPOOXYGENASE ENZYME

In 1976, the 5-lipoxygenase enzyme was identified from rabbit polymorphonuclear leukocytes stimulated with glycogen. The primary immune cells of myeloid origin that produce 5-LOX protein are (1) mononuclear cells like lymphocytes, necrophages, and rhogocytes, and (2) polymorphonuclear leukocytes like neutrophils and eosinophils.

These cells exhibit a critical function in inflammatory and immunological responses. On the other hand, T-cells, endothelial cells, platelets, and erythrocytes are 5-LOX negative. (Sylvie and Davidou, 2009)

CYCLOOXYGENASE ENZYME

The enzyme cyclooxygenase, which comes in at least two isoforms (cyclooxygenase-1 and -2), is involved in the metabolism of arachidonic acid and the creation of proteinoids, including strong proinflammatory prostaglandins. Most mammalian cell types and platelets constitutively produce cyclooxygenase-1. Additionally, the kidney, uterine epithelium, stomach, forebrain, and vascular endothelium also release it. However, cyclooxygenase-1 (not just cyclooxygenase-2) can also be activated at the site of inflammation and has a detrimental role in the animal body. Other models of inflammation generated by carrageenan have further corroborated these findings. First, compared to wild-type mice, mice lacking the cyclooxygenase-1 gene displayed a reduced inflammatory response.

Second, mice deficient in the cyclooxygenase-2 gene displayed an inflammatory response comparable to that of wild-type mice. Thus, these findings have suggested that cyclooxygenase-1 and cyclooxygenase-2 both contribute to the initiation of inflammation. Faseb (1998) Cyclooxygenase-l produces prostanoids, which are crucial for a variety of physiological processes, including the control of platelet aggregation, since thromboxane-2 promotes platelet aggregation and PGh has antiaggregatory qualities. Prostaglandins H and E2 in the alimentary canal prevent the production of stomach acid, have a

continuous vasodilator impact on the gastric mucosa's blood vessels and veins, and cause the production of sticky mucus, which acts as a barrier. Vasodilator prostaglandins, which include prostaglandin-h, prostaglandin E2, and prostaglandin D2, play a major role in the kidney's ability to dilate renal vascular beds, improve organ perfusion, control renal blood flow, and reduce vascular resistance. In every region of the brain, neuronal cells produce cyclooxygenase-1. However, this enzyme is produced in large quantities in the forebrain, where prostaglandins are required for intricate integrative processes. In the early stages of pregnancy, the uterine epithelium also produces cyclooxygenase-1, which may be important for the ovum's development as well as the placenta's creation and angiogenesis needs.

(Dubois et al, 1998)

PROSTAGLANDINS.

By boosting vascular permeability and fortifying the effects of other inflammatory mediators like kinin, serotonin, and histamine, prostaglandins (prostaglandin E2 and prostaglandin b) significantly contribute to the preservation of the inflammatory process. This causes redness, increased blood flow, and plasma exudation in the area of acute inflammation, which ultimately results in oedema. These prostaglandins influence the afferent C fibres, which results in hyperalgesia. Additionally, prostaglandin E2 raises body temperature by acting on neurones in the hypothalamic thermoregulatory network. Synovial fluids from patients with osteoarthritis and rheumatoid arthritis have been found to include elevated amounts of several

prostaglandins, including prostaglandin E2 and prostaglandin B (Negrescu et al, 2024).

NITRIC OXIDE.

One signalling molecule that is essential to the pathophysiology of inflammation is nitric oxide (NO). It has an anti-inflammatory impact when the body is functioning normally. Conversely, NO is regarded as a pro-inflammatory mediator that causes inflammation when it is produced excessively in aberrant circumstances. NOSs help to synthesise and release NO into the endothelium cells by converting arginine to citrulline, which in turn produces NO.(Singh et al, 2024)

CYTOKINES AND CHEMOKINES

The cytokine superfamily of proteins plays a crucial role in the development and control of the immune system and is a vital component of the cell-to-cell signalling network. Interpreting how cytokines and chemotactic cytokines (chemokines) interact with or are mediated by the immune system has advanced significantly in recent years. These interrelated biological signals have amazing powers, including the ability to affect inflammation, T cell subset differentiation, lymphocyte recruitment, haematopoiesis, and growth and development. A thorough list of cytokines and chemokines linked to the immune system is summarised in this chapter. For every cytokine and chemokine, details are provided, including gene cloning and

mapping information, protein properties and expression, receptor usage, source and target cells, key biological activities, and knockout phenotype.

This chapter attempts to demonstrate how this system can preserve broad influence and functional complementation without compromising regulation and specificity of action by grouping cytokines and chemokines into interacting groups with associated physical and/or functional characteristics. A deeper comprehension of these characteristics could result in more sophisticated strategies for reversing aberrant immune responses mediated by cytokines or chemokines, like those that cause autoimmune diseases.(Cohen et al, 1974).

PLATELET ACTIVATING FACTOR

The single PAF receptor (PAF-R) is how platelet-activating factor (PAF), a strong inflammatory mediator, works. The less effective PAF analogue acyl-PAF, which competes for PAF-R, is also produced in large quantities by cells that biosynthesise alkyl-PAF. The plasma form of PAF acetylhydrolase (PAF-AH) breaks down both PAF species. We investigated whether cogenerated acyl-PAF functions as an inhibitor to reduce PAF-R signalling or as a sacrificial substrate to increase inflammatory activation, hence protecting alkyl-PAF from systemic breakdown. Both PAF forms are prothrombotic when isolated in ex vivo tests; however, acyl-PAF inhibited the activation of human platelets expressing canonical PAF-R by alkyl-PAF. Alkyl-PAF causes abrupt mortality in Swiss albino mice, although acyl-PAF boluses can also be given concurrently to counteract this effect. The protective effect of

acyl-PAF against alkyl-PAF-induced death was serially reduced as PAF-AH levels were gradually raised. We infer that abundant acyl-PAF inhibits the effect of alkyl-PAF in a pathophysiological situation, despite the fact that acyl-PAF alone is modestly proinflammatory. These investigations offer proof of acyl-PAF's hitherto unknown function as an inflammatory set-point modulator that controls PAF-R signalling and hydrolysis. (Invent, 1996).

KININS

Kinins are cleaved from kininogens by kallikreins, which are a component of the kinin-kallikrein system. The venom of wasps, bees, and ants also contains kinins, which paralyse their victims by acting as neurotoxins. Kinins are the main mediators of inflammation, which results in leukocyte recruitment, oedema, and discomfort. (Bhoola, 2006)

1.2 ANTI-INFLAMMATORY AGENTS.

Anti-inflammatory medicines are medications or compounds that lessen the body's oedema, pain, and inflammation. They function by preventing the production of molecules that lead to inflammation, such as prostaglandins, which resemble hormones.

They are classified as follows;

1. NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs)

These are drugs that lessen or eliminate pain. A large class of non-opioid analgesic medications is known as NSAIDs. Despite having distinct chemical compositions, they have a number of benefits, including lowering fever and high temperatures, reducing inflammation, and soothing pain. NSAIDs function by reducing the production of prostaglandins, which are crucial for the body's inflammatory response. Therefore, when an injury occurs, the body creates more of these chemicals. Inflammation is decreased by lowering the quantity of prostaglandins at the location of injured tissue.

Mechanism of action.

Nsaids inhibit COX, which promotes the production of prostaglandins, and disrupt the activity of blood platelets, which are essential for blood coagulation. These drugs have anti-clotting qualities as a result. This feature of aspirin may help avoid clogged arteries, which can result in a heart attack or stroke.(Markus and MacGill, 2024)

2. STEROIDAL ANTIINFLAMMATORY DRUGS.

Prescription pharmaceuticals called steroidal anti-inflammatory drugs, sometimes referred to as corticosteroids or glucocorticoids, lower immune system activity and inflammation. Russell (2009) Corticosteroids, sometimes referred to as steroids or glucocorticoids, are prescription drugs that lower inflammation in the body. These are synthetic (man-made) medications that resemble the hormone cortisol, which is produced by your body naturally. Cortisol is typically produced and released by your adrenal glands. (Liu et al, 2006)

3. DISEASE MODIFYING ANTI-RHEUMATIC DRUGS (DMARDS).

DMARDs can also be used to treat autoimmune illnesses, in which the body is attacked by the immune system rather than being protected. By lowering the body's immunological response, DMARDs can lessen joint injury and inflammation. It may take a few weeks for them to begin working, and it may take up to six months for them to become completely effective. It's critical to notify your doctor right away if you see any symptoms of infection because DMARDs can raise your risk of infection. Additionally, they may make obtaining live vaccinations risky. DMARDs come in two varieties: biologic and conventional. Methotrexate, leflunomide, hydroxychloroquine, and sulfasalazine are a few examples of traditional DMARDs. When traditional DMARDs don't work, biologic DMARDs are typically administered. These consist of tocilizumab, tofacitinib, rituximab, abatacept, etanercept, infliximab, and adalimumab. (Sarah et al, 2023)

4. HERBAL MEDICINE.

To assist regulate inflammation in the body, a variety of anti-inflammatory medications are available. Certain herbal medicines contain natural chemicals that may also have anti-inflammatory properties. But study in this field is far less extensive. (circuma longa) Turmeric The turmeric plant's roots are used to make this yellow powder. (Kerry and others, 2024) Turmeric's curcumin is what gives it its yellow hue. Because it inhibits inflammatory cytokines and enzymes, curcumin has anti-inflammatory qualities. This could aid in the treatment of ailments like ulcerative colitis and arthritis. (Kerry, 2024)

The tropical plant ginger (Zingiber officinale) has long been used in traditional medicine. There may be anti-inflammatory qualities to ginger. Many of its constituents have the ability to restrict cyclooxygenase enzyme activity and cytokine synthesis, both of which are factors that contribute to inflammation. People with illnesses like Crohn's disease, ulcerative colitis, psoriasis, rheumatoid arthritis, and systemic lupus erythematosus may find that eating foods containing ginger improves their quality of life. (Aron, 2024) Green tea: Camellia sinensis leaves are the source of green tea. Polyphenols, which are abundant in green, black, and white teas, have potent anti-inflammatory properties. More precisely, the polyphenol epigallocatechin 3-gallate, which is found in green tea, may be very helpful in the treatment of inflammation. Green tea may lower biomarkers for inflammation. This could be useful for treating atherosclerotic cardiovascular disease and other inflammatory conditions. (Kerry, 2024)

1.3 Fadogia cienkowskii.

1.3.1. BOTANICAL CLASSIFICATIONS

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Gentianales

Family: Rubiaceae

Genus: Fadogia

Species: Fadogia cienkowskii

Fadogia cienkowskii is a dicot plant.

English name: No common English name.

Local names (in Nigeria):

- Bakin-haggai (Hausa)

-Ufu-ewureje (Igede tribe of Benue State)

-Ogwu-agu (Igbo)

1.3.2 GEOGRAPHICAL DISTRIBUTION.

Native to tropical Africa, Fadogia cienkowskii is a shrub or subshrub that grows from Guinea to the Transvaal province. It is most frequently found in seasonally dry tropical biomes, and its countries include Cameroon, Cabinda, Zaire (Dem. Rep. Congo), Sudan, Ethiopia, Uganda, Kenya, Tanzania, and Angola. The greatest number of Fadogia species are found in Angola, the Democratic Republic of the Congo, Zambia, Tanzania, and the Central African Republic.

1.3.3 BOTANICAL DESCRIPTION.

The following botanical traits describe the shrub or subshrub Fadogia cienkowskii:

Size: A suffrutex with a potential height of 0.3 to 1.2 meters Stems: A woody rhizome with branches that produces one or more simple stems. Green in colour and oblong-elliptic in shape, leaves have a bitter flavour. They can grow up to 8 cm in length and 2.5 cm in width, and they are grouped in whorls of three at each node. The leaves' undersides are covered in papillae and coarse hairs.

Bloom's: There are usually two to six blooms on the inflorescence, although occasionally there are just one or two. The corolla has a cream or yellow hue.

Fruit: The fruit is popular with children and people traveling through the bush.

Habitat: The plant grows in the savanna and is found in the drier parts of tropical Africa, from Mali to North and South Nigeria.

All portions of Fadogia cienkowskii include alkaloids, and the leafy twigs contain tannins, flavones, and saponins. In complementary medicine, the plant has been used to cure a variety of illnesses, including fever, diarrhoea, inflammation, and general bodily weakness. (Mark and Hyde, 2002)

LITERATURE REVIEW ON Fadogia cienkowskii

1. The wild plant Fadogia cienkowskii is a member of the Rubiaceae family. Investigating the chemical makeup, hepatoprotective properties, and antioxidant properties of the extract and fractions of Fadogia cienkowskii leaves is the aim of this work. Standard procedures were followed for phytochemical analysis; hepatoprotection was assessed in a rat model of liver injury caused by paracetamol, and the antioxidant potential was assessed using both in vitro and in vivo antioxidant models. (Bruce and others, 2021). Therefore, at higher dosages, the ethanol extract and ethyl acetate fraction of F. cienkowskii demonstrated notable hepatoprotection against paracetamol-induced toxicity, surpassing even Silymarin (100 mg/kg). This supported the traditional usage of it to treat liver problems by herbalists in South Eastern Nigeria. (Bruce et al, 2021)

2. Numerous multicellular trichomes, calcium oxalate crystals, stomata, palisade cells, and xylem and phloem arteries were seen in the anatomical sections. The results of the phytomedicinal evaluation showed total ash of 3.85%, moisture content of 3.17%, water soluble extractive of 3.4%, and alcohol soluble extractive of 4.4%, while quantitative microscopical investigation revealed a vein islet number of 5.46mm and stomata index of 18.92. Saponins, alkaloids, flavonoids, carbohydrates, glycosides, terpenes, tannins, and resins were found in the methanol extract of the plant's powdered leaves after phytochemical screening. Fadogia cienkowskii has been found to have traits that have made its application in ethnomedicine inevitable in light of this finding. (Chukwube et al, 2017)

3. Effect of Fadogia cienkowskii ethanol root extract on semen parameters

Rats treated with 200 mg/kg (the highest tested dose) of the extract had a significantly higher sperm count than rats treated with distilled water and other extract doses ($p < 0.000$). Rats treated with extract and control did not vary substantially in the percentage of active, inactive, and living sperm cells ($P = 0.227$, 0.227, and 0.054). When compared to the control group, the extract treatment had no effect on the semen's pH. (Jurbe et al, 2023)

4. Effect of extract on serum biochemical parameters

When comparing rats treated with 100 and 200 mg/kg of the extract to control rats treated with distilled water, there was a notable drop in the activity of alanine aminotransferase and alkaline phosphatase for biochemical measures of liver function. Likewise, aspartate aminotransferase activity was markedly reduced in rats given 200 mg/kg of the extract. Rats administered with 200 mg/kg of the extract had a considerably greater serum total protein concentration than control rats given with distilled water, but there was no significant difference ($p<0.05$) in the serum albumin concentration of rats in any of the groups. (Tobias et al, 2023)

1.4 AIM AND OBJECTIVE.

1. To evaluate the acute toxicity profile of ethanol extract of Fadogia cienkowskii stem bark.

2. To determine the antiinflammatory properties of ethanol extract of Fadogia cienkowskii stem bark.

1.5 JUSTIFICATION OF THE STUDY.

This research is justified by the need for other therapeutic drugs and remedies to treat those conditions with less side effect and better safety and effectiveness, as compared to the current drugs being used for these conditions. This experiment and evaluation of data gathered will prove the therapeutic effect of plant extract Fadogia cienkowskii in the treatment of inflammation.

CHAPTER TWO

2.0 MATERIALS AND METHOD

Equipments used were; Analytical weighing scale (Ohaus Corporation, USA), Beakers (various sizes), Measuring cylinders, Syringes, Orogastric tube etc

2.1 DRUGS AND CHEMICALS

Carrageenan (Sigma-Aldrich, Germany)

Indomethacin was obtained from (Krishat Pharma Industries Limited.)

Dextran (Sigma-Aldrich, Germany)

Cyproheptadine was obtained from(Orange drugs limited)

2.2 Other materials

Cotton wool

Acacia gum

Distilled water

Mortar and pestle

Orogastric tube

Syringe and needle

2.3 PLANT COLLECTION AND EXTRACTION.

The stem bark of Fadogia cienkowskii was collected, identified, and authenticated by Mr. Joseph Azila at the Federal College of Forestry, Jos, Plateau State, Nigeria. A voucher specimen was deposited in the Federal College of Forestry herbarium with voucher number FHJ 258 for future reference.

After removing the debris, the stem bark was dried and thereafter ground to powder using an electric mill. The powdered material was weighed (612g) and stored in an airtight container for further analysis.

The stem bark extract of Fadogia cienkowskii was obtained using the soxhlet extraction method.

The 612g of powdered stem bark was placed in a thimble made of thick filter paper and loaded into the main chamber of the Soxhlet extractor. The extractor was set up on a round-bottom flask that contained 95% ethanol as the extractor solvent and was then fitted with a condenser.

The solvent was heated to 90 degrees Celsius to a reflux, and as the solvent liquor moved up the distillation arm into the condenser, the condensed vapour fell back into the chamber containing the solid material

A rotary evaporator was used to remove the solvent following extraction. After that, the extract was dried in an oven and concentrated. The non-soluble part of the extracted solid in the thimble was thrown away, and the extracted chemical was gathered. After that, the extract was kept dry and cool in an airtight container.

2.4 LABORATORY ANIMALS

Male wistar rats weighing between 180 and 250 grammes and mice were used in all tests. The University of Benin's Animal House Department of Pharmacology and Toxicology provided all of the animals. The animals were acquired in typical habitat settings. They all had free access to rodent feed and water. Every animal was exposed to natural illumination and treated in accordance with accepted experimental procedures that were authorised by the University of Benin's animal ethics committee and pharmacy faculty in Edo state, Nigeria.

2.5.1 ACUTE TOXICITY

The experiment was carried out using (lorkes model,1983) the male wistar mice of (20g-30g) were divided into;

a. Phase 1

3 groups of 3 mice each. The first group received 10mg/kg, the second group received 100mg/kg and the third group received 1000mg/kg of the plant extract.

b. Phase 2

3 groups of 1 mouse each. The first group received 1600mg/kg, the second group received 2900mg/kg and the third group received 5000mg/kg of the plant extract.

After administration they were observed for sedation, convulsions, diarrhea and mortality for 24hours and subsequently two weeks. (Lorkes, 1983)

TEST FOR ANTI-INFLAMMATORY ACTIVITY.

2.5.1 CARRAGEENAN INDUCED PAW OEDEMA.

Five sets of five male wistar rats weighing between 180 and 250 grammes apiece were created. The plant extract was administered orally (p.o.) to the test group at doses of 100, 200, and 400 mg/kg. (Igbe, 2024) Indomethacin 10 mg/kg p.o. was given to the reference group, and 5 ml/kg of distilled water was given to the control group. A Vernier calliper was used to measure the right hind paw's basal paw thickness. One hour later, 0.1 ml of a 0.1%w/v carrageenan suspension was injected into the right hind paw's subplantar tissue. For five hours, the paw thickness was measured hourly with a Vernier calliper. (Winter et al, 1962).

2.5.2 DEXTRAN INDUCED PAW OEDEMA

Five sets of five male wistar rats weighing between 180 and 250 grammes apiece were created. The plant extracts were administered orally (p.o.) to the test group at doses of 100, 200, and 400 mg/kg. (Igbe, 2024) The control group was given 5 mg/kg of distilled water, and the reference group was given 10 mg/kg of cyproheptadine. A Vernier calliper was used to measure the right hind paw's basal paw thickness. After an hour, the right hind paw's subplantar tissue received an injection of 1.5%w/v dextran suspension. A Vernier calliper was then used to measure the paw thickness every hour for five hours.(Glauce et al, 1998).

2.6 STATISTICAL ANALYSIS.

The mean +_ SEM was used to express the experiment's data. Two-way analysis of variance (ANOVA) and the turkey post hoc test (difference between two means) were used to analyse the data using Graph Pad Prism 6. Data were deemed distinct at $p<0.05$ significance levels.

CHAPTER 3

RESULTS

3.1 The percentage yield of ethanol extract of Fadogia cienkowskii stem bark was 3.143%.

3.2 Acute Toxicity Studies.

The extract did not show any mortality and this shows that it is non toxic.

Table 1: Acute toxicity studies of Fadogia cienkowskii ethanol stem bark extract in mice at different doses.

PHASE 1				
Treatment	**Dose**	**Number of death**	**Mortality**	**Total number of mice**
1	10mg/kg	0 of 3	0	3
2	100mg/kg	0 of 3	0	3
3	1000mg/kg	0 of 3	0	3
PHASE 2				
Treatment	**Dose**	**Number of death**	**Mortality**	**Total number of mice**
1	1600mg/kg	0 of 3	0	3
2	2900mg/kg	0 of 3	0	3
3	5000mg/kg	0 of 3	0	3

3.3 Effect of Fadogia cienkowskii extract on dextran induced paw edema.

In the dextran-induced paw edema (fig 3.1) the stem bark extract of Fadogia cienkowskii shows a dose dependent decrease in antiinflammatory activity. At a dose of 200 and 400mg/kg, the extract significantly ($p<0.01$) inhibits dextran-induced paw edema for two hours (1-3h) compared with the distilled water group. The extract inhibitory effect was greater at a dose of 100mg/kg.(1-3h)

Fig 1.

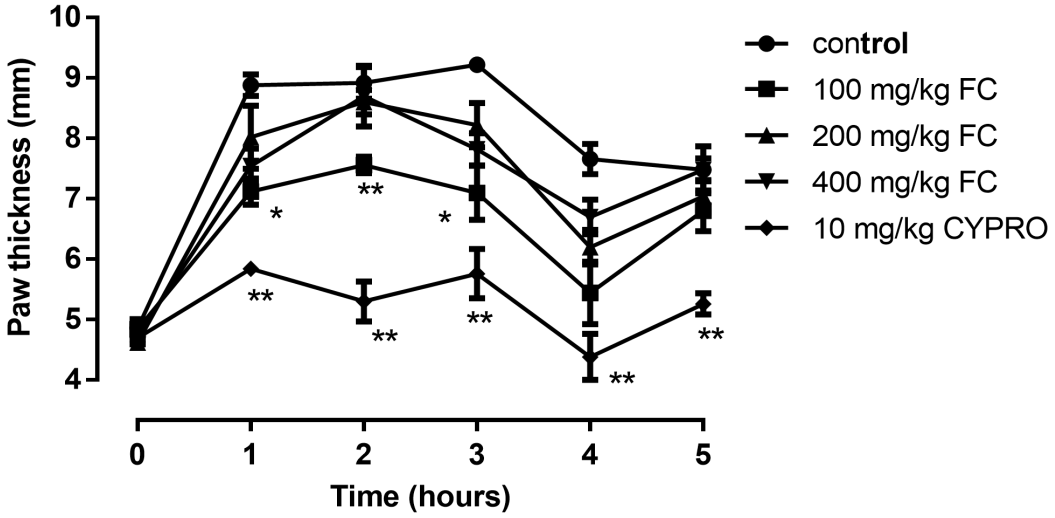
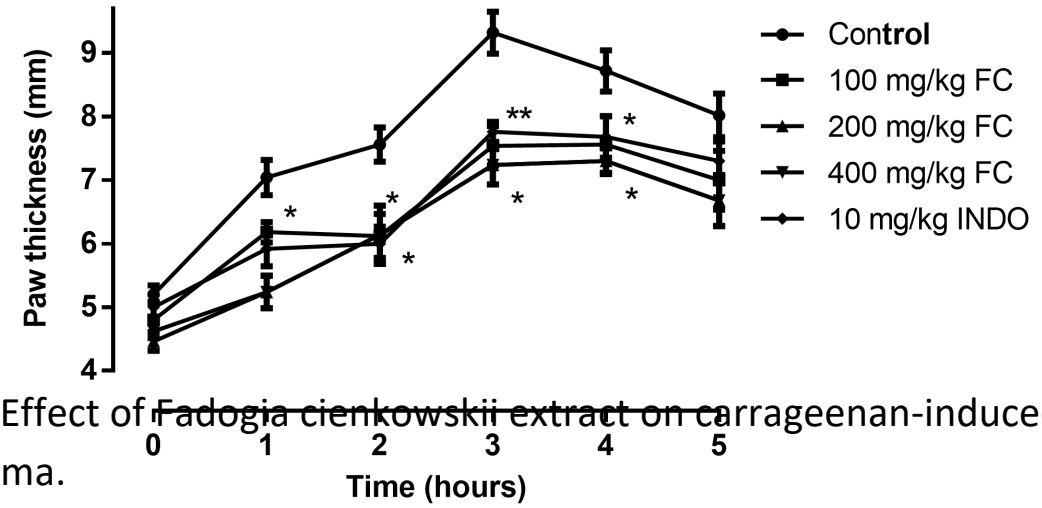


Fig 1. The effect of extract on paw thickness in dextran-induced edema **p<0.01, **p<0.05 as compared to the control (n=5 for each group)



3.4 Effect of Fadogia cienkowskii extract on carrageenan-induced paw edema.

In the carrageenan-induced paw edema (fig 1) the ethanol stem bark extract of Fadogia cienkowskii shows a dose dependent decrease in antiinflammatory activity. At a dose of 100, 200 and 400mg/kg, the extract significantly ($p<0.05$) inhibits carrageenan induced paw edema for three hours (2-5h) compared with the distilled water group (control).

Fig. 2

Fig 2. The effect of extract on paw thickness in carragenan-induced *p<0.05, **p<0.01 as compared to the control (n=5 for each group)

CHAPTER 4

DISCUSSION

The side effects and undesirable effects of the anti-inflammatory medications now on the market for the treatment of various inflammatory disorders have significantly boosted the hunt for a novel anti-inflammatory agent from medicinal plant resources. This study examines the stem bark extract of Fadogia cienkowskii's acute toxicity profile and anti-inflammatory properties. This study was conducted as a result of reporting on the subject. When no other toxicological information is available, acute toxicity assays offer initial insight into a material's harmful nature. Such data can be utilised for:(i) handle situations where a significant amount of the material is accidentally consumed; (ii) identify potential target organs that require closer examination and/or additional testing in repeated-dose toxicity studies; and (iii) choose dosages for short-term and sub-chronic toxicity. According to acute toxicity assessments, the Fadogia cienkowskii stem bark extract used in the study was non-toxic at all dosages (10, 100, and 1000 mg/kg for the first phase) and (1600, 2900, and 5000 mg/kg for the second phase). Numerous inflammatory mediators contribute to the formation of carrageenan-induced paw oedema, a well-defined model of acute inflammation that has been widely utilised to assess the anti-edematous properties of natural items.

It has been suggested that free radicals are crucial to the acute inflammatory response brought on by carrageenan. In 1991, Dawson et al. (Wills et al., 1969) Carrageenan is an irritant that promotes the release of pro-inflammatory and inflammatory mediators. Biphasic oedema was caused by injecting carrageenan into the rat paw's subplantar surface. The release of histamine, serotonin, bradykinin, and to a lesser extent prostaglandins made by cyclooxygenase enzymes (COX) is responsible for the early phase that is seen at around one hour,

while neutrophil infiltration and the continuation of prostaglandin production are responsible for the delayed phase that occurs after one hour. (Lu et al, 2009) The delayed phase of carrageenan-induced acute inflammation also involves the release of pro-inflammatory cytokines such interleukin-1 β (IL-1 β), tumour necrosis factor (TNF-α), and neutrophil-derived free radicals, nitric oxide (NO) (Dawson et al, 1991). Given that the carrageenan model essentially mimics the action of prostaglandins, the fact that the extract reduced the oedema in the second phase raises the possibility that it also decreased the synthesis of cyclooxygenase. (Rosa et al, 1971)

According to reports, histamine and serotonin produced by mast cells are the primary mediators of dextran-induced paw oedema (Lo et al, 1982). Due to the vasodilation caused by these mediators, blood plasma is distributed into the tissue space on a net basis. The tissue swells (oedema) as a result of the increased fluid collecting. Compared to carrageenan-induced oedema, the extract significantly reduced detran-induced oedema, indicating that it may similarly disrupt histamine release.

CHAPTER 5

CONCLUSION

The stem bark extract of Fadogia cienkowskii possess a dose dependent anti inflammatory properties in acute inflammation (carragenan and dextran-induced edema). The anti inflammatory activity may be by blocking the release of inflammation mediators (histamine, serotonin, bradykinin...) and also by inhibiting the synthesis (via inhibition of cyclo oxygenase pathway).

Fadogia cienkowskii has no potential for acute toxicity.

These results therefore surports the claim that Fadogia cienkowskii has antiinflammatory activity may be used in ethnoamedicine for treatment of acute inflammation.

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APPENDIX

ORGANOLEPTIC EVALUATION OF DRIED POWDERED STEM BARK OF Fadogia cienkowskii.

Colour; brown

Taste; characteristic

Texture; fibrous

Odour; characteristic.

PERCENTAGE YIELD OF Fadogia cienkowskii STEM BARK

Weight of Powdered stem bark = 612g

Weight of extract = 21g

Percentage yield =21/612 × 100/1

= 3.43%

PHYSICAL CHARACTERISTICS OF EXTRACT

Colour : Greenish brown

Odour : Characteristic

Taste : characteristic

Texture. : smooth