ANALGESIC, ANTI-INFLAMMATORY, ANTIPYRETIC AND TOXICITY STUDIES OF METHANOL LEAF EXTRACT OF *CULCASIA ANGOLENSIS* (ARACEAE) IN MICE AND RATS

BY

DANIEL NOCK DOSHU

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,
FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

DECEMBER, 2019

ANALGESIC, ANTI-INFLAMMATORY, ANTIPYRETIC AND TOXICITY STUDIES OF METHANOL LEAF EXTRACT OF *CULCASIA ANGOLENSIS* (ARACEAE) IN MICE AND RATS

BY

Daniel Nock DOSHU,
B.Pharm (ABU) 1990
P16PHPG8013

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN PHARMACOLOGY

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,
FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

DECEMBER, 2019

DECLARATION

I declare that the work in this dissertation entitled **"**Analgesic, Anti-inflammatory, Antipyretic and Toxicity Studies of Methanol Leaf Extract of *Culcasia angolensis* (Araceae) in Mice and Rats" has been carried out by me in the Department of Pharmacology and Therapeutics, under the joint supervision of Prof. J. A. Anuka and Dr Jamilu Ya'u. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation has been previously presented for another degree or diploma at this or any other institution.

Daniel Nock DOSHU	_____	_____
Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled "**ANALGESIC, ANTI-INFLAMMATORY, ANTIPYRETIC AND TOXICITY STUDIES OF METHANOL LEAF EXTRACT OF *CULCASIA ANGOLENSIS* (ARACEAE) IN MICE AND RATS**" byDaniel NockDOSHU, meets the regulations governing the award of degree of Master of Science in Pharmacology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. J. A. Anuka Chairman, Supervisory Committee	Signature	Date
Dr. Jamilu Ya'u Member, Supervisory Committee	Signature	Date
Dr. M. G. Magaji Head of Department	Signature	Date
Prof. S. A. Abdullahi Dean, School of Postgraduate Studies	Signature	Date

ACKNOWLEDGEMENT

I thank God Almighty who helped me through this program, which has been a desire in my heart for many years. Words may fail me to express my gratitude to God.

My supervisors; Prof. J. A. Anuka and Dr. Jamilu Ya'u. I sincerely appreciate your constant encouragement, guidance and corrections that helped in standardizing and accomplishing this fit. It is indeed a great pleasure and honour working under your supervision. I must say thank you for your patience and understanding.

I immensely appreciate the Head of Department, Dr. M. G Magaji, the P. G. Coordinator, Dr S.B. Anafi and the Seminar Coordinator, Dr Oluronkoba for all their assistance. I am grateful to all the lecturers of the Department of Pharmacology and Therapeutics most especially Dr Idris Maje who has been my Star in this mission. Your impact had helped me at the course of this study. Thank you for your advice, suggestions and guidance.

I also warmly and gratefully acknowledge the timely assistance and ready to help attitudes of the entire administrative, technical and Animal House Staff of the Department of Pharmacology and Therapeutics for providing a conducive and friendly environment and facilities that enhanced the smooth conduct of this research work.

I appreciate the assistance of Department of Pharmacognosy and Drug Development, laboratory staff (especially Mallam M. Kabiru) and Department of Human Anatomy laboratory (especially Mr. A. Bamidele).

I show my deepest love and gratitude to my beloved family. I am indebted to my dear wife and children for their unflinching support all round and always. Yourfaith in me has been a source of inspiration and encouragement.

I must appreciate my father, Joshua Dogo (Walin Nok) and members of his family for your encouragement, support and prayers.

It's my privilege to express my profound acknowledgement towardsthe young pharmacists and other course mate who helped in one way or the other. Thank you all and may GOD almighty bless and increase you.

Finally, I must sincerely thank His Excellency, the Governor of Kaduna State, Mal. Nasiru Ahmed El-Rufai, OFR for the privilege permission given me to undertake this study. I am grateful to the Head of Service Kaduna State, the Commissioner of Health, the Permanent Secretary in the Ministry of Health, the Director of Pharmaceutical Services, Pharm John Bulus Magaji and the entire Staff and management of the Kaduna State Ministry of Health, for all the encouragement.

Thank you all.

DEDICATION

This work is dedicated to God Almighty.

TABLE OF CONTENTS

CHAPTER THREE

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

ABU - Ahmadu Bello University

ACEI- Angiotensin-Converting Enzyme Inhibitors

ACTH- Adrenocorticotrophic Hormone

ALP - Alkaline phosphatase

ALT - Alanine aminotransferase

ANOVA - Analysis of variance

AST - Aspartate aminotransferase

°C- Degree Centigrade

CAM- Complementary and Alternative Medicine

CAT - Catalase

Cl^- - Chloride ion

CGRP- Calcium Gene Related Peptide

CNS- Central Nervous System

COX-1- Cyclooxygenase-1

COX-2- Cyclooxygenase -2

CRPS- Complex Regional Pain Syndrome

DNA - Deoxyribonucleic acid

EPA- Eicosapentaenoic Acid

ER- Endoplasmic Reticulum

g - Grams

g/dL- Grams per decilitre

GSH - Glutathione

GPx - Glutathione peroxidase

GFR- Glomerular Filtration Rate

GGT- Gamma glutamyl transpeptidase

GIT- Gastro-Intestinal Tract

H- Hour(s)

Hb - Haemoglobin

HCO_3^- - Bicarbonate ion

H & E - Haematoxylin and Eosin

i.p - Intraperitoneal

IU/L - International units per Litre

IASP- International Association for Study of Pain

ICAM-1 - Intracellular Adhesion Molecule-1

IL-1- Interlukin-1

K^+ - Potassium ion

Kg- Kilogram

LD_{50} - Lethal dose in 50% of population

LTs- Leukotrienes

LPO - Lipid peroxidation

Mg- Milligram

mL- Millilitre

MDA - Malondialdehyde

mEq/L - Milliequivalents per litre

mg/dL - Milligram per decilitre

mg/kg - Milligram per kilogram

MFOs - Mixed function oxidase system

mmol - Millimoles

mmol/L - Millimoles per litre

n - Number of animals in a group

Na^+ - Sodium ion

ng/mL -	Nanogram per millilitre
nmol/mL -	Nanomoles per millilitre
NAPBQI-	N-acetyl-P-benzoquinone Imine
NSAIDs-	Non-Steroidal Anti-Inflammatory Drugs
OECD -	Organisation of Economic Cooperation and Development
PCM -	Paracetamol
PGs-	Prostaglandins
<	Less than
≤	Less than or equal to
P -	Probability
PCV -	Packed cell volume
p.o -	Per os
RBC -	Red blood cell
RNA -	Ribonucleic acid
SEM -	Standard error of mean
TNF -	Tumor Necrosis Factor
USA -	United States of America
WBC -	White blood cell
WHO -	World Health Organisation
µL -	Microlitre
µmol/L -	Micromole per litre
% -	Percentage
e.g -	Examples
e.t.c -	eccetera

LIST OF APPENDICES

ABSTRACT

The plant *Culcasia angolensis* is a robust forest climber of the family araceae, found in tropical Africa, Sierra Leone, Cameroon, Angola, DR Congo, Nigeria and Ivory Coast. The plant has been reportedly used for medicinal purposes including treatment of rheumatic pain, wound healing, dislocations and bruises. The present study investigated the analgesic, anti-inflammatory, antipyretic properties and toxicity profile of the plant in swiss albino mice and wistar rats. Acetic acid induced writhes test, hot plate-induced pain, formalin induced inflammation and carrageenan induced inflammation models in rodents were used to evaluate the analgesic and anti-inflammatory properties of the extracts. Phytochemical and acute toxicological screenings were also conducted. The median lethal dose was above 5000 mg/kg in mice and rats for the methanol leaf extract. The histological changes at 5000 mg/kg were slight glomerular necrosis and tubular damage in the kidney, slight vascular congestion, kupfer cell hyperplasia and lymphocyte hyperplasia in liver, lymphocyte hyperplasia on spleen and slight alveolar congestion on lungs. The methanol leaf extract of *Culcasia angolensis* at doses of 125, 250 and 500 mg/kg significantly ($p<0.05$) inhibited the acetic acid induced abdominal writhes in mice in a dose dependent manner. The extract at 500 mg/kg showed activity comparable to 20 mg/kg piroxicam. The methanol leaf extract of *Culcasia angolensis* also significantly ($p<0.05$) and dose dependently increased the reaction time in the thermally induced pain model which was comparable to the standard morphine (5 mg/kg) at 60, 90 and 120 minutes. The methanol leaf extract of *Culcasia angolensis* significantly ($p<0.05$) decreased formalin induced paw edema in a dose dependent manner. The leaf extract (500 mg/kg) exhibited better anti-inflammatory effects than ketoprofen (10 mg/kg), the standard anti-inflammatory drug used. The extract decrease the rectal temperature in a dose dependent manner which was comparable to that of pcm (300 mg/kg). These findings suggest that the methanol leaf extract of *Culcasia angolensis*, possesses analgesic, anti-inflammatory and antipyretic activities that justify the ethnomedical use in the treatment of painful and inflammatory conditions by the herbal practitioners.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Pain is defined by the International Association for the Study of Pain (IASP, 2015) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (Loeser and Treede, 2008). Pain can also be defined as multidimensional, subjective and unpleasant experience that is allied to tissue damage comprising sensory experiences that include; time, intensity, space, emotion, cognition and motivation (Maze *et al*., 2000). It may vary in intensity (mild, moderate or severe), quality (sharp, burning or dull), duration (transient, intermittent or persistent) and referral (superficial or deep, localized or diffuse). Pain follows both sensory and psychological mechanisms. However, pain is beyond sensation, it comprises of perception and subjective interpretation of the discomfort (Maze *et al.,* 2000). Pain plays an important role in drawing attention to tissue injury from harmful stimuli and reflexes that are elicited to protect the injured part of the body (Arome *et al*., 2016).

Damage caused by mechanical, thermal, chemical and electrical stimuli through peripheral receptors triggers pain sensation to nociceptors in an organism (Guyton and Hall, 2006). Perception of pain is a normal physiological response that is mediated by nervous system and is used for diagnosing various diseases such as diabetes, arthritis and cancer that are normally associated with chronic pain (Apkarian *et al*., 2005).

Inflammation (Latin, *inflammatio*), on the other hand, is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants (Ferrero-Miliani *et al*., 2007). Inflammation is a protective immunovascular

response that involves immune cells, blood vessels, and molecular mediators (Bairy *et al.,* 2015). The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function (Abbas and Lichtman, 2009). Redness and heat result from an increase in blood flow, swelling is associated with increased vascular permeability and pain is the consequence of activation and sensitization of primary afferent nerve fibers (Calixto *et al.,* 2003). Certain conditions, such as rheumatoid arthritis, osteoarthritis, inflammatory bowel diseases, retinitis, multiple sclerosis, psoriasis and atherosclerosis appears to have no resolution and a chronic state of inflammation develops that may last for life (Neville *et al.*, 2004). When conditions that induce inflammation are persistent or resolution mechanisms fail, a state of chronic inflammation ensues that can lead to loss of normal physiological functions (Hotamisligil and Erbay, 2008).

Fever on the other hand, also known as pyrexia or a febrile response, is defined as a body temperature above the normal range (36.5-37.5^{o}C) due to an increase in the temperature regulatory set-point (Axelrod and Diringer, 2008). Fever results from the release and conversion of arachidonic acid from cellular lipid membranes into prostaglandin E2, through the action of the cyclooxygenase enzymes COX-1 and COX-2 (Purssell and While, 2013). Symptoms of fever include; sweating, chills sensation of cold and other subjective sensations (Guyton and Hall, 2000). High temperature with absence of these symptoms may indicate a sign of a serious illness (Saper and Breder, 1994). A number of different microorganisms and other substances can cause fever and are collectively termed as pyrogens (Shalini and Donna, 2006). Products released by bacterial cell membranes such as lipopolysaccharide, toxins and breakdown of protein products in an organism body can cause the set point of the hypothalamic thermostat to increase

(Guyton and Hall, 2000). Under normal health conditions the range for oral temperature is between 33.2 – 38.2°C, for the armpit 35.5 – 37.0°C, the rectum 34.4 – 37.8°C, while for tympanic membrane 35.4-37.8°C (Sund-Levander *et al*., 2002). Fever is associated with sickness conditions, such as depression, anorexia, sleepiness, lethargy, inability to concentrate and hyperalgesia (Kelly *et al*., 2003).

Pain, fever and inflammation are beneficial to the immune system. However, they cause a lot of suffering and discomfort to the victims affecting the quality of life and therefore need to be managed. Non-Steroidal Anti-inflammatory Drugs (NSAIDs) are commonly used to manage inflammation, fever and pain (Barar, 2009). Opoid analgesics are choice drugs for severe or chronic malignant pain (Richard *et al*., 2008). The mechanism of action of the NSAIDs involves inhibition of cyclo-oxygenase (COX) enzyme which results in disruption of prostaglandins synthesis (Burke *et al*., 2006). However, non-steroidal anti-inflammatory drugs and opioid analgesics that are normally used to treat severe pains manifest a great number of adverse effects (Beg *et al.,* 2011). Despite progress made in medical science and in the production of new synthetic conventional drugs for management of pain, fever and inflammation, there is still need for development of more cost-effective and improved remedies with minimum side effects. Studies by World Health Organization (WHO) indicate that these compounds/drugs used in the management of pain, inflammation and fever predisposes an individual to a lot of adverseeffectsafter long term use including gastric irritation, ulceration, prolonged bleeding, renal failure, interstitial corrosion, and pruritus (Beg *et al.,* 2011). Reduction in ligament formation, tendon, cartilage healing and delay in muscleregeneration in many studies have beenassociated with NSAIDs (Almekinders, 1999). Conventional drugs used

to manage pain, inflammation and fever only provide symptomatic relief and the greatest disadvantage lies in their toxicity to the liver, kidney and reappearance of symptoms after discontinuation (Shah *et al*., 2006).

In this regard, herbal medicines have been employed in Complementary and Alternative Medicine (CAM) for treatment of pain, fever and inflammation as well as diseases related to these conditions. Many traditionally used medicinal plants are known to possess antipyretic, analgesic and anti-inflammatory properties but only a small percentage are included in health care systems after clinical research to manage these conditions (Singh *et al.,* 2008). In general, natural products and in particular, medicinal plants are believed to be an important source of novel chemical substances with potential therapeutic capabilities. Considering that most of anti-inflammatory, analgesic, anti-malarial and antipyretic synthetic drugs such as aspirin, morphine, chloroquine and artemisinin were derived from plant products, the search for plant species with anti-inflammatory, antipyretic and analgesic properties should be viewed as a fruitful strategy in search of new drugs (Gupta *et al*., 2006).

Culcasia angolensis has been used traditionally by the NgaringNok Community in Jaba Local Government, Kaduna State, Nigeria, in management of pain, malaria, fever and inflammation. Though there are some ethnobotanical studies conducted on this medicinal plant, there has been no scientifically evaluated data about its potential as a good source of alternative medicine. It is against this background that pharmacological screening of analgesic, antipyretic and anti-inflammatory potential of methanol leaf extract of *Culcasia angolensis* in animal models was investigated.

1.2 Statement of Research Problem

Pain is the most common symptom of injuries and diseases (Haddad, 2007). Virtually all known disease conditions are accompanied by pain (Donkor *et al.,* 2013). Pain imposes significant financial burden due to its long-term treatment (Bhangoo and Swanson, 2012). It is one of the most common conditions limiting efficiency and diminishing quality of life (Caraceni *et al.,* 2002; Mert *et al.,* 2013). Pain is the main reason for visiting the Emergency Department in virtually every hospital (Lewen*et al.,* 2010; Nanyak *et al.,* 2013).Several epidemiological studies from different countries have reported wide prevalence rates for chronic pain ranging from 12-80% of the population (Alamgeer *et al.,* 2013). Pain affects all populations, regardless of age, sex, income, race/ethnicity or geographical location; it is not distributed equally across the globe. Pain can lead to depression, inability to work, disrupted social relationships and suicidal thoughts (Goldberg and McGee, 2011).

Extremely high body temperatures destroy body cells, particularly nerve cells (Walter and Carraretto, 2015). Destruction of nerve cells is particularly dangerous because they rarely regenerate and when they do, it is extremely slow (Gerstner and Huff, 1977). The pathologic findings of death due to hyperpyrexia are localized haemorrhages and degeneration of cells throughout the body (Yiu and He, 2006). Therefore, there is need to control fever.

On the other hand, despite the protective nature of inflammation, it may cause damage on body tissues and organs. Therefore, there are instances where control of excessive inflammation would provide significant benefit to the health and well-being of the individual (Mburu *et al*., 2008). Unresolved inflammatory processes may be involved in

the pathogenesis and progression of many inflammatory diseases, including asthma, atherosclerosis, cancer, rheumatoid arthritis, multiple sclerosis, heart disease, gouty arthritis, rhinitis and ischaemia–reperfusion injury (Iwalewa *et al*., 2007; Medzhitov, 2008; Medzhitov, 2010; Alessandri*et al*., 2013). The costs of unrelieved pain and inflammatory diseases can result in longer hospital stays, increased rates of re-hospitalization, increased outpatient visits, and decreased ability to function fully leading to lost income. As such, patient's unrelieved chronic pain and inflammatory problems often result in an inability to work and maintain sound health (Strigo *et al.,* 2000).

Herbal medicine possess many safe and effective products that could be useful in various disorders and are, therefore, the preferred alternative to replace or complement conventional synthetic drugs (Hassan *et al.,* 2013).Recently, there has been a remarkable development in medical science, however, treatment and management of many serious indicators of ill health including pain, fever and inflammation is still problematic and complex (Adedapo *et al*., 2009).

1.3 Justification

Pain, fever and inflammation cause suffering and discomfort among the victims (Kariuki *et al*., 2012). Pain, when untreated can negatively affect all aspects of daily life, including physical activities, school attendance, sleep patterns, family interactions and social relationships and can lead to distress, anxiety, depression, insomnia, fatigue or mood changes, such as irritability and negative coping behaviour (WHO, 2012). Studies by WHO indicates that the NSAIDs used in management of these conditions manifest a lot of side effects (Robotin and Penman, 2006; Beg *et al.,* 2011). These drugs are not universally affordable. Therefore, use of herbal medicine makes it more attractive to

healthcare (Schmidt*et al*., 2008) as it is relatively affordable, more closely conforms to the patient‘s ideology, eases concerns about the adverse effects of synthetic drugs and satisfies a desire for more personalized healthcare (Canter and Ernst, 2004).

The validation of folkloric claims of therapeutic efficacy of medicinal plants supports tropical conservations of plant resources. Scientifically, the employment of beneficial plants as phytomedicine in primary health care results in development of potential bioactive constituents which provide novel lead compounds and precursors in drug development. So also, isosteres are discovered and isolated compounds are utilized as evaluative, investigative and research tools in drug development and testing processes.

This is therefore, a challenge to the research sector to find alternative approaches of managing pain, fever and inflammation (Sen *et al.,* 2010). Although *Culcasia angolensis* is traditionally used among the Ngaring Nok Community of Jaba Local Government Area, Kaduna State, Nigeria to manage pain, fever, inflammation, malaria and other ailments in the traditional system of medicine, an extensive search on the literature reveals that no data has been documented about the medicinal use of the plant in the management of pain, fever and inflammation.

1.4 Theoretical Framework

In recent times, focus on plant research has increased all over the world, with more than thirteen thousand plants studied between 1996 and 2000 (Dahanukar *et al*., 2000). There is sufficient evidence showing immense potential of medicinal plants being used in various traditional systems. Medicinal herbs have been used for the relief of pain throughout history thus practitioners of traditional medicine have enjoyed patronage and success owing to analgesic prototypes available (Sani *et al*., 2009).

1.4.1Models for the studies

1.4.1.1 Toxicity studies

Toxicity refers to the ability of a chemical agent to cause injury. Chemical substances may evoke one or both of two toxic effects. The first is an acute effect which occurs shortly after contact with a single dose of poison. The second is chronic effect which occurs however when an organism is exposed to repeated small and non-lethal doses of potentially harmful substances (Hassel, 1982). Toxicity is categorized as acute, sub-acute and chronic types.

1.4.1.2 Phytochemical screening

Basic phytochemical screening is designed to detect the presence or absence of some classes of plant metabolites by subjecting them to reactions with reagents that could yield observable coloured products. Some of the reactions involve formation of complexes between the organic metabolites and heavy metals resulting to appearance of coloured precipitate (Evans, 1996; Sofowora, 1993).

1.4.1.3 Acetic acid induced writhing

Acetic acid induced abdominal constriction in mice is a widely used model for evaluation of peripherally mediated analgesic agent (Gene *et al.,* 1998). Abdominal constriction responses are found to partly involve local peritoneal receptors (Bentley *et al.,* 1981). The method is also associated with prostanoids such as increase levels of PGE_2 and $PGEF_2\alpha$ in peritoneal fluids (Derardt *et al*., 1980); thus increasing the sensitivity of nociceptors and perception of pain. Acetic acid induced abdominal constriction method is very sensitive and detects antinociceptive effects of substance at a dose that is not feasible using other methods such as flick tail (Sutharson *et al*., 2007).

1.4.1.4 Hot plate (thermal sensitivity) test method

Hot plate test is the most common test of nociception that is based on a phasic stimulus of high intensity (Mandegary *et al*., 2004). Pain induced by thermal stimulus is specific for centrally mediated nociception. The paws of mice and rats are very sensitive to heat at temperatures which are not damaging to the skin. The responses are jumping, withdrawal of the pawsand licking of the paws. The latency time is increasedwhen centrally acting analgesics are administered (Stein, 1995) whereas peripheral analgesics like acetylsalicylic acid and phenylacetic acid do not generally affect these responses (Gislason *et al*., 2009).

1.4.1.5Evaluation of effect of extract by interaction with naloxone

Naloxone is a specific antagonist of opioid receptors. Administration of an antagonist will result in the blockade of these receptors, thus prevent the analgesic agent from binding to elicit effect (Younos *et al*., 1990). This results in prolonging responses in tail immersion test and decreasing reaction time in hot plate test.

1.4.1.6 Brewer's yeast induced pyrexia

This is a suitable model for screening natural or synthetic drugs and compound for their antipyretic effect. Injection of Brewer's yeast subcutaneously induces pyrexia by increasing the synthesis of prostaglandins (Devi *et al.,* 2003). The method described by Loux *et al.,* (1998) was used to investigate the antipyretic activities of the extracts.

1.4.1.7 Formalin induced inflammation test

This is a commonly used primary test for the screening of new anti-inflammatory agents. It is biphasic with the first phase (neurogenic phase, 1-2 hrs) occurring due to release of histamine or serotonin and the second phase (inflammatory phase) of oedema due to

release of prostaglandins or through the release of inflammatory mediators (Ishfaq *et al.*, 2004, Tanko *etal*., 2008). Both phases can be inhibited especially by centrally acting substances and the second phase can also be inhibited for peripherally acting substances. Activity in this model suggests the activation of opioid receptors, hence centrally mediated activities (Gaertner *et al*., 1999).

1.4.1.8 Carrageenan-induced paw oedema

The method used is based on the fact that an aqueous solution of carrageenan (1%) injected into the sub planter surface of the left hind paw of the rat triggers an inflammatory response which is characterized by an increase in the paw diameter (Niemageer *et al.,* 1964). The rats paw diameter are measured using a veniers caliper to determine the percentage inhibition (anti-inflammatory action) produced by the plant extracts.

1.5Aim and Objectives of the Study

1.5.1 Aim

The aim of the study is to investigate the analgesic, anti-inflammatory, antipyretic and toxic effects of methanol leaf extract of*Culcasia angolensis* in Swiss albino mice and Wistar rats

1.5.2 Specific objectives

1. To evaluate the acute toxicity of the methanol leaf extract of *Culcasia angolensis*
2. To evaluate the subchronic toxicity of the methanol leaf extract of *Culcasia angolensis*
3. To assess the analgesic, anti-inflammatory and antipyretic effects of the methanol leaf extract of *Culcasia angolensis*

1.6 Research Hypothesis

The methanol leaf extract of *Culcasia angolensis* possessesanalgesic, anti-inflammatory and antipyretic activities and has no toxic effects on liver, kidney, lung, heart and spleen of the body.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pain

Pain is defined as an unpleasant, subjective, sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (Merskey and Bogduk, 1994).Pain is seen as a multidimensional phenomenon with sensory, physiological, cognitive, affective, behavioural and spiritual components. The affective component (constituting the emotions), behavioural component (constituting the behavioural responses to pain), beliefs, attitudes (constituting both spiritual and cultural attitudes about pain and its control) all alter the way pain is experienced (sensory component) through modification of transmitting noxious stimuli to the brain constituting the physiological component (Besson, 1999).

2.1.1 Causes

There are several causes of pain which include infections (such as viral, bacterial or fungal infections), drugs (such as antiretroviral drugs), damageto the nerve cells (such as peripheral neuropathy) and diseases (such as diabetes, hypertension, cancer, sickle cell) (Wallace, 1992).

2.1.1.1 Infections

Infection with pathogenic organisms such as bacteria, viruses and some fungi are common causes of pain (Berry *et al*., 2001). Pathogenic organisms like bacteria can be invasive or non-invasive. Noninvasive bacteria produce exotoxins interfering with normal secretory and absorptive properties of the intsestinal lumen leading to irritation, inflammation and net fluid loss while noninvasive bacteria do not invade the mucosal

cells. The invasive bacteria (e.g Salmonella and Shigella) penetrate the intestinal muscosa to cause inflammation, necrosis, diarrhoea and systemic complications such as fever (Tal *etal*., 2007).

Infections caused by viruses are common causes of pain in both children and adults. Headache, oral cavity pain, abdominal pain, neuromuscular pain, chest pain, earache, odynophagia (pain while swallowing), myalgia and athralgia. The pain experienced depends on the stage of the infection. In early HIV, most of the pain is from the opportunistic conditions, thus, considered somatic and transient. At the late stages, both somatic and neuropathic pains occur. Fungal infections can be both topical and systemic. Topical fungal infections cause skin pain which can result from sores, rashes and blisters that can hurt and itch (Melzack and Casey, 1968).

2.1.1.2 Drugs

Drugs can also induce painful complications from their adverse effectse.g.Diaper dermatitis, which can result from an adverse effect of antiretroviral therapy (ART). Different drugs induce different and specific form of pain, examples, zidovudine induces muscle pain, efavirenz induces headache and stavudine induces abdominal pain. Nucleoside reverse transcriptase inhibitors (specifically stavudine and didanosine) induce neuropathic pain (Vranken, 2009). Chemotherapy in cancer treatment is also a cause of pain which can be either chronic (headache, neuropathic pain) or acute pain or both. Non-steroidal anti-inflammatory drugs such as ibuprofen cause headache, gastrointestinal ulcerations and bleeding (Gislason *et al*., 2009).

2.1.1.3 Diseases

Diseasesare the main causes of pain as there is virtually no disease that is notassociatedwith pain, ranging from mild to moderate to severe pain. Examples of diseases associatedwith pain include sickle cell, diabetes, cancer and hypertension among others (Olurishe and Mati, 2014).

Sickle cell disease (SCD) is a genetic disorder associated withabnormal haemoglobin, haemoglobin S (Hbs) in the red blood cells. The RBC becomes rigid and crescent shaped (i.e. sickled). When large numbers of sickled red blood cells accumulate, they hinder blood flow, which results in painful vaso-occlusive crises or episodes. It results in ischaemia leading to tissue damage and cell necrosis. All these events cause pain. This is a form of episodic (acute) pain occurring in the arms, legs, abdomen, chest and back. Pain associated with SCD is described as aching, tiring and uncomfortable (Thienhaus and Cole, 2002; Cousins *et al*., 2004).

Cancer is another disease associated with both acute and chronic pain. It has been observed in developing countries that most cancer pains are associated with diagnostic and therapeutic procedures, and treatment. Pain associated with diagnosis occurs when the cancer disease recurs and it is resistant to treatment (Baumann, 2002). However, in developing countries like Nigeria, cancer is presented at an advanced stage with poor accessibility to chemotherapy and radiotherapy. In such cases pain results from the progression of the cancer itself. The cancer mass can produce pain by tissue distension, compression or infiltration. Inflammation due to infection, necrosis or obstruction can also cause pain (Paice, 2003).

Acute pain in cancer results from direct invasion of anatomical structures in tumour through nervous tissue compression, distension and inflammation (Danesh *et al*., 2004). It can also result from investigative procedures such as bone marrow aspiration and lumbar puncture. Chronic pain is caused by the tumor growth itself or through investigative and therapeutic procedures such as limb amputation or chemotherapy (Tsang *et al.,* 2008). Brain tumors and lymphomas can cause headache, while leukemia, lymphoma, bone sarcomas and neuroblastoma can cause diffuse bone and joint pain. Neuropathic pain is caused by injury to the nervous system and it is severe. It is usually described as burning, tingling, sharp or shooting (Satoskar *et al*., 1999).

2.1.2Pathophysiology and types of pain

Pain classification system encompasses the pathophysiology of pain, thus there are several classification systems for pain and these include the followings:

2.1.2.1 Classification based on pathophysiology

Based on the pathophysiology of pain; it is divided into two major types; nociceptive and neuropathic.

Nociceptive pain:

This type of pain results when tissue injury activatesspecificpain receptors called nociceptors sensitive to noxious stimuli (Giordano, 2005). Nociceptors can respond to heat, cold, vibration, stretch stimuli and chemical substances released from tissues in response to oxygen deprivation, tissue disruption or inflammation. Nociceptive pain can besubdivided into somatic and visceral pain depending on the location of activated nociceptors (Urch and Suzuki, 2009).

Somatic pain iscaused by the activation of nociceptors either on tissue surfaces (skin,mucosa of mouth, nose, urethra, anus e.t.c) or deep tissues such as bone,joint, muscle or connective tissue. Examples cut and sprains; causing tissue disruption results in surface somatic pain, while muscle cramps due to pooroxygen supply results in deep somatic pain (Spanswick and Main, 2000).

Visceral pain on the other hand, is caused by activation of nociceptors located in the viscera (Stein, 1995). Visceral consist of the internal organs of the body enclosed in a cavity such as thoracic and abdominal organs. Visceral pain can occur due to infection, distension from fluid or gas, stretching or compression (usually from solid tumors) (Urch and Suzuki, 2009).

Neuropathic pain is caused by structural damage and nerve cell dysfunction in the peripheral or central nervous system (CNS) (Vranken, 2009). This results from any process that causes damage to the nerves such as metabolic, traumatic, infectious, ischemic, toxic or immune mediated pathological conditions (Paice, 2003). Neuropathic pain can also result from nerve compression or abnormal processing of pain signals by the brain and spinal cord. This pain can be either peripheral or central (Mayer and Liebeskind, 1974). Peripheral neuropathic pain arises as a direct consequence of a lesion or disease affecting the peripheral nerve and dorsal root ganglion, while central neuropathic pain arises as a direct consequence of a lesion or disease affecting the CNS (Vranken, 2009). Sensory dysfunction suggestive of neuropathic pain includes allodynia, hypoalgesia, hyperalgesia, paraesthesia, dysthesia, hyperesthesia and hypoesthesia (Nagasako *etal*., 2003).

Allodynia is a form of pain due to stimulus that does not provoke pain, such as a light touch eliciting severe pain (Ramer *et al*., 1998). Hyperalgesia refers to an increased pain response to a normally painful stimulus (tactile or thermal) such as hyperalgesia to cold (Besson, 1999). Paraesthesia refers to abnormal sensation to a stimulus that is normally not unpleasant such as tingling, prickling or numbness. Hypoalgesia refers to diminished pain response to a normally painful stimulus (tactile or thermal). Dysethesia refers to unpleasant sensation which may be spontaneous or evoked. Hyperesthesia refers to increase sensitivity to stimulation while hypoesthesia refers to decrease sensitivity to stimulation (Paice, 2003).

Mixed pain occurs when neuropathic pain coexists with nociceptive pain. In certain diseases, mixed pain can occur to consist of somatic, visceral and neuropathic pain all at the same time, or each separately at different times (Vranken, 2009). Examples include trauma that damages tissue and nerves, burns (affecting skin and nerve endings), and cancer that causes external nerve compression and nerve damages by infiltration (Wallace 1992).

2.1.2.2 Classification of pain based on duration of pain

Based on duration, pain is divided into acute and chronic. Acute pain lasts less than thirty days while chronic pain last more than three months. Symptoms and causes of the two may overlap and pathophysiological factors can be independent of duration, thus making this classification problematic.

Acute pain has sudden onset and immediate feeling following injury, is severe and with short duration. It results from tissue injury stimulating nociceptors and disappears when injury heals (Besson, 1999).

Chronic pain is continually present when healing is expected to be complete. It can be an extension of acute pain and persists for long periods or recur due to persistence of noxious stimuli or repeated exacerbation of injury. Chronic pain can arise without any identifiable pathophysiology or medical illness. Chronic pain can affect quality of life including physical activities, school attendance, sleep patterns and family interactions. It can lead to anxiety, distress, insomnia, fatigue and mood changes (Merskey and Bogduk, 1994; Tsang *etal*., 2008).

Episodic or recurrent pain occurs intermittently over a long period of time. There is pain free period in between painful episodes. Painful episodes can fluctuate in intensity, quality and frequency at times and are unpredictable. Examples of episodic pain include migraine, episodic sickle cell disease pain and recurrent abdominal pain (Derman *et al*., 2009).

Breakthrough pain is characterized as temporary increase in severity of pain over and above pre-existing base line pain level. It is of sudden onset, severe and of short duration. It occurs unexpectedly and independently of any stimulus, without a preceding incident or an obvious precipitating factor. Example is break through pain in cancer (Chapman *etal*., 2008).

Incident pain or pain due to movement is a pain that has identifiable cause. It is induced by simple movements such as walking or by physical movements that exacerbate pain such as weight bearing, coughing or urination. Diagnostic or therapeutic procedures can also cause incident pain (Moulin *et al*., 2007). End of dose pain occurs when usual loading or maintenance dose of analgesic drugs fall below the minimum effective dose at the end of dosing interval (Leonard *et al*., 2006).

2.1.2.3 Classification of pain based on etiology

This classification is commonly based on the underlying disease being malignant or non-malignant. This classification has little relevance to treatment and mechanism of pain.

2.1.2.4 Anatomical classification of pain

Pain can be classified based on the body location (example head, back or neck) or anatomical function of the affected tissue (example; myofascial, rheumatic, skeletal, neurological and vascular). This classification address physical dimension but does not include mechanism. This classification can be useful for diagnosis but not for clinical management of pain (Giordano, 2005).

2.1.2.5Classification of pain based on specific diseases

Pain can be classified based on diseasessuch as HIV/AIDS, cancer, sickle cell diseases pain and diabetic pain. Pain in HIV/AIDS can be acute or chronic. Acute pain includes oral cavity pain, abdominal pain, headaches, neurological and muscular pain. Chronic pain includes neuropathic pain and wasting syndrome (Berry *et al*., 2001). Pain in sickle cell disease is classified into episodic (acute) occurring due to vaso-occlusive episodes (sickle cell crisis) and persistent SCD pain resulting from a vascular necrosis due to poor blood oxygenation (Merskey and Bogduk, 1994).

Other types of pain include phantom pain and psychogenic pain. Phantom pain is a form of pain from a part of the body that has been lost or from which the brain stops receiving signals. It is a type of neuropathic pain. Phantom limb pain is a common experience of amputees (Kooijman *et al*., 2000). Psychogenic pain also called psychalgia or somato form is a pain caused by increased or prolonged mental, emotional or behavioural factors.

Headache, backache and stomachache are sometimes diagnosed as psychogenic (Thienhaus and Cole, 2002).

2.1.3Epidemiology

Pain is the main reason for visiting the emergency department in more than 50% of cases and is present in 30% of family practice visits all over the world (Hasselstrom *et al*., 2002). Several epidemiological studies from different parts of the world have reported prevalence rates for chronic pain, ranging from 12-80% of the population. It becomes more common as people approach death (Perquin *et al*., 2000). Chronic pain is a general complaint in the world and more common in industrialized countries constituting major public health and socioeconomic problem. Prevalence of pain in the general population ranges from 10% to 50% depending on the population studied and the perception of pain (Bishaw, 2007). Data from U.S.A. suggests that chronic pain is responsible for more than 150 billion dollars spent on health care and disability related costs. In Nigeria, for individual experiencing pain, the human cost is incalculable, but can only be evidenced in decreased quality of life, activity limitation, reduced functional capacity and increased financial burden arising from increased use of health services and medication (Igumbar *et al*., 2011).

2.1.4Pain pathway

It consists of afferent nociceptive fibresthat travel back to the spinal cord where they form synapses in its dorsal horn. These nociceptive fibres (located in the periphery) is a first order neuron. The cells in the dorsal horn are divided into physiologically distinct layers called laminae. Different fibre types form synapses in differernt layers, and use either glutamate or substance P as the neurotransmitter. Aδfibre form synapses in laminae

I and V, C fibres connect with neurons in lamina II, Aβ fibres connect with laminae I,III and V (Jessel *et al*., 1991). After reaching the specific lamina within the spinal cord, the first order nociceptive project to second order neurons andcross the midline. The second order neurons then send their information via two pathways to the thalamus; the dorsal column mediallemniscal system and the anterolateral system. The first is reserved more for non-regular painful sensation, while the lateral is reserved for pain sensation. Upon reaching the thalamus, the information is processed in the ventral posterior nucleus and sent to the cerebral cortex in the brain. As there is an ascending pathway to the brain that initiates conscious realization of pain, there is also adescending pathway which modulates pain sensation. The brain can request the release of specific hormones or chemicals that can have analgesic effects which can reduce or inhibit pain sensation. The area of brain that stimulates the release of these hormones is the hypothalamus (Field *et al*., 1998).

The effect of descending inhibition can be shown by electrically stimulating the periaqueductal grey area of the midbrain. The periaqueductal grey area of the midbrain in turn projects to other areas involved in pain regulation, such as the nucleus raphe magnus (which also receivessimilar afferents from the nucleus reticularis paragigantocellularis). In turn, the nucleus raphe magnus projects to the substantia gelatinosa region of the dorsal horn and mediates the sensation of spinothalamic inputs. The periaqueductal grey area of the midbrain also contains opioid receptors which explains one of the mechanisms by which opioids such as morphine and diacetylmorphine exhibit analgesic effect (Selbst and Fein, 2006).

Nociceptor neuron sensitivity is also modulated by a large variety of mediators in the extracellular space (Hucho and Levine, 2007). Peripheral sensitization represents a form of functional plasticity of the nociceptor. The nociceptor can change from being simply a noxious stimulus detector to a detector of non-noxious stimuli. The result is that low intensity stimuli from regular activity initiates a painful sensation. This is commonly known as hyperalgesia. Inflammation is one common cause that results in sensitization of nociceptors. Normally hyperalgesia ceases when inflammation resolves, however, sometimes genetic defects and/or repeated injury can result in allodynia (a complete non-noxious stimulus like light touch causes extreme pain). Allodynia can also be caused when a nociceptor is damaged in the peripheral nerves (Ramer *etal*., 1998). This can result in de-afferentation, which means the development of different central processes from the surviving afferent nerve. With thissituation, surviving dorsal root axons of the nociceptors can make contact with the spinal cord, thus changing the normal input (Field *et al*., 1998).

2.1.5Pain thresholds

In pain science, thresholds are measured by gradually increasing the intensity of a stimulus such as electric current or heat applied to the body. The pain perception threshold is the point at which the stimulus begins to hurt and the pain tolerance threshold is reached when the subject acts to stop the pain (Melzack and Wall, 1996). Differences in perception and tolerance thresholds are associated with, among other factors, ethnicity, genetics and sex. For example,people of Mediterranean origin report some radiant heat intensities as more painfulwhile northernEuropeans described it as non-painful (Tsang *et al*., 2008). Italian women tolerate less intense electric shock than Jewish

or Native American women. Some individuals in all cultures have significantly higher than normal pain perception and tolerance thresholds for electric shock, muscle cramp and heat (Melzack and Wall, 1996; Nagasko *etal*., 2003). Women have lower pain perception and tolerance thresholds than men, and this sex difference appears to apply to all ages, including newborn infants (Guinsburg*et al*., 2000). In West Africa, there is variability in pain perceptions among the ethnic groups, with Fulani's having high tolerance threshold (Verra *etal*., 2009).

2.1.6Pain assessment

A person's self report is the most reliable measure of pain, with health care professionals tending to underestimate severity (Prakachin *et al*., 2007). Pain is whatever the experiencing persons says it is, existing whenever he says it does. To assess intensity of pain, a person may be asked tolocate his pain on scale of 0 to 10, with 0 being no pain at all and 10 being the worst pain he has ever felt. Quality can be established by having the patient complete the McGill Pain Questionnaire indicating which words, best describe his pain (Prakachin *et al.,* 2007).

2.1.7Pain as an aid to diagnosis

Pain is asymptom of many medical conditions.Thus, it is important to know the onset, location, intensity, pattern of occurence,exacerbating factors, relieving factorsand quality of pain (Berry *etal*., 2001). This will help the examining physician to accurately diagnose the problem. Forexample, chest pain described as 'extreme heaviness' may indicate myocardial infarction, while chest pain described as 'tearing' may indicate aortic dissection (Slater and De Sanctis,1976).

2.1.8Drug treatment and overview of antinociceptive drugs

Regardless of etiology of pain, the specific goals of pain treatment is to reduce the severity of pain, restore overall movement and health, teach effective stress management and encourage cooperation in group activities. Drug therapy involves the use of opioids, NSAIDs, antidepressants, and adjuvants. Analgesics include paracetamol, NSAIDs such as the salicylates, and opioid drugs such as morphine and oxycodone. They interact with the neurotransmitters and modulators of the pain system and alleviate and control of pain. NSAIDs exert their analgesic effect not only through peripheral inhibition of prostaglandin synthesis but also through a variety of other peripheral and central mechanisms(Gislason *et al*., 2009).

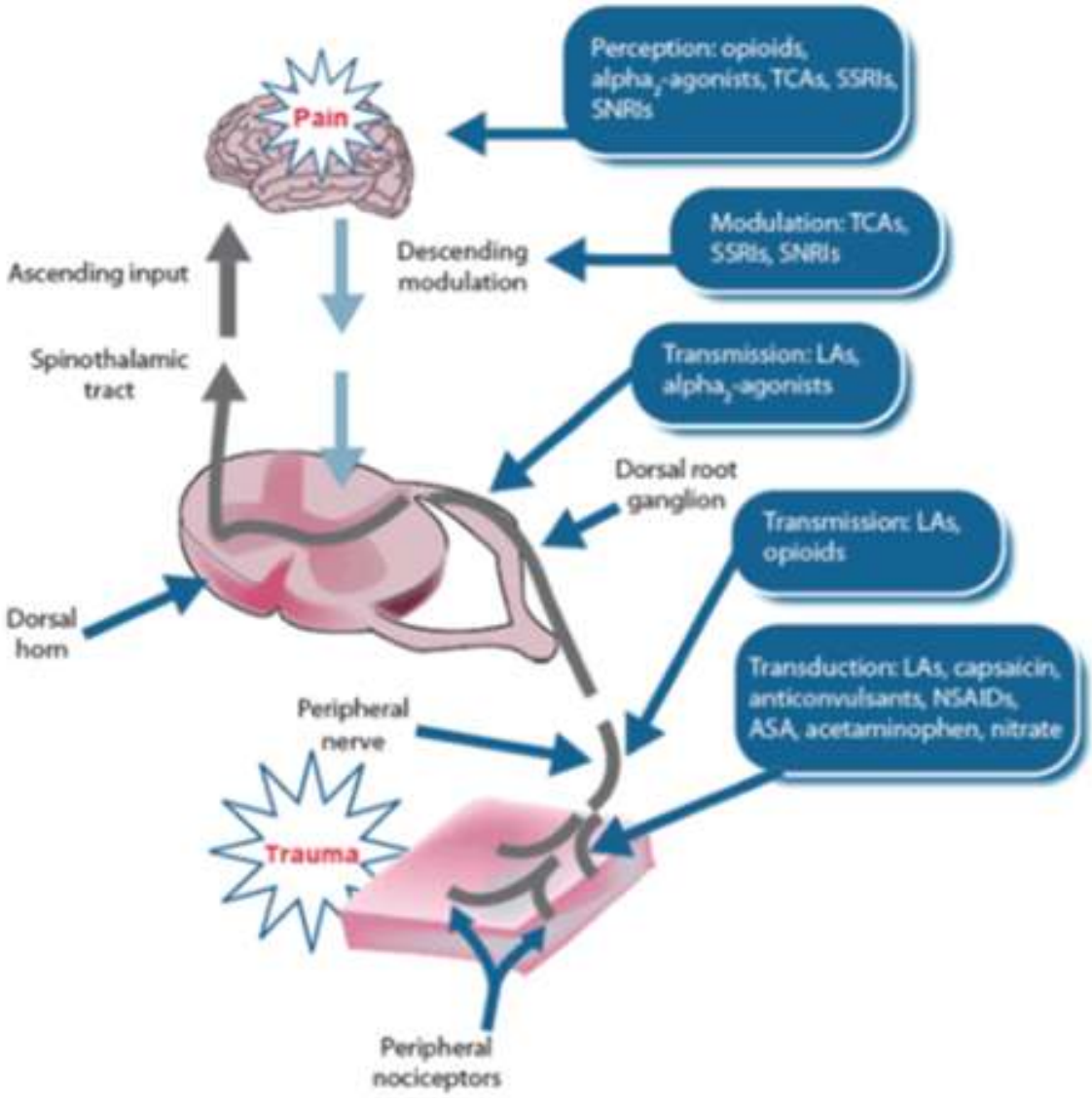


Figure 1: Pathways of Pain Modulation

Source: www.medscape.com

2.1.8.1 Opioids analgesics

Opioid analgesics are used for the treatment of chronic pain. Opioids are effective in chronic malignant pain and moderately effective in non-malignant pain. Opioids do not provide complete analgesia in either acute or chronic pain(Mycek *et al*., 2000).

Opioid analgesics otherwise called narcotic analgesics are natural or synthetic compounds that produce morphine like effects. Opioid analgesics mimic the actions of endogenous opioids peptides, thus act by interacting with *mu, delta* or *kappa* opioid receptors (Stein, 1995). These opioid receptors are coupled to Gi proteins and the actions of the opioids drugs are mainly inhibitory. Opioids close N–type voltage operated calcium channels and open calcium dependent inwardly rectifying potassium channels. This results in hyperpolarization and a reduction in neuronal excitability. Opioids also decrease intracellular cAMP which modulates the release of nociceptive neurotransmitters like substance P (Rang *et al*., 2003).

Although opioids have a broad range of effects, their primary use is to relieve intense pain and anxiety that accompanies the pain, whether from surgery or as a result of injuries or disease such as cancer (Mycek *et al*., 2000). Stimulation of opiate receptors of the CNS can lead to nausea and vomiting (activation of receptors of the brainstem), blunting of pain perception (activation of receptors of spinal cord and thalamus), euphoria (activation of the receptors of the limbic system), and sedation (activation of reticular formation and striatum) (Stein, 1995). Opioid analgesic in use results in changes in the central nervous system. It also results in decrease luteinizing hormone and a consequent decrease in sexual desires. It also results in hormonal imbalances such as reduced secretion of Thyroid Stimulating Hormone (TSH) and increased secretion of

prolactin and growth hormone. Acute respiratory depression occurs due to decreased respiratory centers' sensitivity to carbon dioxide, while in individuals with impaired lungs function and orthostatic hypotension it is due to indirect peripheral vasodilation (Almeida *etal*., 2011).

2.1.8.2 Non- steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAIDS) are a major group of analgesics used for mild to moderate pain (Insel, 1996). Their associated adverse effects of cardiovascular, cerebrovascular and gastrointestinal risks have limited their use (Burke *et al*., 2006).

Non-steroidal anti-inflammatorydrugs act by inhibiting prostanoids biosynthesis. NSAIDs are non-narcotic analgesics which are a group of chemically dissimilar agents with different analgesic, anti-inflammatory and antipyretic activities (Insel, 1996). Prostanoids include prostaglandins (PG) E_2, PGD_2, $PGF_{2\alpha}$, thromboxane A_2 (TXA_2) and prostacyclin (PGI_2). These are special second messengers owing to their ability to cross the cell membrane, diffuse through the extracellular space and interact with high affinity G-protein-coupled receptors located either on the same cell or in neighbouring cells. NSAIDS comprise traditional NSAIDS (tNSAIDS) and NSAIDS selective for cyclo-oxygenase (COX-2). NSAIDS are indicated for pain and stiffness in inflammation such as rheumatoid arthritis and osteoathritis. They act as anti-inflammatory and analgesic agents by inhibiting COX-2 dependent prostanoids in the cells at an inflammatory site and in the spinal cord (Mishra *etal*., 2011). NSAIDS do not inhibit the lipoxygenase pathways of arachidonic acid, and hence do not suppress leukotriene formation. Glucocorticoids suppress the induced expression of COX-2, thus COX-2 mediated

prostaglandin production (Mishra *etal*., 2011). They also inhibit the action of phospholipase A_2, which releases arachidonic acid from the cell membrane (Burke *et al*., 2006). NSAIDS provide only symptomatic relief from pain and inflammation associated with the disease but do not stop the progression of pathological injury to tissues. Although NSAIDS are widely prescribed because of their effectiveness in pain management and improving quality of life, yet adverse effects may be severe. These adverse effects include gastro-intestinal bleeding, ulcer, stomach upset, high blood pressure, fluid retention, renal impairment, heart deseases and allergic reactions such as rashes. Adverse effects of NSAIDS can occur at any time and the chance is greater if used for long durations (Gislason *et al*., 2009).

2.1.8.3Cannabinoids drugs

Some analgesics like cannabinoids exert their action via apoptosis. Apoptosis is the process of programmed cell death that can be induced by either intrinsic factors or extrinsic factors or both morphological changes such as membrane blebbing, cell shrinkage, mitochondrial leakage and nuclear fragmentation (Allison and Samaj, 1995). Molecular changes underlie morphological changes resulting in two pathways of apoptosis (Traversa *etal*., 1995). The intrinsic pathway occurs via mitochondria and the extrinsic pathway occurs through death receptors. Briefly, the intrinsic pathway is initiated by an imbalance in anti-apoptic and pro-apoptic members of Bcl-2 family of proteins that regulate the permeability of the mitochondrial membrane. The imbalance towards the latter lead to cytochrome C leakage into the cytosol. Cytochrome C then combines with pro-caspase 9, ATP and APAF-1 to form the apoptosome. The apoptosome results in the formation of active caspase 9, cleaves pro-caspase 3 into active

caspase 3 and leads to apoptosis. The intrinsic pathway is triggered with ligation of death receptors such as tumor necrosis factor receptor family (CD95), and results in the formation of Death Inducing Signaling Complex (DISC). DISC contains caspase 8 and caspase 10 as the initiator caspases, and these caspases activate caspase 3, resulting in apoptosis. Drugs acting via this mechanism have been shown to be associated with immune functions suppressions (Abbas and Litchman, 2009), lung injury (Ezeonwumelu *etal*., 2012) and super infections (Rang and Dale, 2007). Other agents with analgesic activities include tricyclic antidepressants such as imipramine as well as antiepileptic drugs such as carbamazepine, gabapentin and occasionally phenytoin (Rang *et al*., 1991).

2.1.9Non-pharmacological treatment of pain

Non-pharmacological treatment consist of two main types; less invasive and more invasive options (Thienhaus and Cole, 2002). Non-pharmacological treatments do not rely on medication to alleviate pain. However, in most situations, they are used in addition to pharmacological treatments of pain. Less invasive treatment options include psychiatric approaches, non-invasive stimulatory approaches, psychological approaches and alternative approaches. Psychiatric approaches consist of therapeutic exercise, heat therapy and cold therapy. Therapeutic exercise helps in strengthening weak muscles, mobilizes stiff joints, restoring coordination and balance, promote a sense of well being and helps maintain an appropriate weight. Heat therapyreduces pain from muscles tension or spasms, increases blood flow to the skin, dilates blood vessels, increase oxygen and nutrient delivery to tissues and also decreases joint stiffness by increasing muscle elasticity (Mayer and Liebeskind, 1974). Cold therapy constricts blood vessels near the

skin and helps relieve the pain of muscle tension or spasms. It also helps reduce swelling of an injury(Thienhaus and Cole, 2002).

Non-invasive stimulatory approaches consist of Transcutaneous Electrical Nerve Stimulation (TENS) which is a method of applying gentle electric current to the skin to relieve pain. Itconsists of a small box-shaped device that patients can put in their pocket and it transmits electrical impulses through wires to electrodes taped to the skin inthe painful area. The major disadvantage is that TENS becomes less effective at relieving pain overtime (Mayer and Liebeskind, 1974).

More-invasive approaches arereserved for patients who do not respond to less invasive approaches. Anaesthesiologic approaches include nerve block and epidural steroid injections. Nerve block involves the injection of local anesthetic in the nerves. Although its a temporary block, pain relief can last for a long time (Roberts and Morrow, 2001). In some cases, a solution that can damage the nerve can be injected to produce a more permanent block. Epidural steroid injections can relieve pain by inhibiting the impulses that travel along specific nerves in the body (Giordano, 2005). Epidural steroids injection interrupt the passage of painful impulses through nerves. Invasive stimulatory approaches involves invasive nerve stimulation where electrodes are implanted in the patient's body to send a gentle elecrical current to nerves in the spinal cord or the brain (Mayer and Lebeskind, 1974). Surgical approaches are used in cases where all other approaches have failed. A nerve can be cut close to the spinal cord or bundles of nerves in the spinal cord to interrupt the pathways that send pain signals to the brain (Ramer *etal*., 1998).in most casessurgery relieves pain and the need for pain medication.

2.2 Inflammation

Inflammation is the complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells or irritants (Ferrero-Miliani *etal*., 2007). It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue (Cotran, 1998). Inflammation is different from infection and the two words are not synonyms. Infection is caused by an exogenous pathogen while inflammation is the response of the organism to the pathogen or irritating stimuli (Ferrero-Miliani *et al*., 2007).

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues (Danesh *etal*., 2004, Sharma *et al*., 2010). A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system and various cells within the injured tissue (Abbas and Litchman, 2009). Chronic inflammation is prolonged inflammation which leads to progressive shift in the type of cells, which are present at the site of inflammation, and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process (Doreswamy and Darshan, 2004).

2.2.1 Clinical signs of inflammation

Acute inflammation is a short term process, usually appearing in a few minutes or hours and ceasing once the injurious stimulus has been removed (Adamu *etal*., 2007). It is characterized by five cardinal signs *rubo*r (redness), *calor* (increased heat), tumour (swelling), *dolo*r (pain) and *function laesa* (loss of function) (Cotran*et al.*, 1998).

In the absence of inflammation, wounds and infection may never heal and progressive destruction of the tissue will compromise the survival of the organism. An inflammation that is not monitored can lead to many diseases such as hay fever and rheumatoid arthritis. For this reason, inflammation is closely monitored by the body (Dhannanjayan and Thangam, 2003).

2.2.2 Mechanism of inflammation

2.2.2.1 Acute inflammation

The process of acute inflammation is initiated by cells already present in all tissues, mainly resident macrophages, dendritic cells, histiocytes, Kupffer cells and mastocytes (Cox *etal*., 2012). These cells present on their surfaces certain receptors named *pattern recognition receptors* (PRRs), which recognize molecules that are broadly shared by pathogens but distinguishable from host molecules, collectively referred to as pathogen-associated molecular patterns (PAMPs). At the onset of an infection, burn, or other injuries, these cells undergo activation (one of their PRRs recognizes a PAMP) and release inflammatory mediators responsible for the clinical signs of inflammation. Vasodilation and its resulting increased blood flow cause the redness (*rubor*) and increased heat (*calor*). Increased permeability of the blood vessels results in exudation (leakage) of plasma proteins and fluid into the tissue (edema), which manifests itself as swelling (*tumor*). Some of the released mediators such as bradykinin increase the sensitivity to pain (*dolor*). The mediator molecules also alter the blood vessels to permit the migration of leukocytes, mainly neutrophils, outside of the blood vessels (extravasation) into the tissues (Czock *etal*., 2005). The neutrophils migrate along a

chemotactic gradient created by the local cells to reach the site of injury(Sharma *et al*., 2010). The loss of function (*functio laesa*) is probably the result of a neurological reflex in response to pain (Gale*et al*., 1985).

In addition to cell-derived mediators, several cellular biochemical cascade systems consisting of preformed plasma proteins act in parallel to initiate and propagate the inflammatory response. These include the complement system activated by bacteria, coagulation and fibrinolysis systems activated by necrosis, example is seen in a burn or a trauma (Abbas and Litchman, 2009).

2.2.2.2 Chronic inflammation

Acute inflammation is characterized by marked vascular changes, including vasodilation, increased permeability and increased blood flow, which are induced by the actions of various inflammatory mediators (Giordano, 2005). Vasodilation occurs first at the arteriole level, progressing to the capillary level, and brings about a net increase in the amount of blood present, causing the redness and heat of inflammation (Abramson and Melton, 2000). Increased permeability of the vessels results in the movement of plasma into the tissues, with resultant *stasis* due to the increase in the concentration of the cells within blood - a condition characterized by enlarged vessels packed with cells (Adamu*etal*., 2007). Stasis allows leukocytes to marginate (move) along the endothelium, a process critical to their recruitment into the tissues (Duke, 1992). Normal flowing blood prevents this, as the shearing force along the periphery of the vessels moves cells in the blood into the middle of the vessel (Cotran*et al.,* 1998).

2.2.3 Plasma-derived mediators of inflammation

The followings are plasma-derived mediators of inflammation:

Bradykininis produced by kinin system. It is a vasoactive protein that is able to induce vasodilation, increase vascular permeability, cause smooth muscle contraction and induce pain (Danesh *etal*., 2004).

Complement C3 is produced by complement system and it cleaves to produce *C3a* and *C3b*. C3a stimulates histamine release by mast cells, thereby producing vasodilation. C3b is able to bind to bacterial cell walls and act as an opsonin, which marks the invader as a target for phagocytosis (Rainsford, 2009).

Complement C5ais produced by complement system. It stimulates histamine release by mast cells, there by producing vasodilation. It is also able to act as a chemoattractant to direct cells via chemotaxis to the site of inflammation (Rainsford, 2009).

Factor-XII (Hageman Factor) is produced by liver. It is a protein that circulates inactively, until activated by collagen, platelets or exposed basement membranes via conformational change. When activated, it in turn is able to activate three plasma systems involved in inflammation; the kinin system, fibrinolysis system and coagulation system (Crawford, 1994).

Membrane attack complexis produced by complement system. It is a complex of the complement proteins C5b, C6, C7, C8, and multiple units of C9. The combination and activation of this range of complement proteins forms the membrane attack complex, which is able to insert into bacterial cell walls and causes cell lysis with ensuing death (Abbas and Litchman, 2009).

Plasmin is produced by fibrinolysis system. It is able to break down fibrin clots, cleave complement protein C3 and activate Factor XII.

Thrombin is produced by coagulation system. It cleaves the soluble plasma protein fibrinogen to produce insoluble fibrin, which aggregates to form a blood clot. Thrombin can also bind to cells via the PAR1 receptor to trigger several other inflammatory responses, such as production of chemokines and nitric oxide (Baumann, 2002).

2.2.4 Function of inflammation

Inflammation is basically a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process (Serhan, 2008). The leakage of water and production of a proteinous substance in the injury areas bring about release of humoral factors including antibodies into the area of injury (Danesh *etal*., 2004). The migration of leucocytes to the local site brings about destruction of the injurious agent. In certain situations where the cause of inflammation is due to infection, such as rheumatoid arthritis and rheumatoid fever, this may be uncontrolled by the body's immune system and may require the use of anti-inflammatory drugs and other adjuvant for treatment (Rainsford, 2009).

2.2.5 Morphologic patterns of inflammation

Specific patterns of acute and chronic inflammation are seen during particular situations that arise in the body, such as when inflammation occurs on an epithelial surface, or pyogenic bacteria are involved.

1. **Granulomatous inflammation:**Characterized by the formation of granulomas. They are the result of a limited but diverse number of diseases which include tuberculosis, leprosy, sarcoidosis and syphilis (Porth, 2007).
2. **Fibrinous inflammation:** Inflammation resulting in a large increase in vascular permeability allows fibrin to pass through the blood vessels. If an appropriate

procoagulative stimulus is present, such as cancer cells (Cotran, 1998), a fibrinous exudate is deposited. This is commonly seen in serous cavities, where the conversion of fibrinous exudate into a scar can occur between serous membranes, limiting their function. The deposit sometimes forms a pseudomembrane sheet. During inflammation of the intestine, pseudomembranous tubes can be formed.

3. **Purulent inflammation:** Inflammation resulting in large amount of pus, which consists of neutrophils, dead cells, and fluid. Infection by pyogenic bacteria such as *Staphylococci* is characteristic of this kind of inflammation. Large localized collections of pus enclosed by surrounding tissues are called abscesses (Ferrero-Miliani *et al*., 2007).
4. **Serous inflammation:**Characterized by the copious effusion of non-viscous serous fluid, commonly produced by mesothelial cells of serous membranes but may be derived from blood plasma. Skin blisters is an example of this pattern of inflammation (Parakrama *etal*., 2005).
5. **Ulcerative inflammation:** Inflammation occurring near an epithelium can result in the necrotic loss of tissue from the surface, exposing lower layers. The subsequent excavation in the epithelium is known as an ulcer (Crawford, 1994).

2.2.6 Inflammatory disorders

Inflammatory abnormalities are a large group of disorders that underlie a vast variety of human diseases. The immune system is often involved with inflammatory disorders, demonstrated in both allergic reactions and some myopathies, with many immune system disorders resulting in abnormal inflammation. Non-immune diseases with etiological origins in inflammatory processes include cancer, atherosclerosis and ischaemic heart disease(Cotran, 1998). A large variety of proteins are involved in inflammation and any

one of them is open to a genetic mutation which impairs or otherwise deregulates the normal function and expression of that protein.

Examples of disorders associated with inflammation include: acne vulgaris, rheumatoid arthritis, asthma, sarcodoisis, autoimmune diseases, celiac disease, vasculitis, transplant rejection, inflammatory bowel diseases, glomerulonephritis and chronic prostatitis.

2.2.7 Resolution of inflammation

The inflammatory response must be actively terminated when no longer needed to prevent unnecessary damage to tissues (Cotran, 1998). Failure to do so results in chronic inflammation and cellular destruction. Resolution of inflammation occurs by different mechanisms in different tissues. Mechanisms that serve to terminate inflammation include:

1. Short half-life of inflammatory mediators*in vivo.*
2. Production and release of transforming growth factor (TGF) beta from macrophages.
3. Production and release of Interleukin 10 (IL-10) (Serhan, 2008).
4. Production of anti-inflammatory lipoxins(Greenhalgh, 1998).
5. Down regulation of pro-inflammatory molecules such as leukotrienes.
6. Up regulation of anti-inflammatory molecules such as the Interleukin 1 receptor antagonist or the soluble tumor necrosis factor receptor (TNFR).
7. Apoptosis of pro-inflammatory cells (Jiang *et al*., 2005).
8. Desensitization of receptors.
9. Increased survival of cells in regions of inflammation due to their interaction with the extracellular matrix (ECM) (Teder *etal*., 2002).
10. Down regulation of receptor activity by high concentrations of ligands

11. Cleavage of chemokines by matrix metalloproteinases (MMPs) might lead to production of anti-inflammatory factors (Serhan and Chiang, 2005).
12. Production of resolvins, protectins or maresins.

2.2.8 Management of inflammation

The treatment of patients with inflammation involves three primary goals. First are the relief of pain which is often the presenting symptom and the major continuing complaining of the patient. Secondly is the arrest of tissue damaging processes and thirdly is to improve the quality of life of the patient (Cousins *etal*., 2004). NSAIDs are often used in the reduction of inflammation which results in the relief of pain for a significant period (Sharma *et al*., 2010). Furthermore, most of the NSAIDs are appropriate for the treatment of both acute and chronic inflammation condition. NSAIDs possess analgesic, anti-inflammatory and antipyretic properties (Ezeonwumelu *etal*., 2012). The term non-steroidal word is used to distinguish these drugs from steroids, which also have among other effects a similar eicosanoid depressing anti-inflammatory action (Mishra *etal*., 2011). These steroids are also used in alteration or modulation of inflammatory conditions in some cases. Example includes Prednisolone, Dexamethasone, Triamcinolone, Prednisone and Budesonide.

2.2.8.1 Prednisolone

Is a synthetic glucocorticoid, a derivative of cortisol, which is used to treat a variety of inflammatory and auto-immuneconditions. It is the active metabolite of the drug prednisone(Davis *etal*., 2000) and is used especially in patients with hepatic failure because these individuals are unable to metabolize prednisone into prednisolone.

Medical Uses:

Prednisolone is a corticosteroid drug (corticosteroids inhibit the inflammatory response to a variety of inciting agents and it is presumed, delay or slow healing) with predominant glucocorticoid and low mineralocorticoid activity. This makes it useful for the treatment of a wide range of inflammatory and auto-immune conditions (Czock *et al*., 2005) such as asthma (Field *et al*., 1998), uveitis, pyoderma gangrenosum, rheumatoid arthritis, ulcerative colitis, pericarditis, temporal arteritis,Crohn's disease, Bell's palsy, multiple sclerosis, (Thrower, 2009), cluster headaches, vasculitis, acute lymphoblastic leukemia and autoimmune hepatitis (Lambrou *et al*., 2009), systemic lupus erythematosus, Kawasaki disease and dermatomyositis. It is also used for treatment of sarcoidosis even though the mechanism is unknown (Miura *et al*., 2011).

2.2.8.2Budesonide

Is a glucocorticoidsteroid for the treatment of asthma, chronic obstructive pulmonary disease and non-infectious rhinitis (including hay fever and other allergies). It is also used for treatment and prevention of nasal polyposis in addition to its use for Crohn's diseaseinflammatory bowel disease(Czock *et al*., 2005).

Medical uses

- Ulcerative colitis (colon inflammation): Budesonide assists in the induction of remission in patients with active ulcerative colitis (Habal and Huang, 2010).

- Crohn's disease: Treatment of active Crohn's disease involving the ileum and/or ascending colon inflammation; maintenance of remission (for up to 3 months) of Crohn's disease (mild-to-moderate) involving the ileum and/or ascending colon (Lichtenstein *et al*., 2009).

- Asthma**:** Budesonide is nebulized for maintenance and prophylactic treatment of asthma including patients who require oral corticosteroids and those who may benefit from systemic dose reduction (Svedmyr *et al.,* 1999).

2.3 Toxicology

Toxicology has been defined as the study of the adverse effects of xenobiotics. Modern Toxicology goes beyond the study of the adverse effects of exogenous agents to the study of molecular biology, using toxicant as tools. Historically, Toxicology formed the basis of therapeutics and experimental medicine. Toxicology from (1900 to the present) continues to expand and develop by assimilating knowledge. A recent addition to the field of Toxicology (1975 to the present) is the application of the discipline to safety evaluation and risk assessment (Lorke, 1983).

Toxicology, like medicine, is both a science and an art. The science of Toxicology is defined as the observation and data gathering phase, whereas the art of Toxicology are used to develop extrapolations and hypotheses to explain the adverse effects of chemical agents in situations where there is little or no information (Casarette *et al*., 1996).

2.3.1 Spectrum of toxic dose

Poison can be defined as any agent capable of producing a deleterious response in a biological system, seriously injuring functions or producing death. "All things are poisons and nothing is without poison, solely the dose determines whether a thing is a poison or not" (Grandjean, 2016). Among chemicals, there is a wide spectrum of doses needed to produce deleterious effects, serious injury or death. Some chemicals produce death in microgram doses and are commonly thought as being extremely poisonous. Other chemicals may be harmless after doses in excess of several grams. It should be noted,

however, the measures of acute lethality such as LD_{50} may not accurately reflect the full spectrum of toxicity or hazard associated with a drugs or chemical (Casarette *et al*., 1996).

2.3.2 Duration and frequency of exposure

Toxicologists usually divide the exposure of animals to chemicals into four categories which include:

1. Acute exposure: This is exposure to a chemical for less than 2 hours and examples of exposure routes are intraperitoneal, intravenous, subcutaneous, oral and dermal application.
2. Sub-acute exposure: refers to repeated exposure to a chemical for one month or less.
3. Sub chronic exposure: - refers to exposure for 1 to 3 months
4. Chronic exposure: This is exposure to a repeated dose (low) for a prolong period of time (Casarette *et al*., 1996).

2.3.3 Descriptive animal toxicity test

Two main principles underline all descriptive animals to toxicity testing:

The first is that the effects produced by a compound in laboratory animals, when properly qualified are applicable to humans. On the basis of dose per unit of body surface, toxic effects in humans are usually in the same range as those in experimental animals. On a body weight basis, humans are generally more vulnerable than experimental animals.

The second principle is that exposure of experimental animals to toxic agents in high doses is necessary and valid method of discovering possible hazards in humans. Toxicity tests are not designed to demonstrate that a chemical is safe but to characterize the toxic effects a chemical can produce (Casarette *et al*., 1996).

2.3.4 Mechanism of toxicity

Depending primarily on the degree and route of exposure, chemicals may adversely affect the function or the structure of living organisms (Lorke, 1983). The qualitative and quantitative characterizations of these harmful or toxic effects are essential for an evaluation of the potential hazard posed by a particular chemical (Garba *etal*., 2011). An understanding of the mechanisms of toxicity is of both practical and theoretical importance. Elucidation of the mechanisms of chemical toxicity has led to a better understanding of fundamental physiological and biochemical processes. Pathological conditions such as cancer and Parkinson's disease are better understood because of the studies on the mechanism of toxicity of chemical carcinogens (Casarette *et al*., 1996).

2.3.5Pathophysiology of the liver damage

Hepatic responses to chemical exposure depend on the intensity of the exposure, the population of cells affected and whether the exposure is acute or chronic. Acute poisoning with carbon tetrachloride causes lipid accumulation rapidly and before necrosis is evident some chemicals produce a very specific type of damage (Casarette *et al*., 1996). The pathophysiology is in two phases; the cholestasis and necrotic phase.

Cholestasis is characterized by an accumulation of compounds that cannot be excreted because of occlusion or obstruction of the biliary tree. Hence, the serum concentration of substances (bile pigments, enzymes, bile salts) that normally are present within or eliminated via bile will increase in cholestatic conditions. Alkaline phosphatase (AP), Gamma glutamyl transpeptidase (GGT) and conjugated bilirubin, all of which require a clear biliary tree for elimination, will be elevated (Crawford, 1994).

Conversely, necrosis of hepatocytes following a viral or toxic injury to the liver (e.g.

acetaminophen overdose or viral hepatitis) will cause primarily an elevation of enzymes found within the hepatocyte such as the amino transferases. In hepatocellular disease, the serum levels of GGT and AP do not rise to the same degree as the amino transferases (Nandi *et al*., 1998).

Most drugs-induced liver damage results from the direct toxic effects of drugs or their metabolite as described above. However, hypersensitivity reactions are sometimes involved. A typical example is halothane-induced hepatic necrosis. Trifloroacetyl chloride, a reactive metabolite of halothane couples to macromolecules to form an immunogene. Most patients with halothane-induced liver damage have antibodies that react with halothane-carrier conjugates. There is evidence from rabbit experiments that halothanes-proteins antigens can be expressed on the surface of hepatocytes. Destructions of the cells occur by type II hypersensitivity reactions involving killer T-cells (type III reactions can contribute). Enflurane may also cause antibody-mediated liver damage, and apparent cross sensitization with halothane is reported (Kenna *et al.,* 1993).

2.3.6 Nephrotoxicity

Drug-induced nephrotoxicity is a common clinical problem with non-steroidal anti-inflammatory drugs and angiotensin- converting enzymes inhibitors (ACE I) as essentially the commonest causes of acute renal failure (Murray and Bratter, 1993). This is usually caused by the principal pharmacological actions of these drugs, which although well tolerated by healthy people, cause renal failure in patients with diseases that jeopardize glomerular filtration rate (GFR). GFR depends solely on vasodilator prostaglandin biosynthesis (Gislason *etal*., 2009). This is inhibited by NSAIDs and hence these drugs reduce renal perfusion in such patients. Similarly, in patients with bilateral

renal artery stenosis, GFR depends on angiotensin-II-mediated efferent arteriolar vasoconstriction (which is inhibited by ACE I) (First, 1996). Acute renal impairment occurs on starting an ACE I drug and is reversible if the drug is discontinued promptly. Additionally, NSAIDS indirectly depress renin and aldosterone secretion by inhibiting renal prostaglandin I_2 biosynthesis, and ACEI depress angiotensin II-stimulated aldosterone secretion. Reduced aldosterone can cause hyperkalaemia, especially if GFR is also reduced (Rang and Dale, 2007).

2.4 Fever/Pyrexia

Fever, also known as pyrexia or a febrile response, is defined as a body temperature above the normal range (37^oC) due to an increase in the temperature regulatory set-point (Axelrod and Diringer, 2008). As a person‘s temperature increases, there is, in general, a feeling of cold and once the new temperature is reached, there is a feeling of warmth (Srivastava*et al.,* 2013). Elevated body temperature can be caused by abnormalities in the brain itself or by toxic substances in the temperature-regulating centres (Sharma *et al.,* 2013*).* Fever results from various causes such as liver disease, tumours, infections and toxic drugs (Sharma *et al.,* 2013).

2.4.1 Signs and Symptoms of Fever

A fever is usually accompanied by sickness behaviour, which consists of lethargy, depression, anorexia, sleepiness, hyperalgesia, and the inability to concentrate (Kelly *et al.,* 2003).

2.4.2 Pathophysiology of Fever/Pyrexia

Temperature is ultimately regulated in the hypothalamus. A trigger of the fever, called a pyrogen, causes release of prostaglandin E2 (PGE2). PGE2 in turn acts on the

hypothalamus, which creates a systemic response in the body, causing heat-generating effects to match a new higher temperature set point. In many respects, the hypothalamus works like a thermostat. (Fauci *et al.,* 2008) When the set point is raised, the body increases its temperature through both active generations of heat and retention of heat. Peripheral vasoconstriction both reduces heat loss through the skin and causes the person to feel cold. Norepinephrine increases thermogenesis in brown adipose tissue, and muscle contraction through shivering raises the metabolic rate. If these measures are insufficient to make the blood temperature in the brain match the new set point in the hypothalamus, then shivering begins in order to use muscle movements to produce more heat. When the hypothalamic set point moves back to baseline either spontaneously or with medication, the reverse of these processes (vasodilation, end of shivering and nonshivering heat production) and sweating are used to cool the body to the new, lower setting.This contrasts with hyperthermia, in which the normal setting remains, and the body overheats through undesirable retention of excess heat or over-production of heat. (Fauci *et al.*, 2008) Hyperthermia is usually the result of an excessively hot environment (heat stroke) or an adverse reaction to drugs. Fever can be differentiated from hyperthermia by the circumstances surrounding it and its response to anti-pyretic medications (Cotran, 1998; Beard and Day, 2008).

2.5Screening Models for Pain, Pyrexia and Inflammation

2.5.1 Screening models for pain

Pain models have been classified into: Thermal, electrical, mechanical and chemical stimuli according to the kind of stimuli applied. The neuronal basis of this models is not

clearly known, however they are used to predict analgesic activity of new substances (Parle and Yadav, 2013).

2.5.1.1 Test based on thermal stimuli

2.5.1.1.1 Tail flick test using radiant heat

It is a model used for screening analgesic agents' response in animals. When thermal radiation is applied to the tail/paw of the animal it triggers the animal to withdraw its tail/paw from the thermal source. Tail withdrawal from the heat source is referred to as "tail flick latency". Time taken for the animal to withdraw its paw/tail from the heat source in this model is timed and recorded (Smith *et al*., 1943).

2.5.1.1.2 Hot plate test

This involves placing an animal in an open-ended cylindrical space with the surface consisting of hot metal or boiling liquid by a thermode. The time taken before paw licking and jumping from the heat source is recorded, this is called reaction time. Both licking of the paw and jumping from heat source are considered as supra-spinally integrated response. The model is used for assessing new analgesics agents (Parle and Yadav, 2013).

2.5.1.2 Test based on mechanical stimuli

The hind paw and the tail of animals are ideal sites for applying nociceptive mechanical stimuli. In this model the tail or paw is jammed between two plane surfaces and the pressure of increasing intensity is applied until the animal begins a response behavior of withdrawing its tail or hind paw from the two planes. The vocal reaction taken by the animal to withdraw tail or hind paw from the two plane surfaces is timed and recorded (Green *et al*., 1951).

2.5.1.3 Test based on electrical stimuli

2.5.1.3.1 Electrical stimulation of the tooth-pulp

Electrical current is applied to the tooth-pulp of the laboratory animal in this model. This produces behavioral characteristic reaction such as head flick, biting, chewing, and licking of the tooth pulp due to induction of pain. Time taken for the above observation is timed and recorded (Parle and Yadav, 2013).

2.5.1.3.2 Electrical stimulation of the tail

In this model electrical current of increasing intensity is applied to the tail of the rat or mice. This will generate some observed reflex movement in the tail with increasing electrical currents. Time taken for the animal to withdraw its tail from electrical stimuli is timed and recorded. Morphine or drugs falling in the same class as morphine are effective in this model (Parle and Yadav, 2013).

2.5.1.4 Test based on chemical stimuli

2.5.1.4.1 Formalin-Induced pain test

This model is sensitive to peripherally acting analgesics agents. Nociceptive effect of formalin is considered biphasic. The initial phase is mediated by serotonin (5-HT), histamine and kinin. The second phase is mediated by prostaglandins (Turner, 1965). When formalin is injected into the hind paw of the animal it elicits a painful behavior such as licking of the paw, bitting and lifting. The time taken by the animal to lick, lift and bit the paw is timed and recorded (Parle and Yadav, 2013).

2.5.1.4.2 Acetic acid-induced pain test

In this model acetic acid or phenylquinone is used to induce pain in mice or rats by injecting these irritants into the peritoneal cavity. The animal responds by characteristics

such as stretching of hind paw, turning of trunk, abdominal musculature contraction and stomach touching the floor. This model is sensitive to peripherally acting analgesics.

This agent irritates the serous membrane andtherefore elicite stereotype behaviour. The number of abdominal writhes within a given period is timed and recorded (Parle and Yadav, 2013).

2.5.2 Screening models for pyrexia

2.5.2.1 Turpentine-induced test

Turpentine induces fever slowly reaching its peak at 11th hour after its injection. Macrophages release protein signals such as interleukin-1 and interleukin-6 to counteract the pyrogen (Leon, 2002). Interleukin acts on the temperature regulating hypothalamus to increase body temperature. Stimulating the hepatocystes to secrete acute phase proteins and increasing number of circulating eosinophils and neutrophils begin to neutralize turpentine (Cartmell *et al*., 2000). Turpentine injection into experimental animals induces a persistently high fever pattern (Kuochung *et al*., 2006).

2.5.2.22, 4-Dinitrophenol (DNP)-induced test

2, 4-Dinitrophenol (DNP) is known to induce febrile response through uncoupling oxidative phosphorylation. This then would result in fast consumption of energy without generating adenosine triphosphate causing the release of calcium from its mitochondrial stores and subsequently prevent the uptake of calcium. This leads to free intracellular calcium, muscle contraction and hyperthermia and Energy proton gradient would then be lost as heat (Kumar *et al*., 2002).

2.5.2.3 Brewer's yeast-induced test

Brewer's yeast induces pyrexia after injection subcutaneously into the experimental animal.When subcutaneously injected into the experimental animal; brewer's yeast binds to an immunological protein called lipopolysaccharide-binding protein and these results to release of various endogeneous cytokine factors such as phagocyte and interleukin-1; interleukin will act on T lymphocytes and this will in turn result in hypothalamus producing prostaglandins (Dubey and Maheswari, 2005). Brewer's yeast is also known to induce TNFα and prostaglandins (Kluger, 1991).

2.5.3 Screening models for inflammation

2.5.3.1 Carragenaan-induced hind paw oedema test

Carrageenan has widely been used as a harmful agent for inducing inflammation in laboratory animals and for screening compounds possessing anti-inflammatory activities. When injected into experimental animal this phlogistic agent produces a severe inflammatory reaction (Marzouk *et al*., 2010). Freshly prepared solution of carrageenan of between 1-3% is commonly used and is injected into experimental animal at a dose of 50-150 ul (Naude *et al*., 2010). Carrageenan is known to induce oedema in mice models in two phases and is dependent on age and weight of the experimental animal (Vinegar *et al*., 1969). The first phase (0-2 hours) is due to release of inflammatory mediators such as; serotonin and histamine, resulting in sensitization of central nociceptor neurons.The lysosome, protease, prostaglandins and bradykinin are released majorly in the second phase (D'Amour *et al*., 1965).The second phase (2.5-5 hours) of oedema is sensitive to clinically used anti-inflammatory drugs.

During the second phase prostaglandins play a major role in inflammatory reaction and can stimulate the nociceptors and thus induce pain (Dacie, 1958). The initial phase is due to release of inflammatory mediators such as serotonin and histamine while the second phase is mediated by lysosome, protease, bradykinin and prostaglandins (Vinegar *et al*., 1969).

2.5.3.2 Xylene-induced ear oedema test

Application of xylene to the ear of laboratory animal induces a neurogeneous oedema that is partly related to substance P. Substance P is released from neurons in the midbrain in response to stress and is an undecapeptide of central and peripheral nervous system. When substance P is released from sensory neurons in the periphery, it causes plasma extravasations and vasodilatation resulting into inflammation (Kou *et al*., 2005).

2.5.3.3 Formalin-induced paw oedema test

This technique is based on the ability of the drug to hinder inflammation produced in the hind paw of the mice or rat after injection with formalin. Nociceptive effect of formalin is biphasic, consisting of two phases. The first phase is mediated by the release of histamine, serotonin (5-HT), and kinin while the second phase is majorly mediated by prostaglandins. The size of oedema is measured using string or a vernier caliper and recorded (Turner, 1965).

2.5.3.4 Cotton pellet granuloma test

In this model, laboratory animal is subcutaneously implanted with cotton pellets in the dorsal region to induce granulomas lesion. This model is used to asses proliferative phase of inflammation. Inflammation involves the proliferation of macrophages, neutrolphils and fibroblast which are responsible for granuloma formation (Winter and Porter, 1957).

2.6 Herbal Management of Pain, Fever and Inflammation

2.6.1 Pain

Throughout history man has used different forms of therapy to relief pain. Morphine for example was isolated from a medicinal plant *Papaver somniferium* (De souza, 2011). The search of herbal plants with analgesic activities, used as pain relievers should be viewed as a successful search for new pain relieving drugs (Elisabetskey *et al.,* 1995). Considering that most of anti-inflammatory, analgesic, anti-malarial and anti-pyretic synthetic drugs such as aspirin, morphine, artemisinin, atrophine and chloroquine were derived from the plant products (Gupta *et al*., 2006). White Willow Bark (*Salix alba*) has been used traditionally in management of inflammation, mild feverish colds, influenza, headache, arthritic conditions and muscle spasm (Vadivelu *et al*., 2011). Its anti-inflammatory, antipyretic and antiuricosuric activities have been attributed to flavonoids, tannins and salicylates. Salicin extract when taken at a dose level of 120-240 mg on daily basis can reduce back pain in some patients (Vadivelu *et al*., 2011). *Harpagophytum procumbens* (devil's Claw) has traditionally been used to manage symptoms associated with pain such as, low back pain, osteoarthritis, rheumatoid arthritis, gastrointestinal disorders, gout, myalgia, chronic low back pain and lumbago. Inhibition of both cyclooxygenase and lypoxygenase inflammatory pathways according to current research have been linked to herpagoside (Chrubasik *et al.*, 2004; Vadivelu *et al*., 2011).

The analgesic activities of ginger and ibuprofen showed no significant difference in management of pain indicating that herbal extract do have antinociceptive activity (Bliddal *et al*., 2000). The antinociceptive activity of ginger is assumed to be through inhibition of COX 2 and lipooxygenase (Srivastava and Mustafa, 1989). Likewise,

Mworia *et al.,* (2015) demonstrated antinociceptive activities of leaf extract of *Carissa spinarum* on acetic acid-induced pain test in Swiss albino mice models.The extract showed dose dependent response with 100 mg/kg body weight having the highest inhibition percentage compared to 50 mg/kg body weight.A similar study conducted by Kariuki *et al*. (2012) on root extract of *Toddalia asiatica* exhibited antinociceptive activity in adult Swiss albino mice when pain was induced using acetic acid, hot plate and tail flick test in laboratory animals.

2.6.2 Pyrexia

Though medicinal plants from time immemorial have been used as source of antipyretic agents to manage fever, the emergence of synthetic drugs however, resulted in neglect of their use. However, due to its availability, low cost and fewer side effects herbal medicine is gaining its popularity (Sharma *et al*., 2010). Treatment of fever dates back to 400 B.C years ago when Greek Hippocrates prescribed an extract from the willow bark and leaves (Rao and Knaus, 2008). Tumeric (*Curcuma longa*) is an ancient spice that has been used traditionally as condiment, medicine, and flavouring agent. Its medicinal properties are attributed to the compound curcuminoid. Curcuminoids, a major phytochemical compound in the plant is believed to exert it antipyretic activity by inhibiting both 5-lipooxygenase and cyclooxygenase enzymes (Chandra and Gupta, 1972).

According to Vadivelu *et al*. (2011), white Willow Bark (*Salix alba*) contains heavy concentration of salicin and glycoside a precursor for aspirin. The therapeutic application of *Salix alba*in traditional system of medicine hasspanned over centuries in management of headache, mild feverish colds, arthritic conditions, influenza and inflammation. The

antipyretic activities of *Salix alba*has been atributed to flavonoids, tannins and salicylates present in the extract (Vadivelu *et al*., 2011).

In a study conducted by Lapah *et al., (*2014) on aqueous extract of *phragmanthera capitata*, the extract exhibited antipyretic activity in Sprague dawley rats. Dose level of 100 mg/kg and 200 mg/kg body weight extract reduced body temperature significantly when compared to reference drug. In a similar study conducted by (Mwonjoria *et al.,* 2011) on antinociceptive and antipyretic activities of methanolic root extract of *Solanum incanum* (linneaus) in animal model using brewer's yeast induced-pyrexia demonstrated significant antipyretic activities. The antipyretic activities of 50 mg/kg and 100mg/kg body weight were comparable to reference drug (Mwonjoria *et al.,* 2011).

2.6.3 Anti-inflammatory

For centuries, mankind has used herbal medicine for relieving inflammation and pain (Sen *et al*., 2010). *Boswellia serrata* is among many herbal plants used in ayurvedic medicine and it has been used traditionally in management of pain associated conditions such as osteoarthritis, tendonitis and rheumatoid arthritis. Boswellic acids in *Boswelia serrata* in a number of laboratory studies about its anti-inflammatory activity have shown to exert its effects by inhibitingleukotrienes an inflammatory mediator (Vadivelu *et al*., 2011). The principle components that have been associated with anti-inflammatory activities are boswellic acid, α-boswellic and β-boswellic (Vadivelu *et al*., 2011). Tumeric (*Curcuma longa*) is an ancient spice and condiment that has been used as herbal medicine in India and china. Its medicinal value is attributed to curcuminoid. Curcuminoids exert is activity by inhibiting 5-lipooxygenase and cyclooxygenase

enzymes resulting in a well-established anti-inflammatory activity (Chandra and Gupta, 1972).

Camellia sinensis commonly known as tea plant is an important herbal plant. The leaves and buds produce tea, the most consumed beverage in the world. The anti-inflammatory activity of green tea has been attributed to high content of polyphenols/catechins and mainly epigallocatechin-3-gallate. Potential of green tea in management of arthritis on collagen type-II-induced arthritis in mice has been reported (Curtis *et al*., 2004). Likewise, study carried out by Mwangi *et al*. (2014) on leaf extract of *Caesalpinia volkensii* and *Maytenus obscura* exhibited anti-inflammatory activities in animal models. In addition, a study by Onasanwo *et al*. (2012) on methanolic, hexane, dichloromethane and chloroform leaf extracts of *Anacardium occidentalis* demonstrated strong anti-inflammatory and analgesic activity in animal models. Furthermore, a study conducted by Ravi *et al*. (2009) on methanolic bark extract of *solanum nigrum* berries demonstrated a dose dependent response in reducing the inflamed hind paws of rats.

2.7 *Culcasia angolensis* Welw, ex schott

2.7.1 Description of the plant

Culcasia angolensis commonly called Hyrmim in Ham language by the people of Jaba Local Government Area of Kaduna state, Nigeria, andbelongs to the family Araceae. Itis a robust forest climber with stems that can grow more than 30 metres long (Plate I). They have a thick tough stem, about 6cm in diameter that adheres to host trees by means of clasping roots (Burkil, 1985).It is commonly found in tropical Africa Sierra Leone, Cameroon, Angola, DR Congo, Nigeria and Ivory Coast (Burkil, 1985).Stem climbing to a height of 80–100 ft., 1/3– 1/2 in. thick, not tubercled. Leaves large, 2–6 in. distant,

glabrous; petioles 4–10 in. long, sheathing up to 1–2 in. from the top; apex of sheath prominent, rounded; blade 7–15 in. long, 4–7 1/2 in. broad, unequal-sided, elliptic-oblong, cuspidate-acute, or shortly acuminate, broadly rounded or subtruncate at the base (Plate II); primary lateral veins 10–15 on each side of the midrib, prominent beneath; no glands. Peduncles numerous in a stout terminal bracteate raceme, 1 3/4–3 1/2 in. long, moderately stout. Bracts 3–4 in. long, about 1–1 1/4 in. broad, oblong, obtuse or acute, with 2 wing-like keels down the back. Spathe about 2 1/2 in. long and 2 in. broad, elliptic, obtuse, apiculate, expanded, deeply concave, very shortly convolute at the base, green (Mann), soon falling off; margins revolute. Spadix shorter than the spathe, sessile, clavate; female part about 5 lin. long, 1/4 in. thick; male part 1 1/4–1 1/2 in. long, 4–6 lin. thick near the obtusely rounded apex. Ovaries about 25–28, depressed-globose, 2-celled; stigma large, discoid. Anthers densely crowded, in groups of 4. Berries red (Burkil, 1985).

2.7.2 Taxonomy

The genealogy of the plant is as follows;

Kingdom: Plantae

Clade: Angiosperms

Clade: Monocots

Order: Alismatales

Family: Araceae

Subfamily: Aroideae

Genus:*Culcasia*

Species:*Culcasia angolensis* Welw. ex schott

2.7.3 Ethnobotanical use

The whole plant is harvested from the wild for local medicinal use. The plant is recognized in Ivory Coast as toxic and abortifacient; the leaves of the plant causes uterine contractions and so it is effective in treating menstrual problems, aiding child birth, or causing an abortion (Bown, 2000). In Nigeria the plant is used for the treatment of pain and inflammation resulting from fracture and woundas shown in Plate I (S. Whayh, 18 September 2019, Personal discussion).

Plate I:*Culcasia angolensis* whole plant in its natural habitat at Ngaring Nok, Jaba Local Government Area, Kaduna state

Plate II:*Culcasia angolensis* leaves under shade drying

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Collection

The plant leaves were collected from Ngaring Nok in Jaba LGA, Kaduna State Nigeria in April, 2017 and was identifiedby Mr. Namadi Sanusi of the Herbarium unit of theDepartment of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria by comparing with a specimen voucher number 01676 previously deposited in the herbarium.

3.1.1 Preparation of extract

The plant leaves were cleaned anddried under shade with intermittent weighing until a constant weight was obtained. The leaves were size-reduced using mortar and pestle. The powdered materials 1500 g was extracted with 2 L of 70%$^{v}/_{v}$ methanol usingcold maceration for 72 hours. The extract was concentrated under reduced pressure at a temperature of 45^{o}C. The extract was stored in desiccators until needed for the work. Aqueous solutions were freshly prepared for each study using distilled water.

3.2Animals

Wistar rats (150-200grams) and Swiss albino mice (20-25 grams) of either sex wereobtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria. They were housed in standard propylene cages and kept under natural day and light cycle. The animals were fed on standard laboratory animal diet and water *ad libitum*. Food was withdrawn during the experimental hours to verify pharmacological outcomes (OECD Guidelines 423, 2008). All experimental protocols were approved by

the Ahmadu Bello University Committee on Animal Use and Care(Approval No: ABUCAUC/2018/086).

3.3 Drugs and Chemicals

The chemicals and drugs used for the studies include; Acetic acid(Ranbaxy Laboratories Ltd, Punjab); Ketoprofen (Lek; Slovenia); Piroxicam (Pfizer laboratories, Pakistan);Paracetamol (Vitabiotics, Nigeria); Methanol leaf extract of *Culcasia angolensis* (MECA); Concentrated hydrochloric acid(BDH Limited Poole, England); Chips of magnesium metal(BDH Limited Poole, England);Concentrated sulphuric acid(BDH LimitedPoole, England); Strong lead subacetate solution;Chloroform(Sigma Chemical Co. USA); Ferric chloride(BDH Ltd Poole, England); Glacial acetic acid(Searle Essex, England); Acetic acid anhydride (BDH Ltd Poole, England) and Strong ammonia (BDH Ltd Poole, England).

3.4 EquipmentandOtherMaterials

Animal cages, pestle and mortar, syringes (1mL, 2mL, 5mL and 10mL), filter paper, pair of scissors, mettler balance p165, hot plate (MR 2002), soxhlet apparatus (Gallenkamp), measuring cylinders, separating funnel,beakers, test tubes, funnel, test tube holders, crucible, water bath (Gallenkamp Cat No:H1054), retort stand, water drinkers, weighing mettle balance p165.

3.5 Phytochemical Screening of the Leaf Extract of*Culcasia angolensis*

Phytochemical screening was carried out on the methanol leaf extractof *Culcasia angolensis* as follows:

3.5.1Test for flavonoids (shinoda and sodium hydroxide tests)

About 0.5 g of the extract was dissolved in 2 mL of 50% methanol. Few magnesium chips and 3 drops of hydrochloric acid were added and the pink or tomato red colour within few minutes indicates the presence of flavonoids. Also, few drops of aqueous sodium hydroxide were added to about 5 mL solution of extract or each of its fractions; a yellow colouration indicates the presence of flavonoids(Evans, 1996).

3.5.2Test for cardiac glycosides

Keller Killiani's test: Glacial acetic acid (1 mL) was added to about 3mL of the solution containing2 mg of the extract in a test tube held at 45^{o}; then two drops of concentrated H_2SO_4 was added along the side of the test tube.Formation of purple ring colour at the interface wasobserved for the presence of cardiac glycosides(Evans, 1996).

3.5.3Test for saponins

Methanol extract (0.5 g) was shaken with 3mL of distilled water for 30 seconds and allowed to stand for 30 minutes. Formation of honey comb which persisted for more than 30 minutes was observedfor the presence of saponins(Sofowora**,** 1993).

3.5.4Test for tannins

Lead sub-acetate test: Methanol extract (0.5g) was dissolvedin 2mL of distilled water; 3 drops of lead sub-acetate solution was then added and observed for formation of black-green coloured precipitate which indicated the presence of tannins (Evans, 1996).

3.5.5Test for steroids and triterpenes

Liebermann-Burchard's test: Powdered plant material (0.5 g) was extracted with 5mL of methanol then filtered. The filtrate was evaporated to dryness on a water bath at 100^{o} C. The residue obtained was shaken with 2 mL chloroform and then filtered into a

cleanedand dried test tube. Acetic acid anhydride 2 mL was added to the filtrate and shaken, and then 1mL of concentrated sulphuric acid was also added carefully down the zone of contact of the two liquids.A brownish-red colour was observedimmediately at interphase and violet blue-green at the upper layer later. Red colour observed indicated the presence of triterpenes while blue-green colour indicated steroids (Evans, 1996).

3.5.6Test for alkaloids

Methanol extract (0.5 g) was treated with 5mls of 1% aqueous HCl and then heated. It was filtered and the filtrate was divided into four portions in four test tubes **(**Sofowora,1993).

a. Dragendoff's reagent: Two drops of dragendoff's reagent were addedto test tube 1 and rose red precipitateobserved indicated the presence of alkaloids.
b. Wagner's reagent: Two drops of Wagner's reagent were addedto test tub e 2 and brownish precipitate observed indicated the presence of alkaloids.
c. Mayer's reagent: Two drops of Mayer's reagent were addedto test tube 3 andcreamy precipitate observed indicated the presence of alkaloids.
d. Picric acid reagent: Two drops of picric acid were added to test tube 4 and yellowish precipitate observed indicated the presence of alkaloids.

3.6 Acute Toxicity Studies in Swiss Albino Mice and Wistar Rats (LD_{50})

3.6.1Selection of animals

Female rats of average weight 175 g and female micenulliparous and non pregnant of average weight 23 g were used. Animals were kept and maintained under natural day and night cycle. Animals were housed in cages and fed with conventional rodent laboratory diet with unlimited drinking water.

3.6.2Fixed dose method of acute toxicity study in rats and mice

Lethal dose (LD_{50}) determination was conducted using Organization for Economic Co-operation and Development (OECD 423, 2008) guidelines in rats and mice. In this method, two groups each of threeanimals were fasted prior to dosing (food but not water was withheld overnight for the rats and for 3 hours for mice). The fasted body weight was determined for each animal and the dose was then calculated according to the body weight. Food wasthen further withheld for 3-4 hours in rats and 1-2 hours in mice after the methanol leaf of *C. angolenis* had been administered in a single oral dose using an orogastric canula. A start dose of 2000 mg/kg was used for each animal in the first phase. Animals dosed in the first phase were observed for 48 hours. There was no death in either case and the test proceeded to the second phase. The same procedure was used but at a dose of 5000 mg/kg. Animals were observed individually atleast once during the first 30minutes after dosing then four hourly during the first 24hours, and then daily for 14days. Observations included changes in skin and fur, eyes and mucous membranes, somator activity and behavioural patterns such as tremors, convulsions, salivation, diarrhoea, lethargy, sleep coma and death.

3.7PharmacologicalStudies

3.7.1 Acetic acid induced writhes test

The acetic acid induced writhes test in mice as described by Koster *et al.*, (1959) was employed. Thirtyswiss albino mice were divided into five groups of six mice each. The first group received 10mL/kg of normal saline oral and this served as negative control. Group 5 received piroxicam 20mg/kg as positive control. Group 2, 3 and 4 received 125, 250 and 500 mg/kg oral doses of the methanolleaf extract of *Culcasia*

*angolensis*respectively. Sixtyminutes aftertreatment, mice in all groups were treated with acetic acid (0.6% v/v,10 ml/kg body weight i.p.). Mice were then placed in individual cages. The number of abdominal writhes (stretching of abdomen with involvement of at least one of the hind limb) was counted for each mouse for a period of 10minutes.A reduction in the number of writhes as compared to the vehicle treated animals was considered as evidence for the presence of analgesia and expressed as percent inhibition of writhes.

Percentage Inhibition (%)=

$$\frac{\text{Mean No. of writhes (control)} - \text{Mean No. of writhes (Test)}}{\text{Mean No. of writhes (control)}} \text{ X } 100$$

3.7.2 Hot plate (thermal sensitivity) test in mice

The method previously described by Eddy and Leimbach (1953) was employed for the study. Mice were grouped into five groups of six mice each. The first group served as control and received 10 mL/kg normal saline orally. Groups 2, 3 and 4 received125, 250 and 500 mg/kg of methanolleaf extract of C. *angolensis*orally respectively using an orogastric canula, while the fifth group received 5 mg/kgmorphine solutionorally. Sixty minutes after treatment, each mouse was placed on a hot plate (Gallenkamp thermostat) which was set and maintained at 55± 1^{o}C. The pain response latency was determined using a stop watch. The time it took up to the point the animal either licked the paw, fluttered any of the paws or jumped off the hot plate was recorded and taken as latency or reaction time. A cut-off time of 20 sec was used to avoid paw tissue damage. The latency was observed and recorded at 60, 90, 120 and 150 minutes after acclimatization of the

animals. The prolongation of the latency time was taken as an analgesic response (per cent maximum possible effect (%MPE).

$$\%MPE= \frac{\text{Test} - \text{Baseline}}{\text{Cutoff} - \text{Baseline}} X100$$

Where;

Test = latency to respond after treatment

Baseline = latency to respond prior to treatment and

Cut-off (20 sec) = preset time at which the test was ended in the absence of a response.

3.7.3 Formalin induced inflammation

The method previously described by Dubuisson and Dennis 1977, modified by Tjolsen *et al.*, (1992) was employed for the study. Thirtyfemale Wistar rats were divided into five groups of six rats each. Group 1 was treated with 10mL/kg normal saline orally (negative control); group 2 was treated with10mg/kg ketoprofen orally; while group 3, 4 and 5 received methanol leaf extract of *Culcasia angolensis*orally at doses of 125, 250 and 500mg/kg respectively using an orogastric canula. Inflammation was induced in rat hind paw by injecting formalin 0.1 mL (1% w/v formalin in 0.9% normal saline) into the subplantar surface of the left hind paw 60 minutes after treatment. Paw linear diameter (mm) was measured using a digital vernier caliper (MR 2002) at0, 1, 2, 3, 4 and 5hours after formalin injection (Gaertner *et al*., 1999).

3.7.4 Carrageenan induced paw oedema

The acute anti-inflammatory study wascarried out using the carrageenan induced paw oedema in rats method as previously described by Winter *et al.*,(1962). Thirty wistar rats were randomly selectedand divided into fivegroups of rats (n=6). Group 1 were administered normal saline (10 mL/kg)and group 2,3, and 4 were administered the

methanol leaf extracts of Culcasia angolensis, (125, 250 and 500mg/kg) respectively using an orogastric canula. Group 5 were administered ketoprofen (10 mg/kg).Sixty minutes post treatment, each rat was injected with 0.1 mL of 1% carrageenan into plantar surface of rat right hind paw. The hind paw oedema was measured and recorded at times 0, 1, 2, 3, 4 and 5 hours using vernier caliper to determine the diameter of the oedema. The increase in paw diameter (oedema index) for each rat was calculated as the difference in paw diameter before carrageenan injection and after carrageenan injection at each time interval, while the percent inhibition of oedema was calculated for each group with respect to its vehicle-treated control group using the following relationship:

$$\frac{\text{Mean increase in paw colume of control} - \text{Mean increase in paw colume of treated}}{\text{Mean icrease in paw volume of control}} \text{ X } 100$$

3.7.5 Brewer's yeast induced pyrexia in rats

The method described by Loux *et al*., (1972) was used for the antipyretic study. Twenty five Wistar rats of either sex were selected and randomized into five groups of 5 rats each. The rectal temperature of each animal was taken using the rectal thermometer. Fever was induced in each rat by injecting 20mL/kg of 20% suspension of Brewer's yeast subcutaneously on the back of each rat just below the nape of the neck. Food was immediately withdrawn after the yeast administration. Eighteen (18) hours after, the rectal temperature of each rat was then taken and recorded, it was repeated after 30 minutes and only rats with at least 0.5°C rise in body temperature were used for the study. Group I was administered 10mL/kg body weight normal salineorally; groups II, III, IV were given 125, 250, and 500mg/kg body weight methanol leaf extract of

Culcasia angolensis respectivelyorally using an orogastric canula. While group V was administered 5 mg/kg acetaminophen *i.p*. Rectal temperature of each rat was taken at 0, 1, 2, 3 and 4 hours respectively. Changes in rectal temperature in extract treated groups were compared with the standard antipyretic agent and the negative control.

3.7.6 Sub chronic toxicity study

The study was carried out in accordance with OECD 407 (2008) guidelines. Twenty four male Wistar rats were deprived of food for 24 hours, and divided into four groups of six rats each. Group 1, which served as negative control received normal saline 10mL/kg while rats in groups 2, 3 and 4 were administered, graded doses of the methanol leaf extract of Culcasia angolensis 125, 250, and 500 mg/kg body weight respectively orally daily using an orogastric canula for 28 days. The rats were allowed free access to food and water throughout the duration of the experiment and were observed daily for general signs of toxicity and mortality. Rats were then euthanized with chloroform on the 29th day of the experiment. Blood samples were collected from the jugular vein for estimation of biochemical and haematological parameters. Liver, kidney and lungs were harvested for the determination of organ weight ratio and histopathology.

The relative organ weight for each animal was calculated using the formulae:

$$\frac{\text{Absolute organ weight}}{\text{Body weight of rats on sacrifice day (g)}} \text{X } 100$$

3.7.6.1 Biochemical studies

Blood samples were collected into plain bottles allowed to clot and centrifuged at 3500 rpm for 10 minutes. The sera were separated, stored at -4°C and used for the evaluation

of biochemical parameters which include alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels and serum bilirubin, serum urea nitrogen, creatinine, chloride, sodium, potassium, and bicarbonate using Commercial Kits from Reckson Diagnostics P Ltd, India.

3.7.6.2 Haematological studies

Blood samples were collected into EDTA bottles for estimation of packed cell volume (PCV), haemoglobin concentration (HBG), platelets (PLT), white blood cells (WBC) and differentials, Mean corpuscular haemoglobin concentration (MCHC) using automated haematological machine (Cell-DynTM Abbot, US).

3.7.6.3 Histological studies

The organs harvested from the rats were fixed in 10% buffered formalin for 48 hours. They were then processed routinely, and the tissues were embedded in paraffin wax. Histological sections were cut at 5-6 µm and stained with haematoxylin and eosin; and were then examined microscopically for pathological lesions by a histopathologist (Arthur and John, 1978). Lesions were observed and assessed. Photomicrographs of representative lessions were taken at various magnifications.

3.8 Data Analysis

Results are expressed as mean ± standard error of mean. Data obtained were presented as tables, line graphs and bar charts while histological findings were presented as photomicrographs. One way analysis of variance (ANOVA) followed by *post hoc* test wasused in the analysis of single point data if normally distributed, while Kruskal-Wallis wasused in analysis of single point data if not normally distributed. Results were considered significant at $p \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Extraction Yield of *Culcasia angolensis* Leaf

A sticky black solid residue of 107.6g (7.2% w/w) was obtained from 1,500g crude leaf of *C. angolensis* powdered sample.

4.2 Phytochemical Constituents

Preliminary phytochemical screening of *C. angolensis* leaf extract showed the presence of glycosides(cardiac glycosides), triterpenes, saponins, tannins, flavonoids, steriods and alkaloids, while anthraquinones were absent (Table 4.1).

Table 4.1: Phytochemical Constituents of Methanol Leaf Extract of *C. angolensis*

Phytoconstituents	**Result**
Alkaloids	+
Flavonoids	+
Glycosides	+
Cardiac glycosides	+
Anthraquinones	-
Saponins	+
Steroids	+
Triterpenes	+
Tannins	+

Key: (-) = Absent, (+) = Present

4.3 Estimation of the Median Lethal Dose(LD_{50}) of Methanol Leaf Extract of *C. angolensis*

Acute oral administration of methanolleaf extract of *C. angolensis* showed no observable behavioural signs of toxicity .There werenodeath at doses up to 5,000mg/kg and thus, the oral LD_{50} of the extract was estimated to be greater than 5,000 mg/kg body weight in Swiss albino mice and Wistar rats.

4.4Effect of Methanol Leaf Extract of *Culcasia angolensis* on Acetic Acid Induced Writhes Test in Swiss Albino Mice

The methanol leaf extract of *C. angolensis* significantly ($p<0.05$) decreased the number of acetic acid-induced writhes in mice in a dose dependent manner. The effect of the methanol leaf extract of *C. angolensis* at 500mg/kgshowedsignificant ($p<0.01$) decrease in number of acetic acid-induced writhes comparable to piroxicam at a dose of 20 mg/kg (Figure 4.1)

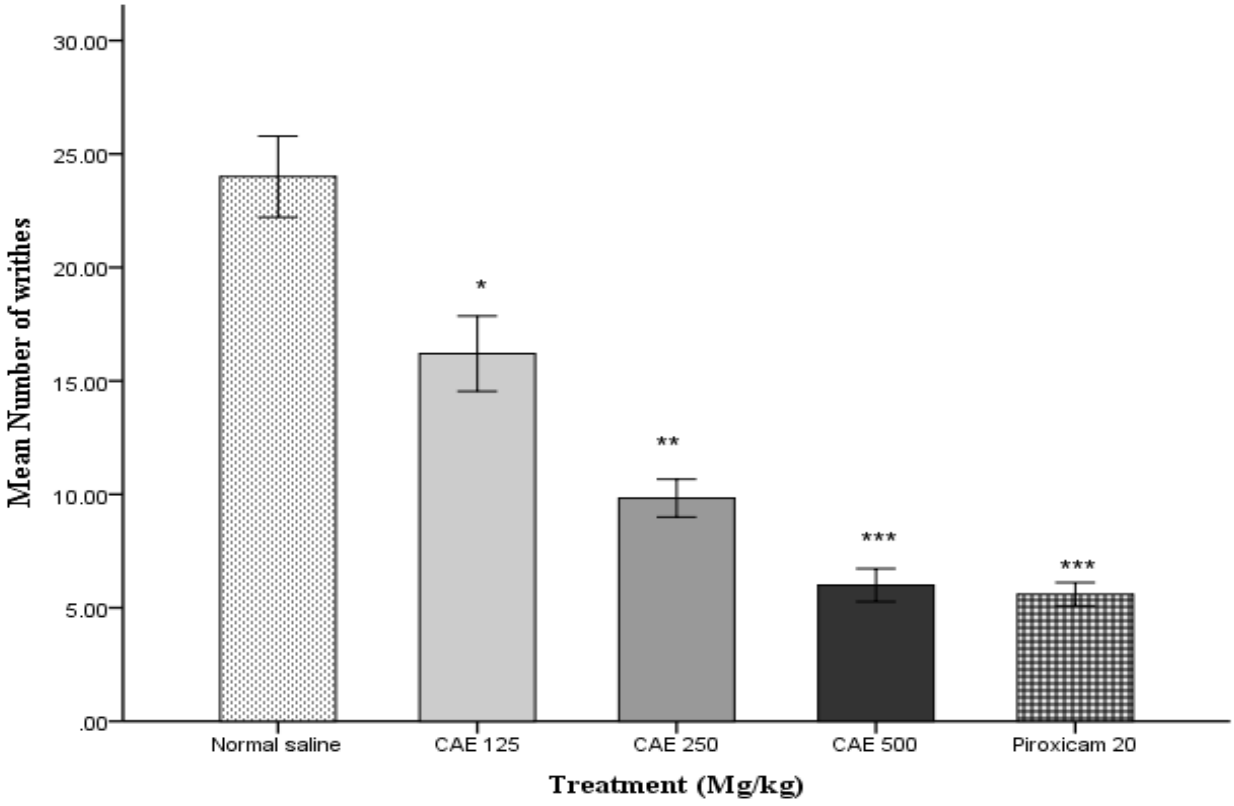


Figure 4.1 Effect of Methanol Leaf Extract of *Culcasia angolensis* on Acetic Acid Induced Writhes Test in Mice

Data was analyzed using one way ANOVA followed by Bonferoni Post Hoc test and presented as mean ± SEM, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $P \leq 0.001$, significant statistical difference as compared with Control group, n=6, CAE= methanol leaf extract of *C. angolensis,* PIR = piroxicam 20 mg/kg

4.5 Effect of the Methanol Leaf Extract of *Culcasia angolensis* on Hot Plate (Thermally Induced) Pain

The methanol leaf extract of *Culcasia angolensis* significantly ($p<0.05$) increased the mean reaction time in a dose dependent manner. The methanol leaf extract of Culcasia angolensis at the doses of 125, 250 and 500 mg/kg body weight showed significantly ($p<0.01$)increased the mean reaction timecomparable to morphine (5 mg/kg) at 120 and 150 minutes post treatment time (Table 4.2).

Table 4.2: Effect of the Methanol Leaf Extract of *Culcasia angolensis* on Hot Plate (Thermally Induced) Pain in Swiss Albino Mice

Groups (mg/kg)	Mean reaction time ± SEM (secs) 0 min	60 mins	90 mins	120 mins	150 mins
D/W(10 mL)	1.63±0.10	1.44±0.22	1.34±0.23	1.36±0.28	1.49±0.19
CAE(125)	1.67±0.14	3.25±0.39	3.32±0.14**	3.01±0.24*	3.02±0.29*
CAE(250)	1.81±0.25	2.82±0.14	3.02±0.16*	3.28±0.31**	3.29±0.18**
CAE(500)	2.08±0.20	2.99±0.19	2.69±0.19	3.87±0.27**	3.81±0.24**
Mor (5)	1.73±0.19	3.37±0.20**	3.07±0.41**	3.73±0.16**	3.72±0.27**

Data was analyzed using repeated measures ANOVA followed by Bonferroni post hoc test, * = P≤0.05, ** = P≤ 0.01 significant statistical increase in mean reaction time compared with time zero; n=6. Values are Mean ± SEM. D/W= distil water, CAE= *Culcasia angolensis* methanol leaf extract, Mor = morphine 5 mg/kg

4.6Effect of the Methanol Leaf Extract of *C. angolensis* on Formalin Induced Inflammation in Wistar Rats

Injection of 0.6% v/v formalin produced local oedema which was significant ($p<0.001$) compared to time zero in all the groups. The extract significantly ($p<0.001$) decreased formalin-induced paw oedema in dose dependent manner (125, 250, and 500 mg/kg body weight) at the fourth and fifth hours treatmenttime comparable to the peak of oedema (Table 4.3).

Table 4.3: Effects of Methanol Leaf Extract of *C. angolensis* on Formalin Induced Inflammation in Wistar Rats

Treatment (mg/kg)	Mean Paw Diameter (millimetre) 0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
D/W 10 mL	2.30±0.06	2.67±0.10	3.12±0.05	3.11±0.05	3.01±0.06	2.9±0.06
CAE (125)	2.22±0.05	2.65±0.06	2.71±0.04	2.72±0.04	2.53±0.03**	2.41±0.01*
CAE (250)	2.35±0.07	2.76±0.02	2.77±0.02	2.85±0.06	2.55±0.04**	2.40±0.05**
CAE (500)	2.20±0.09	2.83±0.03	2.82±0.04	2.73±0.03	2.51±0.06**	2.31±0.04**
Ket (10)	2.23±0.05	2.65±0.06	2.74±0.09	2.41±0.04	2.31±0.02**	2.14±0.01**

Data was analyzed using repeated measures ANOVA followed by Dunnet Post Hoc test, *= $p<0.05$, **= $p<0.001$, significant statistical decrease in mean paw oedema size as compared to time zero; n= 6. Values are Mean ± SEM. CAE = *Culcasia angolensis* methanol leaf extract. Ket= ketoprofen 10 mg/kg, hrs= hours

4.7Effect of the Methanol LeafExtract of *Culcasia Angolensis* on Carrageenan Induced Paw Oedema in Wistar Rats

The extract significantly ($p<0.001$) decreasedcarrageenan-induced paw edema in a dose dependent manner (125, 250, and 500 mg/kg body weight) at the fourth hour (24.4%, 51.5%, 60.4%) and fifth hours(27.3%, 54.5%, 63.6%) treatmenttime comparable to the positive control (Table 4.4). The extract decreased paw oedema at all the tested doses with maximum inhibition at the 5^{th}hour. At the 5^{th}hour, the extract had 27.3%, 54.5%, and 63.6% inhibition respectively

Table 4.4: Effect of the Methanol Leaf Extract of *Culcasia angolensis* on Carrageenan Induced Paw Oedema in Wistar Rats

Treatment(i.p)	Mean Paw Diameter in Millimeter 1hr	2hr	3hr	4hr	5hr
D/W 10mL	1.660± 0.144	2.720± 0.188	2.700± 0.407	3.380± 0.120	3.180±0.124
CAE 125mg/kg	1.560± 0.103 **(6.02%)**	2.300± 0.182 **(15.4%)**	2.440± 0.172 **(9.6%)**	2.420± 0.174 **(28.4%)**	2.540± 0.121 **(27.3%)**
CAE 250mg/kg	1.220± 0.139 **(26.5%)**	2.160± 0.199 **(20.6%)**	1.880±0.159 **(30.4%)**	1.640± 0.181 **(51.5%)** ***	1.680± 0.058 **(54.5%)** ***
CAE 500mg/kg	1.420± 0.073 **(14.5%)**	2.360±0.291 **(13.2%)**	1.380± 0.334 **(48.9%)***	1.340± 0.172 **(60.4%)** ***	1.340± 0.157 **(63.6%)** ***
Ketoprofen 10mg/kg	1.340± 0.160 **(19.3%)**	1.020± 0.073 **(62.5%)** ***	1.000± 0.327 **(62.9%)** **	1.000± 0.241 **(70.4%)** ***	1.520± 0.240 **(63.6%)** ***

Data was analyzed using repeated measures ANOVA followed by Bonferoni Post Hoc test, *= $p \leq 0.05$, **= $p \leq 0.01$, ***= $p < 0.001$significant statistical decrease in mean paw oedema size when compared to the negative control. n= 6. Values are Mean ± SEM. CAE= *Culcasia angolensis* extract, D/W =distil water, hr= hours. Figures in parentheses (bold) are percentage inhibition of inflammation

4.8 Effect of Methanol Leaf Extract of *C. angolensis* on Brewer's Yeast Induced Pyrexia in Wistar Rats

There was an elevation in rectal temperature of all the treated rats 18 hours post administration of brewer's yeast. Zero hour was taken as the baseline for all the treatment groups and was compared with 1, 2 and 3 hours. The extract at all the test doses (125, 250 and 500 mg/kg body weight) produced a statistically significant ($P \leq 0.05$) decrease in the rectal temperature of rats at the third hour. However the group treated with 500 mg/kg body weight of the extract produced a statistically significant ($P \leq 0.01$) decrease in rectal temperature compared to paracetamol at the third hour.

Table 4.5: Effect of Methanol Leaf Extract of *C. angolensis* on Brewer's Yeast Induced Pyrexia in Wistar Rats

Treatment	Rectal temperature (°C) Mean ± SEM after 18hr				
(mg/kg)	Initial temp	0hr	1hr	2hr	3hr
D/W 2mL	37.33±0.11	39.93±0.11	39.98±0.14	40.22±0.49	39.87±0.22
CAE 125	37.21±0.20	39.43±0.19	39.40±0.01	39.21±0.15	38.65±0.71*
CAE 250	37.66±0.43	39.61±0.15	39.69±0.41	39.01±0.21	38.54±0.41*
CAE 500	37.44±0.23	39.58±0.11	38.42±0.08	38.30±0.11*	37.66±0.43**
PCM 300	37.32±0.36	39.47±0.17	37.44±0.33**	36.68±0.30**	37.19±0.48**

Data expressed as mean ± SEM using repeated measure ANOVA followed by Dunnet's post hoc test for multiple comparism. *=significant decrease in rectal temperature at P≤0.05, **= significant at P≤0.01 when compared with control n=6, D/W = distil water, CAE = *Culcasia angolensis* methanol leaf extract.

4.9 Subchronic Toxicity Study of 28 days C. *angolensis* Leaf Extract Administration in Wistar Rats

4.9.1Effect of CAE on body weights of Wistar rats

The result of the weekly body weight changes for the subchronic toxicity study revealed that in all the groups, there was a progressive increase in weight. However, the animals that were treated with the extract showed minimal increase in weight gain that was not statistically significant when compared to the control group. In week 1, the mean weight of all the extract-treated groups was almost similar, however, the 500mg/kg extract treated groups showed lesser weight increments in the last two weeks (3 and 4) that was statistically significant when compared to the control group (Figure 4.2).

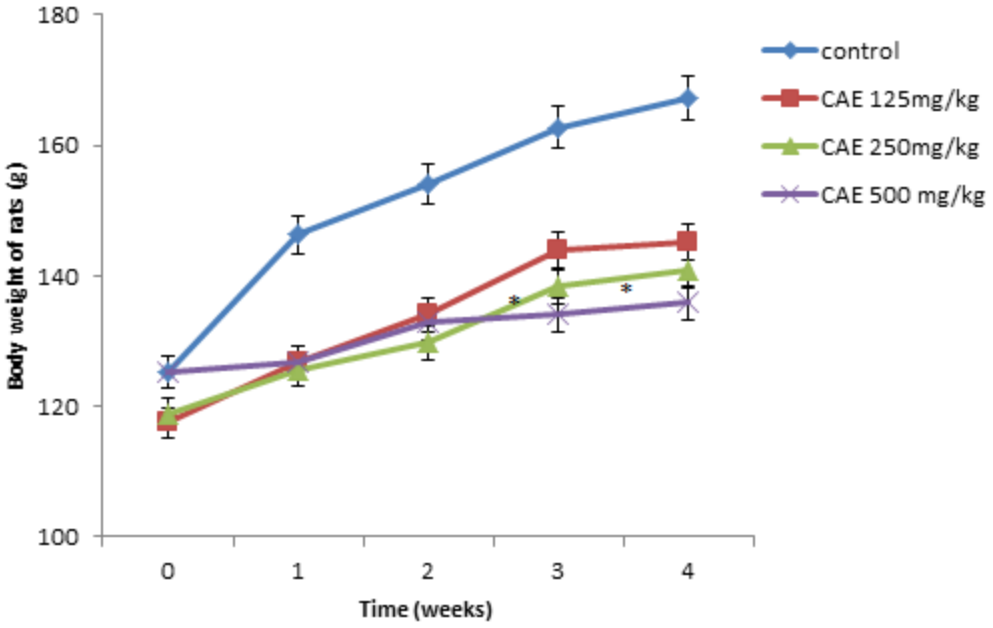


Figure 4.2: Weekly Body Weight Changes of Wistar Rats after 28 days Daily Oral *C. angolensis* Extract Administration

Data is presented as mean ±SEM. n = 6; statistics: Split plot ANOVA and Bonferonni multiple comparison *post hoc* test; * = significance at $P<0.05$ compared to the control group.

4.9.2 Effect of 28 days administration of the methanol leaf extract of *C. angolensis* on relative organ weight of wistar rats

The extract produced no statistically significantchange in the relative organ weight of the selected organs at all doses as shown in table 4.6

Table 4.6: Effect of 28 Days Administration of the Methanol Leaf Extract of *Culcasia angolensis* on Relative Organ Weight of WistarRats

Treatment groups (mg/kg)	Relative Organ weight (%)			
	Liver	**Kidney**	**Lungs**	**Heart**
D/W 10 mL/kg	3.59 ±0.44	0.74 ±0.45	0.91±0.04	0.52 ±0.02
CAE (125)	3.36±0.22	0.71 ±0.78	0.80±0.03	0.46 ±0.05
CAE(250)	3.47±0.09	0.69 ±0.92	0.79 ±0.04	0.49 ±0.04
CAE(500)	3.50±0.14	0.66 ±0.07	0.83 ±0.06	0.53±0.11

n = 6; data expressed asmean ±SEM; statistics: one way ANOVA followed by Dunnet *post hoc* test for multiple comparism. CAE= *Culcasia angolensis* leaf Extract; D/W=distil water

4.9.3Effect of CAE on serum biochemical parameters of liver function

In this study, asignificant ($P \leq 0.05$) decrease in ALT and ALP were observed in animals treated for 28 days with the extract at 250 and 500 mg/kg; however there was a significant ($P \leq 0.05$) and dose-dependent increase in serum ASTlevel. The serum transaminase levels did not vary significantly amongst the extract dose groups. The total protein and albumin were not significantly altered (Table 4.7).

Table 4.7: Effect of 28 Days Administration of Methanol Leaf Extract of CAE on SerumBiochemicalParameters of Liver Function

Liver biomarkers	Treatment groups (per kg)			
	D/W (2 ml)	**CAE 125 mg**	**CAE250 mg**	**CAE 500 mg**
ALT (IU/L)	26.2 ± 4.11	27.2± 7.20	15.1±1.57*	16.3 ± 2.07*
AST (IU/L)	37.67± 0.92	41.68± 0.58*	44.80± 1.67*	59.66± 2.28*
ALP (IU/L)	22.76± 3.38	19.68±4.32	15.80±3.24*	19.21±4.03
Total Protein (g/dL)	6.54±0.24	6.69±0.32	6.44±0.30	6.93±0.34
Albumin (g/dL)	3.14±0.07	3.17±0.08	3.08±0.06	3.03±0.09

n=7 data is expressed asmean ±SEM; statistics: one way ANOVA and Dunnett *post hoc* test; * indicates significance at $P \leq 0.05$ compared to the D/W group; D/W = distil water; ALT= alanine aminotransferase; AST; aspartate aminotransferase; ALP; alkaline phosphatase.

4.9.4 Effect of CAE on serum biochemical parameters of kidneyexcretory functions and electrolytes

This result showed an increase in serum urea level that wasstatistically significant (P<0.05) at 500 mg/kg extract treated group compared to the normal control. There were slight and inconsistent changes in levels of creatinine and other electrolyte assessed.

Table 4.8: Kidney Function Biomarkers and Electrolyte Concentrations in Wistar RatsTreated for 28 days with CAE

Kidney biomarkers	Treatment groups (per kg)			
	D/W (10 ml)	**CAE125 mg**	**CAE250 mg**	**CAE 500 mg**
Urea (mg/dL)	32.2± 1.22	30.80 ± 0.89	31.66 ± 2.70	38.40 ± 1.82*
Creatinine (mEq/L)	0.80±0.05	0.85±0.11	0.88±0.12	0.90 ± 0.60
K^+ (mmol/L)	7.90± 0.10	6.76± 1.20	7.30± 0.36	6.60± 2.27
Na^+ (mmol/L)	220.32± 7.44	221.56±2.99	225.08±6.91	222.98±3.24
Cl^- (mg/dL)	96.67±5.39	98.67±5.26	87.83±3.02	89.40±1.50
HCO_3^- (mg/dL)	88.8±3.30	88.60±2.11	94.50±3.71	89.84±1.82

n = 7; data expressed asmean ±SEM; statistics: one way ANOVA and Dunnett *post hoc* test; * indicates significance at $P \leq 0.05$ compared to the D/W group. D/W=distil water; K^+=potassium ion; Na^+=sodium ion; Cl^-=chloride ion; HCO_3^-=bicarbonate ion.

4.9.5 Effect of CAE on haematological parameters

PCV, Hb and platelet concentrations were dose dependently reduced, but not significantcompared to control. There was an insignificant increase in WBC. A significant increase in lymphocytes was observed at the dose of 500 mg/kg, while the monocytes and eosinophils reduced slightly and consistently, but not statiscally significant. The neutrophils showed similar slight insignificant increase at the lower doses of the extract 125 mg/kg and 250 mg/kg (table 4.4).

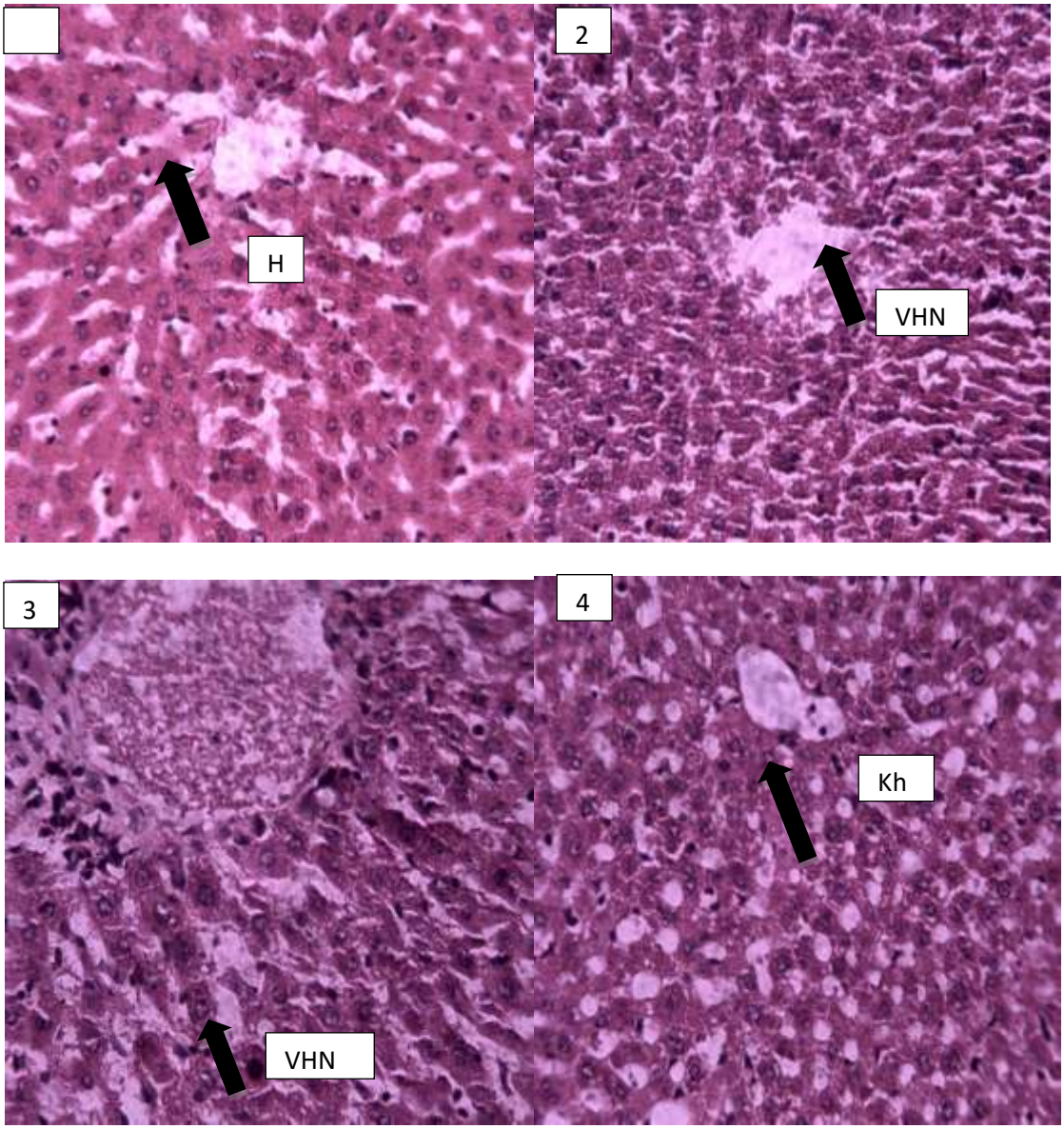
Table 4.4: Effect of Methanol Leaf Extract of *C. angolensis* on Haematological Parameters in Wistar Rats Treated for 28 days

Haematological indices	Treatment groups (mg/kg)			
	D/W (10 ml)	CAE125	CAE250	CAE500
PCV (%)	37.16 ±1.86	36.67 ±3.18	35.00 ±1.97	35.17 ±1.30
Hb (g/dL)	12.72 ±0.33	12.62 ±0.97	11.15 ±0.69	11.12 ±0.51
WBC ($\times 10^9$L)	3.85 ±0.27	4.07 ±0.22	4.27 ±0.10	3.85 ±0.15
RBC ($\times 10^6$L)	5.85 ±0.17	5.98 ±0.15	5.70 ±0.14	5.93 ±0.19
Platelet ($\times 10^5$L)	7.20 ±0.10	7.18 ±0.10	7.18 ±0.11	7.08 ±0.06
Neutrophils (%)	17.33 ±2.08	19.00 ±0.97	18.00 ±1.15	15.00 ±0.97
Lymphocytes (%)	78.17 ±1.17	77.67 ±0.72	78.50 ±0.99	81.33 ±1.38*
Monocytes (%)	2.33 ±0.56	1.67 ±0.21	1.50 ±0.34	2.00 ±0.37
Eosinophils (%)	2.17 ±0.30	1.66 ±0.33	2.00 ±0.36	1.83 ±0.31

n = 7; data expressed asmean ±SEM; statistics: one way ANOVA and Dunnett *post hoc* test; * indicates significance at $P \leq 0.05$ compared to the D/W group; D/W=distil water;PCV=packed cell volume; Hb=haemoglobin; WBC=white blood cell; RBC=red blood cell

4.9.6Effect of CAE on selected organ histology

Histological examination of some selected organs in wistar rats following the administration of methanol leaf extract of *Culcasia angolensis* (125, 250 and 500 mg/kg) body weight for 28 days revealed various findings; **Liver (Plate III):**There were dose-dependent histological changes (slight to moderate hepatocellular necrosis and slight vacuolation) in the liver tissues at 125 and 250 mg/kg treatment groups (Plate III (2,3))however,in the group that received 500 mg/kg of themethanolleaf extract, there was intense vaculation with kupfer cell hyperplasia (Plate III, 4). **Kidney(Plate IV):** The kidneys showed slight glomerular necrosis, and tubular distortion. **Lungs (Plate V):**The lungs showed a dose dependent histological change (slight to moderate alveoli congestion and slight lymphocyte hyperplasia) in all the treated groups.**Heart (Plate VI):**There were no histological changes in the heart muscles of rats in all treated groups. Spleen **(Plate VII):**The spleen histology show normal red and white pulp at extract doses of 125 and 250 mg/kg, however, there were slight lymphocyte hyperplasia at extract dose of 500 mg/kg when compared to the control groupadministerd with normal saline



PlateIII: Photomicrographs of Liver SectionsofWistar RatsFollowing 28 Days Daily Oral Administrations of CAE(H & E stained at × 250 magnifications) 1-4Implies Control, 125, 250 and 500 mg/kg respectively

1: Section showing normal hepatocellular features (H)
2: Section showing slight vacuolation and hepatocellular necrosis (VHN)
3: Section showing slight vacuolation and hepatocellular necrosis (VHN)
4: Section showing intense vacuolation and Kupfer cell hyperplasia (Kh)

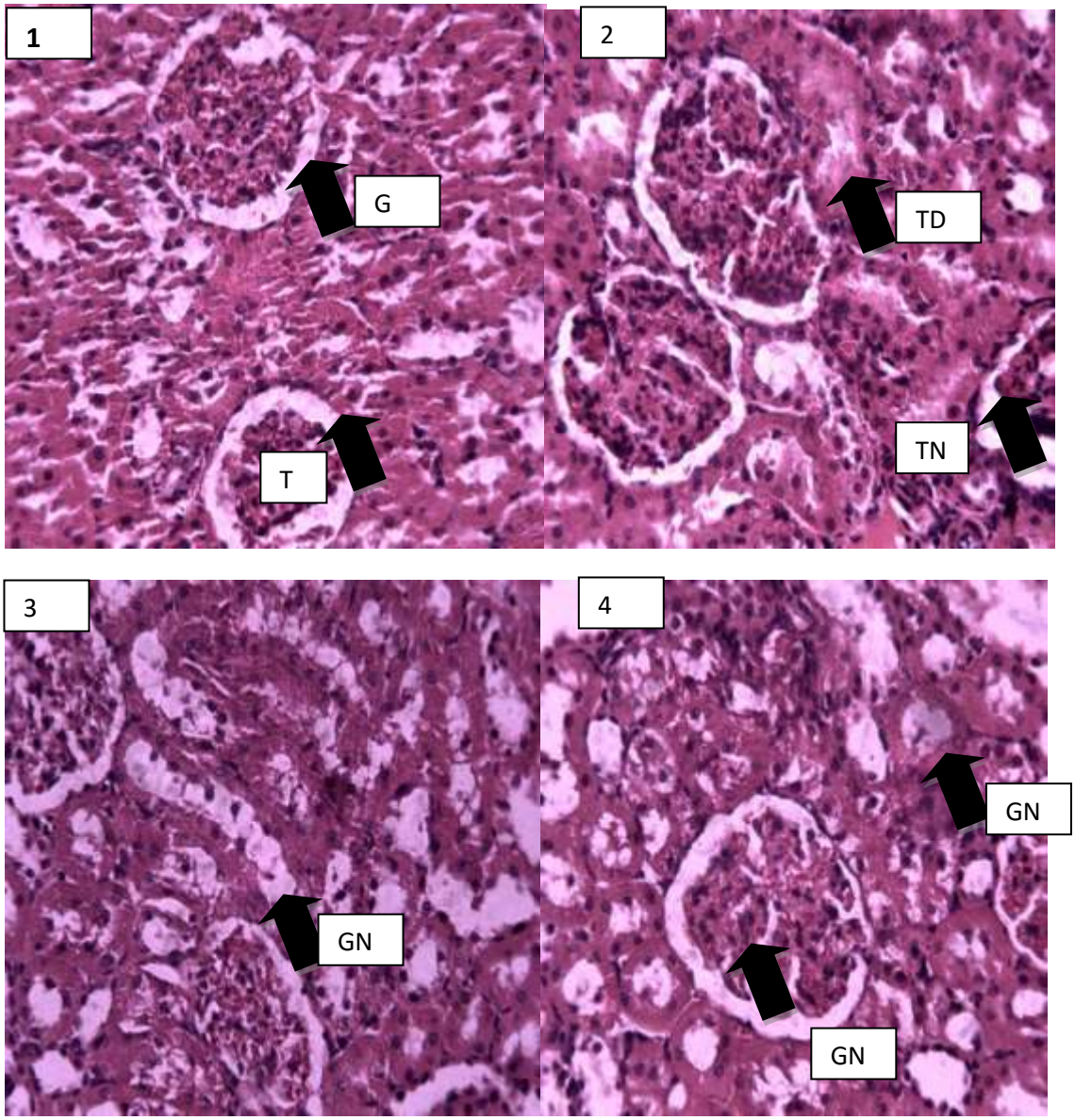


Plate IV:Photomicrographs of Kidney Sections of Wistar RatsFollowing 28 Days DailyOral Administrations of CAE(H & E stained at ×250magnifications)1-4means Control, 125, 250 and 500 mg/kg respectively

1: Section showing normal kidney tubules(T) and glomerulus (G)
2: Section showing slight tubular distortion (TD) and necrosis (TN)
3: Section showing slight glomerular necrosis (GN)
4: Section showing slight glomerular necrosis(GN)

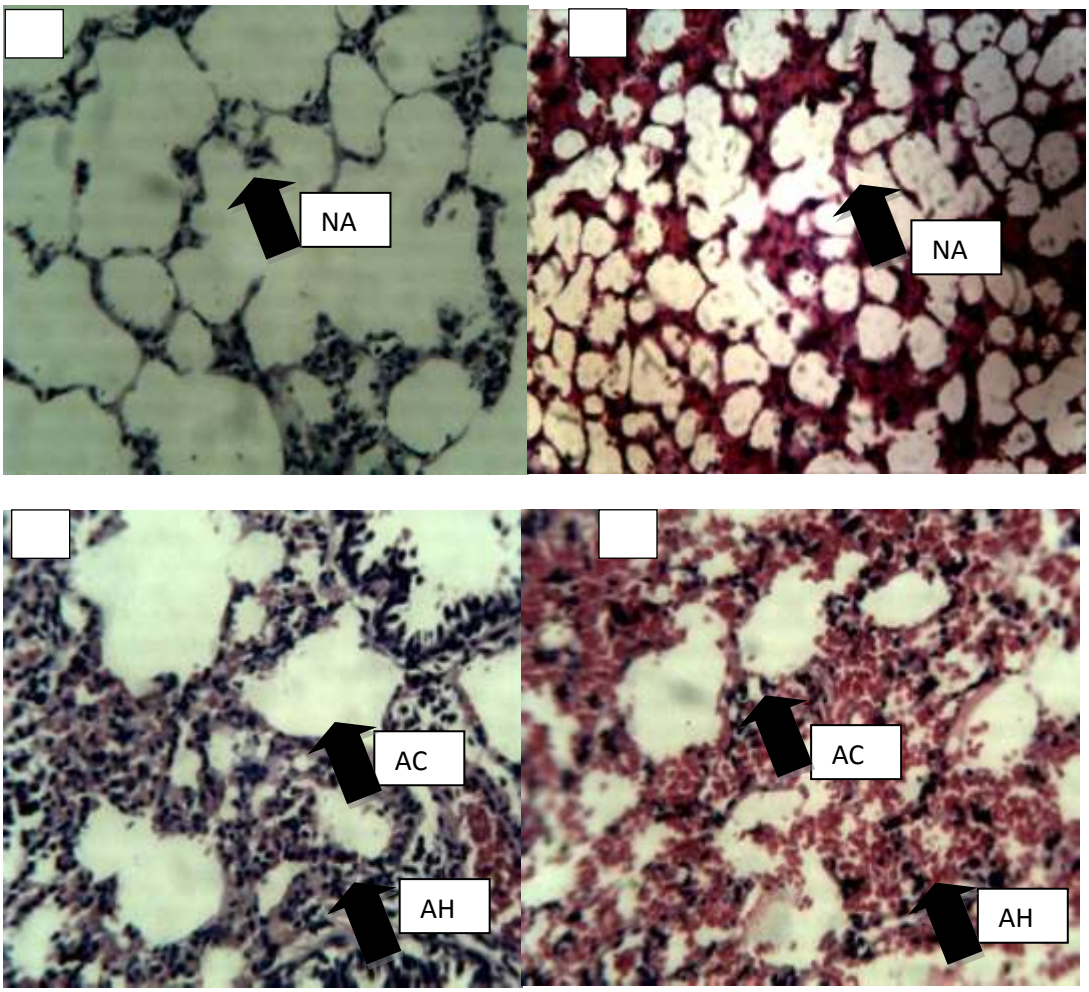


Plate V: Photomicrographs of Lung Sections of Wistar RatsFollowing 28 Days Daily Oral Administrations of CAE(H & E stained at ×250 magnification) 1-4 implies Control, 125, 250 and 500 mg/kg respectively

1: Section showing normal lung alveoli (NA)
2: Section showing normal lung alveoli (NA)
3: Section showing slight alveoli congestion (AC) and slight alveoli hyperplasia (AH)
4: Section showing moderate alveoli congestion (AC) and slight alveoli hyperplasia (AH)

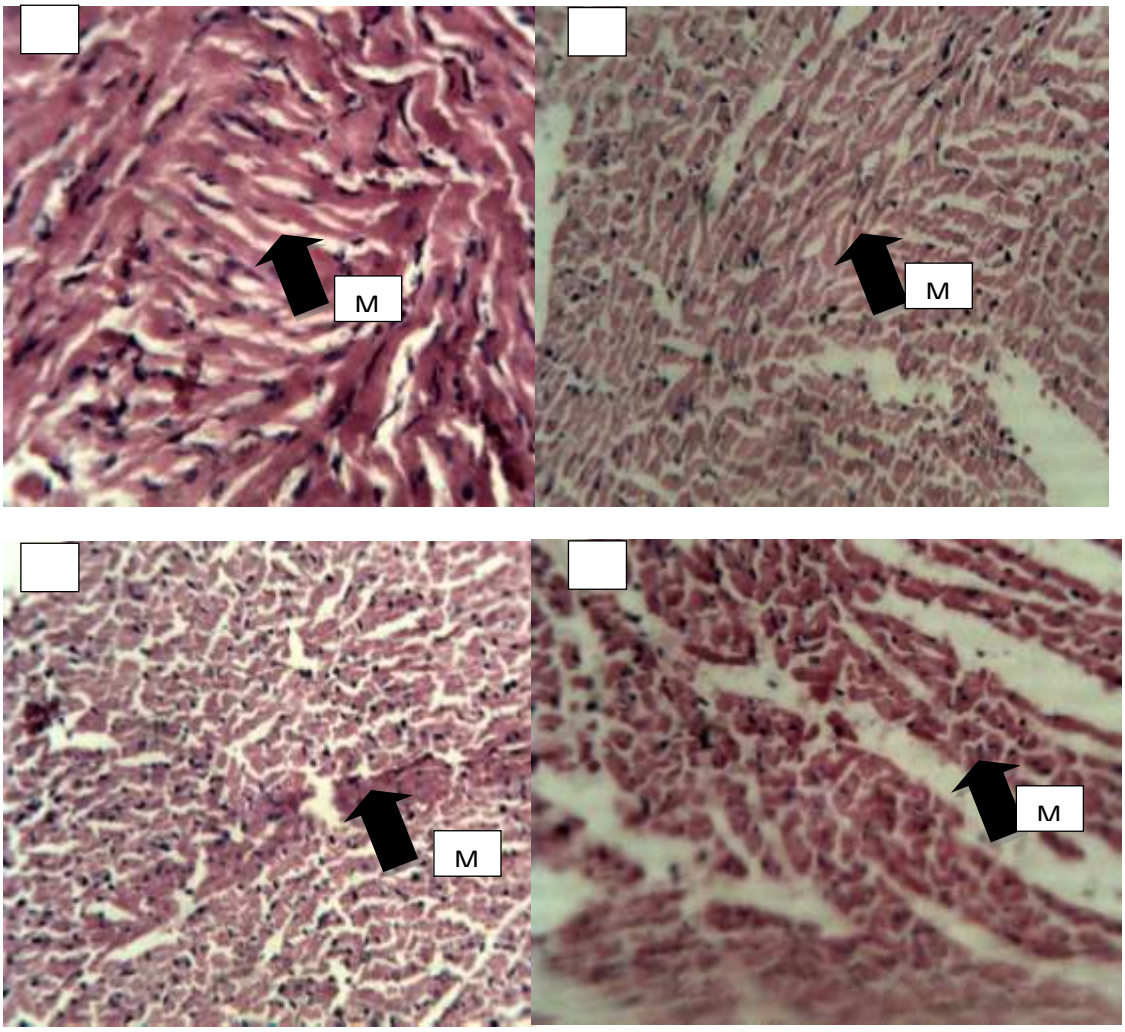


Plate VI: Photomicrographs of Heart Sections ofWistar RatsFollowing 28 Days Daily Oral Administrations of CAE(H & E stained at ×250 magnification) 1-4 implies Control, 125, 250 and 500 mg/kg respectively

(1, 2, 3, 4): Sections showing normal cardiac muscles (M)

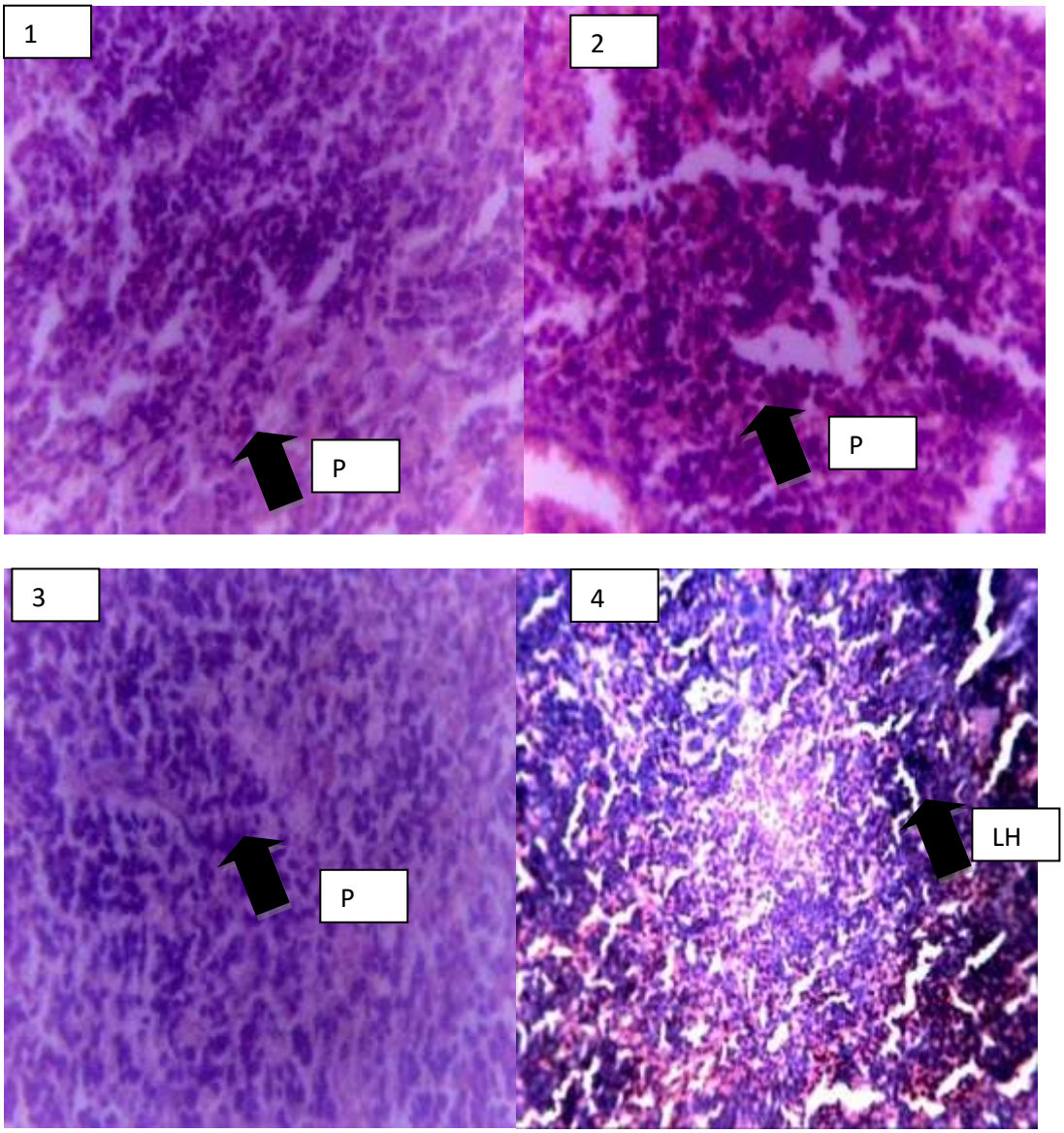


Plate VII: Photomicrographs of Spleen Sections of RatsFollowing 28 Days Daily Oral Administrations of CAE(H & E stained at ×250 magnification) 1-4 implies Control, 125, 250 and 500 mg/kg respectively

1: Section showing normal red and white pulp(P)
2: Section showing normal red and white pulp (P)
3: Section showing normal red and white pulp(P)
4: Section showing slight lymphocyte hyperplasia (LH)

CHAPTER FIVE

5.0 DISCUSSION

The traditional use of *Culcasia angolensis* in the management of pain and inflammation has long been documented in the literature. The present study was designed to investigatethe pharmacological basis for the use of the plant *C. angolensis* for the management of pain and inflammation as claimed in ethno medicine.

The choice of methanol leaf extract of *Culcasia angolensis* for these studies was based on the fact that methanol is a polar solvent and has been found to be good in extracting both polar and non-polar secondary metabolites resulting in an extract that is rich in both organic and inorganic constituents (Chan *et al*., 1995). This is important because the pharmacological activities of most plants extracts used in the treatment of various disease conditions in traditional medicine has been attributed to a combination of constituents rather than a single chemical entity (Saha *et al*., 2013).

The therapeutic or biological activities of any medicinal plant are usually a direct function of the chemical constituents present in the plant and phytochemical constituents often vary with method of extraction and solvent for the extraction (Ayinde and Agbakwuru, 2010). The phytochemical screening of the methanol leaf extract of *Culcasia angolensis* revealed the presence of triterpenoids, glycosides, saponins, flavonoids and alkaloids. These phytochemicals has been reported to possess analgesic and anti-inflammatory properties (Ahmadiani *et al*., 2000; Choi *et al.,* 2005; Reanmongkol *et al.,* 2005; Arrau *etal*., 2010; Amin *etal*., 2012)

Certain flavonoids possess potent inhibitory activity against many enzymes such as protein kinase C, protein tyrosine kinases, phospholipase A_2 and phosphodiesterases (Middleton, 1998). Flavonoids were also reported to be effective as anti-inflammatory agents (Rajnarayan *et al.*, 2001). Thus, the antinociceptive and anti-inflammatory effect exhibited by the methanol leaf extract of*Culcasia angolensis* may be due to the presence of saponins, flavonoids, alkaloids, carbohydrates, tannins or combination of all the phytochemicals found present in the leaf extract.

The acute toxicity study, exposure of the laboratory animals to graded doses of themethanol leaf extract of *C. angolensis*within 24 hour period showed no death at doses up to 5,000 mg/kg body weightoral treatments in wistar rats. The oral median lethal dose (LD_{50}) was thus estimated as greater than 5,000 mg/kg suggesting it to be practically non-toxic on acute oral administration (OECD 423, 2001).This LD_{50} value of 5,000 mg/kg strongly suggests the extract to be relatively non-toxic with acute administration. LD_{50} is a useful index in assessing the safety margin of a substance but it should not be viewed as an absolute value or as being equitable to complete investigation of substance toxicity. LD_{50} may not accurately reflect the full spectrum of toxicity or hazard associated with drug or chemical (Cassarette *et al*., 1996). Even though acute toxicity test represents the first fundamental test in investigation of unknown substances, it should not be regarded as a biological constant since it is affected by several variables such as the animal species, gender, age, route of administration and laboratory conditions (Cassarette *et al*., 1996). The reason for conducting the median lethal dose study is to establish the dose of the administered substance that will kill or cause serious injury to fifty percent of the study population (Cassarette *et al*., 1996). The selected doses for this study were less than

30% of the estimated LD_{50} (Vongtau *et al.,* 2004). Thus it can serve as a guide for the selection of doses to be used for efficacy studies.

Daily administration of the methanol leaf extract of *Culcasia angolensis* for 28 days at graded doses of 125, 250 and 500mg/kg per body weight did not show any statistically significant change in the weight of experimental animals over the period of the study. Body weight changes can be as a result of adverse effects of certain substances and it is a vital index of the general health of experimental animals(Grance *et al.*, 2008; Tucci, 2010). The results obtained in this study suggests that methanol leaf extract of *Culcasia angolensis*treatment marginally increase body weight of the rats inall doses used in the study but the increament was not statically significant as compared to the control group. This could possibly be attributed to loss of appetite leading to decrease intake of food by the rats or the extract may have interferedwith nutrient uptake from the gut or the absorption processes.

The methanol leaf extract of *Culcasia angolensis* produced a significant and dose dependent inhibition of acetic acid induced writhing in swiss albino mice. The abdominal constriction response induced by acetic acid is a sensitive procedure to screen peripherally and centrally acting analgesic agents. The sensitivity of this model is such that it is capable of detecting analgesic activity of compounds at doses that may appear inactive with other models (Aiyelero et al., 2009; Mishra *et al*., 2011; Kakoti *et al*., 2013). Acetic acid is known to stimulate the release of cyclooxygenase enzymes which mediate the conversion of arachidonic acid to prostaglandins which are potent mediators of pain and inflammation (Kuehi and Egan 1980). Non-steroidal anti-inflammatory drugs reduce writhes induced by acetic acid by inhibiting COX in peripheral tissues

therebyblocking the release and/or synthesis of inflammatory mediators. This is achieved by preventing the cyclooxygenase mediated conversion of arachidonic acid to prostaglandin (Smith*et al*., 1998; Donkor *et al*., 2013). The central acting analgesics such as morphine produce their analgesic effect by reducing the sensory effect of the noxious stimulus (Stevenson *et al*., 2006). The methanol leaf extract of *C. angolensis* demonstrated significant level of analgesic activities by effectively inhibiting pain induced by the acetic acid, suggesting the extract may be eliciting its analgesic effect by inhibiting COX in the peripheral tissues, possibly by blocking the release and/or synthesis of inflammatory mediators of prostaglandin origin such as prostaglandin E2 and PF2α

(Cashman, 1996). While other endogenous mediators such as serotonin, histamine and bradykinin may also be acting centrally by reducing the sensory effect of acetic acid. (Wang *et al.*, 2014; Mbiantcha *et al.*, 2011)

The methanol leaf extract of Culcasia angolensis produced a statistically significant increase latency of thermally induced pain in a dose dependent manner. The hot plate thermal model is used to evaluate central antinociceptive effect of drugs (Vogel, 2008; Bhalke and Pal, 2012). The responses are jumping, withdrawal of the paws and licking of the paws. The latency time taken for these responses to occur is prolonged after administration of centrally acting analgesics, whereas peripheral analgesics do not generally affect these responses (Vogel, 2008). The centrally acting agents like morphine activate the release of endogenous peptide and interaction with biogenic amines like epinephrine, norepinephrine, dopamine and serotonin (Winter *et al*., 1962)which are carried to the spinal cord to inhibit the pain muscle transmission within the dorsal horn,

while the involvement of chemical mediators of pain like prostaglandin and prostacyclins is minimised (Katzung, 2005; Bachlav *et al.,* 2009). The ability of methanol leaf extract of *Culcasia angolensis* to increase the latency in the hot plate model indicates that the extract may possess central analgesic activity. Opiates mediate their antinociceptive activities by a receptor specific action on a variety of locations in the brain which include the periaqueductal grey matter, the rostral ventral medulla, and the substantia nigra and within the spinal cord the dorsal horn (Myers and Yaksh, 1968). The opioid receptors (*mu, delta and kappa* receptors) coupled with G-proteins activates a cascade of events involving modulation of the voltage sensitive calcium channel, potassium efflux, reduction in cyclic adenosine monophosphate synthesis (AMP), consequently inhibiting neuronal excitation and in turn diminishing pain stimulus (Kelly*et al.,* 2001).

Formalin test is a useful model, particularly for the screening of new compounds, since it encompasses inflammatory, neurogenic, and central mechanisms of nociception. It is also a valuable model for continuous pain resulting from formalin-induced tissue injury (Tjolsen *etal*., 1992). It provides a more valid model for clinical pain compared to models such as the hot plate and tail flick tests (Bannon and Malmberg,2007). Formalin test differs from most other nociceptive tests in that it is non-specific since it is believed to mediate both neurogenic and inflammatory pain. Formalin is a chemical, little or no restraining of experimental animals is needed during testing and the nociceptive stimulus and response are persistent rather than transient (Bannon and Malmberg, 2007).

Formalin induced inflammatory response is usually bi-phasic in nature (early and late phase or the acute and chronic phase) (Tanko *etal*., 2008). The first phase which usually occurs within 5 minutes is mainly due to the release of histamine, serotonin and kinins as

a result of direct chemical stimulation of nociceptors. The second phase is the chronic phase and lasts for about 20-40 minutes (Vinegar *et al.,* 1969; Chan*et al*., 1995). Centrally acting analgesics such as morphine inhibit both phases, while peripherally acting analgesics such as NSAIDS inhibit only the chronic phase (Vogel, 2008). The anti-inflammatory activity that was elicited by the methanol leaf extract of the plant *Culcasia angolensis* may be due to its ability to inhibit the release of the mediators of inflammation. The ability of the methanol leaf extract of *C. angolensis* to inhibit both phases of formalin induced pain suggests that it may be acting through both peripheral and central mechanisms.

Carrageenan induced inflammation is the most commonly used experimental model for evaluating the anti-inflammatory potency of compounds or natural products (Winter *et al.,* 1962)Carrageenan as a phlogistic agent for testing anti-inflammatory drugs is non-antigenic and devoid of apparent systemic effect. The oedema induced is rapid and gives a more reliable result with anti-inflammatory drugs (Chakraborty *et al.,* 2006). The carrageenan-induced inflammatory process is believed to be a biphasic. The initial phase which begins at the first hour is due to the release of histamine and serotonin while the second phase which occurs at the third hour is attributed to the release of prostaglandins, bradykinins and lysosomes (Brooks and Day, 1991). The anti-inflammatory response was more pronounced at the fourth and fifth hour after sub-plantar injection of carrageenan (1%w/v) as shown in the percentage inhibition calculated. It was also observed that the percentage inhibition of inflammation gradually increased from zero hour up till the fifth hour being highest at the fourth and fifth hour. This indicates that the extract possesses a significant effect against acute inflammation. Although there is inhibition at the first and

second hour, the dose dependent effect from the third hour can possibly be due to the inhibition of COX responsible for prostaglandin biosynthesis. This can be explained by the fact that prostaglandins which are the major mediators of inflammation are released around the third and fourth hour and probably inhibited by the extract. The ability of the extract to inhibit carrageenan induced paw oedema suggests that it possesses a significant effect against acute inflammation.

There was an elevation in rectal temperature of all treated wistar rats 18 hours post administration of brewer's yeast. The extract at all tested doses (125, 250, 500 mg/kg body weight) caused a decrease in rectal temperature compared to the control. However, the group treated with 500 mg/kg body weight of the extract, at the third hour produced a statistical significant ($P \leq 0.01$) decreased in rectal temperature compared to paracetamol, the standard antipyrectic agent. This suggests that the extract posseses significant antipyrectic activity justifying its ethnomedical use in the treatment of fevers.

The liver is the primary organ in the metabolism and biotransformation of drugs, chemical substances and elimination of toxins in living systems and therefore susceptible to injuries by either the substances themselves or their toxic metabolites (Johthy *et al*., 2009). Therefore, a deleterious activity or injury to the liver is assessed through the measurement of plasma concentration of enzymes secreted by the liver and the histological study.

Theserumhaematologyandclinicalbiochemicalanalysis were done to evaluate the possible alterations in hepatic and renal functions influenced by the extract. Liver and kidney function analysis is very important in the toxicity evaluation of drugs and plant extracts as they are both necessary for the survival of an organism (Olorunnisola *et al*., 2012).

High levels of ALT, AST, and alkaline phosphatase are reported in liver diseasesorhepatotoxicity (Brautbar *et al*., 2002).

Liver enzymes are sensitive indicators of liver injury and a moderate elevation of aminotransferases (ALT and AST) in the blood stream or plasma usually suggests chronic hepatitis or biliary obstructions (Karthikeyan *et al*., 2006; Usha *et al*., 2008). In the 28 days subchronic toxicity study, the activity of ALT decreased, while that of AST increased, but none was significant with respect to the control. These slight changes therefore suggest containment within the limits of the normal biochemical or metabolic adjustments of the body system. Although the presence of γ-glutamyltransferase or 5'-nucleotidase which are used to differentiate the hepatic from the extrahepatic sources of alkaline phosphatase (ALP) were not investigated in this study, there was a significant dose-dependent increase in the serum level of ALP in rats treated with methanol leaf extract of *C. angolensis* for 28 days. According to Hall and Cash (2012), moderate increase in ALP, with little or no increase in ALT and AST suggests primary biliary cirrhosis or primary sclerosing cholangitis. The total protein and albumin were not altered in a consistent pattern.

A decrease in total protein and albumin is a sign of reduced synthetic function of the liver or mightbeduetoimpairedhepatocellularfunction.Lowserum albumin content may suggest infection or continuous loss ofalbumin (Tietz, 1994; Yakubu *et al*., 2003).Thus,the insignificant changeinserum concentrationoftotalprotein and albumin inthe*C. angolensis* leaf extracttreatedandcontrolgroupfurtherconfirmsthat the extract does not damage the hepatocellular or secretory functions of the liver at any of the doses tested.

Renal dysfunctioncanbeassessedbyconcurrentmeasurementsof urea,creatinineanduricacidandtheirnormallevelsreflect at reduced likelihood of renal problems (Davis, 2007). In the present study,changesinplasma urea and creatininelevels in *C. angolensis* leaf extract treated groups showed significant differencesindicatingabnormalrenalfunction or renal impairment.

Urea is a non-protein nitrogen compound produced in the liver from ammonia as an end product of protein metabolism and which is often carried in the blood to the kidneys for excretion. Like creatinine,it can be used to measure renal function (Fischbach and Dunning, 2005). Thus, the observed significant increase in serum urea at the 500 mg/kg extract dose in this study could be related to decreased kidney function and subsequent inability to excrete the urea. High serum creatinine level indicates kidney problems, while reduced level of serum creatinine signifies reduced muscle activity as in health problems and normal aging. The effect of the extract on creatinine in this study was neither significant nor in a consistent pattern. Generally, increase in serum creatinine level had been reported to start only when about half of the nephrons are impaired (Van-leeuwen *et al*., 2011)**.**

The insignificant changes observed in the electrolyte levels were not in a consistent manner, but slight decrease in the chloride level occurred at the higher doses of the extract. The liver and kidneys are considered highly useful in toxicity studies because of their involvement in several essential detoxification functions and which exposes them to harmful compounds.

Evaluation of haematological parameters can be used to determine the extent of the deleterious effect of *C. angolensis* leaf extract on the blood of experimental animal. It can

also be used to explain blood relating functions of a plant extract or its products (Yakubu *et al*., 2007). Furthermore, such analysis is relevant to risk evaluation as changes in the haematological system have higher predictive value for human toxicity when the data are translated from animal studies (Olson *et al*., 2000). The non-significanteffectoftheextractontotalredbloodcells,mean corpuscular volume, mean corpuscular Hb, and platelets indicatesthat the extractdoesnotaffecttheerythropoiesis, morphology, or osmotic fragility of the red bloodcells (Guyton, 2000).However, there was a significant increase in the level of lymphocytes at the 500 mg/kg dose group. This increase could suggest that the extract may contain biologically active compound(s) that may have activated the immune system (WHO, 2004) or may suggest toxicity of the extract at that dose level which could also be due to an imbalance in the rate of the lymphocyte synthesis and catabolism (Cooper, 2004). Leukocytesarethefirstlineofcellulardefensethat respond to infectious agents, tissue injury, or inflammatory process.

The histological examination of body organs is one of the golden standards for evaluating treatment related pathological changes (OECD, 2008) and five vital organs were used to assess *C.angolensis* for toxicity. The heart was not affected, while the morphology of liver, kidney spleen and lungs were altered. The histological slides showed a dose dependent hepatocellular necrosis and vacuolations in the liver which may have resulted in the increase in ALP that was seen in the liver function test. Reports have shown that liver ALP is located histochemically in the microvilli of bile canaliculi and on the sinusoids surface of the hepatocytes (Hall and Cash, 2012), thus agents that affect the liver as with the ethanol leaf extract of *C. angolensis* may also cause alterations in the

concentration of this enzyme. Necrosis that was observed in the glomerulus of the kidney may also cause the increase in serum urea concentration, observed in this study, and which probably reduced its filtration ability. Results of the histological studies on the lungs showed that the extract produced a dose dependent alveolar congestion and necrosis compared to the control. It has been established in the present study that the extract possesses good analgesic activity with a possible peripheral activity like NSAIDS.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The methanol leaf extract of *Culcasia angolensis* was found to possess significant analgesic, anti-inflammatory and antipyretic activities at the doses tested, which justify its use in traditional medicine in Nigeria and other African countriesin the management of pain, inflammation and fever conditions. The extract was found to be practically nontoxicwith an LD_{50}>5,000 mg/kg body weight on oral administration. However, the extract was found relatively toxic particularly with prolonged use on the liver and kidney.

6.2 Recommendations

Based on the knowledge acquired in the course of this research, the following recommendations for future work are proposed:

1. Detailed chronic toxicological screening should be conducted on the plant extract
2. Studies should be carried out to isolate, characterize and elucidate the structure of the bioactive constituents responsible for the observed pharmacological effects.
3. Pharmacodynamic and pharmacokinetic studies should be undertaken to establish the exact mechanism of action of both the extract and fractions.
4. Studies should be conducted to elucidate the actual mechanism of action of the extract.
5. Other models such as *Drosophila melanogaster* should be used to conduct this study

6.3 Contribution to Knowledge

1. Oral medial dose of methanol leaf extract of Culcasia angolensis was established to be greater than 5000 mg/kg body weight

2. Toxicological study found that the methanol leaf extract of *Culcasia angolensis* is relatively safe with no observable changes in liver function biomarkers and haematological indices at 125 and 250 mg/kg body weight

3. The methanol leaf extract of *Culcasia angolensis* exerts significant ($p< 0.05$) analgesic activity (3.28±0.31) relative to control (1.36±0.28), anti-inflammoryactivity (1.34±0.172) relative to Ketoprofen (1.00±0.241) and antipyretic activity (37.66±0.43) relative to PCM (37.19±0.48) at 500mg /kg body weight

REFERENCES

Abbas, A.B. & Lichtman, A.H. (2009).Innate Immunity in Saunders (Elsevier).Basic Immunology.*Functions and disorders of the immune system*, 3rd edition.ISBN 978-1-4160

Abdulla, M.A., Lutfi, M.F., Baket, A.O. & Mohammed A.H. (2013).Evaluation of the Anti-inflammatory Effects of Blumea aurita. *Global Journal of Medical Research,* 13(4), 1-8.

Abrahamson, N. & Melton, B. (2000). Leucocytosis: Basics of Clinical Assessment. *American Family Physician*, 62, 2053-2060.

Adamu, A., Abdurrrahman, E.M., Ibrahim, H., Abubakar, M.S., Magaji M.G. & Yaro, A.H. (2007). Effect of Aqueous Methanolic Stem of bark extract of *Maerua angolensis* on acute and sub-acute inflammations. *Nigerian Journal of Pharmaceutical Sciences,* 6(2), 1-6.

Adedapo, A.A., Sofidiya, M.O.& Afolayan, A.J. (2009). Anti-inflammatory and analgesic activities of the aqueous extracts of Margaritaria Discoidea (Euphorbiaceae) stem bark in experimental animal models. *Revista de Biologia Tropical*, 57, 1193–1200.

Adeyemi, O.O., Okpo, S.O. & Onakade, S.A. (2005). Anti-inflammatory activity of the Methanolic extract of Acanthus montanus.*West African Journal of Pharmacology and Drug Reaseach*, 19(1-2), 13-17.

Ahmadiani, A., Hosseiny, J., Semnanian, S., Javan, M., Saeedi, F., Kamalinejad, M.& Serami, S. (2000). Antinociceptive and Anti-inflammatory effects of *Elaeagnus angustifolia* fruits extract. *Journal of Ethanopharmacology*, 72, 287-292.

Aiyelero, O. M., Z. G. Ibrahim, and A. H. Yaro. "Analgesic and anti-inflammatory properties of the methanol leaf extract of Ficus ingens (Moraceae) in rodents." *Nigerian J Pharm Sci* 8 (2009): 79-86.

Alamgeer, U.M., Muhammad, N.V., Hafizullah, K., Safeerah, M., Muhammad, N.H.M., Nazia, T., Abdul-Qayyum, K., Hassen, A., Wasim, K., Ibrahim, J. & Haider, A. (2013).Analgesic and Antiinflammatory Activities of *Thymus serphyllum Linn* in mice.*Polish Pharmaceutical Society- Drug Research*, 72 (1), 113-118.

Alessandri, A.L., Sousa, L.P., Lucas, C.D., Rossi, A.G., Pinho, V. &Teixeira, M.M. (2013). Resolution of inflammation: Mechanisms and opportunity for drug development. *Pharmacology and Therapeutics*, 139, 189–212.

Allison, M. R. & Samaj C. F. (1995). Apoptosis: regulation and relevance to toxicity. *Human Expérimental Toxicology,* 14, 234 – 47.

Almeida, R.N., Navarro, D.S. & Barbosa-Filho, J.M. (2011). Plants with central analgesic activity. *Phytomedicine,* 8(4), 310-322.

Almekinders, L.C. (1999). Anti-inflammatory treatment of muscular injuries in sport.An update of recent studies.*Sports Medicine*, 28, 383-388.

Amin, E., Abouzid, S. & Seida, A. (2012).Phytochemical and Biological Screening on Isoflavonoids from *Dalbergia paniculata* Cultivated in Egypt.*Pharmacologia,* 3(3), 84-89.

Apkarian, A.V., Bushnell, M.C., Treede, R.D.&Zubieta, J.K. (2005).Human brain mechanisms of pain perception and regulation in health and disease.*European Journal of Pain,* 9, 463-484.

Arome, D.A., kapabio, I.S., Onalike, E.I. and Agbafor, A. (2016). Pain and inflammation: Management by conventional and herbal therapy. *Indian Journal of Pain*, 28, 5-12.

Arrau, S., Delporte, C., Cartegena, C., Rodriguez-Diaz, M., Gongalez, P., Silva, X., Cassels, B.K. & Miranda, H.F. (2010). Antinociceptive activity of *Quillaja saponaria* Mol. Saponin extract, quillaic acid and derivatives in mice. *Journal of Ethnopharmacology,* 133, 164-167.

Arthur, S.J. & John, B. (1978). *Acolour Atlas of Histopathological Staining Techniques*. Wolf MED.Pub. Ltd. London, pp 14-20.

Axelrod, Y.K. & Diringer, M.N. (2008).Temperature management in acute neurologic disorders.*Neurologic Clinics*, 26, 585–603.

Ayinde, B. A., & Agbakwuru, U. (2010). Cytotoxic and growth inhibitory effects of the methanol extract Struchium sparganophora Ktze (Asteraceae) leaves. *Pharmacognosy magazine*, *6*(24), 293.

Bachlav, R. S., Gulecha, V. S., & Upasani, C. D. (2009).Analgesic and Anti-inflammatory Activity of Argyreia speciosa roots. *Indian J Pharmacol*, *41*(4), 158-161.

Bairy, K. L., Chogtu, B. & Nayak, V. (2015).Effect of fluticasone propionate with biopolymer on skin inflammatory model in wistar rats.*Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 6, 721-723.

Bannon, A. W., & Malmberg, A. B. (2007). Models of nociception: hot- plate, tail- flick, and formalin tests in rodents. *Current protocols in neuroscience*, *41*(1), 8-9.

Barar, F.K. (2009). *Essentials of Pharmacotherapeutics*.Non-narcotic analgesics and antipyretics, anti-inflammatory agents, drugs for gout, rheumatoid arthritis and migraine. 5th ed., S. Chand & company Ltd, New Delhi. pp. 117-137.

Baumann, T.J. (2002).*Pain Management*. In: Dipiro T.J., Talbert, R.L., Yee, G.C., Matzke, G.R., Wells, B.G. and Posey, M.L. (Eds) Pharmacotherapy: Pathophysiologic Approach 5th edition, McGraw Hill Co. Inc. U.K Pp. 1103-1117.

Beg, S., Swain, S. & Hasan, H. (2011). Systematic review of herbals as potential anti-inflammatory agents: Recent advances, current clinical status and future perspectives. *Pharmacognosy Reviews*. 5, 120–137.

Bentley, G.A., Newton, S.H. & Starr, J. (1981). Evidence for an action of morphine and enkephalins on sensory nerve endings in the mouse peritoneum. *British Journal of Pharmacology*, 73, 325-332.

Berry, P.H., Chapman, C.R., Covington, E.C., Dahl, J.L., Katz J.A., Miaskowski, C., &McLean, M.J. (2001). Pain: current understanding of assessment, management, and treatments. *National Pharmaceutical Council and the Joint Commission for the Accreditation of Healthcare Organizations, VA, USA*, b44.

Besson, J. (1999). The Neurobiology of Pain.*Lancet*, 353, 1610 – 1615.

Bhalke, R.D. and Pal, S.C. (2012). Anti- inflammatory and antinociceptive activity of *Pterospermum acerifolium* leaves. *Asian Journal of Pharmaceutical and Clinical Research*, 5(2): 23-26.

Bhangoo, S.K.& Swanson, G.T. (2012).Kainate receptor signaling in pain pathways.*Molecular Pharmacology*, 83, 307-315.

Bhujbal, S., Patil, K. & Patil, M. (2006). Evaluation of Antipyretic Potentials of *Celosia argentea* Linn Leaf extract. *Planta Indica*, 2, 19-20.

Bishaw, M. (2007).Promoting Traditional Medicine in Ethopia: A brief Historical Review of Government Policy.*Social Science Medicine*, 33,193-200.

Bliddal, H., RosetzskyT. & Schlichting, A. (2000). A randomized, placebocontrolled, cross-over study of ginger extracts and ibuprofen in osteoarthritis.*Osteoarthritis Cartilage Journal.* 8, 9-12.

Brautbar, N., & Williams II, J. (2002). Industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms. *International journal of hygiene and environmental health*, *205*(6), 479-491.

Brook, P.M. and Day, R.O. (1991). Non steroidal anti-inflammatory drugs differences and similarities. *New England Journal of Medicine,* 324: 1716-1725.

Burke, A., Smyth, E. & Fitz, G.A. (2006). Analgesic-Antipyretic Agents; Pharmacotherapy of Gout. In: Brunton, L.L., Lazo, J.S and Parker, K.L. (Eds).*Goodmann and Gilman's, The Pharmacological Basis of Therapeutics*, 11th Ed. Mc Graw Co. Inc. New York, Pp 671-715.

Burkill. H.M, Royal Botanic Garden; Kew (1985-2004).

Burkill,H.M. (1985).The Useful Plants of West Tropical Africa, 2nd Ed. Royal Botanical Gardens, Kew England, 1, 452-453.

Bown, D. (2000). *Aroids: Plants of the Arum family* (No. Ed. 2). Timber press.

Calixto, J.B., Otuki, M.F. & Santos, A.R.S. (2003).Anti-inflammatory compounds of plant origin.Part I. Action on arachidonic acid pathway, nitric oxide and nuclear factor κB (NF-κB).*Planta Medica*, 69, 973–983.

Canter, P. H.& Ernst, E. (2004). Herbal supplement use by persons aged over 50 years in Britain: Frequently used herbs, concomitant use of herbs, nutritional supplements and prescription drugs, rate of informing doctors and potential for negative interactions. *Drugs Aging*, 21, 597–605.

Caraceni, A., Cherny, N., Fainsinger, R., Kaasa, S., Poulain, P., Radbruch, L. & De Conno, F. (2002).Pain measurement tools and methods in clinical research in palliative care.*Journal of Pain and Symptom Management*, 23(3), 239-242.

Cartmell, T., Poole, S., Turnbullp, A.V., Rothwell, N. J.& Luheshi, G. N. (2000). Circulating interleukin-6 mediates the febrile response to localized inflammation in rats. *Journal of Physiology*. 525, 653-661.

Cashman, J. N. (1996). The mechanisms of action of NSAIDs in analgesia.*Drugs*, *52*(5), 13-23.

Cassarette, I., Klaassen, C.D., Amdur, M.O. & Doulls, J. (1996).*Principles of Toxicology* In: Cassarett and Doul's Pharmacology, The Basic Science of Poison Edited by Curtis, D. Klaassen, 5th edition copyright McGraw – Hill (USA) Health Professional Division New York. Pp 13 – 33; 403-414.

Chakraborty, A., Devi, R.K., Rita, S., Sharatchandra, K., Singh T.I. (2006). Preliminary studies on anti-inflammatory and analgesic activities of *Spilanthes acmella* in experimental animal models.*Indian Journal of Pharmacology*, 36: 148-150.

Chan, Y.F., Tsai, H.Y. & Wu, T.S. (2000).Anti-inflammatory and Analgesic Activity of Extracts from the Root of *Angelica pubescens. Planta Medica,* 61, 2-8.

Chandra, D.& Gupta, S. (1972). Anti-inflammatory and anti-arthritic activity of volatile oil of Curcuma longa (Haldi).*Indian Journal of Medical Research,* 60, 138-142.

Chapman, C.R., Tuckett, R.P. & Song, C.W. (2008). Pain and Stress in a Systems Perspective: Reciprocal, neural, endocrine and immune interactions. *Journal ofPain* 9(2), 122-45.

Choi, J., Jung, H., Lee, K. & Park, H. (2005). Antinociceptive and Anti-inflammatory effects of Saponins and Sapogenan obtained from the Stem of *Akebia quinata. Journal of Medicinal Food,* 8(1), 78-85.

Chrubasik, S. (2004). Devil's claw extract as an example of the effectiveness of herbal analgesics. *American Journal of Medicine*. 33, 804–809.

Cotran, Kumar& Collins (1998). *Robbins Pathologic Basis of Disease*. Philadelphia: W.B Saunders Company. ISBN 0-7216-7335-X.

Cousins, M.J., Brennan, F.& Carr, D.B. (2004). Pain relief: A Universal Human Right. *Pain,* 112(2), 1-4.

Cox, W., Abramson, T.L., Lyn Y., Devine, P.G.& Hollon, S.D. (2012).Sterotypes, Prejudice and Depression: The integrated Perspectives. *PerspectivesonpsychologicalSciences*, **7**(5), 427–449.

Crawford, J. M. (1994). The Liver and the Biliary Tract, Cortrans R.S, Kumar V, Robbins SL (eds): Robbins: *Pathological Basis of Disease,* 5th ed Philadelphia: Saunders Pp 831-896.

Curtis, C.L., Harwood, JL., Dent, CM., and Caterson, B. (2004). Drug Discovery Today.*Journal of Drug Discovery and Therapeutics*. 9, 165-174.

Czock, D., Keller, F., Rasche, F.M. & Häussler, U. (2005).Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids.*ClinicalPharmacokinetics*, 44(1), 61-98.

Dacie, J.V. (1958). Practical Hematology, 2nd edition. A Churchill Ltd., London. pp. 38-48.

Dahanukar, R.A., Kurkarni, R.A. & Rege, N.N. (2000).Pharmacology of Medicinal Plants and Natural Products.*Indian Journal of Pharmacology*, 32, S81-S118.

Danesh, J., Wheeler, J.G., Hirschfield, G.M., Eda, S., Eiriksdottir, G., Rumley, A., Lowe, G.D., Pepys, M.B. & Gudnason, V. (2004). C-reactive Protein and other circulating Markers of Inflammation in the prediction of Coronary Heart Disease. *New England Journal of Medicine,* 351, 1387-1397.

Daniel, S.G. & Summer, J.G. (2011). Pain as a global public health priority, BMC *PublicHealth*, 11:770 doi: 10. 1186/1471-2458-11-70.

D'Armour, F.E., Blad, F.R.& Belden, D.A. (1965).Manual for Laboratory work in Physiology, 3rd edition. The University of Chicago Press, Chicago. Illinois. pp.4-6.

Davis, J. B., Gray, J, Gunthorpe, M. J., Hatcher, J. P., Davey, P.T., Overend, P., Harries, M. H., Latcham, J., Clapham, C., Atkinson, K., Hughes, S. A., Rance, K., Grau, E., Harper, A. J., Pugh, P. L., Rogers, D. C., Bingham, S., Randall, A. & Sheardown, S. A. (2000). Vanilloid receptor-1 is Essential for Inflammatory Thermal Hyperalgesia. *Nature,* 405, 183-187.

De Broe, M.E. & Elseviers, M.M. (1998).Analgesic Nephropathy.*New England Journal of Medicine*, 338(7), 446-52.

De Sousa, D.P. (2011). Analgesic-like Activity of Essential Oils Constituents Molecules. *Phytopharmacology*. 16, 223-225.

Derardt,R., Jougney,S., Delevalcee,F.& Falhourt, M. (1980).Release of Prostaglandins E and F in an Algogenic Reactions and its Inhibition.*EuropeanJournal of Pharmacology*, 61(1), 17-24.

Derman, W., Frohlich, E., Grolman, D., Hitchcock, S., Hodgson, E., Kluyts, H., Lundgren, C., Milner, A., Mohr, W., Penfold, P., Raff, M., Thomas, J. & Travers, A. (2009). South African Acute Pain Guidelines: Measurement and Assessment of Acute Pain. *SouthAfricanJournalofAnaesthesiaandAnalgesia*, 5(6), 16-18.

Devi, B. P., Boominathan, R., & Mandal, S. C. (2003). Anti-inflammatory, analgesic and antipyretic properties of Clitoria ternatea root. *Fitoterapia*, 74(4), 345-349.

Dhannanjayan, R. & Thangam, C. (2003).Anti-inflammatory Potential of the Seed of *Carum copticum*, Linn. *Indian Journal of Pharmacology*, 35, 388-391.

Donkor, K., Stephen, A., Jerry, A., Nutifafa, T., Nii, O.M. & Laud, K.O. (2013). Analgesic and anti-inflammatory activities of Asena, a herbal preparation for treatment of arthritis, using rodent models. Medicinal and Aromatic *Plant Research Journal*, 1(2), 20-29.

Doreswamy, R. & Darshan, S. (2004). Patented Anti-inflammatory Plant Drug Development from Traditional Medicine.*Phytotherapy research*, 18, 348-357.

Dubey, R.C.& Maheswari, D.K. (2005).*A text book of Microbiology*; 5th edition published by S.Chand. New delhi pp 67.

Duke, J. (1992). *Handbook of Biologically Active Phytochemicals and their Activities*. CRC Press, Boca, Raton, FL. Pp 47-62.

Eddy, N.B. & Leimbach, D. (1953). Synthetic Analgesics II Dithienylbutenyl and Dithienylbutylamines. *JournalofPharmacologyandExperimentalTherapeutics,* 107, 385-393.

Elisabetskey, E.A., Ahmador, RR., Nunse, D.S.& Carvallo, A.T. (1995). Analgesic activity of Phshotua Colorata Muell.*Journal of Ethnopharmacology*. 48: 77–83.

Evans, W.C. (1996). *Trease and Evans Pharmacognosy*. London Villiere Tindal U.K.., Pp 57–77.

Ezeonwumelu, J.O., Omar, A.N., Ajayi, A.M., Okoruwa, A.G., Tanayen, J.K., Kiplagat, D.M., Okpanachi, O.A., Abba, S., Ezekiel, I., Onchweri, A.N., Okonkwo, C.O. & Byarugba, F. (2012). Phytochemical Screening, Acute toxicity, Anti-inflammatory and Antipyretic Studies of Aqueous Extract of the root of *Flueggavirosa* (Roxb Ex Wild) in Rats.*International Journal of Pharmaceutical and Biomedical Sciences*, 3(3), 128-135.

Fauci, A. (2008). *Harrison"s Principles of Internal Medicine* (17th edition) McGraw – Hill Professional. New York. pp. 117-121.

Ferrero-Miliani, L., Nielsen, O.H., Anderson, P.S. & Girardin, S.E. (2007).Chronic Inflammation.*Clinical ExperimentalImmunology*, 147(2), 227-35.

Field, H.L., Rowbotham, M. & Baron, R. (1998). Post herpetic Neuralgia: irritable nociceptors and de-afferentation. *Neurobiology ofDiseases*, 5 (4), 209-27.

First, M.R. (1996).Renal function. In: L.A. Kaplan and A.J. Pasce, EDS. Clinical Chemistry, Theory, Analysis and Correlation, 3rded New York, Mosby, Inc Pp 486.

Gaertner, M., Muller, L., Roos, J.F., Cani, G., Santos, A.R., Calixto, J.B., Monache, F. & Cechinel Filho, V. (1999). Analgesic triterpenes from *Sebastiania schottiana* root. *Phytomedicine*, 6, 41-44.

Gale, K., Kerasidis, H., & Wrathall, J. R. (1985). Spinal cord contusion in the rat: behavioral analysis of functional neurologic impairment. Gaertner, M., Muller, L., Roos, J.F., Cani, G., Santos, A.R., Calixto, J.B., Monache, F. and Cechinel Filho, V. (1999). Analgesic triterpenes from *Sebastiania schottiana* root. *Phytomedicine*, 6, 41-44.

Garba, I., Abdurahman, E.M., Ibrahim, H., Ibrahim, N.D.G.& Magaji, M.G.(2011). Toxicity and Analgesic effects of *Vernonia amygdalina* Del. (Asteraceae) leaf extract on Mice.*International Journal of Advanced Pharmaceutical and Biological Sciences*, 1(1), 1-4.

Gedif, T. & Hahn, H.J. (2003). The Uses of Medicinal Plants in self-care in Rural Central Ethopia.*JournalofEthnopharmacology*, 87, 155-161.

Gene,R.M., Segura, L., Adzet,T.,Marin, E. & Ingelsias, J. (1998).*Heterothecainuloides*: Inflammatory and Analgesic Effects. *JournalofEthnopharmacology*,60, 157-162.

Gerstner, H. B., & Huff, J. E. (1977).Selected case histories and epidemiologic examples of human mercury poisoning. *Clinical toxicology*, *11*(2), 131-150.

Giordano, J. (2005). The Neurobiology of Nociceptive and Antinociceptive Systems.*PainPhysician*, 8, 277-290.

Gislason, G.H., Rasmussen, J.N., Abildstran, S.Z., Schramm, T.K., Hansen M.L., Fosbol E.L., Sorensen, R., Poulsen, H.E., Kober, L., Madsen, M. & Torp-Perdesen, C. (2009).Increased Mortality and Cardiovascular Morbidity Associated with use of Non Steroidal Anti-inflammatory Drugs in Chronic Heart Failure.*Archives of Internal Medicines*, 169(2), 141-149.

Gogtay, N.J., Bhatt, H.A.,Dalvi, S.S. & Kshirsagar, N.A. (2002).The Uses and Safety of non-allopathic Indian Medicines.*Drugsafety*, 25, 1005-1019.

Goldberg, S.D.& McGee, J.S. (2011).Pain as a global public health priority.*BMC PublicHealth*, 11,770.

Govaerts, R. & Frodin, D.G. (2002). World Checklist and Bibliography of Araceae (and Acoraceae): 1-560. The Board of Trustees of the Royal Botanic Gardens, Kew.

Grance, S. R. M., Teixeira, M. A., Leite, R. S., Guimarães, E. B., de Siqueira, J. M., de Oliveira Filiu, W. F., ... & do Carmo Vieira, M. (2008). Baccharis trimera: effect on hematological and biochemical parameters and hepatorenal evaluation in pregnant rats. *Journal of Ethnopharmacology*, *117*(1), 28-33.

Green, A.F., Young, P.A.& Godfrey, E.L. (1951).A comparison of heat and pressure analgesimetric methods in rats.*Britain pharmacology*. 6, 572-585

Greenhalgh, D. G. (1998). The role of apoptosis in wound healing. *The International Journalof Biochemistry & Cell Biology*, *30*(9), 1019-1030.

Grønhaug, T. E., Glæserud, S., Skogsrud, M., Ballo, N., Bah, S., Diallo, D., & Paulsen, B. S. (2008). Ethnopharmacological survey of six medicinal plants from Mali, West-Africa.*Journal of Ethnobiology and Ethnomedicine*, 4, 1.

Guinsburg, R., Peres, C., Branco, A., Almeida, M.F., Cassia, X., Balda, R., Berenguel, R., Tonelotto, J. & Kopelman, B.I. (2000).Differences in pain expression between male and female newborn infants.*Pain*, 85(2), 127-33.

Gupta, M., Mazumder, U.K., Gomathi, P.& Selvan, V.T. (2006).Antiinflammatory activity of extract of Vernonia amygdalina.*Complementary and Alternative Medicine.* 6, 36-43.

Guyton, A.C.& Hall, J.E. (2006).*Textbook of Medical Physiology*.11th edition. Elsevier Saunders, Philadelphia. pp 429-434.

Guyton, A.C. andHall., J.E. (2000). *Textbook of medical physiology*. 10th edition: Saunders Philadelphia. pp 822–823.

Habal, F.M. & Huang, V.W. (2010).A Decision-Making Algorithm for the Management of Pregnancy in the Inflammatory Bowel Disease Patient.*Ailment Pharmacolgy Therapeutics*, 35(5), 501-15.

Haddad, J. (2007).Molecular regulation of inflammatory pain and hyperalgesia - Is NF-κB the lynchpin? Focus Article and Critical Review.*Experimental and Clinical Sciences International Journal*, 6, 68-92.

Hassel, K. A. (1982). The chemistry of pesticides: their metabolism, mode of action and usesin crop protection.Macmillan London. Pp 8-14.

Hassan, H.S., Sule, M.I., Musa, M.A., Emmanuel, A.A., Ibrahim, H., Hassan, A.S. & Yaro, A.H. (2010). Analgesic and Anti-inflammatory activity of the Saponins

extract of *Carrissa edulis* root in rodents. *InternationalJournalofBiologyandChemicalSciences,* 4(4), 1310-1317.

Hassan, M.M., Khan, S.A., Shaikat, A.H., Hossain, M.E., Hoque, M.A., Ullah, M.H.& Islam, S. (2013). Analgesic and anti-inflammatory effects of ethanol extracted leaves of selected medicinal plants in animal model. *Veterinary World,* 6, 68–71.

Hasselström, J., Liu-Palmgren, J. & Rasjö-Wrååk, G. (2002).Prevalence of Pain in General Practice.*European Journal of Pain*, 6(5), 375-85,385.

Hodge, H. C. & Sterner J.H., (1943). Determination of substances acute toxicity by LD_{50} *BritishAmerican Industrial Hygiene Association,* 10, 93-98.

Hotamisligil, G.S. & Erbay, E. (2008).Nutrient sensing and inflammation in metabolic diseases. Nature *Reviews Immunology*, 8(12), 923 - 934.

Hucho, T.& Levine, J.D. (2007). Signaling pathways in sensitization: towards a nociceptor cell biology. *Neuron,* 55 (3), 365-76.

Igumbar, E.U., Puoane, T.R., Gansky, S.A. &Plesh, O. (2011). Chronic Pain in the Community: A survey in a township in Mthatha, Eastern Cape, South Africa. *JournalofAnaesthesiaandAnalgesia*, 17(5), 103-107.

Insel, P.A. (1996). Analgesic, anti-inflammatory and antipyretic agents and drugs employed in the treatment of gout. Herdman, J.G., Limbird, L.E., Mollinoff, P.B., Ruddon, R.W. &Gilman, A.G. (Eds). *Goodman and Gilmans, The pharmacological Basis of Therapeutics,* 9th edition. McGraw-Hill Co. In: New York, U.S.A Pp. 617-657.

Ishfaq, A., Bukhari, A.D. &Rafeeq, A.K. (2004).Antinociceptive Activity of Methanolic Extracts of St. John's Wort(*Hypericum Perforatum*) preparation.*PakistanJournalofPharmaceuticalSciences*,17(2), 13-19.

Iwalewa, E.O., McGaw, L.J., Naidoo, V. &Eloff, J.N. (2007). Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. *African Journal of Biotechnology, 6*(25),2868-2885.

Jessel, T. M., Kandel, E. R.& Schwartz, J. H., (1991).*Principles of neural science.*Norwalk, C.T: Appleton & Lange. Pp 472-479

Jiang, D., Jiurong, F., Juan, Y., Shuang, C., Suping, L., Yi, P., Glenn, D., Mascarenhas, M. & Marcella, M. (2005).Regulation of Lung injury and repair by Toll-like receptors and hyaluronam.*Natural Medicine,* 11(11), 1173-1179.

Kakoti, B. B., Pradhan, P., Borah, S., Mahato, K., & Kumar, M. (2013). Analgesic and anti-inflammatory activities of the methanolic stem bark extract of Nyctanthes arbor-tristis linn. *BioMed research international*, *2013*.

Kamil, M. (1993).*Glimpses in plant research*. Vol XI, Today and Tomorrow's Printers and Publishers, New York, U.S.A Pp 377-389.

Kariuki, H.N., Kanui, T.I., Yenesew, A., Patel, N.B.& Mbugua, M.P. (2012).Antinociceptive activity of Toddalia asiatica (L) Lam in models of central and pheripheral pain.*Phytopharmacology*. 3, 122-129.

Katzung, B.G. (2005). Basic and Clinical Pharmacology, 6th ed. Appleton and Lange, Connecticut, USA. pp. 297–302.

Kelly, D. J., Ahmad, M., & Brull, S. J. (2001). Preemptive analgesia I: physiological pathways and pharmacological modalities. *Canadian journal of anaesthesia*, *48*(10), 1000-1010.

Kelly, K.W., Bluthe, R.M., Dantzer, R., Zhou, J.H., Shen, W.H., Johnson, R.W.& Broussard, S.R. (2003). Cytokine induced sickness behaviour .*Brain Behaviour and Immunology*. 1: 2-8.

Kenna, J. G., Knight T. L. & Pelt F.N.A.M. (1993). Immunity of halothane metabolite-modified proteins in halothane hepatitis.*A New York Acid Science*, 685.

Kim, H., Son, K., Charg, H. & Kang, S. (2000). Effects of Naturally occurring Flavonoids on Inflammatory Response and their action Mechanism. *Natural Product Science*, 6, 170-178.

Kluger, M.J. (1991). Fever: role of pyrogens and cryogens. *Physiology Review*. 71, 93–127.

Knight, K. (2013).Defining the COX inhibitor Selectivity of NSAIDs: Implication for Understanding Toxicity, 14-20.

Kooijman, C.M., Dijksta, P.U., Geertzen, J.H., Elzinga, A. & Van der Schans, C.P. (2000). Phantom pain and phantom sensations in upper limb amputees: an epidemiological study. *Pain*, 87(1), 33-41.

Koster, R.A., Anderson, M. & De-beer, E.J. (1959). Acetic acid for Analgesic Screening. *FederalProceedings*, 18, 412.

Kou.J., Ni, Y., Li, N., Wang, J., Liu, L.& Jiang, Z.H. (2005).Analgesic and anti-inflammatory activities of total extract and individual fractions of Chinese

medicinal ants Polyrhachis lamellidens.*Biological and Pharmaceutical Bulletin*. 28, 176–180

Koyama, K., Imazumi, T. & Akiba, M. (1997).Antinociceptive Components of *Ganoderma lucidum. Planta Medica*, 63, 117-227.

Kuehl, F. A., & Egan, R. W. (1980).Prostaglandins, arachidonic acid, and inflammation. *Science*, *210*(4473), 978-984.

Kumar, A., Agarwal, K., Maurya, K.A., Shanker, K., Bushra, U.& Tandon, S. (2015). Pharmacological and phytochemical evaluation of Ocimum sanctum root extracts for its antiinflammatory, analgesic and antipyretic activities. Pharmacognosy Magazine. 11, 17-24.

Kumar, S., Bajwa, B.S., Singh, K.& Kalia, A.N. (2013).Anti-inflammatory activity of herbal plants.International Journal of Advances in Pharmacy, Biology and Chemistry. 2, 77-88.

Kumar, S., Baker, K.& Seger, D. (2002). Dinitrophenol-induced Hyperthermia Resolving With Dantrolene Administration.Abstract of North American Congress of Clinical Toxicology. Clinical Toxicology.40 , 599 - 673.

Kuochung, T., Haruko, F., Yasuo, Y.& Yuzo, T. (2006). Effects of turpentine induced fever during the enamel formation of rat incisor. Archives of oral biology. 51: 464-470.

Lambrou, G.I., Vlahopoulus S., Papathanasiou, C., Papanikalaou, M., Karpusas, M., Zoumakis, E. & Zort-zatou-Stathopoulou, F. (2009). Prednisolone exerts late mitogenic and biphasic effects on resistant acute lymphoblastic leukemia cells; Relation to early gene expression. *Leukemia Research,* 5(7), 87-97.

Lapah, P.T., Noa, P.A.& Ogbonna, O.J. (2014).Anti-Pyretic and Analgesic Potentials of Aqueous Extract of Phragmanthera capitata S. Balle in Albino Rats. American *Journal of Pharmacy and Pharmaceutical Sciences*.1, 37-43.

Leon, L.R. (2002). Molecular biology of thermoregulation (Invited Review): cytokine regulation of fever: studies using gene knockout mice. *Journal of Applied Physiology*, 92, 2648-2655.

Leonard, J.P., Daniel, S.B. & Yonoli Xi, M.S. (2006).Increasing Deathfrom Opioid Analgesicsin theUnitedStates.*Journal of Pharmacoepidemiology and Drug safety*, 19(3), 54-67.

Lewen, H., Gardulf, A. & Nilsson, J. (2010).*Documented assessments and treatments of patients seeking emergency care because of pain. Scandinavian Journal of*

Caring Sciences,24(4), 764-771.

Lichtenstein, G.R., Hanaeur, S.B. &Sandborn, W.J. (2009).Management of Crohn's Disease in Adults.*American Journal of Gastroenterology*, 104(2), 465-83.

Loeser, J.D. and Treede, R.D. (2008).The Kyoto protocol of IASP Basic Pain Terminology.*Pain*, 137, 473–477.

Lompo, M., Guissou, I.P., Dubois, J., Dehaye, J.P., Oudraogo, S., Traore, A. & Some, N. (2007). Mechanism of the Anti-inflammatory activity of *Khaya senegalensis* A. Juss, Maliaceae.*International Journal of Pharmacology*, 1-6.

Lorke, D. (1983). A new approach to Acute Toxicity Testing. *ArchivesofToxicology*, 54, 275-287.

Loux J.J., Depalma, P.D.&Yankell, S.L.(1972). Antipyretic Testing of Aspirin in rats. Toxicology and Applied Pharmacology, 22(4), 672-675.

Magaji,M.G.,Anuka,J.A.,Abdu-Aguye, I., Yaro,A.H. & Hussaini,I.M. (2008).Preliminary studieson the Anti-inflammatoryand Analgesic activities of *Securinega virosa*(Euphorbiaceae) in experimental animal models.*Journal of Medicinal Plants Research*, 6(3), 17-25.

Mandegary,A.,Sayyah, M. & Heldari, M.R.(2004). Antinociceptive and AntiInflammatory activity of the seed and root extracts of *Ferula gummosa* Boiss in mice and rats. *Drug Addiction Research Unit*, 12(2), 58-62.

Marzouk, B., Marzouk, Z., Haloui, E., Fenina, N., Bouraoui, A.& Aouni, M. (2010).Screening of analgesic and anti-inflammatory activities of Citrullus colocynthisfrom southern Tunisia.*Jounal of Ethnopharmacology*. 128, 15-19.

Mayer, D.J. & Liebeskind, J.C. (1974).Pain reduction by Focal Electrical Stimulation of the Brain and Anatomical and Behavioural analysis.*BrainResearch*, 68, 73-93.

Maze, M., Hunter, J.C. and Gaeta, R.R. (2000). Conscious sedation and pain. In Carruthers SG, Hoffman BB, Melmon KL, Nierenberg DW (eds.): Melmon and Morrelli's Clinical Pharmacology. McGraw-Hill, NewYork, Fourth edition pp 200.

Mbiantcha, M., Kamanyi, A., Teponno, R. B., Tapondjou, A. L., Watcho, P., & Nguelefack, T. B. (2011). Analgesic and anti-inflammatory properties of extracts from the bulbils of Dioscorea bulbifera L. var sativa (Dioscoreaceae) in mice and rats.*Evidence-Based Complementary and Alternative Medicine, 2011*.

Mburu, A. S., Thurnham, D. I., Mwaniki, D. L., Muniu, E. M., Alumasa, F.& De Wagt, A. (2008). The influence and benefits of controlling for inflammation on plasma ferritin and hemoglobin responses following a multimicronutrient supplement in apparently healthy, HIV+ Kenyan adults. *The Journal of Nutrition*, 138, 613-619.

Medzhitov, R. (2008). Origin and physiological roles of inflammation.*Nature,* 454, 428–435.

Medzhitov, R. (2010). Inflammation 2010: new adventures of an old flame. *Cell,* 140, 771– 776.

Melzack, R. & Casey, K.L. (1968). Sensory, motivational and central control determinants of chronic pain: a new conceptual model. Kenshalo, DR. The Skin Senses. Springfield, Illinois: Thomas. Pp 432 – 435.

Melzack, R. & Wall, P.D. (1996*).The challenge of pain*, 2nd ED. New York: Penguin Books. P. 17-19.

Merskey, H. & Bogduk, N. (1994).*Classisication of Chronic pain.* 2nd Ed. Seattle: International Association for the Study of Pain, Pp 3- 4.

Mert, T., Ocal, I., Cinar, E., Yalcin, M.S. & Gunay, I. (2013).Pain relieving effects of pulsed magnetic fields in a rat model of carrageenan-induced hind-paw inflammation.*International Journal of Radiation Biology*, 90(1), 95-103

Middleton, E. (1998). Effect of flavonoids on immune and inflammatory cell function.*AdvancesinExperimentalandMedicalBiology*, 439, 175-186.

Mishra, D., Ghosh, G., Kumar, P.S. & Panda, P.K. (2011).An Experimental Study of Analgesic Activity of Selective COX-2 Inhibitor with Conventional NSAIDs.*AsianJournalofPharmaceuticalandClinicalResearch,* 4(1), 89-96.

Miura, M., Tamame, T., Naganuma, T., Chinen, S., Matsuoka, M. & Ohki, H. (2011).Steroid pulse therapy for Kawasaki disease unresponsive to additional immunoglobin therapy.*Paediatric and Child Health*, 16(8), 479-84.

Moulin, D.E., Clark, A.J., Gilron, I., Ware, M.A., Watson, C.P., Sessle, B.J., Coderre, T., Morley-Foster, P.K., Stinson, J., Boulanger, A., Peng, B., Finley, G.A., Taenzer, P., Squire, P., Dion, D., Cholkan, A., Gilani, A., Gordon, A., Henry, J., Jovey, R., Lynch, M., Mailis, G.A., Panju, A., Rollman, G.B. & Velly, A. (2007). Pharmacological Management of Chronic Neuropathic – Consensus statement and Guidelines from Canadian Pain Society. *PainResearchManagement*, 12(1), 13-21.

Murray, M.D. & Bratter D.C. (1993).Renal toxicity of the nonsteriodal anti-inflammatory drugs.*Annual Review of Pharmacological Toxicology,* 33, 435 – 465

Musa,A.M., YaroA.H.,Usman,H.,MagajiM.G., Maiha, B.B. & Ibrahim, O.S. (2010). Analgesic and Anti-inflammatory activities of N-butanol soluble fraction of *Cissus cornifolia* Planch. *International Journal of Pure and Applied Sciences*, 4(1), 57-63.

Mwangi, B.M., Gitahi, S.M., Njagi, J.M., Mworia, J.K., Aliyu, U., Njoroge, W.A., Mwonjoria, K.J., Ngugi, M.P.& Mburu, N.D. (2015). Antiinflammatory properties of dichloromethane:methanolic leaf extracts of Caesalpina volkensii and Maytenus obscura in animal models. *International journal of current pharmaceutical research*. 7, 83-87.

Mwonjoria, J.K., Kariuki, H.& Waweru, F.N. (2011). The antinociceptive antipyretic effects of Solanum incanum (Lin.) in animal models, *International Journal of Phytopharmaceuticals*. 2, 22-26.

Mworia, J.K., Gitahi, S.M., Juma, K.K., Njagi, J.M., Mwangi, B.M., Aliyu, U., Njoroge, W.A., Mwonjoria, K.J., Mawia, A.M., Nyamai, D.W., Ngugi, M.P.& Ngeranwa, J.N. (2015) Antinociceptive Activities of Acetone Leaves Extracts of Carissa Spinarum in Mice. *Medicinal and Aromatic Plants*.10: 1- 4.

Mycek, M.J., Harvey, R.A., Champe, P.C. & Fisher, B.D. (2000).Antidepressant drugs.*Lippincott's Illustrated Reviews*: Pharmacology 2nd edition, Lippincott Raven publishers, Philadelphia, Pp 81-142.

Myers, R. D., & Yaksh, T. L. (1968).Feeding and temperature responses in the unrestrained rat after injections of cholinergic and aminergic substances into the cerebral ventricles. *Physiology & Behavior*, *3*(6), 917-928.

Nagasako, E. M., Oaklander, A. L. & Dworkin, R. H. (2003). Congenital insensitivity to pain: anupdate. *Pain*, *101*(3), 213-219.

Naik, D.G., Mujumdar, A.M., Wagole, R.J., Kurkarni, D.K. & Kumbhorjar, M.S. (2000). Pharmacological Studies of *Sterculia foetida* leaves. *Pharmacological Biology*, 1(38), 13-17.

Nandi, J., Banerjee, A., Linscheer, W.G., Chiji, H., Murthy, U.K., Cho, C.& Chan, S.H. (1998).Induction of an ATPase inhibitor protein by propylthiouracil and protection against paracetamol (acetaminophen) hepatotoxicity in the rat.*British Journal of Pharmacology,* 124, 1041-1047

Nanyak, Z.K., Ibrahim, M.G., Ahmed, R., Nduruka, C. & Simi, D. (2013).Antiinflammatory effects of the aqueous extract of *Coffee cenaphora* (Coffe plant) leaves in rats.*Journal of Natural Science Research*, 3(7), 43-49

Naude, P.J., Cromarty, A.D.& Rensburg, C.E. (2010). Potassium humate inhibits carrageenan-induced paw oedema and a graft-versus-host reaction in rats. *Inflammopharmacology*. 18, 33–39.

Neville, A.P., Cliff, J.W. & Ian A. (2004).Journal of Inflammation, http://www.*journalinflammation*.com/content/1/1/1. [cited 2015 Jan 14].

Niemageer, C.J.E., Verbiuggen, F.J.& Jassen, P.A.J.(1964). Effect of various drugs on Carrageenan induced oedema in rat hind paw. *Journal of Pharmacy and Pharmacology*, 16, 310-816.

OECD 423.(2001). Repeated Dose 28-day Oral Toxicity Study in Rodents.OECD Guidelines for the Testing of Chemicals.

OECD 407.(2008). Repeated Dose 28-day Oral Toxicity Study in Rodents.OECD Guidelines for the Testing of Chemicals.

Olorunnisola, O. S., Bradley, G., & Afolayan, A. J. (2012).Protective effect of T. violacea rhizome extract against hypercholesterolemia-induced oxidative stress in Wistar rats. *Molecules*, *17*(5), 6033-6045.

Olurishe, T.O. & Mati, F.G. (2014).Anti-hyperalgesic potentials of *Laggera aurita* in Swiss Albino Mice.*Pakistan Journal of Pharmaceutical Sciences*, 27(1), 169-172.

Onasanwo, S.A., Fabiyi, T.D., Oluwole L.K.&Olaleye, S.B. (2012).Analgesic and anti-inflammatory properties of the leaf extracts of Anacardium occidentalis in the laboratory rodents.*Nigerian Journal of Physiological Sciences*, 27, 65-71.

Paice, J.A. (2003). Mechanism and Management of Neuropathic Pain in Cancer.*Journal of Oncology Support*, 1(12), 107-20.

Parakrama C., Clive, R. & Taylor C. (2005). *GeneralPathology*: The Acute Inflammatory Response; The Host Response to Injury, 3rd Edition McGraw-Hill New York Pp 08385-1495.

Parle, M.& Yadav, M. (2013). Laboratory models for screening analgesics. *International Research Journal of Pharmacy*. 4, 15-19.

Perquin, C.W., Hezebroek-Kampschreur, A., Hunchfeld, J.A., Bohnen, A.M., Van Suijlekom-Smith, L.W., Passchier, J.& Van der Wouden, J.C. (2000). Pain in Children and Adolescents: a common experience. *Pain,* 87(1), 55-8.

Porth, C. (2007). *Essentials of pathophysiology: concepts of altered health states*. Hagerstown, MD: Lippincott Williams & Wilkins. Pp 270.

Prakachin, K.M., Solomon, P.E. Ross, J. (2007). Under estimation of Pain by Health-care Providers: towards a Model of the process of inferring Pain in others. *Canadian Journal of Nurses Research,* 39 (2), 88-106.

Purssell, E.& While, A.E. (2013). Does the use of antipyretics in children who have acute infections prolong febrile illness? A systematic review and metaanalysis.*Journal of Pediatrics*, 163(3),822–827.

Quinette, A.L., Morris, L.D. & Somers, K.G. (2007). The prevalence of low back in Africa: a systematic review. *MuscoskeletalDisorder*, 8(105), 1471-2474.

Rainsford, K.D. (2009). Ibuprofen: Pharmacology, efficacy and safety. *Inflammopharmacology*, 275-342.

Rajnarayan, K., Sripal Reddy, M. and Chaluvadi, M.R. (2001).Bioflavonoids classification, Pharmacological, Biochemical effects and Therapeutic potential.*Indian Journal of Pharmacology;* 33, 2-16

Ramer, M.S., Murphy, P.G., Richardson, P.M. & Bisby, M.A. (1998). Spinal nerve lesion-induced Mechanoallodynia and Adrenergic sprouting in sensory Ganglia are attenuated in Interleukin-6 knockout Mice. *Pain,* 78, 115 – 121.

Rang H.P., Bevan, S. & Dray, A. (1991).Chemical activation for Nociceptive Peripheral Neurons.*British Medical Bulletin,* 47, 534 – 548.

Rang, H.P. & Dale, M.M. (2007). Harmful effects of Drugs In: *Rang and Dales Pharmacology* 6th edition Churchill Livingstone USA Edited by Rang H. P and Dale, M.M. Pp 752 – 764.

Rang, H.P., Dale, M.M. & Ritter, J.M. and Moore, P.K. (2003). Drugs used in affective Disorders. In: *Pharmacology*, 5th edition. Churchill Livingstone. Pp535-549.

Rao, P.N.& Knaus, E.E. (2008).Evaluation of Nonsteroidial antiinflammatory drugs cyclooxygenase inhibition and beyond.*Journal of Pharmaceutical Sciences.* 11, 81-110.

Ravi, V., Saleem, T.S., Patel, S.S., Raamamurthy, J.& Gauthamam, K. (2009).Anti-Inflammatory Effect of Methanolic Extract of Solanum nigrum Linn Berries.*International Journal of Applied Research in Natural Products*. 2, 33-36.

Reanmongkol, W., Subhadhirasakul, S., Thienmontree, S., Thanyanpanit, K., Kalnaowakul, J. & Sengsui, S. (2005). Antinociceptive activity of alkaloid extracts from *Kopsia macrophylla* leaves in mice. *Journal of Science and Technology*, 27 (2), 509-516.

Richard, F., Michelle, A.C.& Luigi, X.C. (2008).*Lippincott's Illustrated Reviews.Pharmacology*.Lippincott Williams & Wilkins.Philadelphia, Fourth edition. 564.

Roberts, J.L.& Morrow J.D (2001).Analgesic, Antipyretic and Anti-inflammatory Agents and Drugs employed in the treatment of Gout. Hardman, J.G. and Limbird,L.E. (eds) *Goodman andGilman's.The Pharmacological basisof Therapeutics*, 10th Ed. Mc Graw Hill Co Inc. New York, Pp 687-731.

Robotin, M.C.& Penman, A.G. (2006). Integrating complementary therapies into mainstream cancer care: which way forward. *Medical Journal of Australia*, 185, 377–379.

Saha, S., Shilpi, J.A., Mondal, H., Hossain, F., Anisuzzman, M., Hasan, M. and Cordell, G.A (2013).Ethnomedicinal, phytochemical, and pharmacological profile of the genus *Dalbergia* L. (Fabaceace).*Phytopharmacology*, 4(2), 291-346

Sani, M., Anuka, J.A., Yaro, A.H. & Mohammed, A. (2009). Antinociceptive and anti-inflammatory of *Cissus Cornifolia* Planch (Vitaceae). *Biological and Environmental Sciences Journal for the Tropics*, 6(3), 56-59.

Santosh, S.B., Sohan, S.C., Anupana A.S., Devanand B.S. & Manohar J.P. (2008).Anti-inflammatory activity of an isolated Flavonoid fraction from *Celosia argentea* Linn. *Journal of Medicinal Plants Research,* 2(3), 052-054.

Saper, C.B.& Breder, C.D. (1994).The neurologic basis of fever.*New England Journal of Medicine,* 330, 1880–1886.

Satoskar, R.S., Bhandarka, S.D. & Ainapure, S.S. (1999). Drug toxicity in man In: *Pharmacology and Pharmacotherapeutics*, Revised 16th edition Mumbai Parakashan India. Edited by R. S. Satoskar Pp 30 – 40; 136; 161-162; 171-172.

Schmidt, B., Ribnicky, D. M., Poulev, A., Logendra, S., Cefalu, W. T., & Raskin, I. (2008).A natural history of botanical therapeutics. *Metabolism*, *57*, S3-S9.

Seibert, K., Zhang, Y., Leahy, K., Hauser, S., Masferrer, J., Perkins, W., Lee, L.& Isakson, P. (1994) Pharmacological and biochemical demonstration of the role of

cyclooxygenase 2 in inflammation and pain. *Proc Natl Acad Sci*USA . 91,12013–7.

Selbst, S.M.& Fein, J.A., (2006). Sedation and analgesia. In: Henretig, F.M., Fleisher, G.R., Ludwig, S., *Textbook of pediatric emergency medicine*.P120-125.

Sen, S., Chakraborty, R.& De, B. (2010). Analgesic and anti-inflammatory herbs: A potential source of modern medicine. *International Journal of Pharmaceutical Sciences and Research*, 11, 32–44.

Serhan, C.N. (2008). Controlling the resolution of acute inflammation; a new genus of dual anti-inflammatory and proresolving mediators.*Journal of Periodontology*, 79(8), 1520-1526.

Serhan, C.N. & Chiang, N. (2005). Novel endogenous small molecules as the checkpoint controllers in inflammation and resolution: entree for resoleomics. *Rheumatoid Disease Clinical North America,* 30, 69 – 95.

Shah, B.S., Nayak, B.S., Seth, A.K., Jalalpure, S.S., Patel, K.N, Patel, M.A.& Mishra, A.D. (2006). Search for medicinal plants as a source of antiinflammatory and anti-arthritic agents. *Pharmacognosy Magazine.* 2, 77–86.

Shalini, M.D.& Dona, S.Z. (2006).Pathopysiology and Management of Fever.*Journal of Supportive Oncology.* 4, 9-16.

Sharma, M., Kaur, R., Puri, S.& Sharma, M. (2013).Quantification of withanolide a from withania somnifera dunal. In tropics of himalaya using hplc with dad detector. *Int. J. Biol. Pharma.Res.* 4(10), 702-705.

Sharma, U.S., Sharma, U.K., Sutar, N., Singh, A. & Shukla D.K. (2010). Anti-inflammatory activity of *Cordia dichotana* Forst F. Seeds extracts. *International Journal of Pharmaceutical Analysis,* 2(1), 01-04.

Shinwari, Z.K. (2010). Medicinal plants research in Pakistan. *Journal of Medicinal Plants Research*. 4, 161-176.

Singh, A., Malhotra, S.& Subban, R. (2008).Anti-inflammatory and analgesic agents from Indian medicinal plants.*International Journal of Integrative Biology*. 1, 57–72.

Singh, S.D.& Majumbar, K. (1995).Analysis of activity of Ocimum sanctum and its possible mechanism of action.*International Journal of Pharmacology*. 3, 188–192.

Slater, E.E. & De Sanctis, R.W. (1976).The Clinical Recognition of Dissecting Aortic Aneurysm.*American Journal of Medicine*, 60 (5), 625-33.

Smith, C. J., Zhang, Y., Koboldt, C. M., Muhammad, J., Zweifel, B. S., Shaffer, A., and Isakson, P. C. (1998). Pharmacological analysis of cyclooxygenase-1 in inflammation.*Proceedings of the National Academy of Sciences*, *95*(22), 13313-13318.

Smith, D.L., D'amour, M.C.& D'amour, F.E. (1943).The analgesic properties of certain drugs and drug combinations.*Journal Pharmacology Experimental Therapeutics*. 77, 184-193.

Sofowora O.A. (1993). *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Limited, Ibadan. Pp 1-2, 150-153.

Sotannemi, E. A., Ahlqvist, R. O. & Pelkonem, R. O. (1997). Histologic changes in the liver and indices of drug metabolism in alcoholics. *European Journal of Clinical Pharmacology*, 11, 295 – 303.

Spanswick, C.C. & Main, C.J. 2000 *Pain Management*: Am interdisciplinary approach. Edinburgh: Churchill Livingstone, Pp 93.

Sparg, S.G., Light, M.E.&Van Staden, J. (2004).Biological activity and Distribution of Plant Saponins.*JournalofEthnopharmacology*, 994, 219-243.

Srivastava, K.C.& Mustafa, T. (1989).Ginger (Zingiber officinale) and rheumatic disorders.*Medical Hypotheses*. 29, 25-28.

Srivastava, S., Singh, P., Jha, K. K., Mishra, G., Srivastava, S. & Khosa, R. L. (2013).Antiinflammatory, analgesic and antipyretic activities of aerial parts of Costus speciosus Koen. *Indian journal of pharmaceutical sciences*, *75*(1), 83.

Stein, C. (1995). The control of Pain in Peripheral Tissues by Opioids.*New England Journal of Medicine*, 332, 1685-1690.

Stevenson, G. W., Bilsky, E. J., & Negus, S. S. (2006). Targeting pain-suppressed behaviors in preclinical assays of pain and analgesia: effects of morphine on acetic acid-suppressed feeding in C57BL/6J mice. *The Journal of Pain*, *7*(6), 408-416.

Strigo, I.A., Carli, F. & Bushnell, M.C. (2000).Effect of ambient temperature on human pain and temperature perception.*Anaesthesiology*, 92, 699-707.

Sund- Levander, M., Forsberg, C.& Wahren, L.K. (2002). Normal oral, rectal, tympanic and axillary body temperature in adult men and women: a systematic literature review. *Journal of Caring Science,* 16, 122- 129.

Sutharson, L., Lila, K.N., Presanna, K.K., Shila, E.B. & Rajan, V.J. (2007).Anti-inflammatory and Antinociceptive Activities of Methanolic extract of the Leaves of *Fraxinus floribunda* Wallic. *African Journal of Biotechnology,* 6 (5), 582-585.

Svedmyr, J., Nyberg, E., Thunqvist, P., Åsbrink- Nilsson, E., & Hedlin, G. (1999).Prophylactic intermittent treatment with inhaled corticosteroids of asthma exacerbations due to airway infections in toddlers. *Acta Paediatrica, 88*(1), 42-47.

Tal, S., Guller, V. & Gurevich, A. (2007).Fever of Unknown Origin in Older Adults.*Clinics in Geriatric Medicine,* 23, 649-668.

Talabi, A.E. (2005). The prevalence of low back pain in school children in Ilorin metropolis Kwara State, Nigeria.2nd International Council for Health, Physical Education, Recreation, Sports and Dance (ICHPER-SD), Africa Scientific Congress, University of Ibadan.

Tanko, Y., Magaji, M.G., Yerima, M., Magaji, R.A. & Mohammed, A. (2008) Antinociceptive and anti-inflammatory activities of aqueous leaves extract of *Ocimumgratissimum* (Labiatea) in rodents.*AfricanJournalofTraditional, ComplimentaryandAlternativeMedicines,* 5(2), 141-146.

Taylor, G.W. (1998). *Back Pain and Injuries in the Athlete*. Handbook of Sports Medicine 2nd EditionPp 45-48.

Teder, P., Jiang, D., Liang, J., Cohn, L., Pure, E., Henson, P.M. & Noble, P.W. (2002).Resolution of Lungs inflammation by CD44.*Science,* 296(5565), 155-158.

Thienhaus, O. & Cole, B.E. (2002).Classification of Pain. Weiner, R.S. *Pain management*: *A Practical Guide for Clinicians*. American Academy of Pain Management, Pp 26.

Thrower, B.W. (2009). Relapse management in multiple sclerosis. *Neurologist*, 15(1), 1-5.

Tjølsen, A., Berge, O. G., Hunskaar, S., Rosland, J. H., & Hole, K. (1992). The formalin test: an evaluation of the method. *Pain*, *51*(1), 5-17.

Traversa, G., Walker, A.M., Ippolitto, F.M., Caffari, B., Capurso, L., Dezi, A., Koch, M., Maggini, M., Alegiani, S.S. & Raschetti, R. (1995).Gastroduodenal toxicity of different Non steroidal anti-inflammatory drugs. *Epidemiology,* 6(1), 49-54.

Tsang, A.,VonKorff, M. & Lee, S. (2008). Common Chronic Pain Conditions in DevelopedandDevoloping Countries: Gender and Age Differences and Comorbidity with Depression-Anxiety disorders. *JournalofPain*, 9(10), 883-891.

Tucci, S., Primassin, S., Ter Veld, F., & Spiekerkoetter, U. (2010). Medium-chain triglycerides impair lipid metabolism and induce hepatic steatosis in very long-chain acyl-CoA dehydrogenase (VLCAD)-deficient mice. *Molecular genetics and metabolism*, *101*(1), 40-47.

Turner, R. (1965). Screening Method in Pharmacology.Anti-Inflammatory Agent.*Academic Press,* New York, NY, USA.13; pp 245.

Udeogaranya, P.O., Okonta, J.M. & Ukwe, C.V. (2005).The Anti-inflammatory effects of the aqueous extract of the root of *Desmodium gangaticum. Nigerian Journal of Pharmaceutical Research,* 4(2), 8-11.

Urch, C.E. & Suzuki, R. (2009).Pathophysiology of Somatic, Visceral and Neuropathic Cancer Pain. In: Sykes, N., Bennett, M.I and Yuan, C.S. *Clinical pain management: Cancer pain*. 2nd Ed. London: Hodder Arnold, Pp 3-14.

Usman, H., Yaro, A.H. & Garba, M.M. (2008). Analgesic and Anti-inflammatory Screening of *Newbouldia laevis* flower in Rodents. *TrendsinMedicalResearch,* 3(1), 10-15.

Vadivelu, N., Urman, R.D.& Hines, R.L. (2011).Essentials of Pain Management.*Springer Science.* 157, 167–169.

Vinegar, R.,Schreiber, W. & Hugo, R. (1969).Biphasic Development of Carrageenan Edema in Rats.*JournalofPharmacologyandExperimentalTherapeutics,* 166, 96-103.

Vogel, H.G. & Vogel, W. H. (1997). Drug Discovery and Evaluation: Pharmacological assays, springer-verlaag, Berling Heidelberg. Pp 204 – 212, 382 – 388.

Vogel, H. G. (2008). Drug Discovery and Evaluation: Pharmacological Assays. Springer-Verlag, Berlin, 3rd edition. pp. 1013-1014.

Vongtau, H. O., Abbah, J., Mosugu, O., Chindo, B. A., Ngazal, I. E., Salawu, A. O., Kwanashie, H. O. and Gamaniel, K. S. (2004). Antinociceptive profile of the methanolic extract of *Neorautanania mitisRoot in Rats and Mice*. *Journal of Ethnopharmacology*, *92*(2-3), 317 – 324.

Vranken, J.H. (2009). Mechanism and Treatment of Neuropathic Pain.*Central Nervous System Agents in Medicinal Chemistry*, 9(1), 73-77.

Wallace, K. (1992). The Pathophysiology of Pain.*Critical Care Nurses*, 15(2), 1-14.

Walter, F.B. (2003). *Medical Physiology*: A Cellular and Molecular Approach. *Elsevier*. Saunders limited. Philadelphia. pp 1300.

Walter, E.& Carraretto, M. (2015).Drug-induced hyperthermia in critical care. *Journal oftheintensive care society*, *16*(4), 306-311.

Walum E. (1998). Acute Oral Toxicity.*Environmental Health Perspectives*, 106, 497-503.

Wang, Y., Chen, P., Tang, C., Wang, Y., Li, Y., & Zhang, H. (2014). Antinociceptive and anti-inflammatory activities of extract and two isolated flavonoids of Carthamus tinctorius L. *Journal of ethnopharmacology*, *151*(2), 944-950.

WHO, Geneva.Winter, C.A.& Poter, C.C. (1957).Effect of alterations in the side chain upon anti-iflammatory and liver glycogen activities of hydrocortisone esters.*Journal of American PharmaceuticalAssociation Science.* 46: 343-383

Winter, C. A. & Porter, C. C. (1957).Effect of alterations in side chain upon anti-Inflammatoryand liverglycogen activities of hydrocortisone esters. *Journal of the American Pharmaceutical Association (Scientific ed.)*, *46*(9), 515-519.

.

Winter, C.A., Riselay, E.A. &Nuss, G.W. (1962). Carrageenan induced Edema in the hind paw of the rats as an assay for anti-inflammatory drugs. *ProceedingsoftheSocietyforExperiementalBiologyandMedicine,* 111, 544-547.

World Health Organization (2012).WHO guidelines on the pharmacological treatment of persisting pain in children with medical illnesses. ISBN9789241548120.

World Health Organization, (2002). Traditional Medicine Strategy 2002–2005.

Yiu, G.& He, Z. (2006).Glial inhibition of CNS axon regeneration.*Nature Reviews Neuroscience,* 7, 617-627.

Younos,C., Rolland, A., Fleurentin, J. & Lanhers,M.C. (1990).Analgesic and BehaviouralEffectof*Morindacitrifolia.PlantaMedica,*56.

APPENDICES

Appendix A: Effect of the Methanol Leaf Extract of *Culcasia angolensis* on Hot Plate(Thermally Induced) Pain in Swiss Albino Mice

Groups (mg/kg)	Mean reaction time ± SEM (secs)				
	0 min	60 mins	90 mins	120 mins	150 mins
D/W (10 mL)	1.63±0.10	1.44±0.22	1.34±0.23	1.36±0.28	1.49±0.19
CAE(125)	1.67±0.14	3.25±0.39	3.32±0.14**	3.01±0.24*	3.02±0.29*
CAE(250)	1.81±0.25	2.82±0.14	3.02±0.16*	3.28±0.31**	3.29±0.18**
CAE(500)	2.08±0.20	2.99±0.19	2.69±0.19	3.87±0.27**	3.81±0.24**
Mor (5)	1.73±0.19	3.37±0.20**	3.07±0.41**	3.73±0.16**	3.72±0.27**

Data was analyzed using repeated measures ANOVA followed by Bonferroni post hoc test, * = P≤0.05, ** = P≤ 0.01 significant statistical increase in mean reaction time compared with time zero; n=6. Values are Mean ± SEM. D/W= distil water, CAE= *Culcasia angolensis* methanol leaf extract, Mor = morphine 5 mg/kg

Appendix B: Effects of Methanol Leaf Extract of *C. angolensis* on Formalin Induced Inflammation in Wistar Rats

Treatment (mg/kg)	Mean Paw Diameter (millimetre)					
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
D/W 10 mL	2.30±0.06	2.67±0.10	3.12±0.05	3.11±0.05	3.01±0.06	2.9±0.06
CAE (125)	2.22±0.05	2.65±0.06	2.71±0.04	2.72±0.04	2.53±0.03**	2.41±0.01*
CAE (250)	2.35±0.07	2.76±0.02	2.77±0.02	2.85±0.06	2.55±0.04**	2.40±0.05**
CAE (500)	2.20±0.09	2.83±0.03	2.82±0.04	2.73±0.03	2.51±0.06**	2.31±0.04**
Ket (10)	2.23±0.05	2.65±0.06	2.74±0.09	2.41±0.04	2.31±0.02**	2.14±0.01**

Data was analyzed using repeated measures ANOVA followed by Dunnet Post Hoc test, *= $p<0.05$, **= $p<0.001$, significant statistical decrease in mean paw oedema size as compared to time zero; n= 6. Values are Mean ± SEM. CAE = *Culcasia angolensis* methanol leaf extract. Ket= ketoprofen 10 mg/kg, hrs= hours

Appendix C: Effect of the Methanol Leaf Extract of *Culcasia angolensis* onCarrageenan Induced Paw Oedema in Wistar Rats

Treatment(i.p)	Mean Paw Diameter in Millimeter 1hr	2hr	3hr	4hr	5hr
D/W 10 mL	1.660± 0.144	2.720± 0.188	2.700± 0.407	3.380± 0.120	3.180±0.124
CAE 125 mg/kg	1.560± 0.103 **(6.02%)**	2.300± 0.182 **(15.4%)**	2.440± 0.172 **(9.6%)**	2.420± 0.174 **(28.4%)**	2.540± 0.121 **(27.3%)**
CAE 250 mg/kg	1.220± 0.139 **(26.5%)**	2.160± 0.199 **(20.6%)**	1.880±0.159 **(30.4%)**	1.640± 0.181 **(51.5%)** ***	1.680± 0.058 **(54.5%)** ***
CAE 500 mg/kg	1.420± 0.073 **(14.5%)**	2.360±0.291 **(13.2%)**	1.380± 0.334 **(48.9%)***	1.340± 0.172 **(60.4%)** ***	1.340± 0.157 **(63.6%)** ***
Ketoprofen 10 mg/kg	1.340± 0.160 **(19.3%)**	1.020± 0.073 **(62.5%)** ***	1.000± 0.327 **(62.9%)** **	1.000± 0.241 **(70.4%)** ***	1.520± 0.240 **(63.6%)** ***

Data was analyzed using repeated measures ANOVA followed by Bonferoni Post Hoc test, *= p≤0.05, **= p≤0.01, ***= p<0.001significant statistical decrease in mean paw oedema size when compared to the negative control. n= 6. Values are Mean ± SEM. CAE= *Culcasia angolensis* extract, D/W =distil water, hr= hours. Figures in parentheses (bold) are percentage inhibition of inflammation

Appendix D: Effect of Methanol Leaf Extract of *C. angolensis* on Brewer's Yeast InducedPyrexia in Wistar Rats

Treatment	Rectal temperature (°C) Mean ± SEM after 18hr				
(mg/kg)	Initial temp	0hr	1hr	2hr	3hr
D/W 2 mL	37.33±0.11	39.93±0.11	39.98±0.14	40.22±0.49	39.87±0.22
CAE 125	37.21±0.20	39.43±0.19	39.40±0.01	39.21±0.15	38.65±0.71*
CAE 250	37.66±0.43	39.61±0.15	39.69±0.41	39.01±0.21	38.54±0.41*
CAE 500	37.44±0.23	39.58±0.11	38.42±0.08	38.30±0.11*	37.66±0.43**
PCM 300	37.32±0.36	39.47±0.17	37.44±0.33**	36.68±0.30**	37.19±0.48**

Data expressed as mean ± SEM using repeated measure ANOVA followed by Dunnet's post hoc test for multiple comparism. *=significant decrease in rectal temperature at P≤0.05, **= significant at P≤0.01 when compared with control n=6, D/W = distil water, CAE = *Culcasia angolensis* methanol leaf extract.