ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF METHANOL EXTRACT AND FRACTIONS OF *LAGGERA AURITA* LINN F (COMPOSITAE) IN MICE AND RATS

By

AISHATU SHEHU

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

AUGUST, 2014

ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF METHANOL EXTRACT AND FRACTIONS OF *LAGGERA AURITA* LINN F (COMPOSITAE) IN MICE AND RATS

By

Aishatu SHEHU, B. Pharm (A.B.U.) 2009

MSc/ Pharm-Sci /2933/11-12

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,

AHMADU BELLO UNIVERSITY ZARIA,

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD

OF A MASTER DEGREE IN PHARMACOLOGY

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,

FACULTY OF PHARMACEUTICAL SCIENCES

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

AUGUST, 2014

DECLARATION

I declare that the work in this thesis titled: **ANALGESIC AND ANTI-INFLAMMATORY EFFECTS METHANOL EXTRACT AND FRACTIONS OF *LAGGERA AURITA* LINN F (COMPOSITAE) IN MICE AND RATS** was performed by me in the Department of Pharmacology and Therapeutics, under the joint supervision of Dr T.O. Olurishe, Dr A.U. Zezi, and Dr A. Ahmed. The information derived from the literature have been duely acknowledged in the text and a list of references provided, No part of this thesis has been previously presented for another Degree or Diploma at this or another University.

SHEHU AISHATU		
Name of the student	Signature	Date

CERTIFICATION

This thesis entitled **ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF METHANOL EXTRACT AND FRACTIONS OF *LAGGERA AURITA* LINN F (COMPOSITAE) IN MICE AND RATS** by AISHATU SHEHU meets the regulations governing the award of the degree of master of science (Pharmacology) of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

_____ _____

Dr T.O.Olurishe, Date
Chairman, Supervisory Committee

_____ _____

Dr A.U. Zezi Date
Member, Supervisory Committee

_____ _____

Dr A. Ahmed Date
Member, Supervisory Committee,

_____ _____

Dr A.U. Zezi Date
Head,
Department of Pharmacology and Therapeutics,
Ahmadu Bello University, Zaria

_____ _____

Prof A.A. Joshua Date
Dean,
School of Postgraduate Studies, Ahmadu Bello University, Zaria

DEDICATION

This work is dedicated to Almighty Allah, then to my loving daughter Hindu (Ummita) Najib

ACKNOWLEDGEMENT

I primarily acknowledge Allah for showing much Mercy and Blessings, Grace and Strength to carry out this work.

My gratitude goes to my supervisors; Dr T.O. Olurishe, Dr A.U Zezi and Dr A. Ahmed for their support, encouragement, sacrifice and constructive criticism. My sincere gratitude goes to my husband Pharm. Najib Bello for his patience, tolerance and endurance, sacrifices and support to achieve this work. I so much acknowledge my mother Hajiya Fatima Mohammed, my Grandmother Hajiya Aishatu Abubakar and my aunties: Hajiya Ladi, Hajiya Larai and Hajiya Sadiya Muhammad. My special thanks to my sisters Sadiya, Aisha (baby) and Hassana, and to my brothers Musa, Isah (Babangida) and Abdurrahman Saidu.

I also wish to acknowledge the support and assistances of Dr Magaji Garba Muhammad, Dr Jamilu Yau, Dr Nuhu Muhammad Danjuma, Mallam Ibrahim Adamu, Dr Bilkisu Bello Maiha, Muhammad Aminu Zayyad, Kamilu Labaran, Danjuma Aguye, Mr John Kaba, Mrs. Ferhat Khan, Aliyu Abdullahi, Nasiru, Idris Maje, Bashir, Abdullahi, Malami Sani, Dr Aliyu Musa, Dr Aminu Musa, Dr Abdulsamad AbdurRahman, Mr John Oduh.

Thanks to all my friends and sisters; Fatima I. Hassan, Rabi'ah Shehu Bidah, Maryam Abdullahi, Sakinah Abdullahi, Asma'u Hamza,Ruqayyah Shehu Hadiza Ma'aji and my brothers Abdullahi Balarabe Nazifi, Salisu Auwal.

ABSTRACT

The plant *Laggera aurita* is an annual or biennial herb of widespread in dry waste places from across Senegal to Southern Nigeria. The plant has been reportedly used for medicinal purposes including treatment of rheumatic pain, healing of cuts and bruises, and as a last resort to phagedenic and chronic ulcers. The present study investigated the analgesic and anti-inflammatory properties of the methanol extract, saponin and flavonoid fractions of the plant in mice and rats. Acetic acid induced writhes test, thermally induced pain and formalin induced inflammation models in rodents were used to evaluate the antinociceptive 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 both the methanol extract and the fractions. 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, slight alveolar congestion on lungs and moderate erosion of stomach epithelium on stomach. The methanol extract, saponin and flavonoid fractions at doses of 200, 400 and 800 mg/kg significantly ($p<0.05$) inhibited the acetic acid induced abdominal writhes in mice dose dependently. Both the extract and fractions at 800 mg/kg showed better activity than 20 mg/kg piroxicam. The methanol extract, saponin and flavonoid fractions of *Laggera aurita* also significantly ($p<0.05$) and dose dependently increased the reaction time in the thermally induced pain model. The methanol extract (800 mg/kg) and saponin fraction (400 and 800 mg/kg) showed better activity when compared to the standard morphine (5 mg/kg) at 60, 90 and 120 minutes. Naloxone, a non specific opioid antagonoist blocked the analgesic effect observed with the thermally induced pain model in mice for both the methanol and

fractions. The methanol extract and saponin fraction of *Laggera aurita* significantly ($p<0.05$) decreased formalin induced paw edema in a dose dependent manner. The saponin fraction (800 mg/kg) exhibited better anti-inflammatory effects than ketoprofen (10 mg/kg), the standard anti-inflammatory drug used. These findings suggest that the methanol extract, saponin and flavonoid fractions of the plant *Laggera aurita* possess analgesic and anti-inflammatory activities that justify the ethnomedical use in the treatment of painful and inflammatory conditions by the herbal practitioners.

TABLE OF CONTENTS

Title Page ----------i

Declaration----------ii

Certification----------iii

Dedication ----------iv

Acknowledgement----------v

Abstract ----------vi

Table of Contents----------viii

List of Tables ----------xvi

List of Plates----------xviii

List of Figures----------xx

List of Appendices ----------xxi

Abbreviations, Definitions and Acronyms ----------xxii

CHAPTER ONE

1.0 INTRODUCTION----------1

1.1 Background Information ----------1

1.2 Role of Traditional Medicine in Healthcare Delivery System----------2

1.3 Justification----------3

1.4 Theoretical Framework----------3

1.4.1 Models for the studies----------4

1.5 Aim of the Study----------6

1.6 Specific Objectives----------6

1.7 Statement of Research Hypothesis----------6

CHAPTER TWO

2.0 LITERATURE REVIEW----------7

2.1 Pain ----------7

2.1.1 Etiology----------7

2.1.2 Pathophysiology and types of pain----------10

2.1.3 Epidemiology of pain----------15

2.1.4 Pain pathway----------16

2.1.5 Pain thresholds----------17

2.1.6 Pain assessment----------18

2.1.7 Pain as an aid to diagnosis----------18

2.1.8 Drug treatment and overview of antinociceptive drugs----------19

2.1.9 Non pharmacological treatment of pain----------23

2.2 Inflammation----------24

2.2.1 Clinical signs of inflammation ----------25

2.2.2 Mechanism of inflammation ----------26

2.2.3 Plasma derived mediators of inflammation ----------27

2.2.4 Function of inflammation ----------29

2.2.5 Morphologic patterns of inflammation ----------29

2.2.6 Inflammatory disorders ----------30

2.2.7 Resolution of inflammation ----------31

2.2.8 Management of inflammation ----------32

2.3 Toxicology----------34

2.3.1 Spectrum of toxic dose----------34

2.3.2 Duration and Frequency of Exposure----------35

2.3.3 Descriptive animal toxicity----------35

2.3.4 Mechanism of toxicity----------36

2.4 The Plant *Laggera aurita* ----------38

2.4.1 Vernacular names----------38

2.4.2 Description of the plant *Laggera aurita*----------39

2.4.3 Ethno medicinal uses----------39

2.5 Plants Used in the Management of Pain----------40

CHAPTER THREE

3.0 MATERIALS AND METHODS----------42

3.1 Plant Collection----------42

3.1.1 Preparation of extract----------42

3.2 Animals----------42

3.3 Drugs and Chemicals----------43

3.4 Equipment and other Materials----------43

3.5 Phytochemical Screening of *Laggera aurita*----------43

3.5.1 Test for carbohydrates and sugars----------44

3.5.2 Test for flavonoids----------44

3.5.3 Test for cardiac glycosides----------44

3.5.4 Test for saponins----------45

3.5.5 Test for tannins----------45

3.5.6 Test for steroids and triterpenes ----------45

3.5.7 Test for alkaloids ----------45

3.6 Fractionation Chart----------46

3.7 Acute Toxicity Studies in Mice and Rats----------48

3.7.1 Selection of animals----------48

3.7.2 Fixed dose method of acute toxicity studies in mice and rat----------48

3.7.3 Histopathological studies----------49

3.8 Antinociceptive Studies----------50

3.8.1 Acetic acid induced writhing test----------50

3.8.2 Hot plate test (thermal sensitivity test) in mice----------50

3.8.3 Formalin induced inflammation test ----------51

3.8.4 Effect of the methanol extract of *Laggera aurita* and its fractions by pretreatment with naloxone assessed by hot plate test----------52

3.9 Data analysis----------52

CHAPTER FOUR

4.0 RESULTS ----------53

4.1 Phytochemical Constituents----------53

4.1.1 Phytochemical constituents of the methanol extract of *Laggera aurita* ----------53

4.2 Acute Toxicity Study ---------- ------55

4.2.1 Median Lethal Dose (LD_{50}) ----------55

4.2.2 Histological studies ----------55

4.3 Effect of *Laggera aurita* on Acetic Acid Induced Writhing Test in Mice ----------74

4.3.1 Effect of methanol extract of *Laggera aurita* on acetic acid induced writhing test in mice ----------74

4.3.2 Effect of saponin fraction of *Laggera aurita* on acetic acid induced writhing test in mice ----------76

4.3.3 Effect of flavonoid fraction of *Laggera aurita* on acetic acid induced writhing test in mice ----------78

4.4 Effect of methanol extract of *Laggera aurita* and its fractions on thermally induced Pain in Mice ----------80

4.4.1 Effect of the methanol extract of *Laggera aurita* on thermally induced pain------80

4.4.2 Effect of saponin fraction of *Laggera aurita* on thermally induced pain----------82

4.4.3 Effect of flavonoid fraction of *Laggera aurita* on thermally induced pain---------84

4.5 Effect of the methanol extract of *Laggera aurita* and its fractions by pretreatment with naloxone assessed by hot plate test----------86

4.5.1 Effect of the methanol extract of *Laggera aurita* by pretreatment with naloxone assessed by hot plate test----------86

4.5.2 Effect of the saponin fraction of *Laggera aurita* by pretreatment with naloxone assessed by hot plate test----------88

4.5.3 Effect of the flavonoid fraction of *Laggera aurita* by pretreatment with naloxone assessed by hot plate test----------90

4.6 Effect of *Laggera aurita* on Formalin Induced Inflammation in Rats ----------92

4.6.1 Effect of the methanol extract of *Laggera aurita* on formalin induced inflammation in rats ----------92

4.6.2 Effect of saponin fraction of *Laggera aurita* on formalin induced inflammation in rats----------94

CHAPTER FIVE

5.0 DISCUSSION ---------- 96

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS----------103

6.1 Conclusion ----------103

6.2 Recommendations ----------103

REFERENCES ----------104

LIST OF TABLES

Table Page

4.1 Phytochemical constituents of the methanol extract of *Laggera* ----------54

4.2 Effect of the methanol extract of *Laggera aurita* on thermally induced pain in mice ----------81

4.3 Effect of the saponin fraction of *Laggera aurita* on thermally induced pain in mice ----------83

4.4 Effect of the flavonoid fraction of *Laggera aurita* on thermally induced pain in mice ----------85

4.5 Effect of the methanol extract of *Laggera aurita* by pretreatment with naloxone assessed by hot plate test----------87

4.6 Effect of the saponin fraction of *Laggera aurita* by pretreatment with naloxone assessed by hot plate test----------89

4.7 Effect of the flavonoid fraction of *Laggera aurita* by pretreatment with naloxone assessed by hot plate test----------91

4.8 Effects of methanol extract of *Laggera aurita* on formalin induced inflammation in rats ---------- 93

4.9 Effect of saponin fraction of *Laggera aurita* on formalin induced inflammation in rats ----------95

LIST OF PLATES

Plate Page

I Photomicrograph of a liver section of mice treated with 2000 mg/kg of methanol extract of *Laggera aurita* ----------56

II Photomicrograph of a liver section of rat treated with 2000 mg/kg of methanol extract of *Laggera aurita* for two days ----------57

III Photomicrograph of a liver section of mice treated with 5000 mg/kg of methanol extract of *Laggera aurita* ----------58

IV Photomicrograph of a liver section of rat treated with 5000 mg/kg of methanol extract of *Laggera aurita* ----------59

V Photomicrograph of a kidney section of mice treated with 2000 mg/kg of methanol extract of *Laggera aurita* ----------61

VI Photomicrograph of a kidney section of rat treated with 2000 mg/kg of methanol extract of *Laggera aurita* ----------61

VII Photomicrograph of a kidney section of mice treated with 5000 mg/kg of methanol extract of *Laggera aurita* ----------63

VIII Photomicrograph of a kidney section of rat treated with 5000 mg/kg of methanol extract of *Laggera aurita* ---------- 64

IX Photomicrograph of a lung section of mice treated with 5000 mg/kg of methanol extract of *Laggera aurita* ----------66

X Photomicrograph of a lung section of rat treated with 5000 mg/kg of methanol extract of *Laggera aurita* ----------67

XI Photomicrograph of a spleen section of mice treated with 5000 mg/kg of methanol extract of *Laggera aurita* ----------69

XII Photomicrograph of a stomach section of mice treated with

5000 mg/kg of methanol extract of *Laggera aurita* ----------71

XIII Photomicrograph of a stomach section of rat treated with

5000 mg/kg of methanol extract of *Laggera aurita* ----------72

LIST OF FIGURES

Figure Page

3.1 Fractionation of Methanol Extract of *Laggera aurita* ----------47

4.1 Effect of Methanol Extract of *Laggera aurita* on Acetic Acid Induced Writhes in Mice ----------75

4.2 Effect of Saponin Fraction of *Laggera aurita* on Acetic Acid Induced Writhes in Mice ----------77

4.3 Effect of Flavonoid Fraction of *Laggera aurita* on Acetic Acid Induced Writhes in Mice ----------79

LIST OF APPENDICES

Appendix		Page
1	Percentage yield of methanol crude extract of *Laggera aurita*	116
2	Percentage yield of saponin fraction of *Laggera aurita*	117
3	Percentage yield of flavonoid fraction of *Laggera aurita*	118

LIST OF ABBREVIATIONS AND ACRONYMS

ACE I:	Angiotensin-Converting Enzyme Inhibitors
ACTH:	Adrenocorticotrophic Hormone
CGRP:	Calcium Gene Related Peptide
CNS:	Central Nervous System
COX-1:	Cyclooxygenase-1
COX-2:	Cyclooxygenase -2
CRPS:	Complex Regional Pain Syndrome
EPA:	Eicosapentaenoic Acid
ER:	Endoplasmic Reticulum
GFR:	Glomerular Filtration Rate
GGT:	Gamma glutamyl transpeptidase
GIT:	Gastro-Intestinal Tract
GSH:	Glutathione
HETE:	Hydroxyeicosatetraeinoic Acid
IASP:	International Association for Study of Pain
ICAM-1 :	Intracellular Adhesion Molecule-1
IL-1:	Interlukin-1
LD_{50}:	Median Lethal Dose
LTs:	Leukotrienes
MELA:	Methanol Extract of *Laggera aurita*
NAPBQI:	N-acetyl-P-benzoquinone Imine
NSAIDs:	Non-Steroidal Anti-Inflammatory Drugs
OECD	Organization for Economic Co-operation and Development
PGs:	Prostaglandins

TNF:	Tumor Necrosis Factor
VCAM-1:	Vascular Adhesion Molecule-1
e.g:	Examples
e.t.c:	eccetera
%:	Percentages

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Pain is an enormous global health problem. It has been estimated worldwide that one in five adults suffer pain and another one in ten adults are diagnosed with chronic pain each year (Daniel and Summer, 2011). Irrespective of age, sex, income, race and location, pain affects all populations. Pain is not equally distributed across the world and it experiences can be acute, chronic, intermittent or in combination.

In Africa, pain in the form of musculoskeletal disorders is the most frequent cause of disability and accounts for 1% of age living with disability in developing world (Tsang *et al*., 2008). However, in developed countries, pain in form of musculoskeletal disorders accounts for 4.3% of age living with disability and at least up to 85% of low back pain occurs in developed countries (Quinette *et al.,* 2007).

Pain produces significant levels of disability affecting the economic and societal status leading to restrictions on daily activities, poor participation and or inability to work (Vranken, 2009). The international studies revealed that chronic pain covered 12% to 41% with prevalence greater in developing countries and more problematic in developed countries (Tsang *et al*., 2008).

In Nigeria, pain steadily increased from 2% at 6-8 years to 28% at 17-18 years (Talabi, 2005). Prevalence of pain increases with chronological age. Pain may be 20% accidental while 80% may result from reasons within the individual. Pain develops gradually and

not suddenly as against accidental pains which are sudden. Generally, degenerative pains are gradual in onset (Talabi, 2005).

Regardless of etiology of pain, the primary goal of therapy is to control pain and can be achieved by both drug and non-drug therapy. Non drug therapy involves maintaining daily programme of stretching and strengthening of general body muscles, flexibility at the harmstrongs and lumbodorsal facia and spine (Taylor, 1998). Other non-drug therapy includes abdominal strengthening, exercise (sit-ups or curls ups), and following correct biomechanical methods when sitting, lifting or bending and sleeping on flat surfaces. Alternative therapies such as acupuncture, guided imagery, chiropractic treatment, yoga, hypnosis, biofeedback, aromatherapy, massage and many others are also used (Mycek *et al*., 2000).

1.2 Role of Traditional Medicine in Healthcare Delivery Systems

Many orthodox drugs were obtained from medicinal plants which are the oldest known health care products. Their importance is still growing although it varies depending on the ethnological, medicinal and historical background of each country (Bishaw, 2007). The use of medicinal plants as a prophylactic or therapeutic agent has existed over the course of history and man has explored the plant kingdom for compounds which are of medicinal value (Gogtay *et al*., 2002). This has been done in order to identify chemical constituents responsible for biological effects isolated or purified in the search for new single entities as pharmaceutical ingredients or characterized and standardized in the search for new multi-component botanical products (Gedif and Hahn, 2003).

1.3 Justification

The use of herbal drugs in the treatment of pain is a common practice in many African countries. Despite the immense technological advancement in modern medicine, many people in developing countries still rely on traditional medicine and healing practices for their daily health care needs (Gedif and Hahn, 2003).

Moreover, the high cost of acquiring synthetic drugs, shortages, side effects and toxicities, limitations in use and ineffectiveness especially due to development of desensitization and dependence by narcotic analgesics (Leonard *et al*., 2006) lead to the investigation of the antinociceptive effect of *Laggera aurita* which have been used in traditional medicinal practices by Sudanese and Nigerian healers to treat pain, fever and jaundice.

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.

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).

1.4.1 Models 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 reaction 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 (Gene *et al.,* 1998). Abdominal constriction responses were 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 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 *et al*., 2008). Both phases can be inhibited especially for 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 (Gaertner *et al*., 1999).

1.4.1.5 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 paws and licking of the paws. The time these responses occur is prolonged when 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).

1.4.1.6 Evaluation 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 the 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.

1.5 Aim of the Study

The aim of the study is to investigate the antinociceptive and anti-imflammatory effects of methanol extract of the plant *Laggera aurita* and its fractions in mice and rats.

1.6 Specific Objectives

The specific objectives of this study are:

1. To evaluate the acute toxicity of the methanol extract, saponin and flavonoid fraction of the plant *Laggera aurita*.
2. To determine the phytochemical in the methanol extract, saponin and flavonoid fractions of the plant *Laggera aurita*.
3. To screen the methanol extract, saponin and flavonoid fractions of the plant *Laggera aurita* for antinociceptive properties.
4. To evaluate the effect of the methanol extract, saponin and flavonoid fractions of the plant *Laggera aurita* by interaction with naloxone.

1.7 Statement of Research Hypothesis

The methanol extract of *Laggera aurita* and its fractions possess neither antinociceptive nor anti-inflammatory properties.

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 Etiology

There are several causes of pain which include infections (such as viral, bacterial or fungal infections), drugs (such as antiretroviral drugs), damages of the nerve (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. Non invasive bacteria produce exotoxins interfering with normal secretory and absorptive properties of the intestinal lumen leading to irritation, inflammation and net fluid loss while non invasive 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 *et al*., 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 are types of pain that can be experienced from HIV/AIDS. 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 side effects *e.g* side effects of antiretroviral therapy (ART) such as diarrhea result in painful conditions such as diaper dermatitis. 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 e.t.c) or acute pain or both. Drugs like non-steroidal anti-inflammatory drugs such as ibuprofen cause headache, gastrointestinal ulcerations and bleeding (Gislason *et al*., 2009).

2.1.1.3 Diseases

Diseases are the main causes of pain as there is virtually no disease that is not associated with pain ranging from mild to moderate to severe pain. Examples of diseases associated with pain include sickle cell, diabetes, cancer and hypertension among others (Olurishe and Mati, 2014).

Sickle cell disease (SCD) is a genetic disorder associated with the presence of abnormal hemoglobin i.e. hemoglobin S (Hbs) in the red blood cells to become 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 ischemia 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 tumor 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).

2.1.2 Pathophysiology 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 activates specific pain 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 be subdivided into somatic and visceral pain depending on the location of activated nociceptors (Urch and Suzuki, 2009).

Somatic pain is caused 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 poor oxygen 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, dorsal root ganglion or dorsal root 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 (Nagasko *et al*., 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 diminish 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 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 *et al*., 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 *et al*., 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 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.

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).

Classification of pain based on specific diseases**:** Pain can be classified based on diseases like HIV/AIDS, Cancer, Sickle cell diseases pain and diabetes 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 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 stop 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).

2.1.3 Epidemiology

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 increase financial burden arising from increased use of health services and medication (Igumbar *et al*., 2011).

2.1.4 Pain pathway

It consists of afferent nociceptive fibres that 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. A ð fibre form synapses in laminae I and V, C fibres connect with neurons in lamina II, A β 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 and cross 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 a descending 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 receives similar 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 (Seibst 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 goes down, 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 *et al*., 1998). This can result in de-afferentation, which means the development of different central processes from the surviving afferent nerve. With this situation, surviving dorsal root axons of the nociceptors can make contact with the spinal cord, thus changing the normal input (Field *et al*., 1998).

2.1.5 Pain 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 painful while northern Europeans 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 *et al*., 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 Fulanis having high tolerance threshold (Verra *et al*., 2009).

2.1.6 Pain Assessment

A person's self report is the most reliable measure of pain, with health care professionals tending to under estimate 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 to locate 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).

2.1.7 Pain as an aid to diagnosis

Pain is a symptom of many medical conditions. Thus, it is important to know the onset, location, intensity, pattern of occurence, exacerbating factors, relieving factors and quality of pain (Berry *et al*., 2001). This will help the examining physician to accurately

diagnose the problem. For example, 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).

2.1.8 Drug 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, Non steroidal anti-inflammatory drugs (NSAIDS), antidepressants, antiseptic drugs and adjuvants.

2.1.8.1 Opioids analgesics

Opioid analgesics are used for treatment of chronic pain (Mycek *et al*., 2000). 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.

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*., 1991).

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). Additives contained in street opioid drugs can cause persistent nerve damage, neuropathy, amblyopic, myelopathy and leukoencephalopathy. Opioid analgesic 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 *et al*., 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). Antidepressants and antiepileptic drugs are used in chronic pain (Rang *et al*., 2003). Antiepileptic drugs have high risk of seizure when stopped abruptly. Adjuvant drugs help potentiate effects of other drugs, thus can hardly be used as a single

agent in pharmacotherapy. Drugs use in the treatment of pain act either by inhibiting prostanoids, non-prostaglandin mediated effects and apoptosis (Mishra *et al*., 2011).

Non-steroidal anti-inflammatory drugs 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 neighboring 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 *et al*., 2011). Aspirin and 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 *et al*., 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 effectiveness in pain management and improving quality of life, yet side effects may be severe. These side effects include stomach problems like bleeding, ulcer, stomach upset, high blood pressure, fluid retention (causing swelling such as around the lower legs, feet, ankles

and hands), kidney problems, heart problems and allergic reactions such as rashes. Side effects of NSAIDS can occur at any time and the chance is greater if used for long durations (Gislason *et al*., 2009).

2.8.1.3 Cannabinoids 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 *et al*., 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 *et al*., 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).

2.1.9 Non-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 therapy reduces 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.

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. It consists 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 in the

painful area. The major disadvantage is that TENS becomes less effective at relieving pain overtime (Mayer and Liebeskind, 1974).

More-invasive approaches are reserved 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 *et al*., 1998). In most cases surgery 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 *et al*., 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 *et al*., 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 *et al*., 2007). It is characterized by five cardinal signs *rubo*r (redness), *calor* (increased heat), *tumor* (swelling), *dolo*r (pain) and *function laesa* (loss of function) (Cotran, 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 *et al*., 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 *et al*., 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.

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 (Adam *et al*., 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, 1998).

2.2.3 Plasma-derived mediators of inflammation

The followings are plasma-derived mediators of inflammation:

Bradykinin is 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 *et al*., 2004).

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).

C5a is 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 complex is 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 *et al*., 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 *et al*., 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 ischemic 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 *et al*., 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 (Cousins *et al*., 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 *et al*., 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 *et al*., 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-immune conditions. It is the active metabolite of the drug prednisone (Davis *et al*., 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 (Miura *et al*., 2011) and dermatomyositis. It is also used for treatment of sarcoidosis even though the mechanism is unknown.

2.2.8.2 Budesonide

Is a glucocorticoid steroid 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 disease (inflammatory bowel disease).

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.

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. The 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."

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 *et al*., 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).

2.3.4.1 Pathophysiology 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 drug 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).

2.3.2.4 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 *et al*., 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 resistible if the drug is discontinued promptly. Additionally, NSAIDS indirectly depress rennin 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 The Plant *Laggera Aurita* Linn F Compositae

2.4.1 Vernacular Names

Igbo (Awka) - *Abanaadene*= vulture's excrement

Yoruba (Ilorin) - *Eru- taba* = Slave of tobacco

Hausa (Zaria) - *Taba-taba* = Tobacco-tobacco

2.4.2 Description of the plant

Laggera aurita is an annual or biennial herb attaining about 1.50 m height. It is wide spread in dry waste places from Senegal to Southern Nigeria. The species is widely dispersed throughout tropical Africa occurring also in damp or even swamp areas. The plant is hardly grazed by livestock and is strongly aromatic (Burkill, 1985).

2.4.3 Ethnomedicinal uses

Laggera aurita is a widely used medicinal plant in African countries like Nigeria, Senegal, Tanzania and Ghana. The leaves are the part of the plant reported to be most commonly used for medicinal purposes, although the plant can be used whole or pulped up. The main indications were to treat rheumatic pain, to heal cuts, and bruises, as an enemas, as an insect repellant, to cure constipation and dysentery and as a last resort to phagedenic and chronic ulcers. The powdered plant or infusions are used for dyspepsia and indigestion in Nigeria (Burkill, 1985).

The antibacterial effect of essential oils of *Laggera aurita* has been established. The methanol, ethylacetate and hexane extract of the plant *Laggera aurita* shows significant antimicrobial activity. The methanol and hexane extract were more active while the ethylacetate extract exhibited good antifungal activity. The analgesic and anti-inflammatory activity of the methanol crude extract were reported by Olurishe and Mati (2014). The membrane stabilizing effect of the ethanol crude extract of the plant *Laggera aurita* has also been demonstrated (Abdulla *et al*., 2013).

The phytochemical constituents isolated from the ethanol crude extract include carbohydrates, tannins, terpenes, sterols, flavonoids, alkaloids, phenols, volatile oil, resin, balsam, cardiac glycosides and phlobatannins.

2.5 Plants Used in the Management of Pain

The use of plants as medicine is an ancient practice especially in developing countries (Biswas *et al*., 2005). It is on this basis that researchers continue to work on medicinal plants in order to produce and develop better medicines for physiological and therapeutic purposes (Usman *et al*., 2008). Many plants have been used traditionally in the management of pain and inflammation (Magaji *et al*., 2008), and their mechanism of action, doses and spectrum of activity of some have been scientifically evaluated. These plants include *Celosia argentea* family Amaranthaceae in which the crude alcoholic extract exhibited antinociceptive activity (Santosh *et al.,* 2008).

The aqueous leaves extract of *Ocimum gratissimum* shows a significant dose dependent antinociceptive effect via acetic acid induced writhing and hot plate method, and also anti-inflammatory activity (Hassan *et al*., 2010). So also, Gossypin, an isolated bioflavonoid from the yellow petals of *Hibiscus vitofolius* has been shown to possess antinociceptive activity similar to morphine and has the advantage of lack of tolerance and dependence liability (Dahanukar *et al*., 2000). The n-butanol soluble fractions of the methanol leaf extract of *Cissus cornifolia* showed analgesic and anti-inflammatory activity via acetic acid induced writhing, hot plate method and carrageenan induced inflammation **(**Musa *et al*., 2010**)**. The flavonoid-rich fraction from *Celosia argentea* possesses significant anti-inflammatory effect when evaluated using carrageenan

induced inflammation and cotton pellet induced granulomatosus models (Bhujbal *et al*., 2006).

Other African medicinal plants with anti-inflammatory activities include *Khaya senegalensis* (Lompo *et al*., 2007), *Acanthus montanus* (Adeyemi *et al.,* 2005) and *Desmodium gangeticum* (Udeogaranya *et al*., 2005).

Some of the anti-inflammatory drugs developed from medicinal plants include Ruxiang or Gummi olibanum, an herbal medicine derived from the gum resin of *Boswellia carterii* of the family Burseraceae which is traditionally used in China to alleviate pain and reduce inflammation, Silymarin capsules from *Silybum marianum* (L.), Milk thistle which is a complex of seven flavonolignans and polyphenols (Doreswamy and Darshan, 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Collection

The plant was collected from Zaria, Kaduna State in December, 2012 and was identified by Mallam Umar Galla of Department of Biological Sciences, Ahmadu Bello University Zaria by comparing with a specimen voucher number 510 previously deposited in the herbarium.

3.1.1 Preparation of Extract

The plant was dried under shade with intermittent weighing until a constant weight is obtained. The stalk part of the plant was removed and whole plant was size-reduced using mortar and pestle. The powdered materials 1500 g was extracted with methanol using soxhlet apparatus at a temperature of 65 °C. The extract was concentrated under reduced pressure and temperature 45°C. The extract was stored in desiccators until needed for the work. Aqueous solutions were freshly prepared for each study using distilled water for both methanol extract and fractions.

3.2 Animals

Wistar strain rats (150-200 grams) and swiss albino mice (20-25 grams) of either sex were obtained from the Animal House facility of the Department of Pharmacology and Therapeutics, 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 420). All experimental protocols were approved by the University animal ethics committee.

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); Naloxone (Pfizer laboratories, Pakistan); Morphine sulphate BP (Martindale Pharmaceuticals, Ramford, Essex); Methanol extract of *Laggera aurita* (MELA); The saponin and flavonoid fractions; Normal saline (Dana Pharmaceuticals limited, Nigeria); Concentrated hydrochloric acid (BDH Limited Poole, England); Chips of magnesium metal (BDH Limited Poole, England); Concentrated sulphuric acid (BDH Limited Poole, 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 Equipment and other Materials

Animal cages, pestle and mortar, syringes (1 ml, 2 mls, 5 mls and 10 mls), 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 balance

3.5 Phytochemical Screening of *Laggera aurita*

Phytochemical screening was carried out on the methanol extract of the plant *Laggera aurita* as follows:

3.5.1 Test for carbohydrates and sugar

Molisch test: Methanol extract (0.5 g) was dissolved in water (3 mls) in a test tube. Three drops of molisch reagent was added then followed by 5 drops of concentrated sulphuric acid along the test tube wall and observed. A purple to violet colour at interface was observed (Evans, 1996).

3.5.2 Test for flavonoids

a. Shinoda's reduction test (Sofowora, 1993): Methanol extract solution (3 mls) was evaporated to dryness and then dissolved in 2 mls of 50% methanol with gentle heating. Four drops of concentrated HCl acid was added; then followed by some chips of magnesium metal and warmed. The solution was observed for the formation of flavonoids.

b. Sodium hydroxide test (Evans, 1996): About 2 mls of filtered methanol extract was dissolved in 2 mls of 10% aqueous sodium hydroxide solution which gave a yellow solution to which dilute HCl was added for formation of colourless solution.

3.5.3 Test for cardiac glycosides

Keller Killiani's test: Glacial acetic acid (1 ml) was added to about 3 mls of the solution containing 2 mg of 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 was observed for the presence of cardiac glycosides (Evans, 1996).

3.5.4 Test for saponins

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

3.5.5 Test for tannins

Lead sub-acetate test: Methanol extract (0.5g) was dissolved in 2 mls of 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.

3.5.6 Test for steroids and triterpenes

Liebermann-Burchard's test: Powdered plant material (0.5 g) was extracted with 5 mls of methanol then filtered. The filtrate was evaporated to dryness on a water bath at 100°C. The residue obtained was shaken with 2 mls chloroform and then filtered into a cleaned and dried test tube. 2 ml of acetic acid anhydride was added to the filtrate and shaken, and then 1 ml of concentrated sulphuric acid was also added carefully down the zone of contact of the two liquids. A brownish-red colour was observed immediately 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).

3.5.7 Test for alkaloids

Methanol extract (0.5 g) was treated with 5 mls 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: to test tube 1, two drops of dragendoff's reagent was added and rose red precipitate observed indicated presence of alkaloids.
b. Wagner's reagent: to test tube 2, two drops of Wagner's reagent was added and brownish precipitate observed indicated presence of alkaloids.
c. Mayer's reagent: to test tube 3, two drops of Mayer's reagent was added and creamy precipitate observed indicated presence of alkaloids.
d. Picric acid reagent: to test tube 4, two drops of picric acid was added and yellowish precipitate observed indicated the presence of alkaloids.

3.6 Fractionation

The methanol extract of *Laggera aurita* (150 g) was dissolved in 400 mls of water then filtered. Filtrate was partitioned with 600 mls of n-hexane solvent four times. Two portions were obtained; aqueous and hexane fractions. The aqueous fraction was partitioned with 600 mls of ethylacetate four times; two layers of ethylacetate and aqueous portions were obtained. The aqueous portion was again partitioned with 600 mls of n-butanol two times; two layers of n-butanol and aqueous portions were obtained. The n-butanol portion was then treated with 200 mls of 2% potassium hydroxide (KOH) solution twice. So also, a layer of KOH and layer n-butanol fractions (saponins) were obtained. The KOH fraction was neutralized with dilute HCl acid, and then followed by 600 mls of n-butanol twice. Two portions, one of n-butanol (flavonoids) and one of salt solution (discarded) were obtained. Diagramatically, it can be represented as follows:

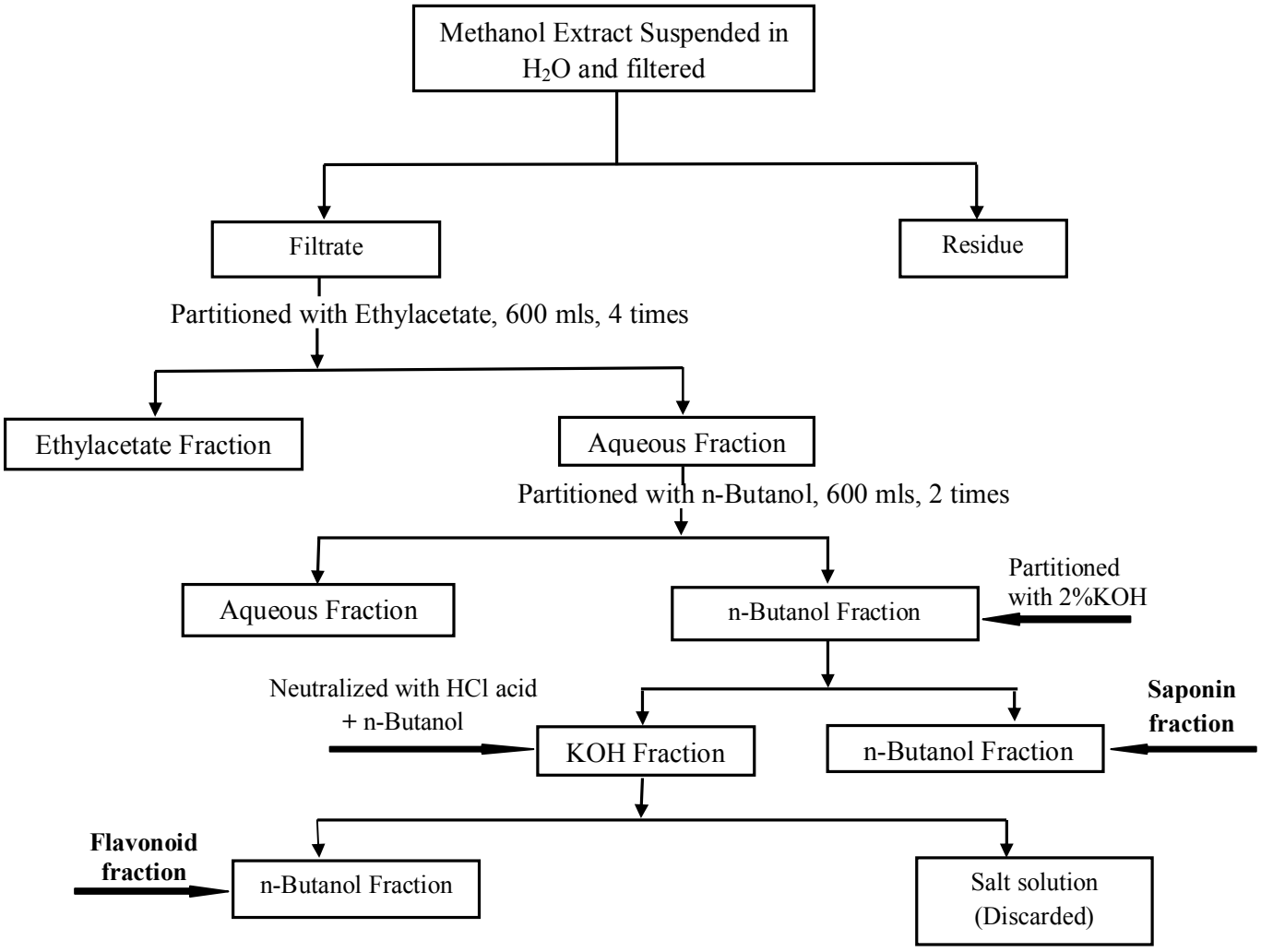


Figure 3.1: Fractionation of Methanol Extract of *Laggera aurita*

Γoxicity Studies in Mice and Rats (LD_{50})

3.7.1 Selection of animals

Female rats and mice nulliparous and non pregnant of average weights 175 g and 23 g respectively 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.

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

LD_{50} determination was conducted using Organization for Economic Co-operation and Development (OECD) guidelines in rats and mice. In this method, two groups each of three animals 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 was then further withheld for 3-4 hours in rats and 1-2 hours in mice after the extract had been administered. The test substance was administered in a single oral dose using an oral 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 after which there was no death 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 at least once during the first 30 minutes after dosing and periodically during the first 24 hours with careful observation during first 4

hours and then daily for 14 days. Observations included changes in skin and fur, eyes and mucous membranes, somator activity and behaviour pattern, autonomic and central nervous systems etc. Animals were also observed for tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Time of onset of toxic symptoms and disappearance were also noted.

Individual weights of animals were determined at least weekly. At the end of the test, surviving animals were weighed and then sacrificed using chloroform. Organs of the animals such as liver, kidney, spleen, lungs, heart and gastrointestinal system were freshly harvested and fixed in 10% v/v formalin for Histopathological studies.

3.7.3 Histopathological studies.

Tissues slices of 3 cm thickness was cut and fixed in 10% buffered formalin saline and put in automatic tissue processor and then fixed in 10% buffered formalin-saline solution for 6 hours. They were then dehydrated for 2 hours in each of the ascending grades of alcohol- 70%, 90% and 100% v/v. The dehydrated tissues were cleaned with toluene for 2 hours after which the tissues were embedded in paraffin wax and left to cool. The blocks were trimmed and sectioned on the microtone at 5 microns. The ribbons of sections were floated in a warm water bath. Suitable sections were selected, attached to slide and dried on a hot plate and stained with Haematoxylin and Eosin stain. Sections were dewaxed in xylene, rehydrated in descending grades of alcohol 100%, 90% and 70% v/v, then stained in haematoxylin for 5 minutes, differentiated in 1% acid alcohol, glued in Scott's tap water and stained with eosin for 3 minutes. Sections were rinsed and dehydrated in ascending grades of alcohol 70%, 90% and

100%, then finally dewaxed in xylene and mounted in a box. The slides were then examined microscopically for pathological lesions (Arthur and John, 1978).

3.8 Antinociceptive Studies

3.8.1 Acetic acid induced writhes test

The acetic acid induced writhes test in mice as described by Koster *et al.,* (1959) was employed. Thirty albino mice were divided into five groups of six mice.

The first group received 10 ml/kg of normal saline orally and this served as control. Group 2 received piroxicam 20 mg/kg as positive control. Group 3, 4 and 5 received 200, 400 and 800 mg/kg oral doses of the methanol extract respectively. Thirty minutes after pretreatment, 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 10 minutes after a 5 minutes latency period.

The above test procedure was repeated for saponin and flavonoid fractions as the test agents.

3.8.2 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 received 200, 400 and 800 mg/kg of methanol extract orally respectively, while the fifth group received 5 mg/kg morphine solution orally. Thirty 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 interval between the time the mouse was placed on the hot plate and the time it jumped off the plate was considered as an index of pain response latency.

The above procedure was repeated using the flavonoid and saponin fractions as the test agents.

3.8.3. Formalin induced inflammation

This involves the measure of acute inflammation induced by subplanter injection of a phlogistic agent which results in increase in the rat hind linear paw diameter (Winter *et al.,* 1963). The phlogistic agent used for this study was formalin (50 µL of 2.5% $^{v}/_{v}$).

Thirty rats were divided into five groups each of six rats each. Group 1 was treated with 10 ml/kg normal saline orally (negative control); group 2 was treated with 10 mg/kg ketoprofen orally; while group 3, 4 and 5 received methanol extract of *Laggera aurita* at doses of 200, 400 and 800 mg/kg respectively. Inflammation was induced in rat hind paw by injecting formalin 0.1 ml (1% w/v in 0.9% normal saline) into the subplanter surface of the left hind paw 30 mins after treatment. Paw linear diameter (mm) was measured using a digital vernier caliper (MR 2002) at 0, 1, 2, 3, 4 and 5 hours after formalin injection.

The above procedure was repeated using flavonoid and saponin fractions as the test agents.

3.8.4 Effect of methanol extract of *Laggera aurita* interacted with naloxone assessed by hot plate test

Four groups of 10 mice each were used. Initially all the animals received naloxone at a dose of 2 mg/ kg i.p. After 15 minutes, the test group (1 and 2) received doses of 400 and 800 mg/ kg methanol extract orally. The control group (3) received normal saline 10 ml/kg orally and the standard group (4) received morphine 5 mg/ kg orally. The index of pain response latency was assessed at 60, 120 and 180 minutes using hot plate method (Younos *et al*., 1990).

The same procedure was repeated using flavonoid and saponin fractions as the test agent.

3.9 Data Analysis

Data was analyzed using one way analysis of variance (ANOVA) for acetic acid tests and repeated measures ANOVA for hot plate (thermal sensitivity) test, effect of extract interacted with naloxone and formalin induced inflammation, followed by Bonferoni post hoc test. Results were considered significant at $P \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Constituents

4.1.1 Phytochemical constituents of the methanol extract

The phytochemical constituents of the methanol extract of *Laggera aurita* are the flavonoids, tannins, steroids, carbohydrates, glycosides, alkaloids and cardiac glycosides. Phytochemical constituents like anthraquinones were absent (Table 4.1).

Table 4.1: Phytochemical constituents of the Methanol extract of *Laggera aurita*

Phytochemical constituents	Result
Flavonoids	+
Alkaloids	+
Glycosides	+
Tannins	+
Saponins	+
Cardiac glycosides	+
Phenols	+
Anthraquinones	-
Carbohydrates	+

Key: + = Present and - = Absent

4.2 Acute Toxicity Study

4.2.1 Median Lethal Dose (LD_{50})

The oral acute toxicity (LD_{50}) was found to be greater than 5000 mg/kg body weight in both mice and rats.

4.2.2 Histological studies

4.2.2.1 Liver

The groups that received normal saline showed normal hepatocytes and intact portal tract in both mice and rats. In the groups that received 2000 mg/kg of the methanol extract of *Laggera aurita*, there was slight vascular congestion in mice (Plate I). However, in rats, there was intense vascular damage with kupfer cell hyperplasia and slight hepatic necrosis (Plate II). In the groups that received 5000 mg/kg of the methanol extract, there was intense vascular congestion with kupfer cell hyperplasia and lymphocyte hyperplasia in mice (Plate III). In rats, there was vascular congestion with lymphocyte hyperplasia (Plate IV).

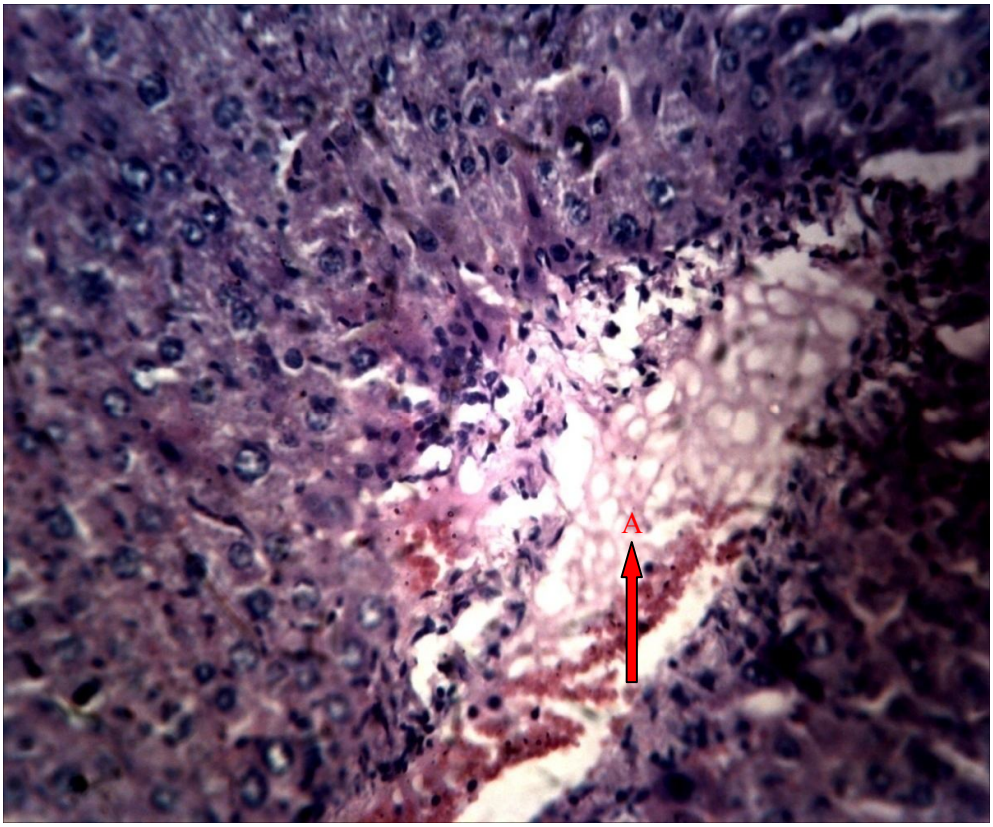


Plate I: Photomicrograph of a liver section of mice treated with 2000 mg/kg of methanol extract of *Laggera aurita* and observed for two days showing A = Slight vascular congestion. H & E stain (× 400)

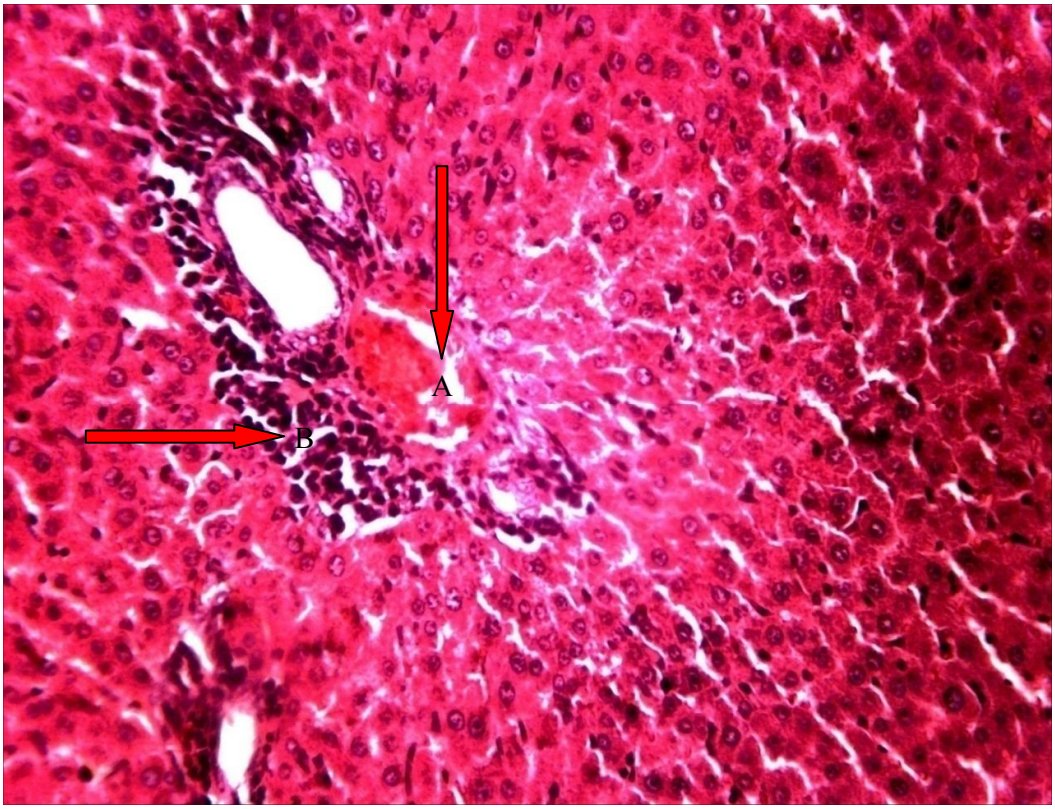


Plate II: Photomicrograph of a liver section of rat treated with 2000 mg/kg of methanol extract of *Laggera aurita* and observed for two days showing A = intense vascular damage with kupfer cell hyperplasia, B = slight hepatic necrosis. H & E stain (× 400)

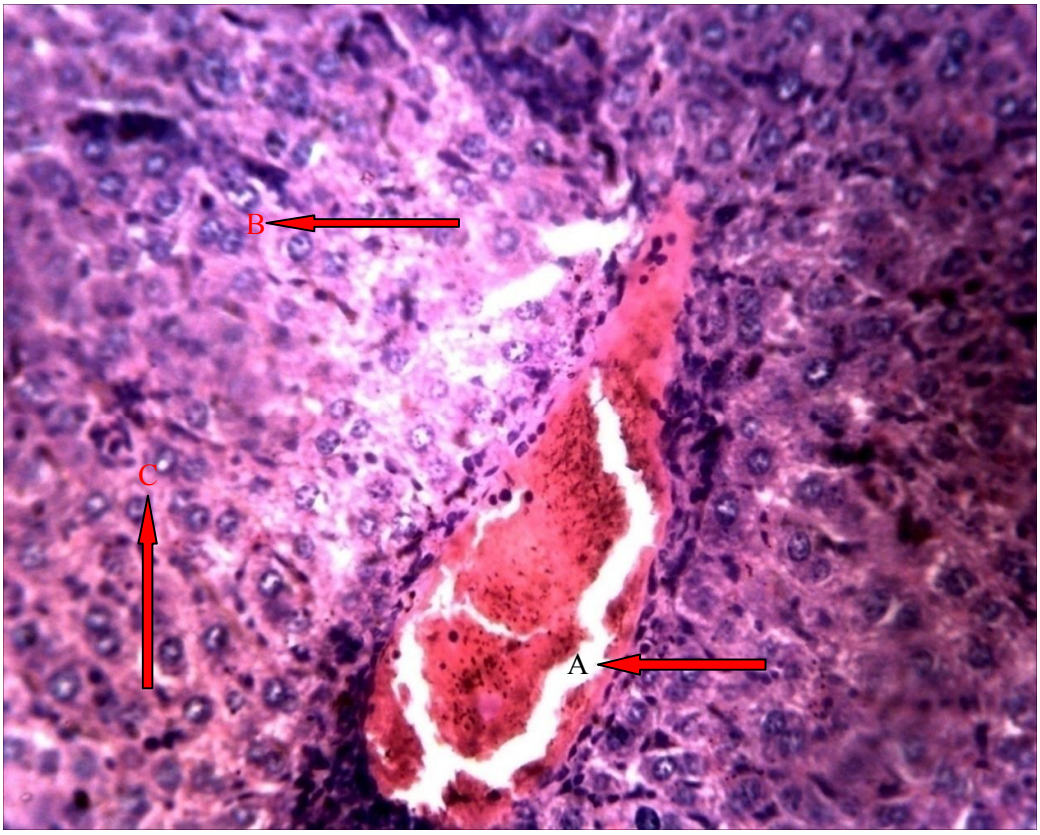


Plate III: Photomicrograph of a liver section of mice treated with 5000 mg/kg of methanol extract of *Laggera aurita* and observed for fourteen days showing A = intense vascular congestion, B = Kupfer cell hyperplasia, C = lymphocyte hyperplasia. H & E stain (× 400)

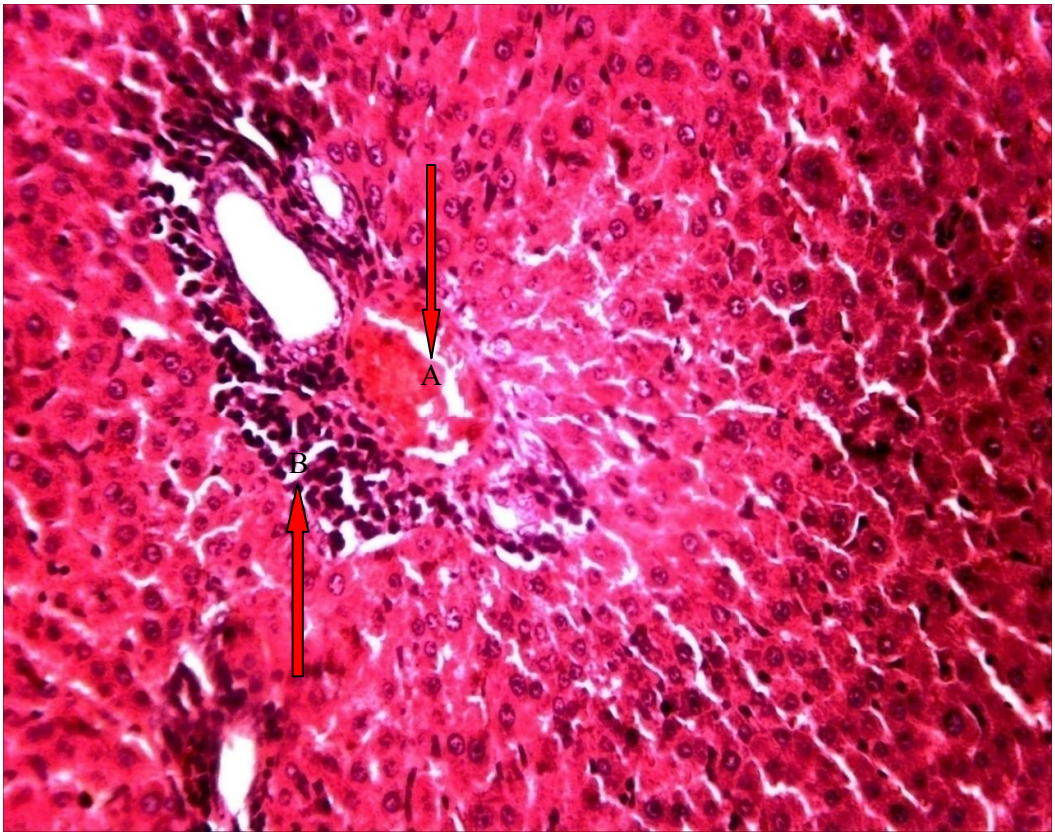


Plate IV: Photomicrograph of a liver section of rat treated with 5000 mg/kg of methanol extract of *Laggera aurita* and observed for fourteen days showing A = vascular congestion, B = lymphocyte hyperplasia. H & E stain (× 400)

4.2.2.2 Kidneys

The normal saline group showed normal renal tissue, normal glomeruli, tubular epithelium, interstitial tissue and vessels. The groups that received 2000 mg/kg of the methanol extract of *Laggera aurita*, showed slight glomerular necrosis in both mice (Plate V) and rats, and tubular distortion in rats (Plate VI). The group treated with 5000 mg/kg showed slight glomerular necrosis with tubular damage in mice (Plate VII) and rats (Plate VIII).

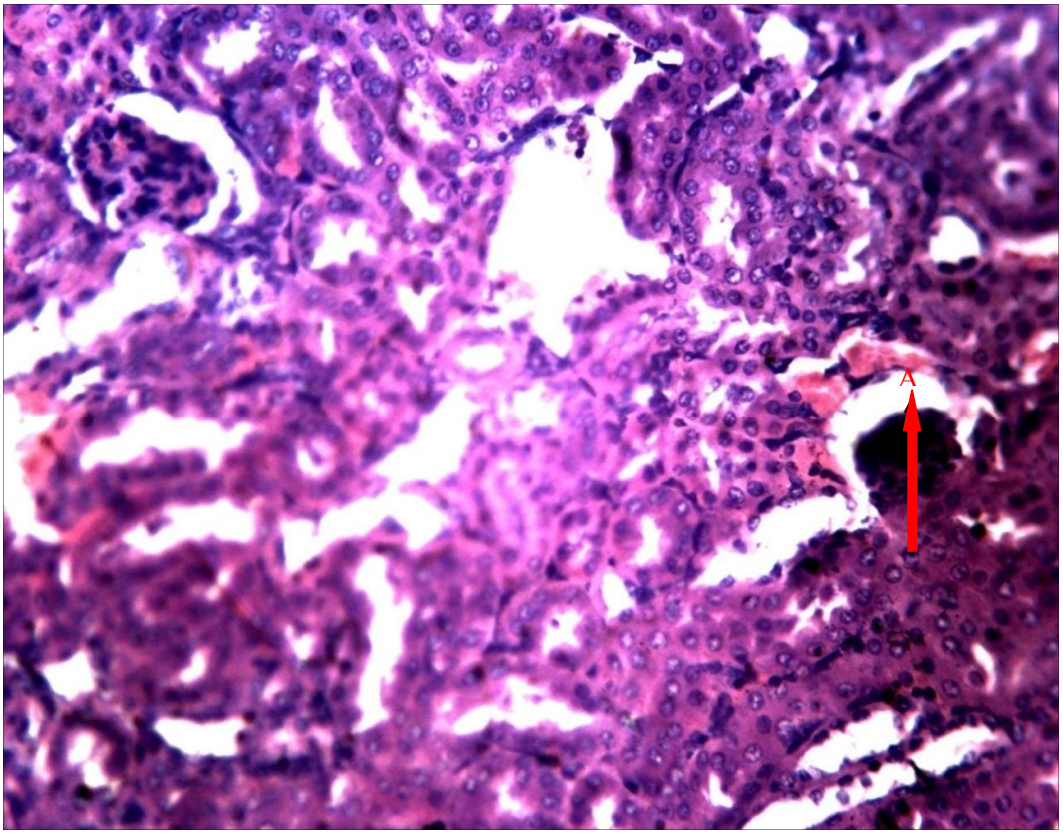


Plate V: Photomicrograph of a kidney section of mice treated with 2000 mg/kg of methanol extract of *Laggera aurita* and observed for two days showing A = slight glomerulus necrosis. H & E stain (× 400)

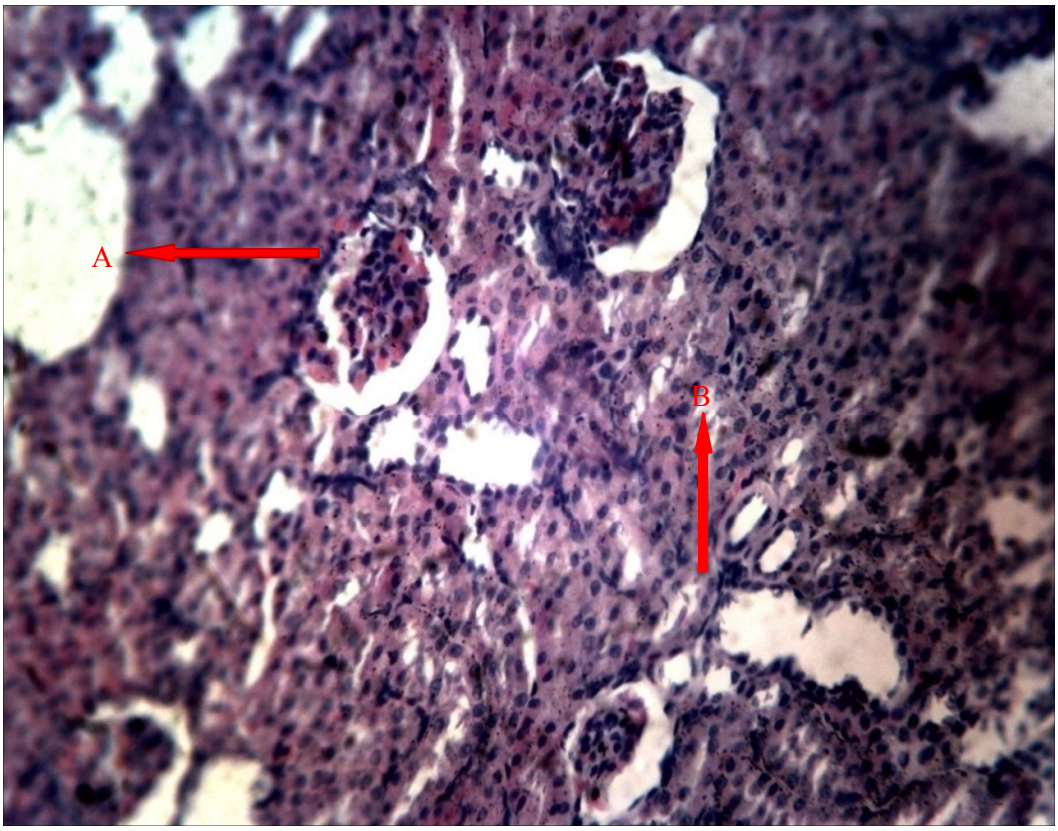


Plate VI: Photomicrograph of a kidney section of rat treated with 2000 mg/kg of methanol extract of *Laggera aurita* and observed for two days showing A = slight glomerulus necrosis, B = tubular distortion. H & E stain (× 400)

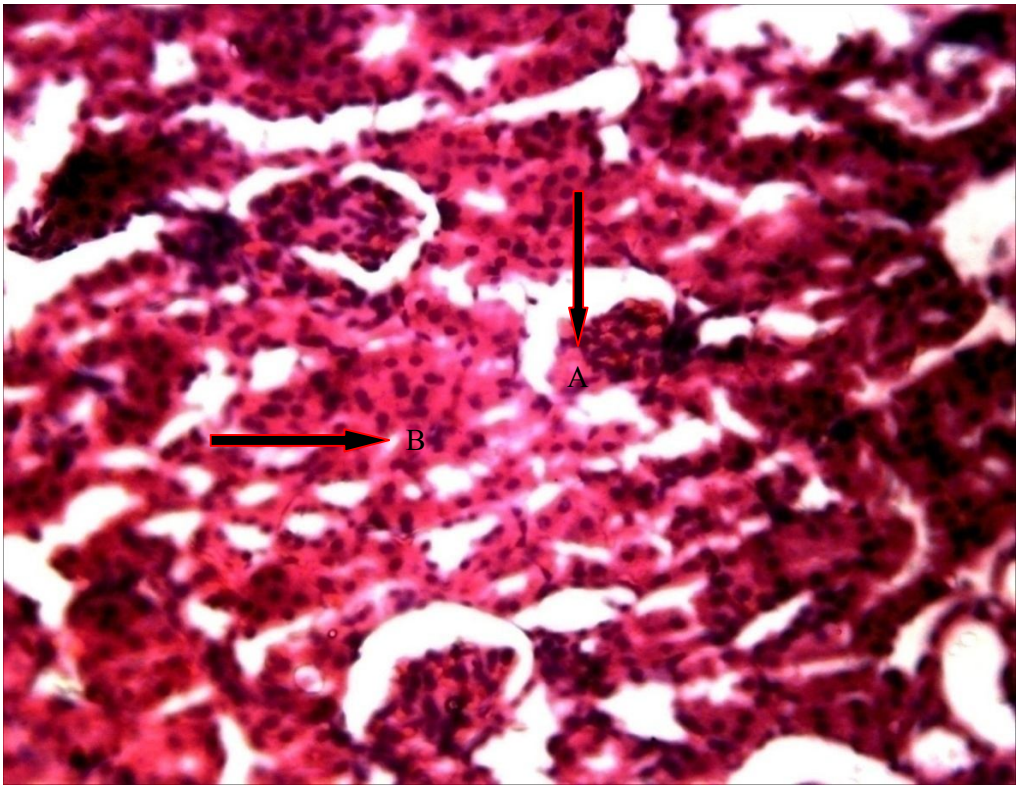


Plate VII: Photomicrograph of a kidney section of mice treated with 5000 mg/kg of methanol extract of *Laggera aurita* and oserved for fourteen days showing A = slight glomerulus necrosis, B = tubular damage. H & E stain (× 400)

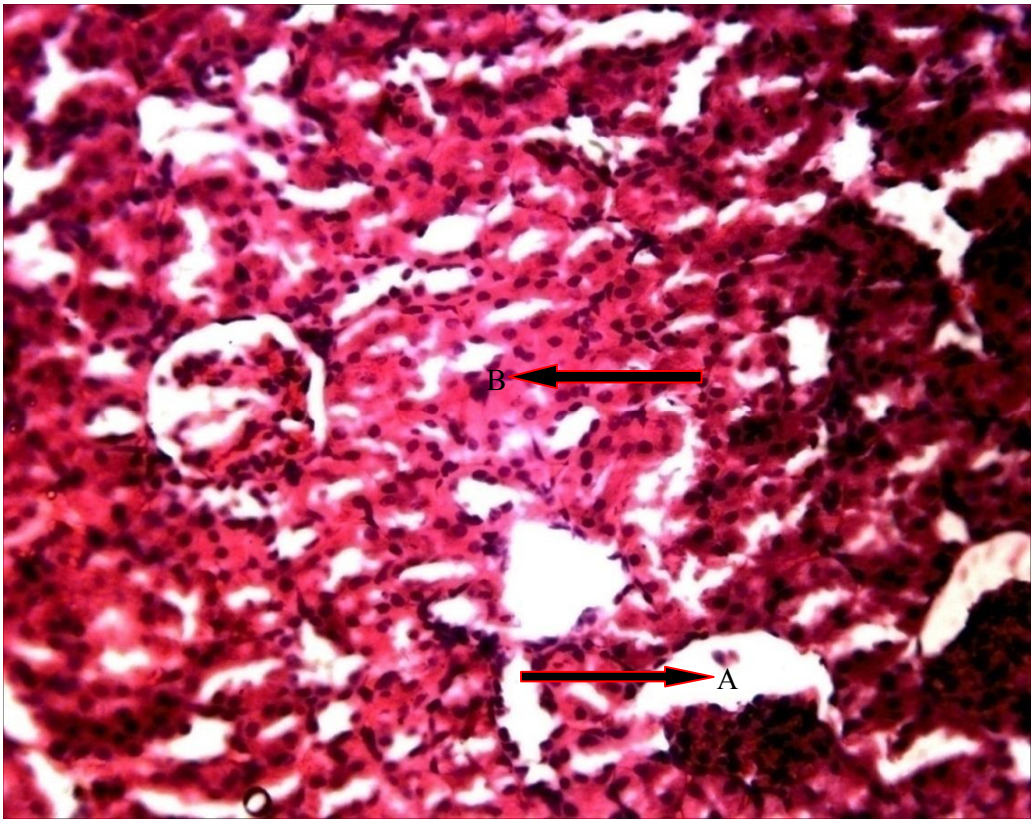


Plate VIII: Photomicrograph of a kidney section of rat treated with 5000 mg/kg of methanol extract of *Laggera aurita* and observed for fourteen days showing A = slight glomerulus necrosis, B = tubular damage. H & E stain (× 400)

4.2.2.3 Lungs

The saline groups showed normal alveoli in both mice and rats. The group that received 2000 mg/kg of the methanol extract also showed normal alveoli in both mice and rats. However, in the groups that received 5000 mg/kg there was slight alveoli congestion in mice (Plate IX), while vacuotation with lymphocyte hyperplasia and congestion were observed in rats (Plate X).

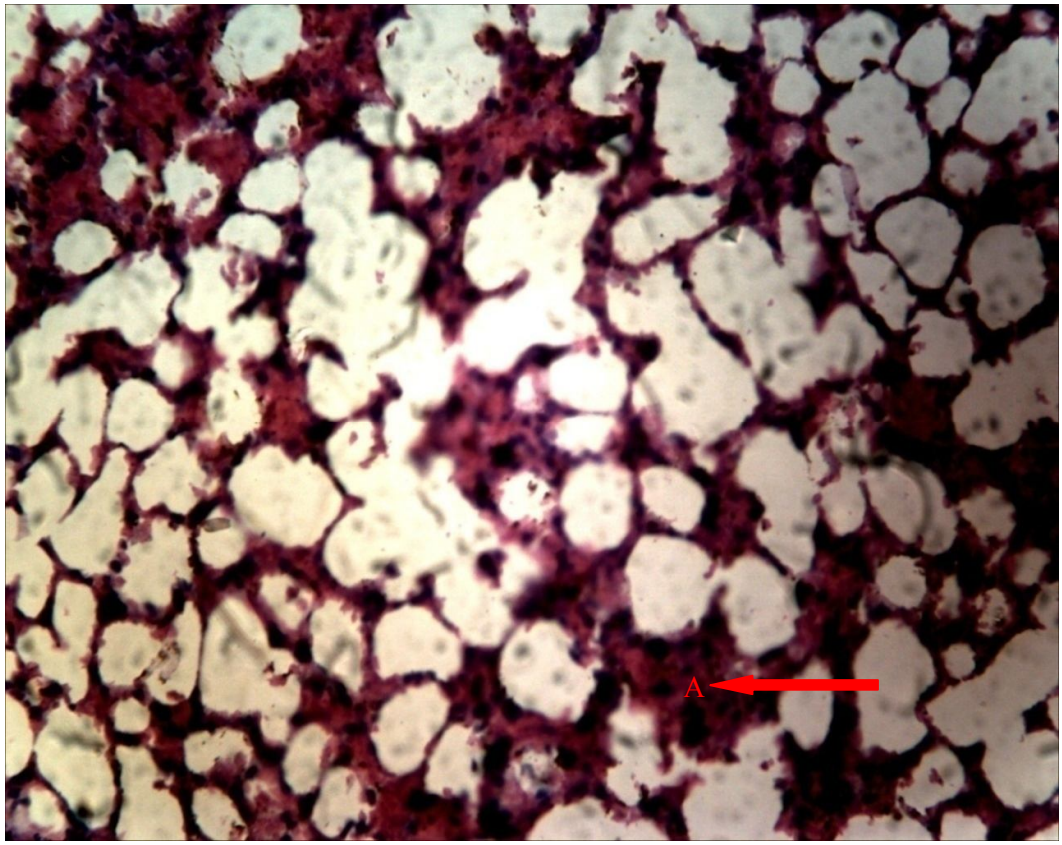


Plate IX: Photomicrograph of a lung section of mice treated with 5000 mg/kg of methanol extract of *Laggera aurita* and observed for fourteen days showing A = slight alveoli congestion. H & E stain (× 400)

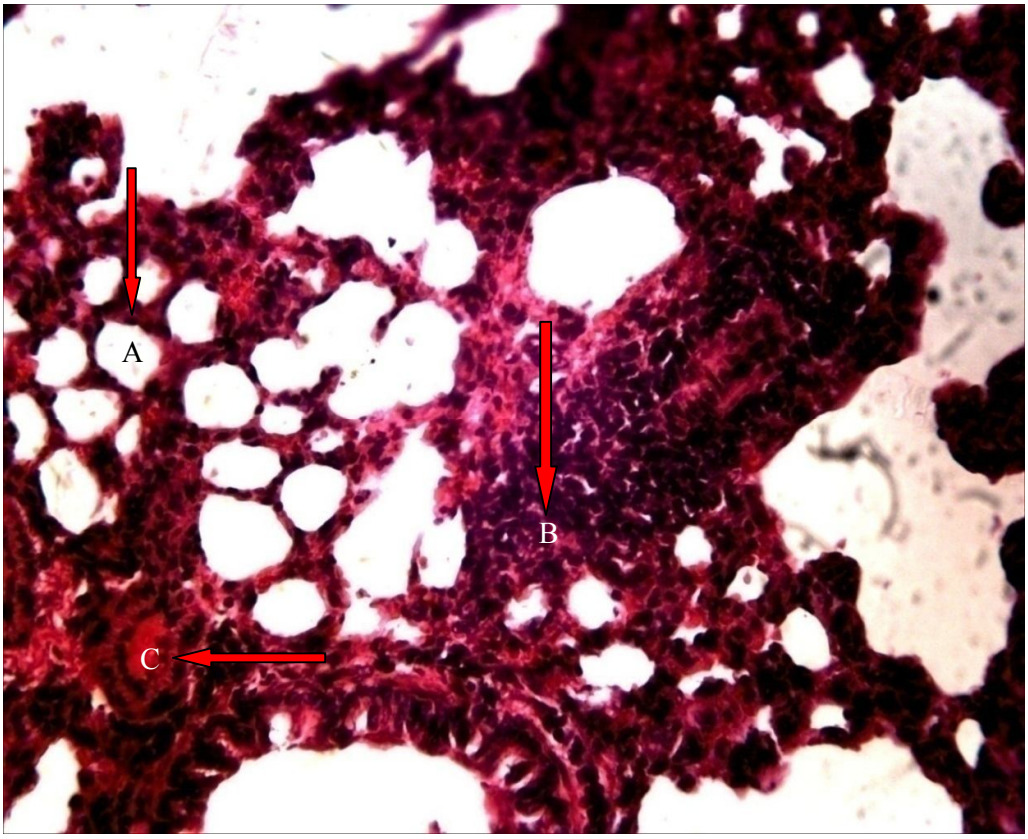


Plate X: Photomicrograph of a lung section of rat treated with 5000 mg/kg of methanol extract of *Laggera aurita* and observed for fourteen days showing A = vacuotation, B = lymphocyte hyperplasia and C = congestion. H & E stain (× 400)

4.2.2.4 Spleen

The saline group showed normal red and white pulp in both mice and rats. The group that received 2000 mg/kg of the methanol extract also showed normal red and white pulp in both mice and rats. However, the mice that received 5000 mg/kg showed lymphocyte hyperplasia (Plate XI).

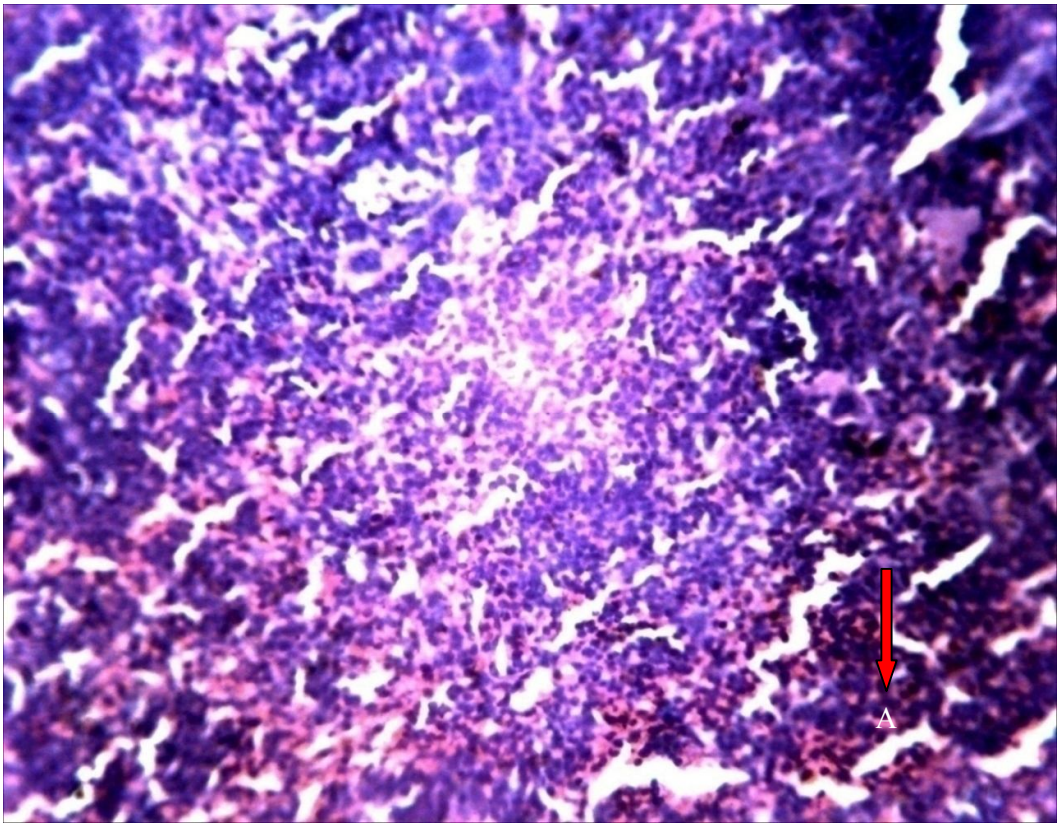


Plate XI: Photomicrograph of a spleen section of mice treated with 5000 mg/kg of methanol extract of *Laggera aurita* and observed for fourteen days showing A = lymphocyte hyperplasia. H & E stain (× 400)

4.2.2.5 Stomach

The normal saline group showed normal mucosa lining in both mice and rats. The group that received 2000 mg/kg of the methanol extract also showed normal mucosa lining in both mice and rats. However, group treated with 5000 mg/kg showed moderate erosion of stomach epithelium in mice (Plate XII) and slight mucosa damage in rats (Plate XIII).

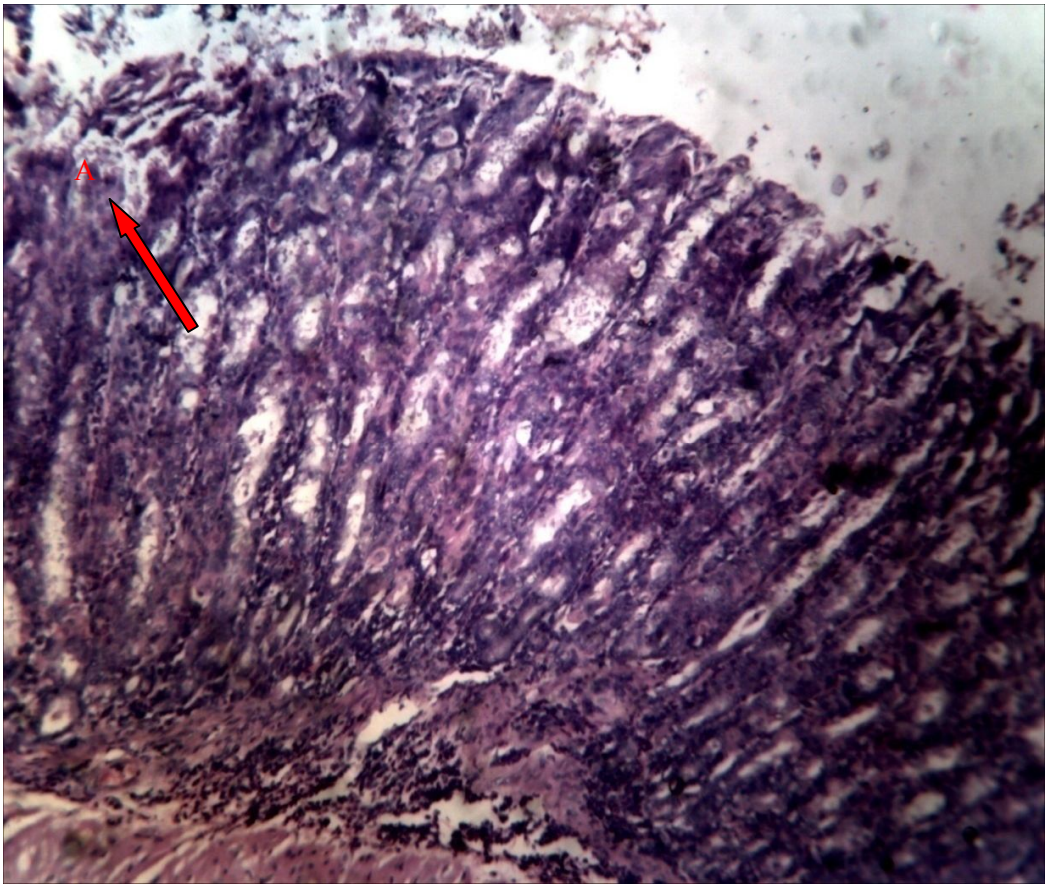


Plate XII: Photomicrograph of a stomach section of mice treated with 5000 mg/kg of methanol extract of *Laggera aurita* and observed for fourteen days showing A = moderate erosion of stomach epithelium. H & E stain (× 400)

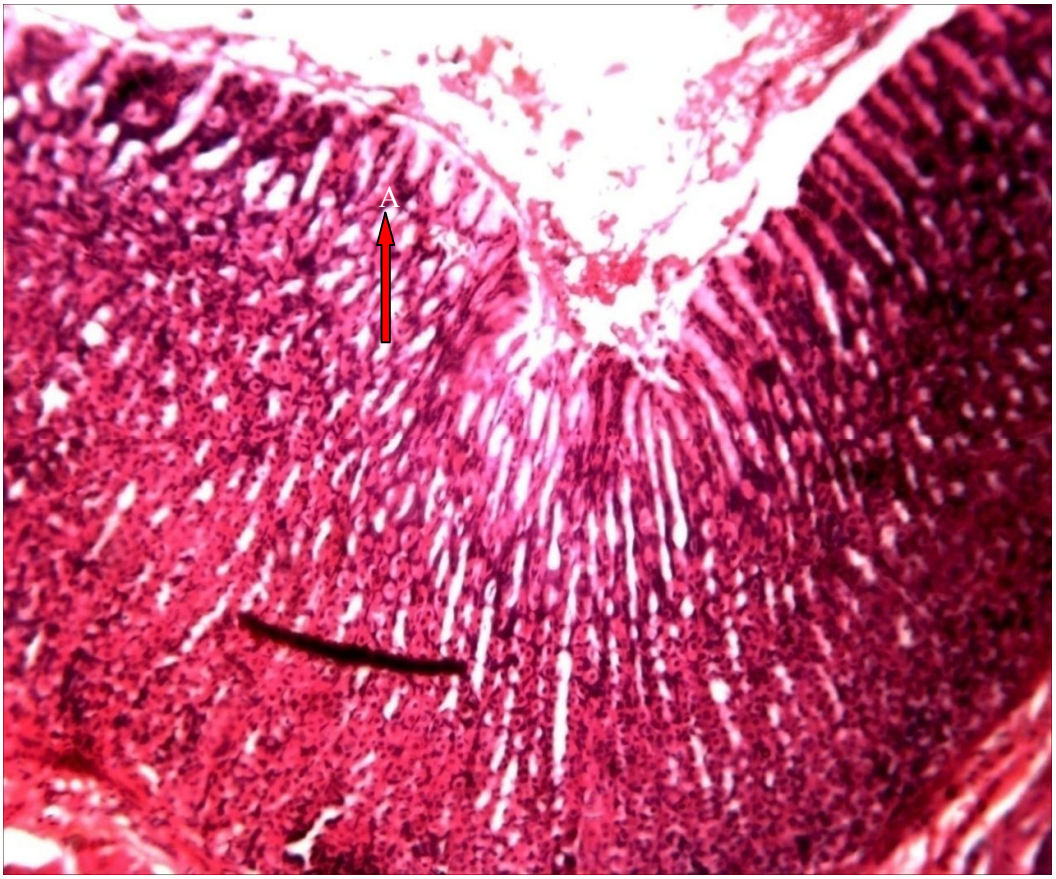


Plate XIII: Photomicrograph of a stomach section of rat treated with 5000 mg/kg of methanol extract of *Laggera aurita* and observed for fourteen days showing A = slight mucosa damage. H & E stain (× 400)

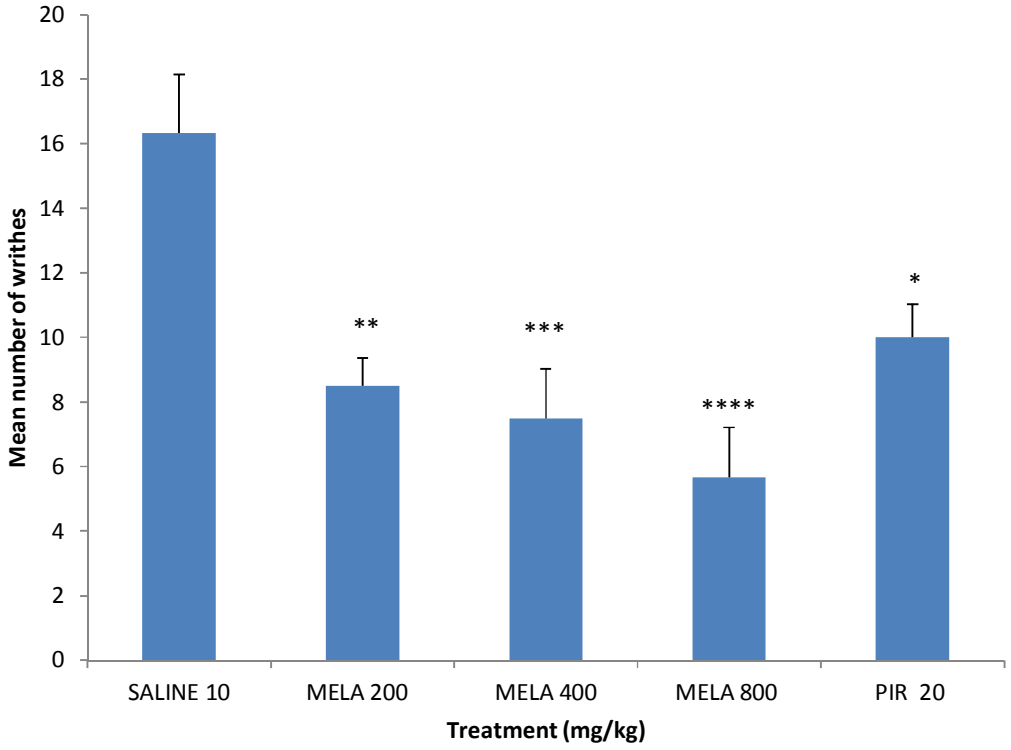
4.2.2.6 Heart

The normal saline group showed normal cardiac muscles in both mice and rats and also the groups that received 2000 mg/kg and 5000 mg/kg of methanol extract showed normal cardiac muscles in both mice and rats.

4.3 Effect of *Laggera aurita* on Acetic Acid Induced Writhes Test in Mice

4.3.1 Effect of methanol extract of *Laggera aurita* on acetic acid induced writhes test in mice

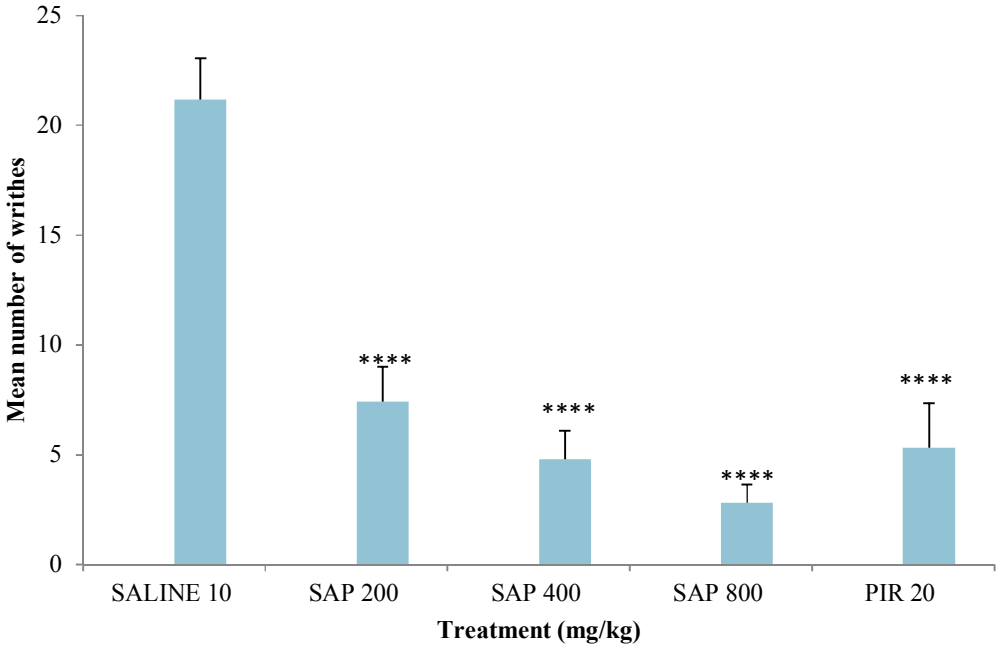
The methanol extract of *Laggera aurita* significantly ($p<0.05$) decreased the number of acetic acid-induced writhes in mice in a dose dependent manner. The effect of the methanol extract of *Laggera aurita* at all doses tested showed significant ($p<0.01$) decrease in number of acetic acid-induced writhes than piroxicam at a dose of 20 mg/kg (Figure 4.1).



Data was analyzed using one way ANOVA followed by Bonferoni Post Hoc test and presented as mean ± SEM, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$ significant statistical difference as compared with Control group, n=6, MELA= methanol extract of *Laggera aurita,* PIR = piroxicam 20 mg/kg

4.3.2 Effect of saponin fraction of *Laggera aurita* on acetic acid induced writhes test in mice

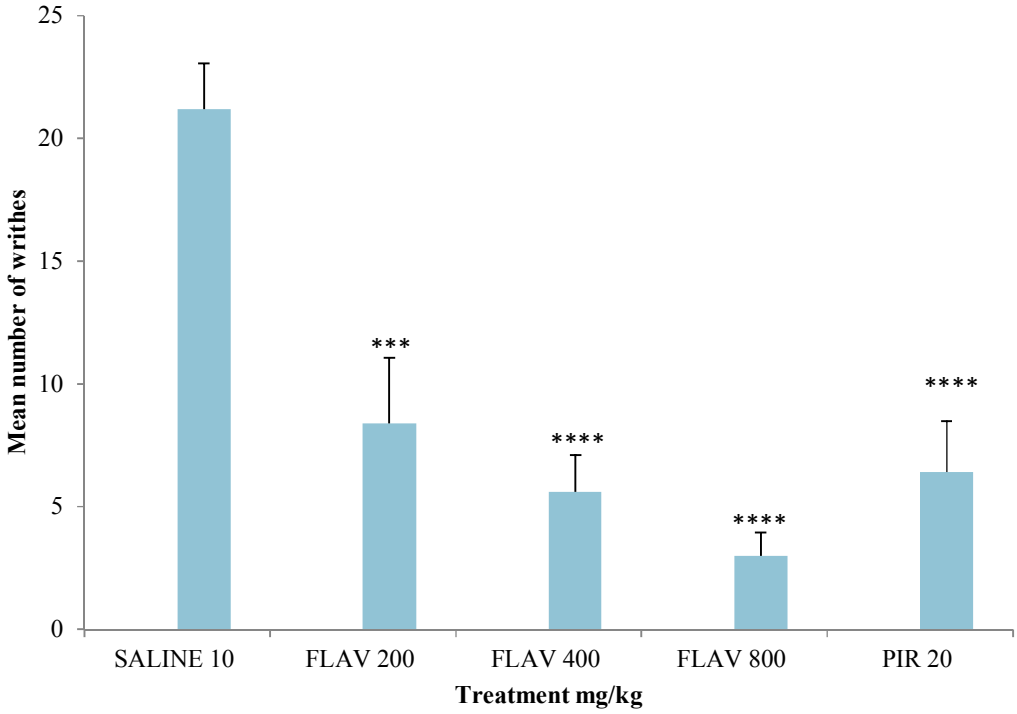
The saponin fraction of *Laggera aurita* significantly ($p<0.0001$) decreased the number of acetic acid-induced writhes in mice. The saponin fraction of *Laggera aurita* at 400 and 800 mg/kg body weight showed significant ($p<0.000$) decreased in number of acetic acid-induced writhes than piroxicam at a dose of 20 mg/kg body weight (Figure 4.2).



Data was analyzed using one way ANOVA followed by Bonferroni post hoc test and presented as mean±SEM, **** = P≤0.0001 significant statistical difference as compared with Control group, n= 6, SAP= saponin fraction of *Laggera aurita,* PIR= piroxicam 20 mg/kg

4.3.3 Effect of flavonoid fraction of *Laggera aurita* on acetic acid induced writhes test in mice

The flavonoid fraction of the plant *Laggera aurita* significantly ($p<0.001$) decreased the number of acetic acid-induced writhes in mice. The effect of the flavonoid fraction of the plant *Laggera aurita* at 400 and 800 mg/kg body weight exhibited significant ($p<0.000$) decrease in mean number of writhes than piroxicam 20 mg/kg (Figure 4.3).



Data was analyzed using one way ANOVA followed by Bonferroni post hoc and presented as mean ± SEM , ***= P ≤0.001, **** =P ≤ 0.0001 significant statistical difference as compared with Control group, n =6, FLA= flavonoid fraction of *Laggera aurita,* PIR= Piroxicam 20 mg/kg

4.4 Effect of *Laggera aurita* on Thermally Induced Pain in Mice

4.4.1 Effect of the methanol extract of *Laggera aurita* on thermally induced pain

The extract significantly ($p<0.05$) increased the mean reaction time in a dose dependent manner. The methanol extract at the dose of 800 mg/kg body weight showed significant ($p<0.01$) increased the mean reaction time than morphine at a dose of 5 mg/kg body weight at three and four hours treatment time (Table 4.2).

Table 4.2: Effect of the methanol extract of *Laggera aurita* on thermally induced pain in mice

Groups (mg/kg)	Mean reaction time ± SEM (secs) 0 min	30 mins	60 mins	90 mins	120 mins
Control	2.29±0.10	2.18±0.22	2.19±0.23	2.43±0.28	2.49±0.19
LA(200)	1.67±0.14	3.25±0.39	3.32±0.14**	3.01±0.24*	3.02±0.29*
LA(400)	1.81±0.25	2.82±0.14**	3.02±0.16	3.28±0.31	3.29±0.18
LA(800)	2.08±0.20	2.99±0.19	2.69±0.19	3.87±0.27**	3.81±0.24**
Mor (5)	2.15±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. LA= methanol extract of *Laggera aurita*, Mor = morphine 5 mg/kg

4.4.2 Effect of saponin fraction of *Laggera aurita* on thermally induced pain

The saponin fraction significantly ($p<0.05$) increased the mean reaction time in a dose dependent manner. The saponin fraction at 400 and 800 mg/kg body weight showed significant ($p<0.01$) increase in mean reaction time than morphine 5 mg/kg at time 90 and 120 minutes (Table 4.3).

Table 4.3: Effect of the saponin fraction of *Laggera aurita* on thermally induced pain in mice

Treatment (mg/kg)	Mean reaction Time ± SEM (secs) 0 mins	30 mins	60 mins	90 mins	120 mins
Control	2.60±0.25	2.60±0.25	2.60±0.25	3.00±0.32	3.00±0.32
SAP(200)	5.00±0.95	2.20±0.20	4.20±0.20	5.8±0.37*	6.00±0.63*
SAP(400)	2.20±0.20	4.20±0.37	4.20±0.37**	6.60±1.03**	7.40±0.98**
SAP(800)	2.80±0.49	3.40±0.25	3.60±0.25	5.60±0.93**	8.60±1.69**
Mor (5)	5.80±0.37	3.00±0.00	9.80±0.86*	10.00±1.76*	9.00±1.30*

Data was analyzed using repeated measure 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. SAP= saponin fraction of *Laggera aurita*, Mor = morphine 5 mg/kg,

4.4.3 Effect of flavonoid fraction of *Laggera aurita* on thermally induced pain

The flavonoid fraction significantly ($p<0.05$) increased the mean reaction time in a dose dependent manner. The flavonoid fraction has no significant increase in mean reaction time compared to the standard morphine 5 mg/kg (Table 4.4).

Table 4.4: Effect of the flavonoid fraction of *Laggera aurita* on thermally induced pain in mice

Treatment (mg/kg)	Mean reaction time ± SEM (secs) 0 mins	30 mins	60 mins	90 mins	120 mins
Control	2.60±0.25	2.60±0.25	2.60±0.25	3.00±0.31	3.00±0.32
FLA (200)	3.80±0.37	2.60±0.51	5.80±0.49*	7.40±1.08	8.20±0.86*
FLA (400)	2.60±0.25	2.20±0.20	6.20±0.49*	9.20±1.77	8.60±1.81
FLA (800)	5.60±0.75	2.40±0.25	5.20±0.58	8.20±0.74*	9.20±1.46
Mor (5)	5.80±0.37	3.00±0.00	9.80±0.86*	10.00±1.76*	9.00±1.30*

Data was analyzed using repeated measures ANOVA followed by Bonferroni post hoc test, * = $p<0.05$ significant statistical difference compared with time zero; n = 6.
Values are Mean ± SEM. FLA= flavonoid fraction of *Laggera aurita*,
Mor = morphine 5 mg/kg

4.5 Effect of *Laggera aurita* Interacted with Naloxone and Assessed by Hot Plate Test

4.5.1 Effect of methanol extract of *Laggera aurita* interacted with naloxone and assessed by hot plate test

The methanol extract co-administered with naloxone significantly ($p<0.01$) decreased the reaction time in a dose dependent manner. The effect of the methanol extract at dose of 800 mg/kg body weight co-administered with naloxone showed significant ($p<0.001$) decrease in reaction time than morphine 5 mg/kg body weight co-administered with naloxone at the three hours treatment time (Table 4.5).

Table 4.5 Effect of methanol extract of *Laggera aurita* interacted with naloxone and assessed by hot plate test

Treatment (mg/kg)`	Mean reaction time ± SEM (secs) 0 mins	60 mins	120 mins	180 mins
Control	2.09±0.17	2.00±0.14	1.97±0.09	2.04±0.14
LA (400)	1.75±0.13	1.90±0.13	1.81±0.09	1.69±0.07
LA (800)	2.16±0.19	1.99±0.21*	2.03±0.19*	1.88±0.18**
Mor (5)	2.21±0.12	2.13±0.16	2.05±0.11	1.94±0.10*

Data was analyzed using repeated measures ANOVA followed by Bonferroni post hoc test, * = $p<0.01$, ** = $p<0.001$ significant statistical difference compared with time zero; n=10. Values are Mean ± SEM. LA= methanol extract of *Laggera aurita*, Mor= morphine 5 mg/kg

4.5.2 Effect of saponin fraction of *Laggera aurita* interacted with naloxone and assessed by hot plate test

The saponin fraction co-administered with naloxone decreased the mean reaction time over time though it was not statistically significant. The saponin fraction co-administered with naloxone showed insignificant decrease mean reaction time to morphine co-administered with naloxone (Table 4.6).

Table 4.6: Effect of saponin fraction of *Laggera aurita* interacted with naloxone and assessed by hot plate test

Treatment (mg/kg)	Mean reaction time ± SEM (secs) 0 mins	60 mins	120 mins	180 mins
Control	2.0±0.55	1.60±0.25	2.20±0.20	2.20±0.20
SAP (400)	2.80±0.37	2.80±0.37	2.80±0.20	2.00±0.32
SAP (800)	3.20±0.37	2.80±0.20	2.80±0.37	2.20±0.20
Mor (5)	4.80±1.80	3.20±1.20	3.00±0.32	1.80±0.20

Data was analyzed using repeated measures ANOVA followed by Bonferroni post hoc test, * = $p<0.05$ significant statistical difference compared with time zero, n = 8. Values are Mean ± SEM. SAP= saponin fraction of *Laggera aurita*, Mor = morphine 5 mg/kg

4.5.3 Effect of flavonoid fraction of *Laggera aurita* interacted with naloxone and assessed by hot plate test

The flavonoid fraction co-administered with naloxone decreased the mean reaction time over time but statistically insignificant. The flavonoid fraction co-administered with naloxone showed insignificant decrease mean reaction time to morphine co-administered with naloxone over time (Table 4.7).

Table 4.7: Effect of flavonoid fraction of *Laggera aurita* interacted with naloxone and assessed by hot plate test

Treatment (mg/kg)	Mean reaction time ± SEM (secs)) 0 mins	60 mins	120 mins	180 mins
Control	2.0±0.55	1.60±0.25	2.20±0.20	2.20±0.20
FLA (400)	2.40±0.25	2.20±0.20	2.20±0.20	1.40±0.25
FLA (800)	1.60±0.25	2.40±0.25	1.60±0.25	1.20±0.20
Mor (5)	4.80±1.80	3.20±1.20	3.00±0.32	1.80±0.20

Data was analyzed using repeated measures ANOVA followed by Bonferroni post hoc test, * = $p<0.05$ significant statistical difference compared with time zero, n = 8. Values are Mean ± SEM. FLA= flavonoid fraction of *Laggera aurita*, Mor = morphine 5 mg/kg

4.6 Effect of *Laggera aurita* on Formalin Induced Inflammation in Rats

4.6.1 Effect of the methanol extract of *Laggera aurita* on formalin induced inflammation in rats

The injection of 0.6% v/v formalin in the paws of rats produced local edema which was significant as compared to time zero in all the groups ($p<0.001$). The red colour denotes peak of edema and blue colour denotes significant statistical decrease in mean paw edema size. The peak of inflammation was reached in the third hour in normal saline and extract dose 200 mg/kg. Mean while for ketoprofen 10 mg/kg and extract doses 400 and 800 mg/kg; peak of edema was reached at the second hour. The extract significantly ($p<0.001$) decreased formalin-induced paw edema in dose dependent manner (200, 400, and 800 mg/kg body weight) at the fourth and fifth hours treatment time comparable to the peak of edema (Table 4.8).

Table 4.8: Effects of methanol extract of *Laggera aurita* on formalin induced inflammation in rats

Treatment (mg/kg)	Mean Paw Diameter (millimetre) 0 hrs	1 hrs	2 hrs	3 hrs	4 hrs	5 hrs
Control	2.24±0.06	2.78±0.10$^{\#\#}$	3.18±0.05$^{\#\#}$	3.4±0.05$^{\#\#\#\#}$	3.03±0.06$^{\#\#\#\#}$	2.7±0.06$^{\#}$
LA (200)	2.08±0.05	2.76±0.06$^{\#\#}$	2.73±0.04$^{\#\#}$	2.88±0.04$^{\#\#}$	2.57±0.03**$^{\#\#}$	2.46±0.01*$^{\#\#}$
LA (400)	2.19±0.07	2.86±0.02$^{\#\#}$	2.87±0.02$^{\#\#}$	2.86±0.06$^{\#\#\#}$	2.65±0.04**	2.34±0.05**
LA (800)	2.21±0.09	2.86±0.03$^{\#}$	2.82±0.04$^{\#}$	2.78±0.03$^{\#\#}$	2.46±0.06**	2.33±0.04**
Ket (10)	2.21±0.05	2.55±0.06$^{\#\#}$	2.69±0.09$^{\#\#}$	2.32±0.04$^{\#\#}$	2.3±0.02**	2.19±0.01**

Data was analyzed using repeated measures ANOVA followed by Bonferoni Post Hoc test, *= $p<0.05$, **= $p<0.0001$, significant statistical decrease in mean paw edema size (blue) as compared to the peak of edema (red), # = $p<0.05$, ## = $p<0.01$, #### = $p<0.000$ significant statistical increase in mean paw diameter as compared to time zero; n= 6. Values are Mean ± SEM. LA= methanol extract of *Laggera aurita*, Ket= ketoprofen 10 mg/kg, hrs= hours

4.6.2 Effect of saponin fraction of *Laggera aurita* on formalin induced inflammation in rats

The injection of 0.6% v/v formalin in the paws of rats produced local edema which was significant as compared to time zero in all the groups ($p<0.001$). The red colour denotes peak of edema and blue colour denotes significant statistical decrease in mean paw edema size. The peak of inflammation was reached in the fourth hour in normal saline group. Mean while for ketoprofen 10 mg/kg and extract doses 200, 400 and 800 mg/kg; peak of edema was reached at the third hour. The extract significantly ($p<0.001$) decreased formalin-induced paw edema size in dose dependent manner (200, 400 and 800 mg/kg body weight) at the fourth and fifth hour comparable to the peak of edema. The extract at 800 mg/kg showed significant ($p<0.01$) decrease in mean paw edema size at the fifth hour to ketoprofen 10 mg/kg (Table 4.9).

Table 4.9: Effect of saponin fraction of *Laggera aurita* on formalin induced inflammation in rats

Treatment (mg/kg)	Mean paw diameter (millimetre) 0 hrs	1 hrs	2 hrs	3 hrs	4 hrs	5 hrs
Control	1.94±0.05	3.82±0.27$^{\#}$	3.88±0.21$^{\#}$	4.12±.13$^{\#\#}$	4.35±.18$^{\#\#}$	3.17±0.16$^{\#}$
SAP (200)	1.95±0.02	3.31±0.03$^{\#\#\#\#}$	3.54±0.09$^{\#\#}$	3.74±0.09$^{\#\#\#}$	3.17±0.92**$^{\#\#\#}$	2.71±0.29**$^{\#\#}$
SAP (400)	1.86±0.69	2.98±0.15$^{\#\#\#}$	3.23±0.11$^{\#\#}$	3.29±0.19$^{\#}$	2.78±0.07$^{\#\#}$	2.34±0.03$^{\#}$
SAP (800)	1.92±0.04	3.19±0.10$^{\#\#}$	3.29±0.05$^{\#\#}$	3.61±0.09$^{\#\#\#}$	2.67±.09**$^{\#\#}$	2.49±0.73**$^{\#}$
Ket (10)	1.96±0.03	2.89±0.10$^{\#}$	3.22±0.02$^{\#\#\#\#}$	3.49±0.09$^{\#\#}$	2.85±.08**$^{\#\#}$	2.49±0.12

Data was analysed using repeated measures ANOVA followed by Bonferroni post hoc test, * = $p<0.05$, ** = $p<0.01$, significant statistical decrease in mean paw edema size (blue) as compared to the peak of edema (red), #= $p<0.05$, ## = $p<0.01$, ### = $p<0.001$, #### = $p<0.000$ significant statistical increase in mean paw diameter as compared to time zero; n = 6. Values are Mean ± SEM. SAP= saponin fraction, Ket= ketoprofen 10 mg/kg

CHAPTER FIVE

5.0 DISCUSSION

The biological or pharmacological actions of plant extracts are known to be due to presence of specific phytochemical constituents. Analgesic and anti-inflammatory effects have been observed and reported with flavonoids (Amin *et al*., 2012) and tannins (Ahmadiani *et al*., 2000). There are also reports on alkaloidal analgesic effects (Reanmongkol *et al.,* 2005), and with saponins (Choi *et al.,* 2005, Arrau *et al*., 2010). 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-inflammation (Rajnarayan *et al.,* 2001). Thus, the antinociceptive and anti-inflammatory effect exhibited by the plant *Laggera aurita* may be due to the presence of saponins, flavonoids, alkaloids, carbohydrates, tannins or combination of all found present in the plant.

Flavonoids have also been reported to inhibit inflammatory process by inhibiting enzymes involved in prostaglandin synthesis (Duke, 1992; Kamil, 1993; Kim *et al*., 2000). Thus, the significant analgesic and anti-inflammatory effect of the extract may be due to the presence of flavonoids. So also, the alkaloids and saponins present might have contributed to the analgesic activity of the extract. However, the better analgesic effect mediated by the saponin and flavonoid fractions may suggest the predominant contribution of saponin and flavonoid to the antinociceptive effect of the plant.

The oral LD_{50} for both methanol extract and its fractions were above 5000 mg/kg, showing the relative safety of both extract and fractions. 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 a substance. LD_{50} may not accurately reflect the full spectrum of toxicity or hazard associated with drug or chemical (Cassarette *et al*., 1996).

The Organization for Economic Co-operation and Development (Walum, 1998) classification of acute systemic toxicity based on oral LD_{50} as $>500 \leq 2000$ mg/kg as non toxic or harmful. Based on this classification, the oral LD_{50} greater than 5000 mg/kg established in both mice and rats indicated relative oral safety. Other toxicity scales (Hodge and Sterner, 1943) reported that compound with an oral LD_{50} of between 500 – 2000 mg/kg should be considered practically non toxic.

Acetic acid induced writhing test is usually employed to screen for peripheral analgesic activity (Bentley *et al*., 1981, Gene *et al.,* 1998). Intraperitoneal administration of acetic acid to mice leads to the release of prostaglandins like PGE_2 and $PGF_{2\alpha}$ (Tanko *et al*., 2008) which have been implicated as mediators of pains and inflammations (Santosh *et al*., 2008). Aspirin and it derivatives which exhibited analgesic activity similar to the non steroidal anti-inflammatory agents were detected using this method. However, this model may not be able to indicate mechanism of analgesic activity of the extract because of the presence of other agents such as antihistamines (Naik *et al.,* 2000) and myorelaxant (Koyama *et al.,* 1997) which are able to reduce pain induced by acetic acid. Thus, activity shown by methanol extract and its fractions suggest that analgesic effects may be due to their action on visceral receptors sensitive to acetic acid, or inhibition of production of algogenic substances, or the inhibition at the central level of painful messages transmission or combination of all the three mentioned above. The

analgesic activity shown by the methanol extract of *Laggera aurita* and its fractions may be due to inhibition of peripheral pain mediators.

The thermally induced pain model is utilized as a standard method for the evaluation of centrally mediated analgesia. Narcotic agents like morphine, pentazocine and codeine mediate their analgesic effect through this mechanism (Leonard *et al*., 2006). The analgesic activity observed in the methanol extract of *Laggera aurita* and its fractions as may be due to it activity via central mechanism similar to the narcotic analgesic agents (Vogel and Vogel, 1997). This centrally mediated effect which is through interaction with opioid recepotrs (Leonard *et al*., 2006) was blocked by prior administration of naloxone. Naloxone is a specific antagonist of opioid receptors (Stein, 1995). The results suggest that methanol extract and its fractions have central analgesic effect, as evidenced by decreased in mice response. Central opioid mediated analgesia is confirmed by the blocking effect of naloxone (Almeida *et al*., 2011).

The formalin induced inflammatory response is usually bi-phasic in nature (Tanko *et al*., 2008). The first phase is mainly due to the release of histamine, serotonin and kinins (1-3 hours) while the second phase is mainly attributed to the release of prostaglandins (Vinegar *et* al., 1969; Chan *et al*., 1995). Anti-inflammatory activity was elicited by the methanol extract and saponin fractions of the plant *Laggera aurita*. This may be due to its ability to inhibit the release of the mediators of inflammation. The methanol extract and saponin fraction of the plant *Laggera aurita* inhibited both phases of inflammation.

Different types of saponins isolated from plants like *Phytolocca americana, Madhuca longifolia* and *Carissa edulis* have reportedly exhibited significant analgesic and anti-

inflammatory activity (Singh *et al*., 1992, Hassan *et al*., 2010). Similarly, other types of saponins extracted from *Hedera helix* and *Hedera colchica* exerted anti-inflammatory activity by blocking bradykinin (Sparg *et al*., 2004) and other anti-inflammatory mediators such as the prostaglandins (Gepdiremen *et al*., 2005). Thus, the saponin fraction might have acted via blockade of both bradykinin and prostaglandins to elicit their effect.

So also, several flavonoids isolated from medicinal plants have been shown to possess significant anti-inflammatory activity (Kim *et al*., 2000, Amin *et al*., 2012). For example, an isolated flavonoid fraction from *Celosia argentea* Linn showed significant dose dependent anti-inflammatory activity (Santosh *et al*., 2008). This was supported by other workers who found that flavonoids inhibit phosphodiesterases which are involved in cell activation. The effect is dependent on biosynthesis of protein cytokines that mediate adhesion of circulating leucocytes to the sites of injuries (Duke, 1992, Abramson and Melton, 2000). Thus the anti-inflammatory activity of *Laggera aurita* may also be due to it ability to inhibit phosphodiesterases involved in cell activation and mediate adhesion of circulating leucocytes at inflammatory sites.

Some analgesic drugs act by inhibiting prostanoids biosynthesis. They act as analgesic and anti-inflammatory agents by inhibiting COX-2 dependent prostanoids in the cells at inflammatory site (Mishra *et al*., 2011) and in the spinal cord (Ramer *et al*., 1998). They also inhibit the action of phospholipase A_2, which releases arachidonic acid from the cell membrane (Burke *et al*., 2006).These classes of drugs are usually associated with side effects like stomach problems such as bleeding, ulcer, stomach upset (Traversa *et al*., 1995), kidney problems, heart problems (Gislason *et al*., 2009), high blood pressure

and fluid retention (Knight, 2013). Thus, the histological changes observed in the organs from animals exposed to 2000 and 5000 mg/kg may be resultant from inhibition of prostanoid biosynthesis.

Many therapeutic agents cause liver damage, manifested clinically as hepatitis or (in less severe cases) only as abnormal levels of some hepatic enzymes (e.g. increase plasma aspartate transaminase activity). For example, paracetamol, isoniazid, iproniazid and halothane cause hepatotoxicity by mechanism of self damage. Drug usually induced self damage or death by reactive metabolite of the drug, involving non-covalent and or covalent interaction with target molecules. Cell death is often via triggering of apoptosis rather than by acute necrosis (Allison and Samaj, 1995). Non covalent interaction includes lipid peroxidation (where peroxiradicals produces hydroperoxides). It also involves generation of cytotoxic oxygen radicals and reaction causing depletion of glutathione (which results in oxidative stress). So also, modification of sulfhydrate on key enzymes and structural proteins are other form of non covalent interaction (Sotannemi *et al*., 1997). Thus, the histological changes observed in the liver of animals treated at 2000 and 5000 mg/kg may be via apoptosis or self damage involving covalent or non covalent interactions or both.

Covalent interaction involves adduct formation between the metabolites and cellular macromolecules (Boobis *et al*., 1989). A typical example is seen in Paracetamol, where it metabolite N-acetyl-p-benzoquinone imine (NAPQI) react with cellular macromolecules. Mild drug induced abnormalities of liver functions are not uncommon but the mechanisms of liver injury are often uncertain. Hepatotoxicity caused by toxic doses of Paracetamol is clinically important as it was fourth most common cause of

death following self poisoning in United Kingdom (Boobis *et al*., 1989). Thus, the hepatotoxic effect seen with methanol extract of the plant must be taken into cognisance. Liver damaged can also be produced by immunological mechanisms (Rang *et al*., 2003) or by hypersensitivity reactions. A typical example is halothane induced hepatic necrosis (Kenna *et al*., 1993). Thus, the toxic effect seen in the liver with methanol extract may be through immunological mechanisms or hypersensitive reactions.

Drug induced nephrotoxicity is a common problem. Indeed, Non-steroidal anti-inflammatory drugs (NSAIDs) and angiotensin converting enzyme inhibitors (ACE-I) are causes of acute renal failure as a result of their principal pharmacological actions (Gislason *et al*., 2009, Mishra *et al*., 2011).

Analgesic nephropathy is the third type of renal damage in which NSAID caused interstitial nephritis it is possible that such analgesic related nephritis is usually related to inhibition of renal prostaglandins synthesis, but its pathogenesis is not understood. Thus, the effect of methanol extract observed on the kidney suggest that it act in similar way to NSAIDs to produce renal damage by inhibiting renal prostaglandin synthesis or inhibiting glumerular filtration (Casserete *et al*., 1996).

The histopathological changes observed in both first and second phase of acute toxicity studies in mice showed effect on the organs especially in the second phase. Thus, the moderate erosion of stomach epithelium and kidney problems observed in the toxicological studies suggests that the methanol extract may be acting via

cycloxygenase 1 and 2 blockades. Agents acting via this mechanism are usually associated with stomach ulcerations and renal damages (De Broe and Elseviers, 1998).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The methanol extract of *Laggera aurita* and its fractions have been shown to possess significant analgesic and anti-inflammatory activities which may be responsible for it use in painful and inflammatory conditions.

The results of histopathological studies showed effects on kidney and liver at dose of 2000 mg/kg and on kidney, liver, spleen, lungs and stomach at dose of 5000 mg/kg. Hence this suggests that the extract of the plant *Laggera aurita* has toxic effect on the organs mentioned at high doses.

6.2 Recommendations

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

1. Detailed toxicological screening should be conducted.
2. Studies should be carried out to isolate, characterize and elucidate the structure of the bioactive constituents responsible for the observed pharmacological effects.
3. Pharmacodynamics and pharmacokinetics studies should be undertaken to establish the exact mechanism of action of both the extract and fractions.

REFERENCES

Abbas, A.B. and Lichtman, A.H. (2009). "Innate Immunity". Basic Immunology Functions and disorders of the Immune System, 3rd Ed. Philadelphia, In Saunders Elseviers United States, Pp 168-182.

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

Abrahamson, N. and 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. and 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.

Adeyemi, O.O., Okpo, S.O. and 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. and Serami, S. (2000). Antinociceptive and Anti-inflammatory effects of *Elaeagnus angustifolia* fruits extract. *Journal of Ethanopharmacology*, 72, 287-292.

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

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

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

Arrau, S., Delporte, C., Cartegena, C., Rodriguez-Diaz, M., Gongalez, P., Silva, X., Cassels, B.K. and 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. and John, B. (1978). *A colour Atlas of Histopathological Staining Techniques*. Wolf MED.Pub. Ltd. London, pp 14-20.

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.

Bentley, G.A., Newton, S.H. and 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., Convington, E.C., Dahl, J.L., Katz, J.A., Miaskowski, C. and Mclean M.J. (2001). Pain: Current Understanding of Assessment, Management and Treatments. National Pharmaceutical Council, Inc 1894 Preston White Drive Reston, VA 20191 www.npcnow.org.

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

Bhujbal, S., Patil, K. and 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.

Biswas, T., Gupta, M., Achari, B. and Pal, B.C (2005). Hopane-type Saponins from *Glinus lotoides* Linn. *Phytochemistry,* 66, 621-626.

Boobis, A. R., Fawthrop, D. J. and Daven, D. S. (1989). Mechanism of cell death. *Trends in Pharmaceutical Sciences,* 10, 275 – 80.

Burke, A., Smyth, E. and 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. (1985). The Useful Plants of West Tropical Africa, 2nd Ed. Royal Botanical Gardens, Kew England, 1, 452-453.

Cassarette, I., Klaassen, C.D., Amdur, M.O. and 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.

Chan, Y.F., Tsai, H.Y. and Wu, T.S. (1995). Anti-inflammatory and Analgesic Activity of Extracts from the Root of *Angelica pubescens. Planta Medica,* 61, 2-8.

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

Choi, J., Jung, H., Lee, K. and 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.

Cotran, K. Collins (1998). *Robbins Pathologic Basis of Disease.* Philadelphia: W.B Saunders Company pp 152-170.

Cousins, M.J., Brennan, F. and 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. *Perspectives on psychological Sciences*, **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.

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

Dahanukar, R.A., Kurkarni, R.A. and 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. and 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. and Summer, J.G. (2011). Pain as a global public health priority, BMC *Public Health*, 11:770 doi: 10. 1186/1471-2458-11-70.

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. and Sheardown, S. A. (2000). Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature,* 405, 183 – 187.

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

Derardt, R., Jougney, S., Delevalcee, F. and Falhourt, M. (2000). Release of Prostaglandins E and F in an Algogenic Reactions and its Inhibition. *European Journal of Pharmacology*, 51, 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. and Travers, A. (2009). South African Acute Pain Guidelines: Measurement and Assessment of Acute Pain. *South African Journal of Anaesthesia and Analgesia*, 5(6), 16-18.

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

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

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

Eddy, N.B and Leimbach, D. (1953). Synthetic Analgesics II Dithienylbutenyl and Dithienylbutylamines. *Journal of Pharmacology and Experimental Therapeutics,* 107, 385-393.

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. and Byarugba, F. (2012). Phytochemical Screening, Acute toxicity, Anti-inflammatory and Antipyretic Studies of Aqueous Extract of the root of *Fluegga virosa* (Roxb Ex Wild) in Rats. *International Journal of Pharmaceutical and Biomedical Sciences*, 3(3), 128-135.

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

Field, H.L., Rowbotham, M. and Baron, R. (1998). Post herpetic Neuralgia: irritable nociceptors and de-afferentation. *Neurobiology of Diseases*, 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, 3rd ed New York, Mosby, Inc Pp 486.

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. and 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. and Hahn, H.J. (2003). The Uses of Medicinal Plants in self-care in Rural Central Ethopia. *Journal of Ethnopharmacology*, 87, 155-161.

Gene, R.M., Segura, L., Adzet, T., Marin, E. and Ingelsias, J. (1998). *Heterotheca inuloides*: Inflammatory and Analgesic Effects. *Journal of Ethnopharmacology,* 60, 157-162.

Giordano, J. (2005). The Neurobiology of Nociceptive and Antinociceptive Systems. *Pain Physician*, 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. and 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. and Kshirsagar, N.A. (2002). The Uses and Safety of non-allopathic Indian Medicines. *Drug safety*, 25, 1005-1019.

Greenhalgh, D.G. (1998). "The role of apoptosis in wound healing". *International Journal Biochemical Cell Biology,* 30(9): 1019–1030.

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

Habal, F.M. and 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.

Hassan, H.S., Sule, M.I., Musa, M.A., Emmanuel, A.A., Ibrahim, H., Hassan, A.S. and Yaro, A.H. (2010). Analgesic and Anti-inflammatory activity of the Saponins extract of *Carrissa edulis* root in rodents. *International Journal of Biology and Chemical Sciences,* 4(4), 1310-1317.

Hassel, K.A. (1982). The Chemistry of Pesticides, their Metabolism, Mode of Action and Use in Crop Protection.Pp 8-14.

Hasselstrom, J., Liu-Palmgren, J. and Rasjo-Wraak, G. (2002). Prevalence of Pain in General Practice. *European Journal of Pain*, 6 (5), 375-85.

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

Hucho, T. and 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. and Plesh, O. (2011). Chronic Pain in the Community: A survey in a township in Mthatha, Eastern Cape, South Africa. *Journal of Anaesthesia and Analgesia*, 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. and 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. and Rafeeq, A.K. (2004). Antinociceptive Activity of Methanolic Extracts of St. John's Wort (*Hypericum Perforatum*) preparation. *Pakistan Journal of Pharmaceutical Sciences*, 17(2), 13-19.

Jessel, T., Kandel, M., Eric, R., Schwartz, R and James, H. (1991). *Principles of neural science.* Norwalk, C.T: Appleton & Lange Pp 472-79.

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

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

Kenna, J. G., Knight T. L. and Pelt F.N.A.M. (1993). Immunity of halothane metabolite-modified proteins in halothane hepatitis. *A New York Acid Science*, 685 – 661.

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

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. and 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. and De-beer, E.J. (1959). Acetic acid for Analgesic Screening. *Federation Proceedings*, 18, 412.

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

Lambrou, G.I., Vlahopoulus S., Papathanasiou, C., Papanikalaou, M., Karpusas, M., Zoumakis, E. and 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.

Leonard, J.P., Daniel, S.B. and Yonoli Xi, M.S. (2006). Increasing Death from Opioid Analgesics in the United States. *Journal of Pharmacoepidemiology and Drug safety,* 19(3), 54-67.

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

Lompo, M., Guissou, I.P., Dubois, J., Dehaye, J.P., Oudraogo, S., Traore, A. and 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. *Archives of Toxicology,* 54, 275-287.

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

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

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

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

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

Middleton, E. (1998). Effect of flavonoids on immune and inflammatory cell function. *Advances in Experimental and Medical Biology*, 439, 175-186.

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

Miura, M., Tamame, T., Naganuma, T., Chinen, S., Matsuoka, M. and 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. and

Velly, A. (2007). Pharmacological Management of Chronic Neuropathic – Consensus statement and Guidelines from Canadian Pain Society. *Pain Research Management*, 12(1), 13-21.

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

Musa, A.M., Yaro A.H., Usman, H., Magaji M.G., Maiha, B.B. and 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.

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

Naik, D.G., Mujumdar, A.M., Wagole, R.J., Kurkarni, D.K. and 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. and 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.

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

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

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

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 and 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.

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

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

Rajnarayan, K., Sripal R.M. and Chulvadi, M.R. (2001). Bioflavonoid Classifications, Pharmacological, Biochemical effect and Therapeutic potentials. *Indian Journal of Pharmacology*, 33, 2-16.

Ramer, M.S., Murphy, P.G., Richardson, P.M. and 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. and Dray, A. (1991). Chemical activation for Nociceptive Peripheral Neurons. *British Medical Bulletin*, 47, 534 – 548.

Rang, H. P., Dale, M.M. and Ritter. J.M. and Moore, P.K. (2003). Drugs used in affective Disorders. In*: Pharmacology*, 5th edition Churchill Livingstone, Pp 535-549.

Rang, H.P. and 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.

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

Roberts, J.L. and 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 and Gilman's. The Pharmacological basis of Therapeutics*, 10th Ed. Mc Graw Hill Co Inc. New York, Pp 687-731.

Sani, M., Anuka, J.A., Yaro, A.H. and 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. and 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.

Satoskar, R.S., Bhandarka, S.D. and 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.

Seibst, S.M. and Fein, J.A. (2006). Sedation and Analgesia, In: Henretig, F.M., Fleisher, G.R., Ludwig, S., *Textbook of pediatric emergency medicine* 2nd edition Pp 120-125.

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. and 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.

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

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

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

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

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

Sutharson, L., Lila, K.N., Presanna, K.K., Shila, E.B. and 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.

Tal, S., Guller, V. and 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. and Mohammed, A. (2008) Antinociceptive and anti-inflammatory activities of aqueous leaves extract of *Ocimum gratissimum* (Labiatea) in rodents. *African Journal of Traditional, Complimentary and Alternative Medicines,* 5(2), 141-146.

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

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

Thienhaus, O. and 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.

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

Tsang, A., Von Korff, M. and Lee, S. (2008). Common Chronic Pain Conditions in Developed and Devoloping Countries: Gender and Age Differences and Comorbidity with Depression-Anxiety disorders. *Journal of Pain*, 9(10), 883-891.

Udeogaranya, P.O., Okonta, J.M. and 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. and 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. and Garba, M.M. (2008). Analgesic and Anti-inflammatory Screening of *Newbouldia laevis* flower in Rodents. *Trends in Medical Research,* 3(1), 10-15.

Verra, F., Mangano, V.D. and Modiano, D. (2009). Genetics of Succeptibility to Plasmodium falciparum: From Classical malaria resistance genes towards genome wide association studies. *Parasite Immunology*, 31(5), 234-253.

Vinegar, R., Schreiber, W. and Hugo, R. (1969). Biphasic Development of Carrageenan Edema in Rats. *Journal of Pharmacology and Experimental Therapeutics,* 166, 96-103.

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

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 Q*, 15(2), 1-14.

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

Winter, C.A., Riselay, E.A. and Nuss, G.W. (1963). Carrageenan induced Edema in the hind paw of the rats as an assay for anti-inflammatory drugs. *Proceedings of the Society for Experiemental Biology and Medicine,* 111, 544-547.

Younos, C., Rolland, A., Fleurentin, J. and Lanhers, M.C. (1990). Analgesic and Behavioural effect of *Morinda citrifolia. Planta Medica,* 56, 430-434.

APPENDICES

Appendix 1

Calculation of Percentage yield of the methanol crude extract of *Laggera aurita*

One thousand five hundred grams of crude drug was extracted using 7.5 litres of methanol

First extraction:

Weight of crude drug extracted = 1500 g

Weight of crude extract obtained = 228.03 g

$$\text{Percentage yield} = \frac{\text{Weight of crude extract}}{\text{Weight of the crude drug}} \times 100$$

$$= \frac{228.03}{1500} \times 100$$

$$= 15.202\%$$

Appendix 2

Calculation of Percentage yield of the saponin fraction of *Laggera aurita*

Weight of crude extract = 150 g

Weight of saponin rich fraction = 2.26 g

$$\text{Percentage yield} = \frac{\text{Weight of saponin fraction}}{\text{Weight of the crude drug}} \times 100$$

$= 2.26/150 \times 100$

$=1.51\%$

Appendix 3

Calculation of Percentage yield of the flavonoid fraction of *Laggera aurita*

Weight of crude extract = 150 g

Weight of flavonoid fraction = 0.73 g

$$\text{Percentage yield} = \frac{\text{Weight of Flavonoid fraction}}{\text{Weight of the crude drug}} \times 100$$

$$= 0.73/150 \times 100$$

$$= 0.487\%$$