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PHYTOCHEMICAL ANALYSIS AND THE ANTI-INFLAMMATORY ACTIVITIES OF DICHLOROMETHANE FRACTION OF METHHANOL EXTRACT OF CRATEVA ADANSONII

BY

CHILOKWU CHINELO ADAORA
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FACULTY OF NATURAL SCIENCES
CARITAS UNIVERISTY, AMORJI-NIKE
EMENE, ENUGU.

SUPERVISOR: MR. O.M. EZENWALI

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CERTIFICATION

I certify that this research work was carried out by **CHILOKWU CHINELO, ADAORA** with Reg. No. Bc/2009/273, of the Department of biochemistry, Faculty of Natural Science; Caritas University Amorji-Nike Emene Enugu State.

…….. ……..
Mr. M.O. Ezenwali Date
(Project Supervisor)

…… ……
Mr. M.O. Ezenwali Date
(Head of Department)

…… ……..
External Examiner Date

DEDICATION

This project work is dedicated to my creator, God Almighty, to my lovely parents, to my Grand father Mr. Joseph Okonkwo, whom i am happy that God has kept alive all this years and to my supervisor M.O. Ezenwali.

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My gratitude goes to God Almighty for his blessing in my life. I am grateful for his endless love, guidance and protection and his grace upon me and my family.

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I pray that Almighty God will reward you all Amen.

ABSTRACT

Inflammation is a complex biological response of vascular tissue to harmful stimuli such as pathogen, damage cells or irritants (Ryan & Majno, 1983). The urgency generated by increased rate of stroke, atherosclerosis attribute due to prolonged use of cyclooxygenase-1 and Cyclooxygenase- 2 inhibitors have accelerated anti-inflammatory drug research over the last decade while synthetic pharmaceutical agents continued to dominate research (Ryan & Majno, 1983). Attention increasingly has been directed to natural products. These are often more affordable and available and sometimes are perceived as more effective than conventional anti- inflammatory drugs.

Anti-inflammation was carried out using 12 rats which was divided into three groups of 4 rats each. Group 1 and 2 served as the negative and positive control respectively. Group 3 received 25mg/kg b.w. of the dichloromethane fraction of methanol extract of

Crateva adansonii showed significance anti-inflammatory activity when compared with the standard difference used.

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CHAPTER ONE
INTRODUCTION AIM AND OBJECTIVES

Inflammation is one of body's natural ways of protecting itself. It is also an essential reaction of the body to infection. Too little of an inflammatory response, and the body is unable to repel microbial invasions or heal injuries. Too much of an inflammatory response, and the immune system begins attacking the body's own organs eventually leading to chronic disease (Sears, 2000).

Anti-Inflammatory refers to the property of a substance or treatment that reduces inflammation. There are obvious clinical markers of inflammation which include pain, redness, fever, swelling and loss of function (Pai et al. 2004). However if inflammation is allowed to continue unchecked it may results in neurodegenerative disease or cancer (Pai et al. 2004). A variety of safe and effective anti-inflammatory agents are available including non-steroidal anti-inflammatory drugs, corticosteroids.

Non-steroidal anti-inflammatory drugs (NSAID are usually indicated for the treatment of acute and chronic inflammation. NSAIDS work by reducing the production of prostaglandins (Bayness & Marek, 2005). Prostaglandins are chemicals that promote inflammation, pain and fever. The enzymes that produce prostaglandins are phospholipase$A_{2,}$ PGH_2synthase and Cyclooxygenase (Cox). There are two types of COX enzymes, cyclooxygenase-1 (Cox-1) and cyclooxygenase-2 (Cox-2). NSAIDS reduce the production of prostaglandin by inhibiting COX enzymes resultant effect is that, therefore, inflammation, pain and fever are reduced. Some herbs and plants possess anti-inflammatory and they include:

S/No	**Common Name**	**Botanical Name**	
1	Andiroba oil	*Carapa guianensis*	It is rich in omega-3 fatty acids that promote skin healing from cuts and may slow growth of skin cells in psoriasis and age spots. It relieves pain and swelling

2	Alfalfa	*Medicago sativa L.*	It serves as an oxidant in the blood stream. They are staple of salads and contain nutrient, the leaves hold the best healing potential and contain phytoestrogens that could be beneficial in menopausal and breast feeding women.
3	Aloe Vera Gel	*Aloe vera*	The leaf juices of the aloe plant have important medicinal uses. It stimulates collagen synthesis and skin regeneration after a burn; it helps heal acne, improve the appearance of wrinkles and hydrate damaged skin.
4	Apple	*Malus domestica*	Other names include apple cider vinegar, wine vinegar. The best of the medicinal compounds are contained in the apple peel. It helps in relieving arthritis, apple contains malic and tartaric acids, salts of potassium, sodium, magnesium and iron.

Crateva adansonii belonging to the family *Capparacea* and phylum Magnoliophyta is small tree of forest and

savanna woodland, often on river-banks, widely distributed in Nigeria and across Africa. The leaves are applied externally to relieve pain in joint, the fresh juice of leaves is used for the relief of ear-ache, eye infection and anodyne in toothache. Powder of bark is used in rheumatism, itch, epilepsy and asthma (Sivarajan & Balachandran, 1994).

1.2 *Crateva adansonii* as a plant.

The flowering tree *Crateva adansonii* is called the sacred garlic pear and temple plant. The tree is sometimes called the spider tree because the showy flowers bear long, spidery stamens. It is native to Japan, Australia, much of south East Asia and several south pacific Islands. It grows in forest and savanna woodland, often on river-banks from Senegal to N Nigeria, and across Africa to Zaire, Tanganyika and Madagascar. The plants common name is amakarode in Igbo, In Yoruba, it is egun-orun and in Hausa, it is ungududu. The tree

attains approximately 15m height. The trunk is irregular, seldom straight, but is worthy of cultivation as an ornamental for its dense masses of white flowers borne at the ends of all the shoots. In the bush, owing to grass burning which it survives and repeated stripping of its leaves, the tree is often stunted. The wood is soft and yellow and strong-smelling when cut. The leaves are however eaten in soups or mixed with cereals. They are boiled and added to mixture called in Hausa "Kwado" containing a paste of locust beans, Parkia Spp. (Leguminosae Mimisoideae). The Yoruba consumes the leaves as a potherb.

In upper volt they are the ingredient of sauces. To some people the leaves are taken only in time of dearth and they are sold in northern markets. The bark is widely used for stomach-troubles in Nigeria. In Jebel Marra a bark-paste is used as a poultice on swellings.

Crateva adansonii is a decidious plant with three-palmate leaves. The leaves are arranged opposite one

another. They are elliptic with entire margins. The flowers are pale green. The plants bloom from March to May. The flowers are arranged in racemes. The fruits are berries. It can withstand temperatures above 1^{o}c and 2^{o}c (Burkill, 1985).

Taxonomy

Family:	Capparaceae
Genus:	Crateva
Species:	Crateva adansonii DC
Phylum/Division:	Magnoliophyta
Sub division:	Magnoliophytina
Classes:	Rosopsida
Sub class:	Dilleniidae
Super order:	Violanae
Order:	Capparales

The medicinal effect of *Crateva adansonii* plant bark is includes general healing, leprosy, stomach troubles. For the leaf, it is used in eye treatments, liver, for leaf; a pain-killer, for roots, dropsy, swellings, edema, gout,

veneral diseases. Powder of bark is used in rheumatism, itch, epilepsy and asthma.

1.3 Research Aim and Objectives

Aim:

To study the anti-inflammatory effect of dichloromethane fraction extracts from *Crateva adansonii* on rats.

Objectives:

i) To determine the anti-inflammatory effect of *Crateva adansonii* extracts in methanol on rats.

ii) To determine the phytochemicals present in *Crateva adansonii* extract in methanol on rats.

CHAPTER TWO

LITERATURE REVIEW

2.0 **Definition of Inflammation**

Inflammation is one of the body's natural ways of protecting itself. It includes many chemical reactions that help to fight off infections, to increase blood flow to places that need healing and to generate pain as a signal that something is wrong with the body. The consequences of this can range from discomfort, extreme pain, damage to tissues which can be very serious, and scaring which can be very disfiguring (Talukder, 2006).

2.1 Characteristics of inflammation.

It is characterized by five (5) cardinal signs in acute inflammation:

a. Pain

b. Swelling

c. Redness

d. Heat

e. Loss of function (Ruth, 2009)

Redness and heat are due to increased blood flow to the inflamed site. Swelling is caused by accumulation of fluid; pain is due to release of chemicals that stimulate nerve endings. Loss of function has multiple causes (Parakrama & Taylor, 2005). These five signs appear when acute inflammation of internal organs may not result in the full set. Pain only happens where the appropriate sensory nerve endings exist in the inflamed area e.g. acute inflammation of the lung (pneumonia) does not cause pain unless the inflammation involves the parietal pleura, which does have pain-sensitive nerve endings. (Parakrama Chandrasoma, Clive R. Taylor 2005).

2.2 Causes of Inflammation

Inflammation can be caused by a number of factors that can damage cells but it is broadly divided into:-

1. **Physical agents:** it can be car accident injury or assault or environmental agent like

severe cold and heat (burns), ultraviolet ionizing radiations.

2. **Chemical agents: -** for example, drugs, venom, iodine carbon monoxide.
    - **i.** ORGANIC: Microbial toxin and organic poison such as weed killers, insecticides etc.
    - **ii.** INORGANIC: Acid, alkalis, iodine.
3. **Ischemia:**- Lack of or restricted blood supply which may eventually lead to death of tissue (necrosis).
4. **Immune: -** autoimmune disorder and allergies.
5. **Microbes:-** Bacteria, protozoa, virus, fungi
6. **Antigens:** It stimulates immunological functions

2.3 Types of Inflammation

There are two basic types of inflammation

i) Acute inflammation

ii) Chronic Inflammation

i. Acute Inflammation is of short duration, which could be anything from a few minutes to a few days which makes it an immediate response such inflammation is caused by foreign substance or by physical damage. The major cells involved in acute inflammation are neutrophils (primarily), basophils (inflammatory response) and eosinophils (response to helmith worms and parasites), mononuclear cells (monocytes, macrophages), the primary mediators are vasoactive, amines and eicosanoids. The outcomes of acute inflammation include; resolution, abscess formation, chronic inflammation (Talukder, 2006).

ii. Chronic Inflammation, on the other hand is long lasting. It may persist for weeks, months or even years which makes it a delayed response. Chronic inflammation may follow on from acute inflammation or exist by itself. An acute inflammation will become chronic if the immune system is unable to rid the body of the offending foreign agent or if the agent is constantly able to re enter the body. In the case of persistent infections, such as tuberculosis and autoimmune disease chronic inflammation will arise without the person first going through the acute inflammation stages.

The major cells involved in chronic inflammation include mononuclear cells, (monocytes, macrophages, lymphocytes, plasma cells), fibroblasts. The primary mediators are IFN-Y and other cytokines, growth factors, reactive oxygen species, hydrolytic enzymes. The outcomes include Tissue destruction, fibrosis, and necrosis (Talukder, 2006).

2.4 Chemical Mediators of Inflammation

Chemical Mediator of Inflammation can be defined as any messenger that acts on blood vessels, inflammatory cells or other cells to contribute to an inflammatory response. Inflammatory mediators are soluble, diffusible molecules that act locally at the site of tissue damage and infection, and at more distant sites.

Once leukocytes have arrived at a site of infection or inflammation they release mediators which control the later accumulation and activation of other cells. However, in inflammatory reactions initiated by the immune system, the ultimate control is exerted by the

antigen itself, in the same way as it controls the immune response itself.

For this reason the cellular accumulation at the site of chronic infection, or in autoimmune reactions (where the antigen cannot ultimately be eradicated), is quite different from that at sites where the antigenic stimulus is rapidly cleared (Talukder, 2006).

Early phase mediators are produced by mast cells and platelets. They are especially important in acute inflammation and include mainly histamine, serotonin and other vasoactive substances. Platelets may contribute to inflammatory responses resulting as a consequence of tissue injury through a variety of mechanisms including:

1. The release of vasoactive amines and other permeability factors,
2. The release of lysosomal enzymes.
3. The release of coagulation factors which lead to localized and generalized fibrin deposition, and

4. The formation of platelet aggregates which result in the blocking of vessels and capillaries.

To the early phase mediators also belong chemo attractants (e.g. C5a) and cytokines such as IL-1, IL-6, and TNF-&. Late phase mediators are responsible for the regulation of vascular events occurring later- from about 6-12 hours after initiation inflammation. The later vascular events are mediated, at least in part, by products of arachidonic acid.

The chemical mediators of inflammation have considerable functional redundancy of the mediators by inflammation. This explains why certain patients may have complete absence of humoral component (e.g. complement component (3), yet minimal problems with increased susceptibility to infection.

The chemical mediators of inflammation are summarized below:-

Function	Mediators
1. increased vascular permeability of small blood	Histamine, serotonin, bradykinin, C3a, C5a,

vessels	PGE2, LTc4,/ Prostacyclins, activated Hageman factor, fibrino peptides
2. Vasoconstriction	TXA_2, LTB_4, LTC_4, C5a, N-formyl Peptides.
3. Smooth muscle contraction	C3a, C5a, histamine, LTB_4, LTC_4, TXA_2, serotonin, PAF, bradykinin.
4. Increased endothelial cell stickiness.	IL-1, TNF-&, MCP, endotoxin, LTB_4,
5. Mast cell degranulation	C5a, C3a,
6. Phagocytes, stem cell proliferation, Recruitment from bone marrow Adherence / aggregation chemotaxis	IL-3, G-CSF, GM-CSF, M-CSF CSFS, IL-1 IC3b, IgG, Fibronectin, lectins. C5a, LTB_4, IL -8 and other chemokines
7. Pyrogens	IL-1, TNF &, PGE_2, IL-6
8. Pain	PGE_2, bradykinin

2.4.0 Properties of Mediators

In this section we emphasize general properties of the mediators of inflammation and highlight only some of the more important molecules. We also touch upon some of the mechanisms that limit and terminate inflammatory reactions:

- Mediators may be produced locally by cells at the site of inflammation, or may be derived from

circulating inactive precursors (typically synthesized by the (liver) that are activated at the site of inflammation.

Cell-derived mediators are normally sequestered in intracellular granules and are rapidly secreted upon cellular activation (e.g. histamine in mast cells) or are synthesized de novo in response to a stimulus (e.g., prostaglandins and cytokines produced by leukocyte and other cells). Plasma protein-derived mediators (complement proteins, kinins) circulate in an inactive form and typically undergo proteolytic cleavage to acquire their biologic activities.

- Most mediators act by binding to specific receptors on different target cells.

  Such mediators may act on only one or a very few cell types, or they may have diverse actions, with differing outcomes depending on which cell type they affect. Other mediators (e.g. lysosomal proteases, ROS) have direct enzymatic and / or

toxic activities that do not require binding to specific receptors.

- The actions of most mediators are tightly regulated and short lived.

  Once activated and released from the cell, mediators quickly decay (e.g. arachidonic acid metabolities), are inactivated by enzymes (e.g. kininase inactivates bradykinin), are eliminated (e.g. antioxidants scavenge toxic oxygen metabolites) or are inhibited (e.g. complement regulatory proteins block complement activation).

2.4.1 Histamine

Histamine is an organic nitrogen compound involved in local immune responses as well as regulating physiological function in the gut and acting as a neurotransmitter (Mariet, 2001). Histamine triggers the inflammatory response. As part of an immune response to foreign pathogens, histamine is produced by basophils and by mast cells found in nearby connective

tissues. Histamine increases the permeability of the capillaries to white blood cells and some protein, to allow them to engage pathogens in the infected tissues. (Giuseppe et al. 2003) Histamine are known to be stored in mast cells.

Most histamine in the body is generated in granules in mast cells or in white blood cells called basophils. Mast cells are especially numerous at sites of potential injury- the nose, mouth and feet, internal body surfaces and blood vessels. Non-mast cell. Histamine is found in several tissues, including the brain, where it functions as a neurotransmitter. Another important site of histamine storage and release is the enterochromaffin–like (Ecc) cell of the stomach

Mechanism of action

Histamine exerts its action by combining with specific cellular histamine receptors. The four histamine receptors that have be discovered in humans and animals are designated H1 through H4 and are all G-

protein-coupled receptors (GPCR). Histamine biology is a series of weak interactions. In all of the known physiological reactions, the histamine backbone is unchanged (Noszal, et al. 2004). In the H2 receptor mechanism, histamine is protonated at the end-chain amine group. This amine group interacts with aspartic acid in the transmembrane domains of cells. The other nitrogen in the molecule interacts with threonine and aspartic acid in different transmembrane domains. This is a three-pronged interaction. It brings the transmembrane domains close to each other, causing a signal transduction cascade (Noszal, B., kraszni M., Racz, A. 2004).

Properties

Molecular formula - $C_5 H_9 N_3$

Molar Mass – 111.15g mol^{-1}

Melting point - 83.5^{o}C (182.3^{O}F)

Boiling point – 209.5^{o}C (409.1^{o}F)

Solubility in water – Easily soluble in cold water, hot water (http://www.science/ab.com)

Solubility – Easily soluble in methanol, very slightly soluble in diethyl ether (http://www.sciencelab.com), easily soluble in ethanol.

The H1 receptors mediate acute vascular effects together with smooth muscle constriction in the bronchi (histamine act as "Spasmogen") and the stimulation of eosinophil chemotaxis. The H_2 receptors mediate a number of anti-inflammatory effects, including the inhibition of eosinophil chemotaxis, but cause the vasodilation and the H3 receptor is mainly involved in the control of histamine release by different producing cells.

2.4.2 Serotonin (5 –Hydroxy tryptamine)

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter. Biochemically derived from tryptophan, serotonin is primarily found in the gastrointestinal (GI) tract, platelets, and in the central

nervous system (CNS) of animals including humans. It is popularly thought to be a contributor to feelings of well-being and happiness (Young, 2007). Serotonin is also capable of increasing vascular permeability, dilating capillaries and producing contraction of nonvascular smooth muscle. Most serotonin is stored in the dense gastrointestinal tract and central nervous system but a large amount is also stored in the dense granules of platelets.

Serotonin secreted from the enterochromaffin cells eventually finds it way out of tissues into the blood. There, it's actively taken up by blood platelets, which store it. When the platelets bind to a clot, they disgorge serotonin, where it serves as a vasoconstrictor and helps to regulate hemostasis and blood clothing. Serotonin also is a growth factor for some types of cells, which may give it a role in wound healing. Serotonin plays an important part in the regulation of learning, mood, sleep and vasoconstriction (constriction of blood vessels).

Experts say serotonin also might have a role in anxiety, migraine, vomiting and appetite Alterations in serotonin levels in the brain may affect mood.

Serotonin levels change with the season:-Serotonin transporter protein, a brain chemical that lowers serotonin levels around brain cells, is more active during the winter meaning that serotonin levels are lower. Researchers from the university of Toronto and the Medical University of Vienna believe that this mechanism explains why some people feel miserable during the winter.

In addition to animal, serotonin is found in fungi and plants (kang et al. 2009). Serotonin is presence in insect venoms and plant spines serves to cause pain, which is a side effect of serotonin injection. Serotonin is produced by pathogenic amoeba, and its effect on the gut causes diarrhea. Its widespread presence in many seeds and fruits may

serve to stimulate the digestive tract into expelling the seed.

In animals, including humans, serotonin is synthesized from the amino acid L-tryptophan hydroxylase (TPH) and amino-acid decarboxylase (DDC). The TPH-mediated reaction is the rate limiting step in the pathway. TPH has been shown to exist in two forms: TPH1, found in several tissues and TPH2, which is a neuron-specific Isoform (Cote et al. 2003).

Properties

Molecular formula - C_{10} H_{12} N_2O

Molar mass - 176.215g/mol

Appearance – white powder

Melting point – 121- 122^{oc} (ligroin) (Pietra, s;

Farmaco, Edi zione scientifia 1958)

Boiling point - 416 ± 30.0^{oc} (at 760 Torr)

Solubility in water – Slightly soluble.

2-4-3 CYTOKINES

Cytokines are soluble (glyco) proteins, non immunoglobulin in nature released by living cells of the host, which act non enzymatically in picomolar to nanomolar through specific receptors to regulate host cell function. In Greek cyto means cell and kinos means movement, cytokines are also small signaling molecules used for cell signaling

They can be classified as proteins, peptide, or glycoproteins. Cytokines make up the fourth major class of soluble intracellular signalling molecules alongside neurotransmitters, endocrine hormones, and autocoids. They possess typical hormonal activities.

1. They are secreted by a single cell type, react specifically with other cell types (target cells) and regulate specific vital functions that are controlled by feed back mechanisms.
2. They generally act at short range in a paracine or autocrine (rather than endocrine) manner;

3. They interact first with high – affinity cell surface receptors (distinct for each type or even subtype) and then regulate the transcription of a number of cellular genes by little understood second signals. This altered transcription (which can be an enhancement or inhibition) result in changes in cell behaviour .

   Target cells, on which cytokines transform their information signal, may be localized in any body compartment (sometimes a long distance from the site of secretion). Other types of these molecule act mostly as neighbouring cells in the microenvironment where they have been released. These are characterized as local hormones and their secretion is brought about by antocrine (only the cell or organ of secretion is affected) or paracine mechanism

   Each cytokine has a matching cell-surface-receptor. Subsequent cascades of intracellular signaling then alter

cell functions. This may include the up regulation and (or down regulation of several genes and their transcription factors, resulting in the production of other cytokines, an increase in the number of surface receptors for other molecules, or the suppression of their own effect by feedback inhibition.

The effect of a particular cytokine on a given cell depends on the complementary receptor on the cell surface and downstream signals activated by receptor binding; these last two factors can vary by cell type. Cytokines are characterized by considerable "redundancy" in that many cytokines appear to share similar functions.

It seems to be a paradox that cytokines binding to bodies have a stronger immune effect that the cytokine alone. This may lead to lower the therapeutic doses.

Cytokines ore synthesized, stored and transported by various cell types not only inside of the immune system (lymphokines, interleukins, monokines, tumor

necrosis factors, interferons) but also by other cells which are mainly studied in haematology (colony-stimulating factors) oncology (transforming growth factors), and cell biology (peptide growth factors, heat shock and other stress proteins). The main types of cytokines are listed below:-

Cytokine Types	Individual cytokines
i. Lymphokines	MAF (macrophage activating factor, MCF (Macrophage chemotactic factor), LMIF (leukocyte migration inhibition factor), HRFs(histamine releasing factors
ii. Interleukins	IL-1,IL-2,IL-15, IL-14
iii. Tumor necrosis factors	TNF-& (cachectin), TNF-& (lymphotoxin).
iv. Interferons	IFN-&, IFN-B, IFN-Y,IFN-w, IF-T
v. Colony Stimulating factors	G-CSF(granulocyte colony stimulating factors), GM-CSF granulocyte-macrophage CSF), M-CSF (macrophage CSF), multi-CSF(IL-3)
vi. Polypeptide growth factors	aFGF (acidic fibroblast growth factor, bFGF (basic fibroblast growth factor), EGP (epidermal growth factor), NGF(nerve growth factor), PDGF (platelet-derived growth factor), VEGF (vascular endothelial growth factor).

vii. Transforming growth factors	TGF-&,TGF-B
viii. X- chemokines	Il-8, NAP-2 (neutrophil – activating protein 2), PF-4 (platelet factor4), BTG (B-thromboglobulin.
ix. Stress proteins	HSPs (heat shock proteins), GRPs (glucose-regulated proteins), ubiquitin, superoxide dismutase (Mn).

The central role of cytokines is to control the direction amplitude and duration of immune responses and to control the remodeling of tissues, be it developmentally programmed, constitutive, or unscheduled. Unscheduled remodeling is that which accompanies inflammation, infection, wounding and repair. From the point of inflammation view there are two main groups of cytokines: Pro inflammatory and anti-inflammatory pro-inflammatory cytokines are produced predominantly by activated macrophages and are involved in up-regulaton of inflammatory reactions. Anti-inflammatory cytokines belong to the T Cell-derived cytokines and are involved in the down–regulation of inflammatory reactions.

2.4.4 Arachidonic acid

Arachidonic acid (AA, sometimes ARA) is a poly unsaturated omega-6 fatty acid 20:4 (w-6). It is the counterpart to the saturated arachidic acid found in peanut oil. (L. arachis –peanut) (Dorland's medical Didionary – A. 2007). It can be naturally found in some foods or SyntheSized. Some of this kind of element is necessary for nutrition in the body, but too much can be extremely harmful. Arachidonic acid is helpful for facilitating the growth of muscle tissues around the skeleton. Humans get arachidonic acids through their diets.

In chemical structure, arachidonic acid is a carboxylic acid with a 20-carbon chain and four cis-double bonds; the first double bond is located at the sixth carbon from the omega end. Some chemistry sources define aradidonic acid to designate any of the eicosatetraenoic acids. However, almost all writing in biology, medicines and nutrition limit the term to all-cis 5, 8, 11,14 eicosatetraenoic acid. Arachidonic acid is a

polyunsaturated fatty acid present in the phospholipids (especially phosphotidyl-ethanolamine, phosphatidyl choline, and phosphatidylinositides) of membranes of the body's cell and is abundant in the brain, muscles, liver. In resting cells, arachidonic acid is stored within the cell membrane, esterified to glycerol in phospholipids. A receptor – dependent event, requiring a transducing G–protein, initiates a phospholipid hydrolysis and releases the fatty acid into the intracellular medium.

Arachidonic acid is freed from a phospholipid molecule by the enzyme phospholipase A2 (PLA2), which cleaves off the fatty acid, but can also be generated from DAG by diacylglycerol lipase (Baynes, John N; Marek H.,; Dominiczak 2005). Arachidonic acid generated for signaling purposes appears to be derived by the action of phosphatidylcholine specific cytosolic phospholipase A_2 ($CPLA_2$ 85KDG), whereas inflammatory arachidonic acid is generated by the action of a low-

molecular –weight secretary PLA_2 ($SPLA_2$ 14-18 KDa). (baynes , John W.; Marek H. Domini Czak (2005) Arachidonic acid is a precursor in the production of eicosanoids:

- The enzymes cyclooxygenase and peroxidase lead to prostglandin in $H_{2,}$ which in turn is used to produce the prostaglandin, prostacyclin and thromboxanes.
- The enzyme 5 – lipoxygenase leads to 5-HPETE, (5-hydroxy eicosatetraenoic acid, which in turn is used to produce the leukotrienes.

Arachidonic acid is also used in the biosynthesis of anandamide. Some arachidonic acid is converted into hydroxyeicosatetraenoic acids (HETES) and epoxygenase (walter E; PhD. Boron 2003). The production of these derivatives and their action in the body are collectively known as the "arachidonic acid cascade. Through the conversion of arachidonic acid to active components such as the prostaglandin

PGF2&, arachidonic acid is necessary for the repair and growth of skeletal muscle tissue. (Trappe et al. 2001). Metabolism of arachidonic acid is carried out by three distinct enzyme pathways expressed in neuralcells: cyclooxygenase, lipoxygense, and pathnays expressed in neural cells: Cyclooxygenase, lipoxygenase, and cytochrome P450. Several products of these pathways act within neurons to modulate the activities of iron channels, protein kinase, ion pumps and neurotransmitters uptake systems. The newly formed eicosanoid may also exit the cell of origin and act at a distance, by binding to G-protein – coupled receptors present on nearby neurons or glial cells. Finally, the actions of the eicosanoids may be terminated by diffusion, uptake into phospholipids or enzymatic degradation.

Properties

Molecular formula – $C_{20}H_{32}O_2$

Molar mass – 304.47g mol-1

Density – 0.922g/cm^3

Melting point – 49^oC, 224k, - 56^oF

Boiling point – 169 – 171^oC, 442 – 444k, 33k – 340 ^oF (at 0.15mmHg)

Acidity (Pka) – 4.752.

2.4.5 Platelet Activating Factor (PAF)

Platelet-activating factor, also known as a PAF, PAF-acether or AGEPC (acetyl-glyceryl–ether– phosphory (choline) is a potent phospholipid activator and mediator of many leukocyte functions, including platelet aggregation and degranulation, inflammation and anaphylaxis it is also involved in changed to vascular permeability the oxidative burst, chemotaxis of leukocytes, as well as augmentation of arachidonic acid metabolism in phagocytes.

PAF is produced by a variety of cells, but especially those involved in host defense, such as platelet, endothelial cells, neutrophils, monocytes and

macrophages. PAF is continuously produced by these cells but in low quantities and production is controlled by the activity of PAF acetylhydrolases it is produced in larger quantities by inflammatory cells in response to specific stimuli. Phospholipid that is produced especially by mast cells and basophils, causes the aggregation of blood platelets and the release of blood-platelet substance (as histamine or serotonin); several molecular species of platelet-activating factor that vary in the length of the O-alkyl side chain have been identified.

- Its alkyl group is connected by an ether linkage at the c-1 carbon to a 16 – carbon chain.
- The acyl group at the c2 carbon is an acetate unit (as opposed to a fatty acid) whose short length increases the solubility of PAF allowing it to function as a soluble signal messenger
- The C3 has a phosphocholine head group, just like standard phosphatidylcholine.

- PAF is produced by stimulated basophils, monocytes, polymorphonuclear neutrophils, platelets and endothelial cells primarily through lipid remodeling. A variety of stimuli can initiate the synthesis of PAF. These stimuli could be macrophages going through phagocytosis or endothelium cells uptake of thrombin (William, 2010).

2.4.6 FREE RADICAL

In chemistry, free radical is an atom, molecule or ion that has unpaired valence electrons or an open electron shell and therefore may be seen as having one or more dangling covalent bonds.

The formation of radical may involve breaking of covalent bonds homolytically, a process that requires significant amounts of energy. This is known as hemolytic bond dissociation energy. The bond energy between the covalently bonded atoms is affected by the

structure of the molecule as a whole, not just the identity of the two atoms. An example is the production

of superoxide by the electron transport chain. Free radicals can be useful because they help important reactions in the body take place and can be utilized to manufacture pharmaceuticals custom designed plastics and other innovative materials (Oakley, 1988).

2.4.6.1 Nitric oxide (No)

Nitric oxide or nitrogen oxide, also known as nitrogen monoxide, is a molecule with chemical formula No. It is a free radical and is an important intermediate in the chemical industry. Nitric oxide is a by- product of combustion of substances in the air, as in automobile engines, fossil fuel power plants and is produced naturally during the electrical discharges of lighting in thunderstorms. In mammals including human, NO is an

important cellular signaling molecule involved in many physiological and pathological processes. It is a powerful vasodilator with a short half-life of a few seconds in the

blood. Nitric oxide (No) is produced by many cells in the body however, its production by vascular endothelium is particularly important in the regulation of blood flow. Because of its importance in vascular function, abnormal production of No, as occurs in different disease states, can adversely affect blood flow and other vascular functions. Despite being a simple molecule, No is an important biological regulator and is a fundamental component in the fields of neuroscience, physiology and immunology.

Nitric oxide is produced from the amino acid L-arginine by the enzymatic action of nitric oxide synthase (NOS). There are two endothelial forms of NOS. constitutive NOS (CNOs; type III) and inducible NOS (INOS; type II). Co-factors for NOS include oxygen, NADPH,

Vascular actions of No include the following:-

- Anti thrombotic effect –inhibits platelet adhesion to the vascular endothelium.

- Anti-proliferative effect – inhibits smooth muscle hyperplasia
- Anti-inflammatory effect – inhibits leukocyte adhesion to vascular endothelium, scavenges superoxide anion

  Because of the above actions of NO, when its production is impaired or its bio availability is reduced, the following can result
- Vasoconstriction (e.g. coronary, vasospasm, elevated systemic vascular resistance, hypertension).
- Thrombosis due to platelet aggregation and adhesion to vascular endothelium.
- Inflammation due to up regulation of leukocyte and endothetial adhesion molecules.
- Vascular hypertrophy and stenosis (Hrabie, Keefer & Derosa, 2008).

2.4.6.2 Oxygen-derived free radicals.

An atom or atom group having an unpaired electron on an oxygen atom, typically derived from molecular

oxygen For example, one electron reduction of 0_2 produce the superoxide radical, 0_2, other examples include the hydroperoxyl radical (HOO), the hydroxyl radical (HO.), and nitric oxide (No). They have a role in reperfusion injury. Oxygen-derived radicals are generated constantly as part of normal aerobic life. They are formed in mitochondria. Reactive oxygen species are also formed as necessary intermediates in variety of enzyme reactions. Examples of situations in which oxygen radicals are over produced in cells include:

-White blood cells such as neutrophils specialize in producing oxygen radicals, which are used in host defense to kill invading pathogen.

- cells exposed to abnormal environments such as hypoxia or hyperoxia generate abundant and often damaging reactive oxygen species A number of drugs have oxidizing effects

- cells exposed to abnormal environments such as hypoxia generate abundant and often damaging reactive

oxygen species. A number of drugs have oxidizing effects on cells and lead to production of oxygen radicals.

- Ionizing radiation is well known to generate oxygen radicals within biological system. Interestingly, the damaging effects of radiation are higher in well oxygenated tissues than in tissues deficient in oxygen. Antioxidants are known to protect cells from oxidant stress and there are numerous small molecules that function as antioxidants Examples include bilirubin, uric acid, flavonoids and carotenoids (Bowen, 2003).

2.4.7.0 Complement system

There are four major plasma enzyme system which have an important role in haemostasis and control of inflammation. One of them is the complement system. The complement system helps or complements the ability of antibodies and phagocytic cells to clear pathogens from an organism. The complement consists of a number of small proteins found in the blood,

generally synthesized by the liver, and normally circulating as inactive precursors (pro-proteins). When stimulated by one of several triggers, proteases in the system cleave specific proteins to release cytokines and initiate on amplifying cascade of further clearages. The end-result of this activation cascade is massive amplification of the response and activation of the cell-killing membrane attack complex. Over 25 proteins and protein fragments make up the complement system, including serum protein, serosal proteins and cell membrane receptors. They account for about 5% of the globulin fraction of blood serum.

Three biochemical pathways activate the complement system:

i. The classical complement pathway,
ii. The alternative complement pathway and
iii. The lectin pathway.

In the late 19th century, Hans Ernst August Buchner found that blood serum contained a factor or

principle capable of killing bacteria. In 1896, Jules Bordet, a young Belgian scientist in Paris at the Pasteur Institute demonstrated that this principle had two components: one that maintained this effect after being heated, and one that lost this effect after being heated. The heat-stable component was responsible for the immunity against specific microorganisms, whereas the heat-sensitive (heat-labile) component was responsible for the non-specific antimicrobial activity conferred by all normal Serum. The heat-labile component is what is now called complement.

Functions of Complement System

The following are the basic functions of complement:

i. Opsonization: - enhancing phagocytosis of antigens
ii. Chemotaxis: attracting macrophages and neutrophils.

iii. Cell lysis: rupturing membrane of foreign cells.

iv. Clumping of antigen – bearing agents.

Over View

The proteins and glycoproteins that constitute the complement system are synthesized by the liver hepatocytes. But significant amounts are also produced by tissue macrophages, blood monocytes and epithelial cells of the genitourinal tract and gastrointestinal tract. The three pathways of activation all generate homologous variants of the protease C3-convertase. The classical complement pathway typically requires antigen: antibody complexes for activation (specific immune response), whereas the alternative and mannose-binding lectin pathways can be achieved by C3 hydrolysis or antigens without the presence of antibodies (non-specific immune response).

Recent research has suggested that the complement system is manipulated during HIV/AIDS to further damage the body (Sacks et al. 2003).

2.4.7.1. The Clotting System/Coagulation System

This, apart from complement system is one of the four major plasma enzyme systems which are important in control of inflammation.

Coagulation (thrombogenesis) is the process by which blood forms clots. It is an important part of hemostasis, the cessation of blood loss from a damage vessel, where in a damaged blood vessel wall is covered by a platelet and fibrin-containing clot to stop bleeding and begin repair of the damaged vessel. Disorders of coagulation can lead to an increased risk of bleeding (hemorrhage) or obstructive clotting (thrombosis).

Coagulation begins almost instantly after injury to the blood vessel has damaged the endothelium lining the vessel. Exposure of the blood to proteins such as tissue factor initiates changes to blood platelets and the plasma protein fibrinogen, a clotting factor. Platelets immediately form a plug at the site of injury; this is called primary hemostasis. Secondary hemostasis occurs

simultaneously. Proteins in the blood plasma, called coagulation factors or clothing factors, respond in a complex cascade to form fibrin strands, which strengthen the platelet plug.

Platelet activation

When the endothelium is damaged, the normally isolated, under-lying collagen is exposed to circulating platelets, which bind directly to collagen with collagen-specific glycoprotein surface receptors. Activated platelets release the contents of stored granules into the blood plasma. The granules include ADP, serotonin, platelet-activating factor (PAF), Platelets 4 and thromboxane A_2 (TXA_2) which activate additional platelets. The granules' contents activate a G-linked protein receptor, cascade, resulting in increased calcium concentration in the platelets' cytosol. The calcium activates protein kinase C, which activates phosphalipase A_2 (PLA_2). PLA_2 then modified the integrin membrane glycoprotein increasing its affinity to bind

fibrinogen. The activated platelets change shape from spherical to stellate, and the fibrinogen cross-links with glycoprotein acid in aggregation of adjacent platelets completing primary hemostasis.

The Coagulation Cascade

The coagulation cascade of secondary hemostasis has two pathways which lead to fibrin formation. These are the contact activation pathway (also known as the intrinsic pathway), and the tissue factor pathway (also known as the extrinsic pathway). It was previously thought that the coagulation cascade consisted of two pathways of equal importance joined to a common pathway. It is known that the primary pathway for the initiation of blood coagulation is the tissue factor pathway. The pathways are a series of reactions, in which enzymes (inactive enzyme precursor) if a serine protease and its glycoprotein co-factor are activated to be become active components that then catalyze the next reaction in the cascade, ultimately resulting in

cross-linked fibrin. The coagulation factors are generally serine proteases (enzymes), which act by clearing downstream proteins. There are some exceptions. For example, FVIII and FV are glycoproteins and factor XIII is a transglutaminase (Hoffbrand, 2002).

2.4.7.2. The Kinin System

The Kinin-Kallikrein system or simply Kinin system is one of the four major plasma enzymes that has an important role in control of inflammation. It is of blood proteins that play a role in inflammation, blood pressure control, coagulatIon and pain. Its important mediators bradykinin and Kallidin are vasodilators and act on many cell types. They are liberated from precursors molecules, kininogens, by the actions of various protease, collectively known as kininogenases.

There types of Kininogens have been identified.

High molecular weight kininogen (HMWK) is produced by the liver. It acts mainly as a cofactor on coagulation and inflammation, and has no intrinsic catalytic activity.

Low molecular weight kininogen (LMWK): which is produced locally by numerous tissues and secreted together with tissue kalikrein.

T- Kininogen

These molecules are synthesized by hepatocytes and are released into the plasma, where in addition to releasing kinins,

They function as:

i. Cofactors in the coagulation pathway
ii. Inhibitors of cysteine protease enzymes
iii. Part of the acute phase response.

The kinins are potent vasoactive basic peptides and their properties are wide ranging, including the ability to increase vascular permeability, cause vasodilation, pain and the contraction of smooth muscle, and to stimulate arachidonic acid metabolism.

Three different pathways may lead to kinin formation during inflammation:

i) The generation of bradykinin as a result of activation of the Hageman factor and the production of plasma kallikrein,

ii) The production of lysylbradykinin by tissue kallikreins,

iii) The action of cellular proteases in kinin formation (Seth, 2008).

2.4.7.3. The Fibrinolytic System

This is the one of the four major plasma enzyme systems which has an important role in the control of inflammation. Fibrinolysis is a process that prevents blood clots from growing and becoming problematic. This process has two types: primary fibrinolysis and secondary fibrinolysis. The primary type is a normal body process, whereas secondary fibrinolysis is the breakdown of clots due to medicine, a medical disorder, or some other cause. In fibrinolysis, a fibrin clot, the product of coagulation, is broken down. It is main

enzyme plasmin cuts the fibrin mesh at various places, leading to the production of circulation fragment that are cleared by other proteases or by the kidney and liver. Plasma is produced in an inactive form, plasminogen, is the liver. Although plasminogen cannot clear fibrin, it still has no affinity for it, and is incorporated into the clot when it is formed. Once haemostasis is restored and the tissue is repaired, the clot or thrombus must be removed from the injured tissue. This is achieved by fibrinolytic pathway. The end product of this pathway is the enzyme plasmin, a potent proteolytic enzyme with a broad spectrum of activity. Plasma is formed by activation of the proenzyme, plasminogen by either plasma or tissue activators. Two forms of plasminogen are present in the plasma, one has a glutamic acid at the N- terminal of the polypeptide chain, and is called native or glu-plasminogen and the other a lysine. The latter form a rise as a result of partial degradation of the parent molecule by auto cleavage.

Tissue plasminogen activator (t-pA) and urokinase are the agents that convert plasminogen to the active plasmin, thus allowing fibrinolysis to occur. T-pA is released into the blood very slowly by the damaged endothelium of the blood vessel, such that, after several days (when the bleeding has stopped) the clot is broken down. This occurs because plasminogen became entrapped within the clot when it is formed; as it is slowly activated, it breaks down the fribrin mesh. T-PA and urokinase, are themselves inhibited by producing more active forms of both tissue plasminogen activator (tPA) and urokinase.

Triggering of fibrinolysis occur when the plasminogen activator, plasminogen and fibrin are all in close proximity. Both plasminogen and its activator bind avidly to fibrin as the clot forms. This close association prevents inhibition of plasimn activity by inhibitor and allows proteolysis of the fibrin to proceed after the

production of lys-plasminogen (Abelous & Bardier, 2009).

2.5.0 Anti-Inflammatory agents

Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system (Opal, 2000).

2.5.1 Non-Steroidal Anti-Inflammatory Drugs (NSAIDS)

Most NSAIDS act as non selective inhibitors of the enzyme cyclooxygenase (COX), Inhibiting both the cyclooxygenase -1- (COX-1) and cycloxygenase-2 (COX-2) Isoenymes. This inhibition is competitively reversible (albeit at varying degrees of reversibility), as opposed to the formation of prostaglandins and inhibition. Cox catalyzes the formation of prostaglandins and thromboxane from arachidonic acid (itself derived from

the cellular phospholipid bilayer by phospholipaseA_2) On its own, COX enzyme synthesizes prostaglandins, creating inflammation. In whole, the non-steroidal anti-inflammatory drugs prevent the prostaglandins from ever being synthesized, reducing or eliminating pain.

Some common examples of non-steroidal anti-inflammatory drugs are aspirins, ibuprofen and naproxen.

On the other hand, there are analgesics that are commonly associated with anti-inflammatory drugs but have no anti-inflammatory effects. An example is paracetamol called acetaminophen in the US and sold under the brand name of Iylenol. Immune selective Anti-Inflammatory Derivates (IMSAIDS).

(IMSAIDS) are a class of peptides being developed by IMULAN Bioltherapeutics, LLC, which discovered to have diverse biological properties, including anti-inflammatory properties. (MASAIDS work by altering the activation

and migration of inflammatory cells, which are immune cells responsible for.

2.5.1.1 Mechanism of Action of NSAIDS.

COX-1 is a constitutively expressed enzyme with a "house-keeping" role in regulating many normal physiological processes. One of these is in the stomach lining, where prostaglandins serve protective role, preventing the stomach mucosa from being eroded by its own acid. COX-2 is an enzyme facultatively expressed in inflammation, and it is inhabitation of COX-2 that produces the desirable effects of NSAIDS. When nonselective COX-1/COX-2 inhibitors (such as aspirin, Ibuprofen, and naproxen) lower stomach prostaglandin levels, ulcers of the stomach or duodenum internal bleeding can result. NSAIDS have been studied in various assays to understand how they affect each of these enzymes. While the assays reveal differences, unfortunately different assays provide differing ratios.

The discovery of COX-2 led to research to development of selective COX-2 led to research to development of reflective Cox-2 inhibiting drugs that do not course gastric problem of older NSADS

Acetaminophen is not considered an NSAIDS because it has a little anti inflammatory activity. It treats Pain mainly by blocking cox-2 mostly in the central nervous system, but not much in the rest of the body.

However, many aspects of the mechanism of action of NSAIDS remain unexplained, for this reason further cox pathways are hypothesized. The xox-3 pathway was believed to fill some of this gap but recent finding make it appear unlikely that it plays any significant role in humans and alternative explanation models are proposed. Nsaids are also used in the acute pain caused gout because they inhibit urate crystal phagocytosis besides inhibition of prostaglandin synthase. The term "non-steroidal" distinguishes these drugs from steroids, which, among a broad range of other effects, have a

similar eicosanoid –depressing, anti-inflammatory action. As analgesics, NSAIDs are unusual in that they are non-narcotic. The most prominent members of this group drugs are aspirin, Ibuprofen, and naproxen, all of which are available over the counter in most countries.

Antipyretic activity of NSAIDS.

NSAID have antipyretic activity and can be used to treat fever. Fever is caused by elevated levels of prostaglandin E2, which alters the firing rate of neuron within the hypothalamus that control thermoregulation general inhibition of prostanoid biosynthesis (PG2) within the hypothalamus. PGE2 Signals to the hypothalamus to increase the body is thermal set point. Ibuprofen has shown more effective as an antipyretic than acetaminophen (paracetamol) . Arachidonic acid is the precursor substrate for cyclooxygenase leading to the production of prostaglandin F. D & E (Opal, 2000).

2.5.2 Steroidal Anti-inflammatory drugs.

Inflammation is a protective response of the body to infection, Injury, a chronic medical problem like arthritis. Anti-inflammatory medicines are available in two categories, steroidal and non-steroidal, are prescribed to reduce inflammation. In addition, many people take anti inflammatory drugs for treatment of the flu, headaches and other painful conditions.

Steroidal anti-inflammatory are powerful medications, which are based on harmful substances like cortisone adrenaline. These non-steroidal medicines, they can be taken as pills, given through your vein, or injected directly into a joint space Many steroids, to be specific glucocorticoids, reducing inflammation of swelling by binding to gluococorticoids receptors. These drugs are often referred to as corticosteroids.

The steroidal anti-inflammatory can have these side effects. The can be more serious side effects including;

1. loss of bone (if you ae on these for along time, ask your provider about taking calaum supplements

2. Cataracts
3. Problems with your ability to fight infection
4. swelling and weight gain
5. mood changer
6. High blood pressure and
7. Problem with your bone marrow where blood cells are produced inflammatory drugs for the are effective. They slow down or stop the inflammation and the dosage can adjusted in an attempt to reduce the side effect. The steroid can be saving.

Remember to tell your health care provide that you take aspirin or other anti-inflammatory medication as well as acetaminophen, because of possible side effects or interactions with other medications that may be recommended to you (Opal, 2000).

CHAPTER THREE

MATERIALS AD METHODS

3.1 Materials

3.1.1 Equipment/Apparatus used.

The equipment and apparatus used for the research study with the manufacturers name and country include the following.

Equipment	Manufacturers
Beakers	West Germany
Conical flask	Pyrex, England
Chemical balance	Gallen Kamp, England
Filter paper	Whatman No1
Gloves	Neogloves
Grinder (Mill)	Thomas-willy, USA
Laboratory cage	Nigeria (Locally made)
Glass column	
UV light-box	Nigeria (Locally made)
Weighing balance/Electric balance	Wang, Taiwan.
Water bath	Gallen Kamp, England
Test tubes	Pyrex, England
Syringe (5ml and 1ml)	
Measuring cylinders (10ml and 100ml)	Great Britain

Reagent bottles Prove di-sacco title

3.1.2 Chemical, Solvents and Reagents.

The chemicals and reagents used where gotten from BRAIN PHOSPHORYLATIONSHIP (RESEARCH/TRAINING) CENTER. NO 9 Ogui, Enugu /School Biochemical laboratory and they were of analytical grade.

Chemicals/reagents used

Mayer's Reagent.

Olive oil

Benzene

Concentrated ammonium

Fresh albumin

Methanol

Dichloromethane

Glacial acetic acid

Ferric chloride

Concentrated sulfuric acid

Sodium hydroxide

Tween-80

Chloroform

Acetone

Distilled water

Ibuprofen

3.1.3. Animals

The experimental animals used for this research study were Wistar albino rats of both sexes, about 8 to 12 weeks old with average weight of 35.0g-53.0g were obtained from the animal house of the College of Medicine, University of Nigeria, Enugu Campus. The animals were housed in the animal house at Brain-Phosphorylationship Research AND Training Center- NO 9 Ogui Road. 5th floor,Right Wing. Former

ACB Building, Enugu and maintained on standard pellets (poultry feed) and water ad libitum. A short period was allowed for acclimatization.

3.2 Methodology

3.2.1 Collection and preparation of plant materials

Fresh stem barks of *Cratera adnsonii* was collected from Asata Street, Ogui Road, Enugu state, Nigeria in the month of April, 2013. Extracts was obtained from the stem bark, the bark was peeled from the stem with a sharp knife and chipped into pieces, which was air dried under room temperature and ground into powder form using a blender (grinder). The resulting powder was used for extraction.

3.2.2 Extraction

A weighed (660.96 gram) of the plant (bark) powder was mixed with 2500ml of methanol and stirred with magnetic strirrer, before it is left for 48 hours.

After 48 hours, it was filtered with filter cloth, measuring out 1000ml of methanol extract of *Crateva adansonii* which produces a green color. Some of the extracts were evaporated using water bath maintained

at 45^{o}c. The dried extracts were weighed and kept in a well labeled sterile specimen bottles.

3.3 Fractionation

3.3.1 Gel Column chromatography.

A glass wool was inserted into a glass column, then a silica gel was poured into the glass column setting on top of the glass wool, then the methanol extract of *Crateva adansonii* was poured into the glass column, a burette is erected and a dichloromethane is measured out in 500ml and poured into the burette, continually releasing its content into the glass column containing the methanol extract of *Crateva adansonii* and a beaker is placed under the glass column to collect the elute which is colourless and contains the active ingredient from the plant material.

3.4 Identification of phytochemical groups in the extract

The qualitative methods already established to test for classes of compounds in plant extracts by (ciulei, 1964) and (chitravadivu et al. 2009) were used. The substances that were tested for included; alkaloids, saponins, phlobatannins, Anthraquinones, Glycosides, Tannins, Coumarins, Flavonoids, which are reported to have biological activities on animal tissues. They dry extracts of dichloromethane fraction of methanol extract of *crateva adanosonii* bark, was used to determine the compounds.

3.4.1 Preparation of Reagents phytochemical analysis.

1% Hydrochloric acid.

A quantity 1ml of concentrated hydrochloric acid was diluted in distilled water and made up to 100ml with the distilled water.

10% Ammonia solution

A quantity 10ml of concentrated ammonia solution diluted with 90ml of distilled water to make it up to 100ml.

0.1% Ferric chloride.

0.1ml of 5% ferric chloride was diluted in 9.9ml of distilled water to make it up to mark.

3.4.2 Qualitative Phytochemical analysis of the Extract.

This analysis is carried out both on methanol extract of *Crateva adansonii* and dichloromethane fraction of methanol extract of *Crateva adansonii*.

a. Test for alkaloids.

A quantity of 0.4mg of methanol extract of *Crateva adansonii* was stirred with 8ml of 1% HCL and the mixture was warmed and filtered. 2ml of the filtrate was treated with a few drops of potassium mercuric iodide (Mayer's reagent).

Turbidity or precipitation indicates the presence of alkaloids.

b. Test for saponins.

20mg of the extract was boiled in 20ml of distilled water in water bath for five minutes and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for forth formation 3 drops of olive oil was mixed with forth, shaken vigorously and observed for emulsion development.

c. Test for anthraquinones

200mg of the extract was boiled with 6ml of 1% HCL and filtered. The filtrate was shaken with 5ml of benzene, then filtered and 2ml of 10% ammonia solution was added to the filtrate. The mixture was shaken.

The presence of a pink, violet or red color in the ammonical phase indicates the presence of free hydroxyl anthraquinones.

d. Test for glycosides (cardiac glycosides test).

5ml (10mg/ml in methanol) of the extracts was mixed with 2ml of glacial acetic acid having one drop of $Fecl_3$ solution. To the mixture obtained 1ml of concentrated H_2SO_4 was added to form a layer. Presence of brown ring of the interface indicated deoxy sugar characteristics of cardiac glycosides.

e. Test for coumarins

In a small test tube, 300mg of the extract was covered with filter paper moistened with 1N NaOH. The test tube was placed for few minutes in a boiling water bath. After removing the filter paper it was examined under the UV light, yellow fluorescence indicates the presence of coumarins.

f. Test for flavonoids

50mg of the extract was suspended in 100ml of distilled waters and then filtered. 5ml of dilute ammonia solution was added to 10ml of filtrate followed by few drops of concentrated H_2SO_4. Presence of flavonoids was confirmed by yellow colouration.

g. Test for tannins

50mg of the extract was boiled in 20ml of distilled water and filtered. A few drops of 0.1% $FeCl_3$ was added in filtrate and observed for colour change, brownish green or a blue-black colouration indicates the presence of tannins.

h. Test for Resins.

Using colour test

A quantity, 0.12g of the extract was extracted with chloroform and the extract concentrated to dryness. The residue was re-dissolved in 3ml Acetone and 3ml concentration Hcl added. This mixture was then heated in a water bath for 30 minutes. A pink colour that changes to magnet red indicated the presence of resin.

i. Test for Phlobatannins.

50mg of the extract was boiled with 1% aqueons hydrochoric acid, the deposition of a red precipitate indicated the presence of phlobatannins.

3.5 Thin layer chromatography (TLC).

A small sample of the evaporated dichloromethane fraction of methanol extract of *Crateva adansonii* was dissolved with few drops of dichloromethane reagent creating a sample in solution form.

Then a small TLC plate is then marked with a pencil, creating horizontal line towards the end of the TLC plate. The sample is then loaded on the line created on the TLC plate and placed in a beaker containing an amount of the developed TLC solvents enough to stay below, the line where the sample was loaded. It is then covered with a watch glass. The solvent is allowed to diffuse through TLC plate before bringing it out and marking the separated samples with pencil which is then allowed to dry before reading the result.

Result:- Only one band of separation was formed, which means that the sample contains a pure compound.

3.6 Anti-Inflammatory activity test.

The rats paw edema method of winter et al; (1962) was used for this test. The increase in the right hind paw volume induced by the subplater injection of fresh egg albumin (Akah and Njike, 1990; Okoli and Akah, 2000) was used as a measure of acute inflammation. Adult wistar albino rats of either sex where divided into 3 groups of 4 animals in two groups and 3 animals in one group. The control group received equivalent volume of 3% $^{v}/_{v}$ tween-80 administered intraperitoneally, while the last group received 25mg/kg body weight-dose of dichloromethane 4 fraction of methanol extract of Crateva adansonii in 3% Tween-80 administered intraperitoneally.

One hour after administration of test-substances, acute inflammation was induced by injection of 0.1ml of undiluted fresh egg albumin into the subplantar of the right hind paw of rats. The volume of the paw was measure by mercury displacement before and at 30, 60,120,180,240 minutes after egg-albumin injection. Edema formation was assessed in terms of the difference in the zero time paw volume of the injected paw and its volume at the difference times after egg-albumin injection. For each dose of extract, percentage inhibition of edema was calculated using the relation (Perez,1996);

$$\textbf{Inhibition of Edema (\%)} = 100\left(1-\left(\frac{x}{y}\right)\right)$$

where:

x = mean paw volume of treated rats after egg albumin injection

y = mean paw volume of control rats after egg albumin injection

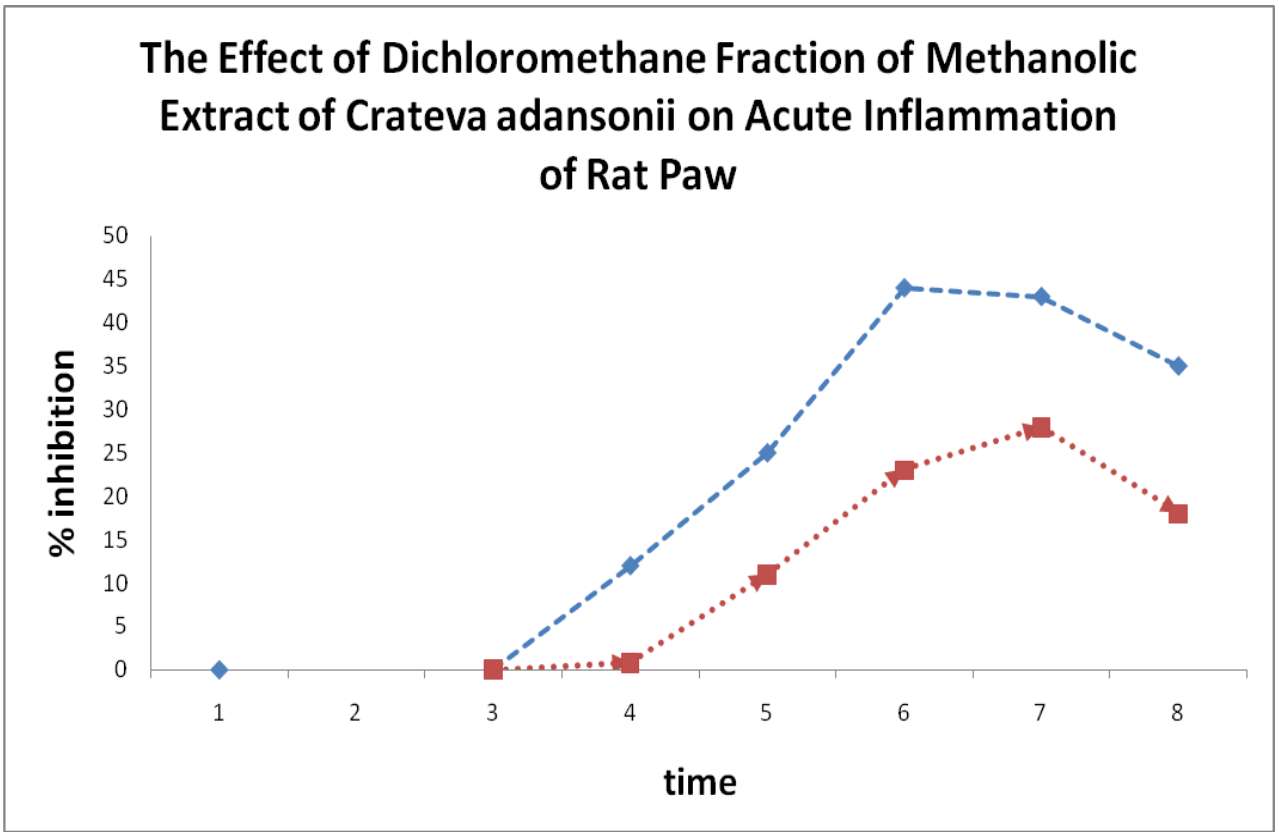
Inflammation = Average paw volume at time (t) – Average paw volume at zero time.

% inflammation =Average inflammation of treated group at time (T)/Average inflammation of control at same time x 100.

CHAPTER FOUR

Graphical Representation of the Anti-inflammatory

The effect of Dichloromethane fraction of Methanol Extract of Crateva adansonii Acute Inflammation of Rat Paw.



Red line: (doted arrow shape) standard-Ibuprofen 100mg/kg.b.w

Blue line: (square dot) Dichloromethane fraction 25mg/kg .b.w.

Figure 1: Percentage Inhibition of Egg-albumin-induced acute inflammation of rat paw.

Table 1: Phytochemical Analysis of *Crateva adansonic*

phytochemical constituent of methanodic Bark Extract of *Crateva adansonic*

S/No	Constituents	Experimental methods	Relative presence in methanol extract
1	Alkaloids	Mayer's test	++
2	Saponins	Emulsion test	+
3	Anthraquiones	Ammonium test	-
4	Glycosides	Sulphuric acid test	+
5	Coumarins	Sodium hydroxide test	-
6	Phlobatannins	Hydrogen chloride test	+
7	Tannins	Ferric chloride test	-
8	Resins	Colour test	+++
9	Flavonoids	Ammonia test	++

+ Present in trace concentration

++ Present in moderately high concentration

+++ Present in very high concentration

- Absent

Table 1: Phytochemical Analysis of Dichloro methane fraction

phytochemical constituents of Dichloromethane fraction of methanolic Bark Extract of *Crateva adansonii*

S/No	Constituents	Experimental methods	Relative presence in methanol extract
1	Alkaloids	Mayer's test	-
2	Saponin	Emulsion test	-
3	Anthraquinones	Ammonia test	-
4	Glycosides	Sulphuric acid test	+
5	Coumarins	Sodium hydroxide test	-
6	Phlobatannins	Hydrogen chloride test	-
7	Tannins	Ferric chloride test	-
8	Resins	Colour test	-

9	Flavonoids	Ammonia test	-

- Absent

+ Present in trace concentration

Chapter five

Discussion

This study investigates the anti-inflammatory activity of *Crateva adansonii* which is used as traditional medicine in rat edema method.

Having used the method of Lorks (1982), Launky (1964) Longe (1996) to qualitatively establish phytochemicals in the plant extract, this study established that methanol extract of *Crateva adasonii* stem bark contains alkaloid, saponin, glycosides, phlobatannins, resin and flavonoid while anthraquinones, coumarins and tannins were absent. The phytochemicals in the dichloromethane fraction of methanol extract of *Crateva adansonii* stem bark indicates the presence of glycoside while alkaloid, saponin, anthraquinones, coumarin, phlobatannin, tamin, resin and flavonoid were absent.

The result from this anti-inflammatory model shows that dichloromethane fraction administered at 25mg/kg displayed visually high anti-inflammatory activity against egg albumin-induced inflammation. The ibuprofen exhibited lower level of inhibition compared to the dichloromethane fraction between 30 to 240 minutes which means that the dichloromethane fraction has higher level of inhibition against egg albumin induced inflammation. Egg albumin has been known to induce acute inflammation as it is macroscopically characterized by swollen pains, heat and redness which are mediated by prostaglandin, cytokinine, chemokine, histamine, etc(Noszal et al. 2004). Therefore the anti-inflammatory activities of dichloromethane fraction of methanol extract of *Crateva adansonii* on egg-albumin edema paw inflammation in all phases suggests a suppression of local systemic manifestation of acute inflammation.

Conclusion

Following the experimental analysis and result, the methanol extract stem bark of *Crateva adansonii* has high significance in the treatment of acute inflammation in white albino rats. It is therefore recommended for such cases of inflammation in these researches. Further researches are hence encouraged on this plant extract.

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APPENDIX I

Tween-80 negative control

Volume of 3% tween- 80= 2.0ml

S/No	Weight(g)	Dosage	Volume	Sex
1	91.37		0.5ml	F
2	90.19		0.5ml	M
3	91.79		0.5ml	F
4	92.03		0.5ml	M
			2.0ml	

Table 1

S/No	0.00	30	60	120	180	240
1	5.20	4.60	4.60	4.60	4.80	4.80
2	5.20	4.60	4.50	4.60	4.60	4.80
3	5.30	4.60	4.40	4.50	4.60	4.70
4	5.20	4.60	4.60	4.70	4.80	4.90

Table II

S/No	0.00	30	60	120	180	240
1	0.80	1.40	1.40	1.40	1.20	1.20
2	0.80	1.40	1.50	1.40	1.40	1.20
3	0.70	1.40	1.60	1.50	1.40	1.30
4	0.80	1.40	1.40	1.30	1.20	1.10

Table III

S/No	0	30	60	120	180	240
1		0.60	0.60	0.60	0.40	0.40
2		0.60	0.70	0.60	0.60	0.40
3		0.70	0.90	0.80	0.70	0.60
4		0.60	0.60	0.50	0.40	0.30
	Y	0.625	0.70	0.625	0.525	0.425

APPENDIX II

Ibuprofen – standard group – positive control.

100mg/kg

Volume – 1.50ml

S/No	Body weight	Dosage	Volume (ml)	Sex
1	82.99	8.299	0.498	F
2	82.09	8.209	0.49	M
3	84.76	8.476	0.51	F
		24.984	1.50ml	

Table I

S/No	0	30	60	120	180	240
1	5.20	4.80	4.60	4.70	4.80	4.80
2	5.20	4.60	4.60	4.80	4.80	4.90
3	5.35	4.70	4.70	4.80	5.00	5.00

Table II

S/No	0	30	60	120	180	240
1	0.80	1.40	1.40	1.30	1.20	1.20
2	0.80	1.40	1.40	1.20	1.20	1.10
3	0.65	1.30	1.30	1.20	1.00	1.00

Table III

S/No	0	30	60	120	180	240
1		0.60	0.60	0.50	0.40	0.40
2		0.60	0.60	0.40	0.40	0.30
3		0.65	0.65	0.55	0.35	0.35
	X	0.62	0.62	0.48	0.38	0.35

Table IV
Inhibition percentage of ibuprofen

S/No	30	60	120	180	240
X	0.55	0.525	0.55	0.30	0.275
Y	0.625	0.70	0.625	0.525	0.425
%	12	25	44	43	35

APPENDIX III

Dichloromethane fraction

25mg/kg.b.w

S/No	Body weight	Dosage	Volume	Sex
1	94.51			M
2	70.85			F
3	94.39			M
4	65.28			M
		23.40	2.0ml	

Table I

S/No	0	30	60	120	180	240
1	5.20	4.60	4.60	4.80	4.80	4.40
2	5.40	4.80	5.00	5.00	5.10	5.20
3	5.30	4.90	4.80	5.00	5.10	5.10
4	5.30	4.70	4.70	5.00	5.00	5.00

Table II

S/No	**0**	**30**	**60**	**120**	**180**	**240**
1	0.80	1.40	1.40	1.20	1.20	1.20
2	0.60	1.20	1.00	1.00	0.90	0.80
3	0.70	1.10	1.20	1.00	0.90	0.90
4	0.70	1.30	1.30	1.00	1.00	1.00

TABELE III

S/N	0	30	60	120	180	240
1		0.60	0.60	0.40	0.40	0.40
2		0.60	0.40	0.40	0.30	0.20
3		0.40	0.50	0.30	0.20	0.20
4		0.60	0.60	0.30	0.30	0.80
	X	0.55	0.525	0.35	0.30	0.275

TABLE IV

INHIBITION PERCENTAGE FOR DICHLOROMETHANE FRACTION

	30	60	120	180	240
X	0.55	0.525	0.55	0.30	0.275
Y	0.625	0.70	0.625	0.525	0.425
%	12	25	44	43	35