ANALGESIC AND ANTI-INFLAMMATORY STUDIES ON METHANOL ROOT EXTRACT OF *ANDROPOGON GAYANUS* KUNTH (POACEAE) IN MICE AND RATS

BY

Suleiman Zandam UMAR B.

Pharm (ABU 2010)

MSc/ Pharm-Sci /43348/2012-2013

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY
IN PARTIAL FULFILLMENT 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

SEPTEMBER, 2016

DECLARATION

I declare that the work in this dissertation entitled **Analgesic and anti-inflammatory studies on methanol root extract of *Andropogon gayanus* Kunth (Poaceae) in mice and rats** was performed by me in the Department of Pharmacology and Therapeutics, under the joint supervision of Prof. N.M. Danjuma and Prof. A.U. Zezi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation has been previously presented for another Degree or Diploma at this or any other University.

Suleiman Zandam UMAR		
Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled ANALGESIC AND ANTI-INFLAMMATORY STUDIES ON METHANOL ROOT EXTRACT OF *ANDROPOGON GAYANUS* KUNTH (POACEAE) IN MICE AND RATS by Suleiman Zandam UMAR meets the regulations governing the award of the degree of Master of Science in Pharmacology of the Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof. N.M. Danjuma		
Chairman, Supervisory Committee	Signature	Date
Prof. A.U. Zezi		
Member, Supervisory Committee	Signature	Date
Prof. N.M. Danjuma		
Head, Department of Pharmacology and Therapeutics	Signature	Date
Prof. K. Bala		
Dean, School of Postgraduate Studies	Signature	Date

DEDICATION

This work is dedicated to Almighty Allah (S.W.A.) and to the memory of my late friend Pharm. Rafiq Lawal Dauda.

ACKNOWLEDGEMENT

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

My gratitude goes to my supervisors; Prof. N.M. Danjuma and Prof. A.U. Zezi for their support, encouragement, sacrifice, understanding and above all, patience. May Allah reward them abundantly.

Special thanks to the Head, Department of Pharmacology and Therapeutics, the entire academic and technical staff, especially Malam Aliyu Ahmed Yunusa, Dr. Sani Malami, Dr. M.G. Magaji, Dr. (Mrs) L.O. Ayanwuyi and Mrs. Aishatu Shehu towards the achievement of this work.

My sincere gratitude goes to my parents Alh. Umaru Zandam and Haj. Sa'adatu Sule Gaya for their prayers, encouragement and advice. I also wish to thank all my siblings, cousins and other relatives for their support and prayers.

In addition, I also wish to acknowledge the support and assistance of my friends Malam Odoma Saidi, Abdullahi Balarabe Nazifi, Muhammad Laminu, Mustapha Halliru, Abdulhakim Abubakar, Amina Yusuf, Ahmad Danbala, Nuruddeen Bakori, Umar I.I, Michael Oraebosi, Abdulrashid Usman, Shehu Sani, Dr. Imran and Mustapha Muhammad.

ABSTRACT

The plant *Andropogon gayanus* is employed in herbal medicine for the treatment of various disease conditions. The dried root of the plant is soaked in milk and taken for the treatment of postpartum pain and bronchitis in North-west Nigeria. In this study the methanol root extract of the plant was screened for phytochemical constituents followed by acute toxicity studies in mice and rats. Analgesic activity was evaluated using acetic acid-induced writhing test in mice, hot plate test in mice and formalin-induced pain test in rats whereas anti-inflammatory activity was evaluated using Carrageenan-induced paw oedema model in rats. Acetic acid induced writhing test in mice is employed in the screening of the involvement of opioid receptors, ATP dependent potassium ion (K^+_{ATP}) channels and α_2-adrenergic receptors in the mechanism of analgesia. Preliminary phytochemical screening revealed the presence of glycosides, saponins, flavonoids, alkaloids and tannins amongst others. Toxicity studies in both rats and mice revealed oral LD_{50} value of > 5000 mg/kg and intraperitoneal LD_{50} value of 1265 mg/kg. The extract at doses of 250, 500 and 1000 mg/kg (*p.o*) produced a significant ($p < 0.05$) inhibition of writhing induced by acetic acid. In the hot plate test the extract at all doses increased the reaction time at different intervals; the difference when compared to the negative control group and to the value at time zero was statistically significant ($p< 0.05$). In formalin-induced pain test, the extract exhibited significant ($p< 0.05$) analgesic and anti-inflammatory activities in first and second phases of the formalin test respectively. The extract significantly ($p< 0.05$) reduced oedema induced by carrageenan in rats. Naloxone, a non specific opioid antagonist significantly ($p< 0.05$ and $p< 0.01$) blocked the analgesic effect observed with administration of acetic acid in the methanol extract and morphine treated mice respectively. However, glibenclamide (K^+_{ATP} channel

blocker) did not inhibit the analgesic effect of the extract, neither did the α_2 receptor blocker, yohimbine. This study suggests that the root of *Andropogon gayanus* contains bioactive constituents that possess analgesic and anti-inflammatory effects, the former supposedly being mediated through the action of opioid receptors.

TABLE OF CONTENTS

CONTENT **PAGE**

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

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

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

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

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

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

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

List of Tables ---------- xiii

List of Plates---------- xiv

List of Figures---------- xv

List of Appendices ---------- xvi

Abbreviations, Definitions and Acronyms ---------- xvii

CHAPTER ONE---------- 1

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

1.1 Statement of Research Problem----------5

1.2 Justification for the Study---------- 6

1.3 Theoretical Framework---------- 8

1.3.1 Phytochemical screening---------- 8

1.3.2 Models for the study---------- 8

1.4 Aim and Objectives of the study---------- 10

1.4.1 Aim of the Study---------- 10

1.4.2 Specific Objectives---------- 10

1.5 Statement of Research Hypothesis---------- 11

CHAPTER TWO ----------12
2.0 LITERATURE REVIEW----------12
2.1 Pain---------- 12
2.1.1 Classification of pain---------- 13
2.1.2 Epidemiology of pain---------- 18
2.1.3 Pain pathways---------- 19
2.1.4 Pain threshold---------- 20
2.1.5 Management of pain----------21
2.2 Inflammation---------- 26
2.2.1 Classification of inflammation---------- 28
2.2.2 Chemical mediators of inflammation---------- 30
2.2.3 Function of inflammation----------31
2.2.4 Resolution of inflammation---------- 32
2.2.5 Inflammatory disorders---------- 33
2.2.6 Management of inflammation---------- 33
2.3 Traditional Medicine---------- 35
2.3.1 Plants used in the management of pain and inflammation---------- 36
2.4 *Adropogon gayanus* Kunth---------- 38
2.4.1 Taxonomy/nomenclature of the plant---------- 38
2.4.2 Ehnomedicinal uses of *Adropogon gayanus*---------- 40
CHAPTER THREE---------- 41
3.0 MATERIALS AND METHODS----------41
3.1 Plant Material---------- 41
3.1.1 Plant collection and identification----------41
3.1.1 Preparation of plant extract---------- 41
3.2 Experimental Animals Husbandary---------- 42

3.3 Drugs and Chemicals---------- 42
3.4 Materials---------- 42
3.5 Preliminary Phytochemical Screening---------- 43
3.5.1 Test for alkaloids----------43
3.5.2 Test for tannins (Ferric chloride test) ----------43
3.5.3 Test for free anthraquinones (Bontrager's test) ---------- 44
3.5.4 Test for saponins (Frothing test) ---------- 44
3.5.5 Test for steroids and triterpenes (Liebermann-Burchard's test) ---------- 44
3.5.6 Test for flavonoids (Shinoda test) ---------- 44
3.5.7 Test for cardiac glycosides (Keller Kiliani's test) ---------- 44
3.5.8 Test for carbohydrates (Molisch's test) ---------- 45
3.5.9 Thin layer chromatographic studies of extract ---------- 45
3.6 Acute Toxicity Studies (Determination of LD_{50}) ---------- 46
3.7 Analgesic studies---------- 46
3.7.1 Acetic acid induced writhing in mice---------- 46
3.7.2 Hot plate method in mice---------- 47
3.7.3 Formalin induced pain test in rats---------- 48
3.8 Anti-inflammatory Studies---------- 48
3.8.1 Carrageenan induced rat paw oedema model---------- 48
3.9 Mechanistic Studies ----------49
3.9.1 Determination of the effects of naloxone co-administration with methanol root extract of *A. gayanus* on acetic acid-induced writhing in mice ---------49
3.9.2 Determination of the effects of glibenclamide co-administration with methanol root extract of *A. gayanus* on acetic acid-induced writhing in mice --------- 50
3.9.3 Determination of the effects of yohimbine co-administration with methanol root extract of *A. gayanus* on acetic acid-induced writhing in mice ---------50
3.10 Statistical Analysis---------- 51

CHAPTER FOUR---------- 52

4.0 RESULTS----------52

4.1. The Plant Extract and Phytochemical constituents of *A. gayanus* ----------52

4.1.1 Thin Layer Chromatography---------- 54

4.2 Acute toxicity studies (LD_{50} Determination) ---------- 57

4.3 Analgesic studies---------- 58

4.3.1 Acetic acid induced writhing test in mice---------- 58

4.3.2 Hot plate test in mice---------- 60

4.3.3 Formalin induced pain test in rats---------- 62

4. 4 Anti-inflammatory Studies---------- 64

4.4.1 Carrageenan-induced Paw Oedema Test in Rats---------- 64

4.5 Mechanistic Studies---------- 66

4.5.1 Effects of naloxone co-administration with methanol root extract of *A. gayanus* on acetic acid-induced writhing in mice---------- 66

4.5.2. Effects of glibenclamide co-administration with methanol root extract of *A. gayanus* on acetic acid-induced writhing in mice---------- 68

4.5.3 Effects of yohimbin co-administration with methanol root extract of *A. gayanus* on acetic acid-induced writhing in mice---------- 70

CHAPTER FIVE---------- 72

5.0 DISCUSSION---------- 72

CHAPTER SIX---------- 78

6.0 CONCLUSION AND RECOMMENDATIONS---------- 78

6.1 Conclusion---------- 78

6.2 Recommendations---------- 78

REFERENCES----------79

APPENDICES---------- 95

LIST OF TABLES

TABLE **PAGE**

Table 4.1 Phytochemical constituents of the methanol root extract of *Andropogon gayanus*---------- 53

Table 4.2 R_f values for the P-Anisaldehyde/sulphuric acid (general) spray chromatogram---------- 56

Table 4.3 Effect of methanol root extract of *A. gayanus* on Acetic Acid-Induced writhing in mice---------- 59

Table 4.4 Effect of methanol root extract of *A. gayanus* on Formalin-Induced Pain in Rats---------- 63

LIST OF PLATES

PLATE		PAGE
Plate I	*Andropogon gayanus* in its natural habitat----------	39
Plate II	Chromatograms of methanol root extract of *Andropogon gayanus*--	55

LIST OF FIGURES

FIGURE **PAGE**

Figure 4.1 Effect of methanol root extract of *Andropogon gayanus* on hot plate-Induced pain test in mice---------- 61

Figure 4.2 Effect of methanol root extract of *Andropogon gayanus* on Carrageenan-Induced paw oedema in rats---------- 65

Figure 4.3 Effect of naloxone co-administration with methanol root extract methanol of *Andropogon gayanus* on acetic acid-induced writhing in mice---------- 67

Figure 4.4 Effect of glibenclamide co-administration with methanol root extract of *Andropogon gayanus* on acetic acid-induced writhing in mice---------- 69

Figure 4.5 Effect of yohimbine co-administration with methanol root extract of *Andropogon gayanus* on acetic acid-induced writhing in mice--- 71

LIST OF APPENDICES

APPENDIX **PAGE**

Appendix A Effect of methanol root extract of *Andropogon gayanus* on hot plate-induced pain in mice---------- 95

Appendix B Effect of methanol root extract of *Andropogon gayanus* on Carrageenan- induced rat paw oedema in ----------96

Appendix C Effect of naloxone co-administration with methanol root extract of *Andropogon gayanus* on acetic acid induced writhing test in mice ---------- 97

Appendix D Effect of glibenclamide co-administration with methanol root extract of *Andropogon gayanus* on acetic acid-induced writhing in mice ---------- 98

Appendix E Effect of yohimbine co-administration with methanol root extract of *Andropogon gayanus* on acetic acid-induced writhing in mice-- 99

LIST OF ABBREVIATIONS AND ACRONYMS

ACTH	Adrenocorticotrophic Hormone
ANOVA	Analysis of variance
AR	Adrenergic receptor
CNS	Central Nervous System
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase -2
EPA	Eicosapentaenoic Acid
GIT	Gastro-Intestinal Tract
GSH	Glutathione
HCl	Hydrochloric acid
IASP	International Association for Study of Pain
IL-1	Interleukine-1
Kg	Kilogram
L.B	Liebermann Burchard
LD_{50}	Median Lethal Dose
LTs	Leukotrienes
MEAG	Methanol Extract of *Andropogon gayanus*
Mg	Milligram
mg/kg	Milligram per kilogram
mg/dL	Milligram per deciliter
mL	Milliliter
mmol/L	Millimole per litre
NAPBQI	N-acetyl-P-benzoquinone Imine
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs

P.O	*Per oral*
PGs	Prostaglandins
SEM	Standard Error of Mean
TLC	Thin layer chromatography
TNF	Tumor Necrosis Factor
W.H.O.	World Health Organisation
e.g.	Examples
etc.	et cetera
%	Percentage

CHAPTER ONE

1.0 INTRODUCTION

The International Association for the Study of Pain defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (Feinberg *et al*., 2012). In other words, it is a highly unpleasant physical sensation caused by illness or injury. It is also defined as the effect produced in consciousness by the arrival of nerve impulses generated by noxious stimuli in the brain (Mishra *et al.,* 2011). It is sometimes interpreted as a suffering that results from the perception of painful stimuli.

Pain is a universal human experience and the most common reason people seek medical care. Pain tells us something is wrong in the structure or function of our body and that we need to do something about it. Because pain is such a strong motivator for action, it is considered one of the body's most important protective mechanisms. Pain is always personal; no two people experience it in the same way, which makes it very difficult to define and treat (Macintyre *et al.,* 2010). It may affect ability to work, relate with family and friends. Activity levels and even sleep may be affected. All of these may become overwhelming and can lead to increasing pain and distress (Chair *et al.,* 2010).

Pain is a multidimensional sensory experience that is unpleasant and associated with hurting and soreness. It may vary in intensity, quality and duration from mild, moderate, or severe, sharp, burning or dull and transient, intermittent, or persistent. It can also be superficial or deep, localized or diffused (Woolf, 2004). Pain can be acute or chronic. Acute pain is usually of short duration and the cause often identifiable such as disease and trauma, while chronic pain persists after healing is expected to be completed, or is

caused by a chronic disease which causes continuous pain and re-occurs at intervals for months or even years (Rajagopal, 2006).

Pain affects all populations irrespective of age, sex, race, income and location. Pain is not equally distributed across the world and the experience of it can be acute, chronic, intermittent or in combination. In developed countries, pain in form of musculoskeletal disorders accounts for 4.3% of people living with disability and at least up to 85% of low back pain occurs in developed countries (Quinette *et al.,* 2007). In Africa, pain in the form of musculoskeletal disorders is the most frequent cause of disability and accounts for 1% of people living with disability in developing world (Tsang *et al*., 2008).

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

In Nigeria, pain prevalence ranges from 2% at 6-8 years to 28% at 17-18 years (Talabi, 2005). Prevalence of pain increases with increasing 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).

Inflammation can be defined as series of changes that occur in a living tissue when it is injured provided that the injury is not of such a degree that causes destruction. It is characterised by redness, swelling, heat and pain and at times, loss of function (Punchard *et al.,* 2004). Inflammation is a defensive mechanism of the body to remove injurious stimuli and initiate healing process for the tissue, but if it runs unchecked, it can lead to onset of certain diseases as vasomotor rhinnorrhoea, rheumatoid arthritis, and atherosclerosis (Sharma *et al.*, 2010).

The heat sensation in inflammation is due to increased movement of blood through dilated vessels into the cooled extremities, which also results in increased redness due to the additional number of erythrocytes passing through the area. The swelling is as a result of increased passage of fluid from dilated and permeable blood vessels into the surrounding tissues, infiltration of cells into the damaged area, and in prolonged inflammatory responses deposition of connective tissue. Pain in inflammation is due to the direct effects of mediators and the stretching of sensory nerves due to oedema (Punchard *et al.*, 2004). Inflammation removes foreign matter from the body, disposes damaged cells, and initiates wound healing. It is controlled by mast cells that are in close proximity to autonomic nerves. Mast cells are constituents of connective tissues containing large granules that contain heparin, serotonin, bradykinin, and histamine (Kumar *et al.*, 2012).

Regardless of the etiology of painful and inflammatory conditions, the primary goal of therapy is to control pain and reduce inflammation which 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 hamstrings and

lumbodorsal facia and spine (Taylor, 1998). Other non-drug therapy methods includes abdominal strengthening, exercise (sit-ups or curls ups), and following correct biomechanical methods when sitting, lifting or bending and sleeping on flat surfaces.

Traditional medicine (TM) according to the World Health Organization (WHO) "is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses (WHO, 1999). TM as practiced in Nigeria and other African countries is involved in the management of diseases such as HIV/AIDs, malaria, tuberculosis, diabetes mellitus, hypertension, fungal infections, cancerous growths, painful and inflammatory conditions such as hemorrhoids among others.

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 ethno pharmacological, 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).

Andropogon gayanus Kunth is a species of grass native to most of the tropical and sub-tropical savannas of Africa. Also known as gamba grass, it is a tufting perennial bunchgrass. Matured plant can grow 4 meters (13 ft) tall and 70 centimeters (2.3 ft) in diameter. Leaves are 30-60 cm long and up to 3 cm wide, with a distinctive white midrib and covered with soft hairs. The root system spreads up to 1 m from the tussock, close to the soil surface. It reproduces from the seed which are contained in a fluffy V-shaped seed head consisting of up to six groups of branches, each containing 2-18 primary branches. It belongs to the family Poaceae and is widely used in Nigeria and other African countries as a source of nutrition to animals. It is also widely used for its various medicinal values among which is pain and inflammation.

1.1 Statement of Research Problem

Several epidemiological studies from different parts of the world have reported prevalence rates for chronic pain, ranging from 12-80% of the population and it becomes more common as people approach death (Perquin *et al*., 2000). 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. In Nigeria, for individual experiencing pain, the human cost has not been estimated, 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). It has been estimated that globally 20% of all adults suffer from pain and that 10% are newly diagnosed with chronic pain each year, making pain an enormous public health problem world over (Goldberg and McGee, 2011).

The use of analgesics also known as pain killers is the most common method in the treatment of pain and inflammation. These medications may cause serious side effects in some individuals. Orthodox drugs used in the treatment of pain include, non-steroidal anti-inflammatory drugs, opioid and non-opioid analgesics (Feinberg *et al.,* 2012). Non-steroidal anti-inflammatory drugs such as aspirin, ibuprofen, diclofenac and naproxen in the treatment of mild to moderate pain and inflammation are limited by their significant side effects such as gastrointestinal tract irritation, blood disorders, liver damage, renal damage, tinnitus, hypersensitivity reactions etc (Mishra *et al*., 2011). Opioid analgesics, though very effective in chronic pain management, are associated with problems such as addiction and tolerance, and side effects such as constipation, weight gain and loss of libido. Opioids are also associated with dependence, and abrupt withdrawal can lead to withdrawal symptoms such as tiredness, diarrhoea, abdominal cramps and sweating (Stannard *et al*., 2007).

1.2 Justification for the Study

Majority of the world population depends on traditional medicine such as herbs for the treatment of various ailments and most of the orthodox medicine in use today were derived from herbal traditions (Ezeonwumelu *et al.,* 2012). The use of traditional medicine is rapidly growing; most people are working in the field of ethno medicine due to accessibility and affordability. Hence, there is need for at first the preliminary acute toxicity studies of this plant to ascertain the acute toxic profile of the plant (Salawu *et al.,* 2009).

The use of herbal drugs in the treatment of pain and inflammation 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) necessitate the investigation of the antinociceptive effect of so many medicinal plants which have been used in traditional medicinal practices to treat pain, fever and jaundice.

The validation of folkloric claims for 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.
Many plants have been claimed to have analgesic and anti-inflammatory properties but for many of such plants, there is no scientific basis for their use to support such claims. Such investigations can provide information for the identification of lead compounds that may be safer and more effective in the management of pain and inflammation thus may have better application in such conditions. *Andropogon gayanus* is widely used in management of pain and inflammatory conditions but its efficacy has not been established, thus prompting the need to go into this research.

1.3 Theoretical Framework

Different procedures and models are in place for the screening of plants used in traditional medicine to evaluate their analgesic and anti-inflammatory properties in order to establish their efficacy.

1.3.1 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 in the formation of coloured solutions or precipitate (Sofowora, 1993; Evans, 1996).

1.3.2 Models for the Study

1.3.2.1 *Acute toxicity studies*

In this study, an acute toxicity test was carried out to determine the level of safety of the methanol root extract of *Andropogon gayanus* using Lorke's method, (1983). The method can be used in different species, via several routes of administration. Result of the median lethal dose (LD_{50}) which is an index of acute toxicity obtained through the oral route, enabled in the selection of doses to be used in the experiments.

1.3.2.2 *Acetic acid induced writhing test*

Acetic acid induced abdominal constriction in mice is a widely used model for evaluation of peripherally mediated analgesia (Vogel, 2008). Pain is induced in rats or mice by intraperitoneal injection of irritants such as phenyl quinine and acetic acid into the peritoneal cavity, where the animal reacts with characteristic stretching and

abdominal movement (Milind and Monu, 2013). Acetic acid causes writhes by increasing the release of prostaglandins in peritoneal fluids as well as lipoxygenase products, which enhances inflammatory pain by increasing capillary permeability (Khan *et al.,* 2010). This method is very sensitive and detects anti-nociceptive effects of substance at a dose that is not feasible using other methods such as flick tail (Sutharson *et al*., 2007). The acetic acid induced writhing test was thought to be a peripheral pain model which is generally used for screening plants and new agents for analgesic properties (Gene *et al.,* 1998). However, it has also been shown that the acetic acid test is a non-specific nociceptive model (Bighetti *et al*., 1999) and is now thought to involve several pathways for nociception including the central and peripheral.

1.3.2.3 *Hot plate test (thermal sensitivity) method*

Hot plate test is the most common test of nociception that is based on a 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 response include but not limited to 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).

1.3.2.4 *Formalin induced pain test*

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

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

1.3.2.5 *Carrageenan induced paw oedema model*

Carrageenan induced rat paw oedema is a well established animal model for evaluating the anti-oedematous effect of drugs or compounds (Sharma *et al.,* 2010). Formation of oedema caused by carrageenan is said to be in two phases; the first hour after carrageenan injection (first or early phase), involves the release of serotonin, histamine and bradykinin while the second or late phase (2 –5 hours) with increased oedema formation that remains up to the fifth hour involves the release of prostaglandins (Khan *et al.,* 2009).

1.4 Aim and Objectives

1.4.1 Aim of the study

The aim of this study is to investigate the analgesic and anti-inflammatory effects of methanol root extracts of *Andropogon gayanus* Kunth in mice and rats.

1.4.2 Specific objectives

1. To conduct preliminary phytochemical screening on the methanol root extract of *Andropogon gayanus*.
2. To determine the LD_{50} of the extract orally (*p.o*) and intraperitoneally *(i.p*) in mice and rats.

3. To evaluate the analgesic properties of the extract using scientific models in mice and rats.
4. To evaluate the anti-inflammatory properties of the extract using scientific model in rats.
5. To establish the possible mechanism of analgesia of the extract.

1.5 Statement of Research Hypothesis

The methanol root extract of *Andropogon gayanus* possesses analgesic and 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 through modification of transmitting noxious stimuli to the brain (constituting the physiological component) (Besson, 1999).

Nociception can be defined as the process by which information about tissue damage is conveyed to the central nervous system. There can be pain without nociception such as phantom limb pain or nociception without pain. There are four classical descriptions of pain processes:

a) Transduction*:* the conversion of the energy from a noxious thermal, mechanical, or chemical stimulus into electrical energy by sensory receptors called nociceptors.
b) Transmission: this involves the transmission of these neural signals from the site of transduction to the spinal cord and brain.
c) Perception: the appreciation of signals arriving in higher structures as pain.

d) Modulation: descending inhibitory and facilitatory input from the brain that influences nociceptive transmission at the level of the spinal cord (Berry *et al.*, 2001).

Perception of pain can be influenced by several psychological factors such as the processes of attention, memory or learning, thought, beliefs, mood, behavioural responses, and ability to cope. Although pain may be an indicator of tissue damage, it may also be experienced in the absence of an identifiable cause. The degree of disability experienced varies in relation to the experience of pain and also in response to pain alleviation methods (Macintyre *et al.*, 2010).

2.1.1 Classification of Pain

The four most commonly used systems for the classification of pain are;

- The pathophysiological mechanism of pain (nociceptive or neuropathic).
- The duration of pain (chronic or acute).
- The etiology of pain (malignant or non-malignant).

2.1.1.1 *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 are cut and sprains; causing tissue disruption and 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 the activation of nociceptors located in the viscera (Stein, 1995). Viscera 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.

Sensory dysfunction suggestive of neuropathic pain includes allodynia, hypoalgesia, hyperalgesia, paraesthesia, dysthesia, hyperesthesia and hypoesthesia (Vranken, 2009).

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). Dyesthesia 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).

2.1.1.2 *Classification based on pain duration*

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).

2.1.1.3 *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 stomach ache are sometimes diagnosed as psychogenic (Thienhaus and Cole, 2002).

2.1.2 Epidemiology of pain

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). 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).. 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). It has been estimated that globally 20 % of all adults suffer from pain and that 10 % are newly diagnosed with chronic pain each year, making pain an enormous public health problem world over (Goldberg and McGee, 2011).

2.1.3 Pain pathways

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).

2.1.4 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). 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).

2.1.5 Management of pain

Pain management or treatment can be broadly divided into two; pharmacologic and non-pharmacologic.

2.1.5.1 *Non-pharmacological treatment of pain*

Pharmacological treatment is the mainstay of pain treatment. However, optimal pain management also includes psychological, physical rehabilitative, and in some cases, surgical treatment strategies. A variety of comfort-producing measures have been implemented for pain management such as, endo-tracheal tube suctioning, repositioning in bed, massage, oral care, and reassurance. Other measures include application of heat or cold, massage, therapeutic touch, guided imagery, and relaxation techniques. This is common for critically ill patients (Helms and Barone, 2008).

Non-pharmacologic measures should supplement, not replace, the use of medications. In addition to supplementing the pain-relieving effects of analgesics, nonpharmacologic approaches offer other advantages. Example, they can improve mood, reduce anxiety, increase patient's sense of control, strengthen coping abilities, assist with sleep, relax muscles, and improve quality of life (Wallace, 1992).

Psychological Approaches:

Psychological interventions used in pain management include contingency management, cognitive behavioural therapy, biofeedback, relaxation, descriptions, and psychotherapy. Patients in whom psychological interventions may be most appropriate include those who express interest in such approaches, manifest anxiety or fear, have inadequate pain relief after appropriate pharmacologic interventions, or experience chronic or recurrent pain. Psychological preparation such as preparation for surgery or for an invasive procedure or psychological intervention such as relaxation may help to control the affective dimension of pain when it is acute. This, in turn, helps minimize the biological stress response as well as emotional distress and suffering that the patient experiences. Methods such as relaxation and imagery are simple and can be taught quickly, whereas others require more time. Patient education materials, example, printed instruction sheets, audiotapes can supplement, but not replace, clinician efforts to instruct patients in these methods (Berry *et al.,* 2001).

Physical Rehabilitative Approaches:

These methods of pain management are appropriate for many types of pain and are essential in patients with chronic non-cancer pain. In addition to relieving pain, such methods can reduce fear and anxiety, improve physical function, and alter physiological

responses to pain. Treatments used in physical rehabilitation include stretching, exercises or reconditioning (to improve strength, endurance, and flexibility), gait and posture training, and attention to ergonomics and body mechanics. Other non-invasive physical treatments for pain include thermotherapy, cryotherapy, counter-irritation, and electro analgesia (Ray, 2001).

Surgical Approaches:

Neurosurgical procedures for managing pain include neurolysis (injection of a chemical or application of heat or cold to destroy neural tissue), neuroaugmentation procedures, and neuroablative surgeries (disruption of neural signals and/or removal of neural structures associated with pain). For example, micro vascular decompression of the trigeminal nerve is sometimes used to manage trigeminal neuralgia (Berry *et al.*, 2001).

2.1.5.2 *Pharmacological treatment of pain*

This is the mainstay of pain treatment. The World Health Organization (WHO) has published a simple and validated three-step approach to pain management that has been shown to be effective in relieving pain. The basic principles behind the three steps of the ladder include selecting the appropriate analgesic for the pain intensity and individualizing the dose by titration of opioid analgesics (Katz *et al.*, 2008).

Step 1:

Treatment of mild pain: this involves the use of non-opioid analgesics with or without adjuvants. Pain can usually be adequately treated with aspirin, acetaminophen, and non-steroidal anti-inflammatory drugs (NSAIDs). These drugs differ from opioids in two important ways: there is a ceiling effect to the analgesia and they do not produce

tolerance or physical dependence. Management of all levels of pain includes a drug from this category, except in case of contraindication. Acetaminophen may be preferable in patients at risk for NSAID side effects such as renal failure, bleeding, gastric ulceration or hepatic dysfunction. NSAIDs may be appropriate for other patients, particularly if there is inflammation.

Step 2:

Mild to moderate pain: this involves the use of a weak opioid or low dose opioid, with a non-opioid, with or without an adjuvant. In the initial treatment of moderate pain, low dose opioid drugs are added to aspirin, acetaminophen, or NSAIDs. For patient convenience, many opioids are marketed as combination products containing one of these agents. It is the daily cumulative acetaminophen dose that limits the dosing of the opioid in combination medications. For this reason separate dosing of the opioid and acetaminophen is preferred (Katz *et al.,* 2008).

Step 3:

Treating Severe pain: this involves the use of strong opioid analgesics with acetaminophen or aspirin, with or without adjuvant.

Drugs used in the treatment of pain:

There are three classes of drugs used in the treatment of pain which include; opioid analgesics, non-opioid analgesics and adjuvant or co-analgesic.

Opioid analgesics (opioids):

This group includes opioid agonists such as morphine-like agonists and agonist-antagonist opioids. Opioids bind to opioid receptors in the central nervous system. They inhibit the transmission of nociceptive input from the periphery to the spinal cord, activate descending inhibitory pathways that modulate transmission in the spinal cord, and alter limbic system activity. Thus, opioids modify sensory and affective aspects of pain (Stein, 1995).

Non-opioid analgesics (non-opioids):

This group includes drugs such as acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin and other salicylic acid derivatives. The primary mechanism of action of NSAIDs is inhibition of the enzyme cyclooxygenase (COX) which blocks prostaglandin synthesis. Acetaminophen appears to act mostly via a central mechanism. All non-opioids have anti-inflammatory, antipyretic, and analgesic effects, but the anti-inflammatory effect of acetaminophen is essentially negligible. The analgesic effect of NSAIDs is prompt, whereas the anti-inflammatory effect is longer (1-2 weeks or more).This latter effect can indirectly relieve some pain by reducing tissue swelling (Berry *et al.*, 2001).

Adjuvant analgesics or co-analgesics:

This is a diverse group of drugs, with primary indications for conditions other than pain, with analgesic properties relevant to some conditions. Commonly used adjuvant analgesics include antiepileptic drugs, tricyclic antidepressants, and local anaesthetics. Antiepileptic drugs such as pregabalin and gabapentin bind to pre-synaptic voltage-gated calcium channels in the dorsal horn, which result in decrease in the release of excitatory

neurotransmitters such as glutamate and substance P. These drugs are effective in the treatment of diabetic neuropathy and post-herpertic neuralgia. The exact basis for analgesic effect is not known, but is not specifically related to their anticonvulsant activity (Moulin *et al.*, 2007).

Tricyclic anti-depressants provide the best evidence in the management of neuropathic pain. The mechanism of analgesic activity is also unclear. They block the reuptake of serotonin and noradrenaline, and block hyperalgesia induced by N-methyl-D-aspartate agonists. They also have sodium channel blocking properties. Their analgesic property is independent of their antidepressant effects. Examples of such drugs include; amitriptyline and imipramine (Moulin *et al.*, 2007).

Local anaesthetics are another type of adjuvant analgesic. They block sodium channels and inhibit the generation of abnormal impulses by damaged nerves to exert their peripheral analgesic effects. When used systemically, they do not produce conduction block as they do with local injection and topical application but may suppress unusual electrical activity in structures associated with pain. Example of such drugs is topical lidocaine (Berry *et al.*, 2001).

2.2 Inflammation

Inflammation is the normal physiological response of the body to injury. The cause of tissue injury is attributed to trauma, autoimmune, microbial, toxins (chemicals) and heat (Sodano and Grisanti, 2010). When tissue is damaged or injured many substances are released leading to changes to the surrounding undamaged tissues. The released substances leads to inflammation; the substances implicated in inflammation are

numerous and include; histamine, serotonin, prostaglandin, leukotriene, lipoxin, platelet activation factor, bradykinin etc. These substances are implicated in inflammation process and are of great significance in therapeutics.

Inflammation is the body's reaction to injury or chemical irritants. It has been characterised based on visual observation by five cardinal signs. These signs include redness (rubor), swelling (tumour), heat (calor) which is only applicable to the body extremities, pain (dolor) and loss of function (functio laesa). Inflammation is described as the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree that can destroy its structure and vitality at once (Punchard *et al.,* 2004).

Inflammation is the means by which the body deals with insult and injury. Insult may be caused: mechanically by pressure or foreign bodies; chemically by toxins, acidity, and alkalinity; physically by temperature, by internal processes such as uremia, and by mircoorganisms such as bacteria, virus, and parasites. Inflammation rids the body of foreign matter, disposes of damaged cells, and initiates wound healing. It is controlled by mast cells that are in close proximity to autonomic nerves. Mast cells are constituents of connective tissues containing large granules that contain heparin, serotonin, bradykinin, and histamine. These substances are released from the mast cell in response to injury and infection, and, by their degranulation, they control most inflammatory processes (Hotamisligil, 2006).

2.2.1 Classification of inflammation

Inflammation is classified into per-acute, acute, sub-acute, chronic and chronic active based on duration (Martin, 2013). Per-acute is characterised by hyperemia, hemorrhage, slight edema and involves few inflammatory cells. If it's due to highly pathogenic agents it can lead to shock and sudden death. It lasts for about 0-4 hours, and is usually very acute due to potent stimulus. Acute inflammation last from 4-6 hours to 3-5 days, there is vascular involvement with neutrophils predominating. It displays the cardinal signs of *rubor, calor, tumor and dolor*. Sub-acute inflammation is a transition between acute and chronic; it last between few days to one week. Chronic inflammation persists over a long period of time resulting in persistent inflammatory stimulus and with some degree of tissue repair. It lasts from weeks to years while chronic active is characterised by chronicity with superimposed features of acute inflammation. It also mixes the chronic time frame with overlapping acute features (Martin, 2013).

2.2.1.1 *Acute inflammation*

This is a rapid or transient response to injury or irritant. Acute inflammation can be caused by infections such as bacterial or viral infections, immune reactions such as reaction to a bee sting and several other stimuli such as tissue necrosis, trauma, radiation, burns and foreign body. It is an immediate response to trauma, usually within seconds to two hours. It is manifested by vascular changes, oedema, and predominantly neutrophilic infiltration (Kumar *et al.,* 2012).

Acute inflammation has three major components: alterations in vascular quality that lead to an increase in blood flow; structural changes in the microvasculature that permit plasma proteins and leukocytes to leave the circulation; and emigration of the leukocytes

from the microcirculation, their accumulation in the focus of injury, and their activation to eliminate the offending agent (Badizadegan, 2003).

In acute inflammation, fluid loss from vessels with increased permeability occurs in different phases: an immediate transient response lasting for less than 30 minutes, mediated mainly by the actions of histamine and leukotrienes on endothelium; a delayed response starting at about 2 hours and lasting for about 8 hours, mediated by kinins, complement products, and other factors; and a prolonged response that is most noticeable after direct endothelial injury, such as after burns (Khan and Solomon, 2007).

2.2.1.2 *Chronic inflammation*

Chronic inflammation can occur either by evolving from an acute inflammation or without an acute phase. It is the persistence of inflammation with attempts of repair resulting from persistence of the injurious agent. Its response is prolonged, usually weeks, months and even years. Chronic inflammation is caused by persisting infection or prolonged exposure to irritants, repeated acute inflammation such as otitis and autoimmune diseases such as rheumatoid arthritis (Kumar *et al.*, 2012).

Chronic inflammation is characterized by infiltration with mononuclear cells (which include macrophages, lymphocytes, and plasma cells), tissue destruction (induced by the persistent offending agent or by the inflammatory cells), healing attempts by connective tissue replacement of damaged tissue, accomplished by proliferation of small blood vessels and, in particular fibrosis. It inevitably causes tissue damage and is accompanied by simultaneous attempts at healing and repair. The exact nature, extent and time course

of chronic inflammation is variable and depends on the balance between the causative agent and the attempts by the body to remove it (Wassung, 2012).

2.2.2 Chemical mediators of inflammation

The inflammatory process begins with chemical a series of inflammatory chemicals that are released in the extracellular fluid. Sources of inflammatory mediators include injured tissue cells, phagocytes, lymphocytes, mast cells and blood proteins, the most important of which are histamine, kinins, prostaglandins, complement, and lymphokines. Some of these mediators may have individual inflammatory role, they all promote dilation of the small blood vessels in the vicinity of the injury. As more blood flows into the area congestion with blood occurs which accounts for the redness and the heat of the inflamed area. They also increase the permeability of local capillaries. As a result, exudates, fluid contain proteins such as clotting factors and antibodies, seeps from the bloodstream into the tissue spaces. This exudate is the cause of the local oedema or swelling which in turn, presses on adjacent nerve endings, contributing to a sensation of pain (Wassung, 2012).

Eicosanoids are short-lived, hormone-like substances present in tissues throughout the body. They function as mediators of a variety of physiological responses such as inflammation, blood clotting, vascular dilation, and immunity. They can be divided into four classes: prostaglandins, leukotrienes, thromboxanes, and prostacyclins. A large part of the inflammatory process is regulated specifically by the prostaglandins and leukotrienes (Percival, 1999; Danesh *et al.*, 2004).

Histamines are chemicals responsible for the itchy nose, watery eyes, or rash that often accompany an allergic reaction. Their function is to help remove the toxin causing the

problem by sneezing, coughing, crying, and scratching. They bring more blood and lymphatic fluid to the site of the invasion, which in turn carries white blood cells to the site and toxins away from it. It can act on sensory neurones to produce itching at low concentration and pain at high concentrations (Danesh *et al.*, 2004).

Kinins observe a number of pro-inflammatory effects including the release of prostanoids, cytokines, and free radicals from a variety of cells. They degranulate mast cells to releases histamine and other inflammatory mediators and also cause plasma extravasation by contraction of vascular endothelial cells (Dray, 1995).

Cytokines are immune system modulators produced by cells throughout the body. They communicate with the brain, signalling an alarm when an intruder is detected. A subclass of cytokines known as leukotrienes or interleukins ensures that the immune response is checked before it begins to attack outlying healthy cells and tissue. They significantly call off the inflammatory response (Wassung, 2012).

2.2.3 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).

2.2.4 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.

2.2.5 Inflammatory disorders

Inflammatory abnormalities are a large group of disorders that underline 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 (Cotran, 1998).

2.2.6 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) and thirdly removal of the causative agent. 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 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.

2.2.6.1 *Prednisolone*

Is a synthetic glucocorticoid, a derivative of cortisol, which is used to treat a variety of inflammatory and auto-immune disorders. 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's disease (Miura *et al*., 2011) and dermatomyositis. It is also used for the treatment of sarcoidosis even though the mechanism is unknown.

2.2.6.2 *Budesonide*

This 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:

1. Ulcerative colitis (colon inflammation): Budesonide assists in the induction of remission in patients with active ulcerative colitis (Habal and Huang, 2010).
2. 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).
3. 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 Traditional Medicine

Traditional medicine as identified by the World Health Organization, is the sum total of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO, 1999).

Traditional healing can be defined as practices designed to promote mental, physical and spiritual well-being that are based on beliefs which date back to the time before the spread of western scientific bio-medicine. It includes a wide range of activities, from physical cures using herbal medicines and other remedies, to the promotion of psychological and spiritual well-being using ceremony, counselling and the accumulated wisdom of elders. Large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs in many developing countries (Martin, 2003).

The primary source of remedies in Traditional Medicine is botanical, although animal and mineral materials have been used. Of the several thousands of remedies available worldwide, about 500 are in common use. Herbal medicines are used after processing, which may be soaking in water or vinegar, or even wine (Li, 2000). Plants and their secondary metabolites have a long history of use in modern medicine and in certain systems of traditional medicine, and are the sources of important drugs such as atropine, codeine, digoxin, morphine, quinine and vincristine (WHO, 2005).

2.3.1 Plants used in the management of pain and inflammation

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 anti-nociceptive 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 anti-nociceptive 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).

2.4 *Andropogon gayanus* Kunth

2.4.1 Taxonomy/Nomenclature of the plant

Kingdom:	Plantae
Subkingdom:	Viridiplantae
Infrakingdom:	Streptophyta
Superdivision:	Embryophyta
Division:	Tracheophyta
Subdivision:	Spermatophytina
Phylum:	Magnoliophyta
Class:	Magnoliopsida
Superorder:	Lilianae
Order:	Poales
Family:	Poaceae
Genus:	*Andropogon*
Species:	*gayanus*
Botanical name:	*Andropogon gayanus* Kunth
Local Names:	English; Gamba grass, Hausa; *Gamba/Tsaure*, Yoruba; *Eruwa ako*



Plate I: A picture of *Andropogon gayanus* in its Natural Habitat

2.4.2 Ethnomedicinal uses of *Andropogon gayanus*

The plant is widely used especially in Africa for its various medicinal properties. The roots of *Andropogon gayanus* are soaked in milk and taken to treat post-partum pain and bronchitis Safana local government Area of Katsina state. In Senegal, the root of the plant is used as purgative (Thomas, 1972). The water extract of the root is used in the treatment of cough and bronchitis in Central African Republic (Haxaire, 1979). The roots together with the leaves are reported to be used to wash wounds and scarification in South-West Nigeria (Adjanohoun *et al*., 1991). The whole plant is used in the treatment of hiccups in Benin Republic (Adjanohoun *et al*., 1991), in Mali it's used in post-partum pain and as creams or lotions in skin infections (Malgras, 1992), and as a nasal decongestant in Niger Republic (Saadou, 1993). The leaves are also used in swelling face, hands and feet in Benin Republic (Verger, 1995), whereas the leaves are also used in the treatment of diarrhoea in Sokoto state of Nigeria (Etuk *et al.*, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Material

3.1.1 Plant collection and identification

The fresh roots of *Andropogon gayanus* was collected in August, 2015 at Samaru, Sabon Gari Local Government Area, Kaduna state. The plant was identified and authenticated by Mallam Namadi Sanusi, a botanist in the Herbarium of the Department of Biological Sciences of Ahmadu Bello University, Zaria and was compared with a voucher specimen number 247.

3.1.2 Preparation of the plant extract

The collected roots were dried under shade for two weeks and size reduced with mortar and pestle. The grinded material was extracted by cold maceration using aqueous methanol (70:30). Two thousand and fifty grams of the powder was extracted in two portions. One thousand grams was first added into Erlnmeyer Flask with 2000 ml of the solvent and kept for 48 hours and shaken intermittently, then filtered. The extract was evaporated to dryness in an evaporator under reduced pressure and controlled temperature (40-60°C). The remaining 1000 g of the powder was also extracted using the same method and solvent. After evaporation, the extract was preserved in a desiccator until needed for the work. Aqueous solutions were freshly prepared for each study using distilled water.

3.2 Experimental Animals Husbandry

Wistar rats and mice of both sexes (120-180g and 17-30g respectively) were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were allowed free access to standard feed and water *ad libitum*. They were kept in clean cages bedded with saw dust which was replaced every three days. The study was conducted according to ethical guidelines on laboratory animal use and care policy, which is in compliance with Ahmadu Bello University Research Policy (Revised 2010).

3.3 Drugs and Chemicals

The following drugs and chemicals were used. They are methanol, normal saline, distilled water, acetic acid (Ranbaxy Laboratories Ltd, Punjab), aspirin, morphine (Martindale Pharmaceuticals, Ramford, Essex), naloxone (Abcam plc, Cambridge), yohimbine (Abcam plc, Cambridge), glibenclamide (Abcam plc, Cambridge), carrageenan (Sigma chemical, Germany), and 10% formalin solution. Reagents used for the phytochemical screening were obtained from the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria. All the reagents were of analytical grade.

3.4 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), digital vernier caliper (Battenfeld Technologies Inc. USA), 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, TLC plates and laboratory record book.

3.5 Preliminary Phytochemical Screening

Phytochemical screening was carried out on the dried extract using simple chemical tests to detect the presence of carbohydrates, alkaloids, saponins, tannins, flavonoids, terpenoids, anthraquinones, glycosides and cardiac glycosides (Evans, 2002). The following methods were used:

3.5.1 Test for alkaloids

To 0.5 g of the extract, dilute HCl (20 ml) was added in a conical flask, heated on a steam bath and then filtered. The filtrate was made alkaline with dilute NH_3 solution and then extracted with chloroform. The $CHCl_3$ extract was concentrated and treated with equal volume of 1% HCl. Dragendoff's reagent and Mayer's reagent 2 ml each were added to the aqueous portion in two separate test tubes. Occurrence of orange-red and cream precipitate respectively indicated the presence of alkaloids (Evans, 2002).

3.5.2 Test for tannins (Ferric chloride test)

To 0.2 g of the methanol extract 5 ml of distilled water was added and allowed to dissolved then filtered. Few drops of ferric chloride solution were added to the filtrate. Formation of a blue-black precipitate indicated hydrolysable tannins while green precipitate indicated the presence of condensed tannins (Evans, 2002).

3.5.3 Test for free anthraquinones (Bontrager's test)

Small portion of the extract was shaken with 10 ml of benzene and filtered. Five milliliters of dilute NH_3 solution was added to the filtrate and shaken. The production of a pink, red or violet colour indicated the presence of free anthraquinones (Evans, 2002).

3.5.4 Test for saponins (Frothing test)

Small quantity of the extract was dissolved in10 ml of distilled water. It was then shaken vigorously for 30 seconds and allowed to stand for 30 minutes. Formation of froth that lasts more than 15 min indicated the presence of saponins (Brain and Turner 1975).

3.5.5 Test for steroids and triterpenes (Liebermann-Burchard's test)

The extract, 0.5 g was dissolved in 5 ml of chloroform and filtered. Equal volume of acetic anhydride was added to the filtrate. One milliliter of concentrated H_2SO_4 was added and the colour change observed immediately and over a period of one hour. Red, pink or purple colour indicates the presence of triterpenes, while blue or blue-green indicated steroids (Evans, 2002).

3.5.6 Test for flavonoids (Shinoda test)

The extract 0.5 g, was dissolved in 5 ml of methanol and warmed on steam bath. Magnesium chips and 5 drops of concentrated hydrochloric acid were added. A red or orange colour indicated the presence of flavonoids (Evans, 2002).

3.5.7 Test for cardiac glycosides (Keller Kiliani's test)

Glacial acetic acid 5 ml was added with one drop of ferric chloride to 5 ml of aqueous solution of the methanol extract. The mixture was kept for one minute then transferred to

a test tube. 1.5 ml of concentrated sulphuric acid was added with a pipette, so that it runs down the wall of the tube and forms a separate layer at the bottom. On standing, a brown colour at the interface indicates the presence of deoxy sugars and a pale green colour in the upper layer indicates the presence of a steroid nucleus (Brain and Turner, 1975).

3.5.8 Test for carbohydrates (Molisch's test)

The extract 0.5 g was dissolved in 3 ml of water few drops of Molisch's reagen were added and carefully small amount of concentrated H_2SO_4 were added from the side of the test tube to form a lower layer. With a soluble carbohydrate, this appears as a purple ring if the sulphuric acid is gently poured in to form a layer below the aqueous solution (Evans, 2002).

3.5.9 Thin layer chromatography (TLC) of methanol root extract of *A. gayanus*

The TLC profile of the extract was obtained on pre-coated TLC plate using chloroform: methanol:acetic acid (18:1:1) solvent system to determine the separation profile of the extract. A 100 mg/ml stock solution of *A. gayanus* was made using methanol. The solution was spotted on the TLC plate using a capillary tube and then developed. The plates were dried and then sprayed with p-anisaldehyde solution, Liebermann- Burchard reagent, Dragendorff's reagent, Aluminum chloride or Ferric chloride each followed by heating at temperature of 110 ^{0}C for ten minutes and the various spots together with distinct colours that appear were observed and inferences were made. Bontrager's reagent was also sprayed on one of the chromatograms and the plate observed under UV light at a wavelength of 254 nm to detect the presence of anthraquinones.

3.6 Acute Toxicity Studies

The median lethal dose (LD_{50}) of the extract was determined using Lorke's method (1983) through the oral and intraperitoneal routes. For the oral route, the study was carried out in two phases in both mice and rats. In phase 1, three groups of three animals each were used. The extract was administered orally in geometrically increasing doses (10, 100 and 1000 mg/kg). The treated animals were observed for four hours post administration for signs of toxicity and for 24 hours for mortality. After 24 hours, phase 2 was initiated. In phase 2, four animals were given specific doses of the extract orally (1200, 1600, 2900 and 5000 mg/kg). The animals were then observed for signs of toxicity for the first 4 hours and mortality for 24 hours. The geometric mean of the lowest lethal dose and the highest non-lethal dose was evaluated as the median lethal dose (LD_{50}) of the extract. The same procedure was repeated in both species of animals using the intraperitoneal routes.

3.7 Analgesic Studies

3.7.1 Acetic acid induced writhing test in mice

Acetic acid induced writhing method described by Koster *et al.,* (1959) was adopted for evaluation of analgesic activity. Writhing is defined as a stretch tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor and turning of trunk (Mishra *et al.*, 2011). Thirty Swiss albino mice of both sexes were divided into five groups. Groups 1 and 5 served as negative control (distilled water, 10 ml/kg) and positive control (Aspirin 300 mg/kg) respectively, while groups 2, 3, and 4 received 250 mg/kg, 500 mg/kg, and 1000 mg/kg of methanol root extract respectively. All administrations were *per oral.*

Sixty minutes after treatment, the mice received 0.6% acetic acid (10 ml/kg) interperitoneally to induce pain. Five minutes after acetic acid injection, the animals were each placed in an observation box, and observed for 10 minutes. The number of writhes produced by each mouse within the 10 minutes period was counted. Percentage inhibition was calculated using the following formular:

$$\% \text{ Inhibition} = \frac{\text{Mean no. of writhes (Negative control)} - \text{Mean no. of writhes (test)}}{\text{Mean no. of writhes (Negative control)}} \times 100$$

3.7.2 Hot plate test in mice

This method was carried out as described by Eddy and Leimback (1953). 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 temperature of the hot plate was set at 45 ± 1°C (Mishra *et al.*, 2011). Thirty swiss albino mice of both sexes used were fasted 12 hours prior to the experiment, and divided into five groups of six rats each. Groups 1 and 5 served as negative control (distilled water 10 ml/kg) and positive control (Morphine, 10 mg/kg) respectively, while groups 2, 3, and 4 received 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the extract. The test and standard drugs were administered orally.

The animals were individually placed on the hot plate and the time until either licking or jumping occurred (reaction time, RT) was recorded using a stop-watch, 60, 90, 120 and 150 minutes after treatment.

3.7.3 Formalin induced pain test in rats

The method described by Dubuisson and Dennis (1977) as modified by Murray *et al*., (1988) was adopted in this experiment. Thirty rats were divided into 5 groups of 6 each. Group 1 was given Distilled water (1 ml/kg, *p. o*) and served as negative control, while group 5 was administered morphine (10 mg/kg, *p. o*). Groups 2, 3 and 4 were administered 250, 500 and 1000 mg/kg doses *(p. o*) of the extract. One hour after the administration, 50 µL of freshly prepared 2.5 % formalin was injected subcutaneously into the plantar surface of the left hind paw of each rat. The observation was made in two phases, phase 1 (neurogenic pain) and phase 2 (inflammatory pain). Phase 1 lasts for 5 minutes from the time of formalin administration while phase 2 covers from 15 minutes to 60 minutes after formalin administration with 10 minutes lag period between the two phases. The severity of pain was monitored based on the following scale:

Rat stands firmly or walks on the injected paw……..Score 0

The injected paw partially elevated or favoured…….Score 1

The injected paw is clearly lifted off the floor……Score 2

The rat licks, chews or shakes the injected paw…….....Score 3

3.8 Anti-inflammatory Studies

3.8.1 Carrageenan induced rat paw oedema test

The anti-inflammatory study was carried out using the method described by Winter *et al.,* (1963). Thirty wistar rats were divided into five groups, 1 and 5 served as negative (distilled water, 1 ml/kg) and positive (Aspirin, 300 mg/kg) controls respectively, while groups 2, 3, and 4 received 250, 500, and 1000 mg/kg of the extract. Treatments were administered 1 hour before carrageenan injection. Carrageenan was prepared as 1% w/v solution in 0.9 % w/v NaCl and 0.1 ml was injected underneath the planter region. The

paw size was then measured with digital vernier caliper at 0, 1, 2, 3, 4, and 5 hours after Carrageenan injection (Sharma *et al.,* 2010).

3.9 Mechanistic Studies on *A. gayanus*

3.9.1 Determination of the effects of naloxone co-administration with methanol root extract of *A. gayanus* on acetic acid-induced writhing in mice

The acetic acid induced writhing test described by Koster *et al.,* (1959) was adopted for the evaluation of possible mechanism of anti-nociception. 30 Swiss albino mice of both sexes were divided into six groups. Group 1served as negative control (distilled water 10 ml/kg, only), group 2 received Naloxone (2 mg/kg, only), group 3 received the extract alone at 500 mg/kg body weight, group 4 received morphine also alone at 10 mg/kg and groups 5 and 6 were treated with the extract at 500 mg/kg and morphine 10 mg/kg 15 min after naloxone 2 mg/kg was administered. All administrations were *per oral* except for naloxone. Sixty minutes after treatment, all the groups were treated with 0.6 % $^{v}/_{v}$ acetic acid (10 ml/kg) intraperitoneally to induce pain except for group 2 in which the acetic acid was administered 15 min after treatment. 5 minutes after acetic acid injection, the animals were each placed in an observation box, and observed for 10 minutes. The number of writhes produced by each mouse within the 10 minutes period was counted. Percentage inhibition was calculated using the following formular:

$$\% \text{ Inhibition} = \frac{\text{Mean no. of writhes (Negative control)} - \text{Mean no. of writhes (test)}}{\text{Mean no. of writhes (Negative control)}} \times 100$$

3.9.2 Determination of the effects of glibenclamide co-administration with methanol root extract of *A. gayanus* on acetic acid-induced writhing in mice

The acetic acid induced writhing test described by Koster *et al.*, (1959) was adopted for the evaluation of possible mechanism of anti-nociception. Twenty four Swiss albino mice of both sexes were divided into four groups. Group 1served as negative control (distilled water 10 ml/kg, only), group 2 received Glibenclamide (5 mg/kg, only), group 3 received the extract alone at 500 mg/kg body weight, while animals in group 4 were treated with the extract at 500 mg/kg 15 min after glibenclamide 5 mg/kg was administered. All administrations were *per oral* except for glibenclamide which was *i.p*. Sixty minutes after treatment, all the groups were treated with 0.6% acetic acid (10 ml/kg) intraperitoneally to induce pain except for group 2 in which the acetic acid was administered 15 min after treatment. 5 minutes after acetic acid injection, the animals were each placed in an observation box, and observed for 10 minutes. The number of writhes produced by each mouse within the 10 minutes period was counted. Percentage inhibition was calculated using the following formular:

$$\% \text{ Inhibition} = \frac{\text{Mean no. of writhes (Negative control)} - \text{Mean no. of writhes (test)}}{\text{Mean no. of writhes (Negative control)}} \times 100$$

3.9.3 Determination of the effects of yohimbine co-administration with methanol root extract of *A. gayanus* on acetic acid-induced writhing in mice

The acetic acid induced writhing test described by Koster *et al.*, (1959) was adopted for the evaluation of possible mechanism of anti-nociception. The animals were divided into four groups with 6 mice in each group. Group 1served as negative control (distilled water 10 ml/kg, only), group 2 received yohimbine (1 mg/kg, only), group 3 received the extract alone at 500 mg/kg body weight, group 4 received 500 mg/kg of the extract 15

min after yohimbine 1 mg/kg was administered. All administrations were *per oral* except for yohimbine which was done intraperitoneally.

Sixty minutes after treatment, all the groups were treated with 0.6% acetic acid (10 ml/kg) intraperitoneally to induce pain except for group 2 in which the acetic acid was administered 15 min after treatment. 5minutes after acetic acid injection, the animals were each placed in an observation box, observed and number of writhes by each mouse was counted for 10 minutes. Percentage inhibition was calculated using the formular below:

$$\% \text{ Inhibition} = \frac{\text{Mean no. of writhes (Negative control)} - \text{Mean no. of writhes (test)}}{\text{Mean no. of writhes (Negative control)}} \times 100$$

3.10 Statistical Analysis

Results were expressed as mean ± standard error of mean and presented as graphs and tables. Data were analysed using one way analysis of variance (ANOVA) for all acetic acid tests followed by Dunnett t-test, repeated measures ANOVA for hot plate (thermal sensitivity) test and carraageenaan induced inflammation, followed by Bonferoni *post hoc* test for multiple comparison and the non-parametric Kruskal Walli's analysis for formalin-induced pain. Results were considered significant at $p \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 The Plant Extract and Phytochemical Constituents of *A. gayanus*

The extract obtained was a greenish-black, sticky sweet smelling solid. The percentage yield of the root of *Andropogon gayanus* using cold maceration technique and 70 % methanol as the solvent of extraction was found to be 1.8 $\%^{w}/_{w}$. The preliminary phytochemical screening using standard tests of the methanol root extract of *Andropogon gayanus* revealed the presence of flavonoids, triterpenes, tannins, saponins, glycosides, alkaloids and cardiac glycosides. Phytochemical constituents like anthraquinones and steroids were absent (Table 4.1).

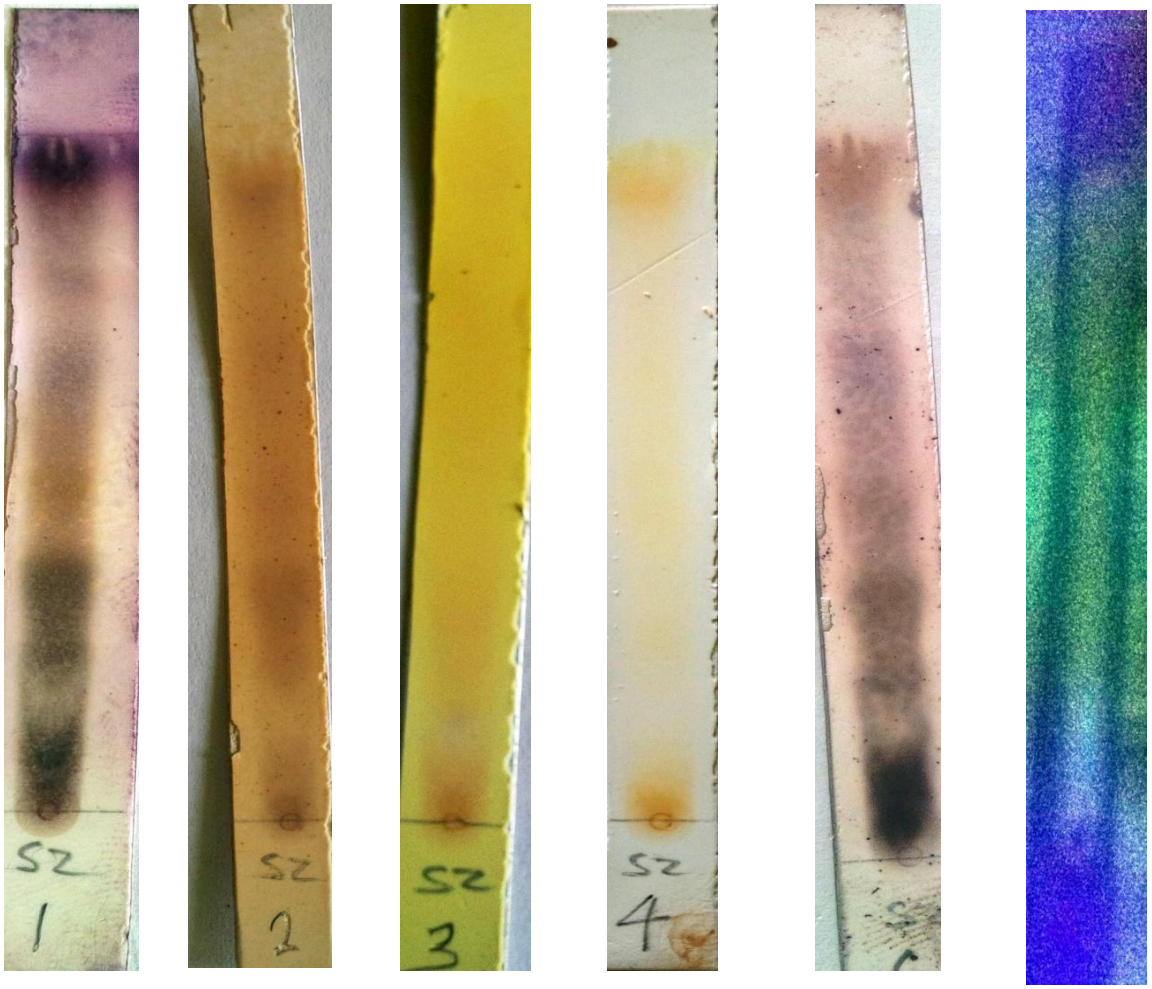
Table 4.1: Phytochemical Constituents of the Methanol Root Extract of *Andropogon gayanus*

Phytochemical Constituent	Test	Result
Carbohydrates	Molisch	+
Anthraquinones	Bontrager	-
Glycosides	Kehling	+
Cardiac glycosides	Kelle-killiani	+
Saponins	Frothing	+
Steroids	Liberman Buchard	-
Triterpenes	Liberman Buchard	+
Tannins	Ferric chloride	+
Flavonoids	Shinoda	+
Alkaloids	Dragendorff	+

Key: + = Present and - = Absent

4.1.1 Thin Layer Chromatography

Results of the thin layer chromatography analysis of the methanol root extract of *A. gayanus* using different spraying reagents revealed the presence of several phytochemical constituents. P-Anisaldehyde/Sulphuric acid is a general spraying reagent which identified nine different spots (Plate IIa). Ferric chloride ($FeCl_3$) is a specific spray for phenolic compounds such as tannins, where a black colour indicates the presence of such. Five different such black spots were identified after the spray (Plate IIb). Dragendorff spray is specific for alkaloidal components and spot 1 was shown to identify the presence of alkaloids (Plate IIc). Bontrager's spray when viewed under UV light at 254 nm wavelength is for anthraquinones and spots 8 and 9 revealed the presence of anthraquinones by showing fluorescent light at these spots (Plate IIf). Aluminium chloride ($AlCl_3$) spray confirmed the presence of flavonoids at spots 2 and 9 (Plate IId). Liebermann Burchard is specific for steroids and triterpenes, where pink, red, or purple colour indicated the presence of triterpenes, steroids are absent in the extract as blue or green colour is not evident on the plate (Plate IIe).



a b c d e f

Plate II: Chromatograms of Methanol Root Extract of *Andropogon gayanus*

Key: a=P-anisaldehyde spray; b=Ferric chloride spray; c=Dragendorff spray; d=Aluminium chloride spray; e=Liberman buchard spray; f=Bontrager's spray.

Table 4.2: R_f Values for the P-Anisaldehyde/Sulphuric Acid Spray Chromatogram

Spot	R_f values
1	0.10
2	0.21
3	0.33
4	0.47
5	0.56
6	0.66
7	0.80
8	0.89
9	0.96

$$R_f \text{ value} = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by the solvent}}$$

4.2 Determination of LD_{50} (Acute Toxicity Studies)

The mice and rats that received the methanol root extract of *Andropogon gayanus* orally did not show signs and symptoms of toxicity after the first four hours of the extract administration. The oral median lethal dose for both mice and rats was found to be greater than 5000 mg/kg. However, the intraperitoneal median lethal dose for both rats and mice was found to be about 1265 mg/kg body weight.

4.3 Analgesic studies

4.3.1 Acetic acid-induced writhing test in mice

The methanol root extract of *Andropogon gayanus* significantly ($p< 0.01$) decreased the number of writhes caused by acetic acid in a non-dose-dependent manner (Table 4.3). Out of the three dose levels of the extract tested, the highest percentage inhibition of writhes (59.54%) was obtained at 500 mg/kg, and the effect of the extract at all doses tested was found to be less than that of the standard (Aspirin) at 300 mg/kg.

Table 4.3: Effect of Methanol Root Extract of *A. gayanus* on Acetic Acid Induced Writhing in Mice

Treatment (mg/kg)	Average number of writhes	Percentage Inhibition (%)
D/Water (10 ml/kg)	28.00 ± 2.21	-
MEAG 250	14.67 ± 2.62**	47.61
MEAG 500	11.33 ± 2.06***	59.54
MEAG 1000	13.67 ± 2.56***	51.18
ASA 300	6.33 ± 0.84***	77.40

Values are presented as Mean ± SEM, * = $p< 0.05$, ** = $p< 0.01$, *** = $p< 0.001$ compared to Distilled water (D/Water) group – One way ANOVA followed by Dunnett's t- test n = 6, ASA = Acetylsalicylic acid (aspirin), MEAG = Methanol root extract of *Andropogon gayanus.*

4.3.2 Hot plate test in mice

The methanol root extract of *A. gayanus* significantly ($p< 0.05$) increased the reaction time at different doses (Figure 4.1). The peak of activity was recorded at 120 min where all doses of the extract tested were able to significantly ($p< 0.05$) increase the reaction time compared to control. Morphine 10 mg/kg as expected, significantly ($p< 0.05$) increased the reaction time at all time levels except at 150 min.

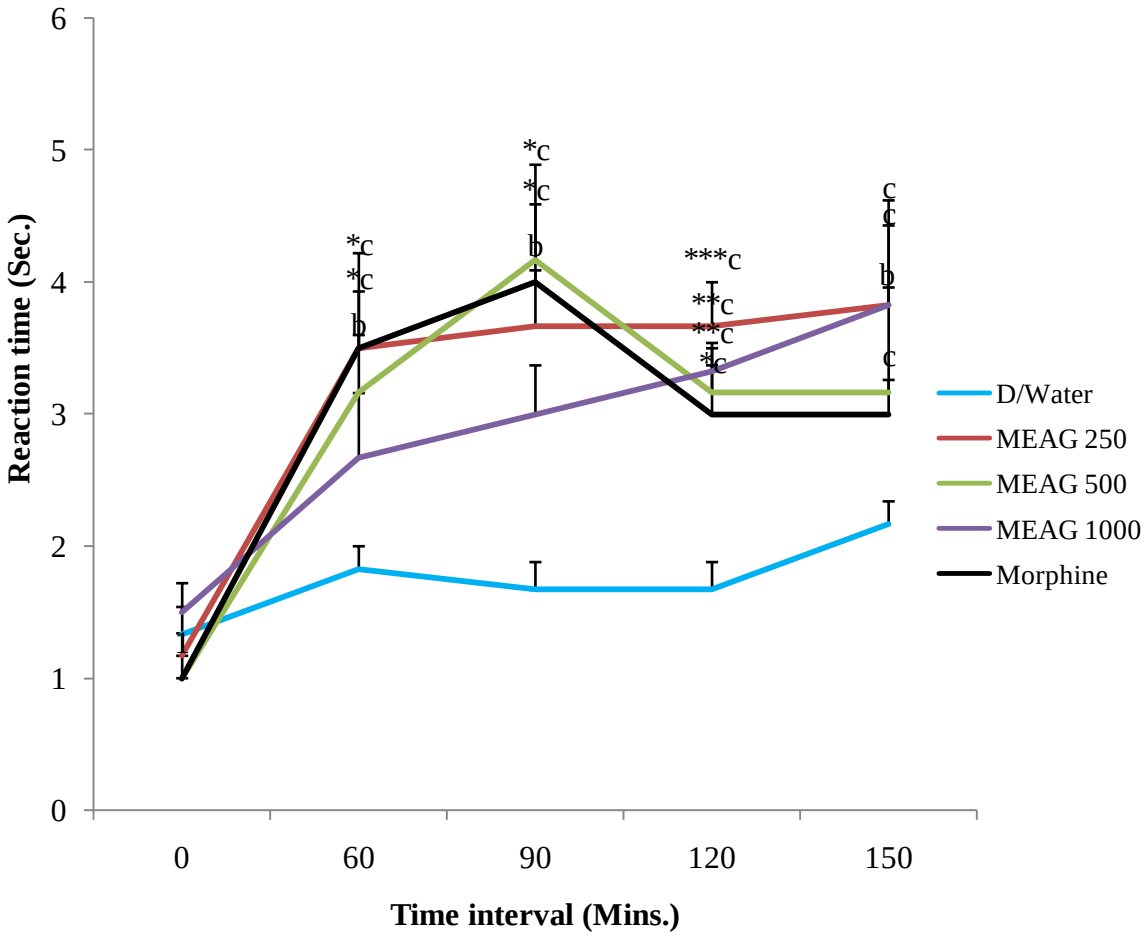


Fig. 4.1: Effect of Methanol Root Extract of *A. gayanus* on Hot Plate Induced Pain in Mice

Values are presented as Mean ± SEM, * = $p< 0.05$, ** = $p< 0.01$, *** = $p< 0.001$ compared to Distilled water (D/Water) group – Repeated measures ANOVA followed by Dunnett's t- test; a, b, and c = $p< 0.05$, $p< 0.01$ and $p< 0.001$ respectively compared to reaction time 0. n = 6, MEAG = Methanol root extract of *Andropogon gayanus.*

4.3.3 Formalin-induced pain test in rats

During both phases of formalin test, the extract at 250 and 500 mg/kg showed insignificant reduction in pain response. Morphine in phase 1 and 2 significantly ($p< 0.01$ and $p< 0.001$ respectively) reduced the severity of pain when compared to the negative control (distilled water). Similarly, the extract at 1000 mg/kg significantly ($p< 0.05$ and $p< 0.01$) decreased pain severity in phase 1 and phase 2 respectively (Table 4.4).

Table 4.4: Effect of Methanol Root Extract of *A. gayanus* on Formalin-Induced Pain in Rats

Treatment (mg/kg)	Mean Pain Scores	
	Phase 1	**Phase 2**
D/Water 1 ml/kg	3.00 ± 0.00	3.00 ± 0.00
MEAG 250	2.17 ± 0.48	2.17 ± 0.54
MEAG 500	2.50 ± 0.22	2.50 ± 0.22
MEAG 1000	2.00 ± 0.23*	1.50 ± 0.22**
Morphine 10	1.67 ± 0.33**	0.83 ± 0.31***

Values are presented as Mean ± SEM, * = $p< 0.05$, ** = $p< 0.01$, *** = $p< 0.001$compared to Distilled water (D/Water) group – Non parametric Kruskal wallis. n = 6, MEAG = Methanol root extract of *Andropogon gayanus.*

4. 4 Anti-Inflammatory Studies

4.4.1 Carrageenan-induced paw oedema test in rats

The sub-plantar injection of 1% carrageenan produced a local oedema in all groups tested with the peak of inflammation at the 3rd hour. The extract as well as aspirin were able to reduce inflammation induced by carrageenan throughout the period of study although the difference in the oedema index was not statistically significant at all times when compared to the negative control. The extract at a dose of 1000 mg/kg and aspirin were able to significantly ($p< 0.05$) reduce inflammation at the 1st hour when compared to distilled water group. At the 5th hour, the extract at all doses tested and aspirin significantly ($p< 0.01$ and $p< 0.001$ respectively) reduced inflammation (Fig. 4.2).

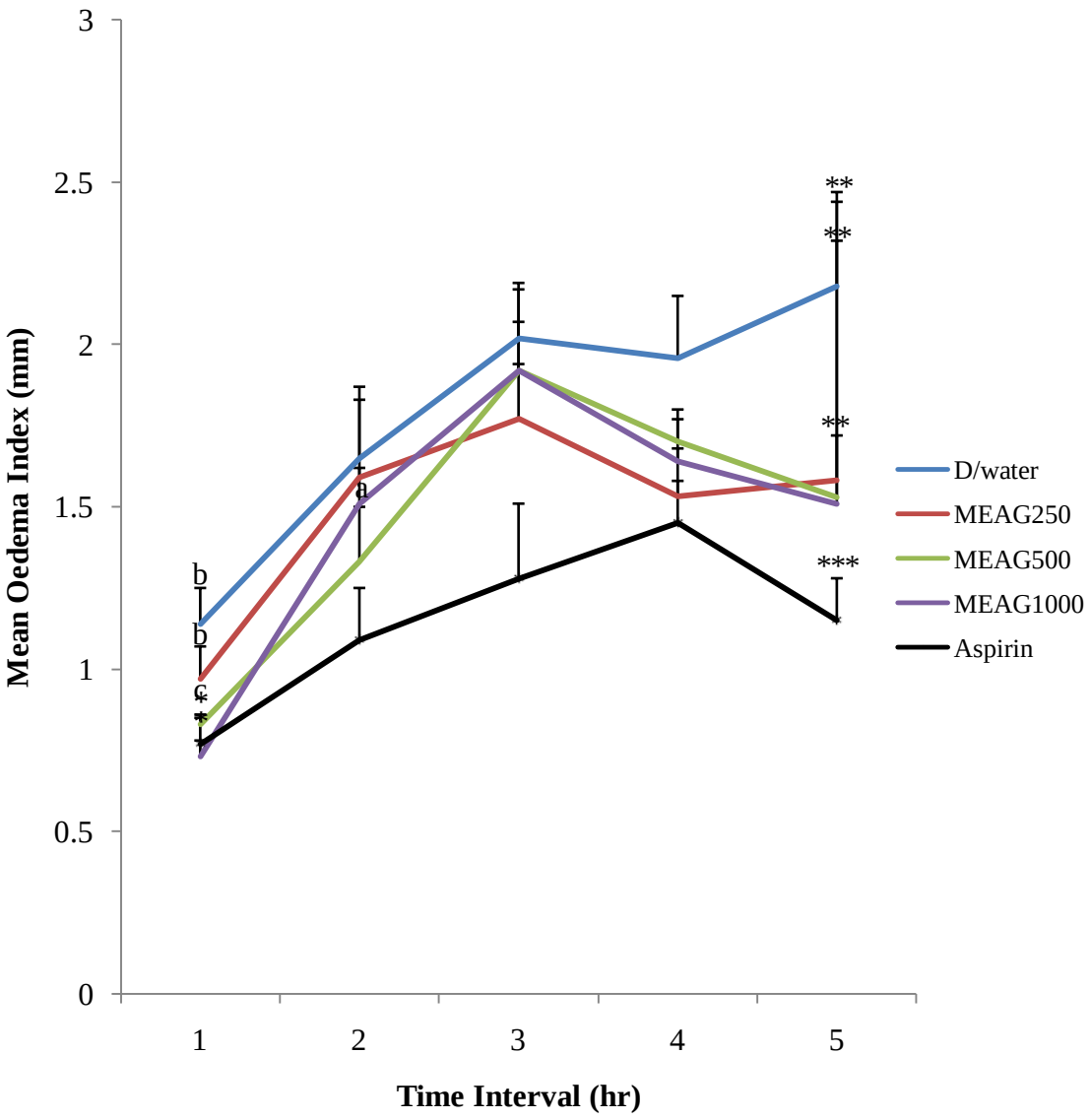


Figure 4.2: Effect of Methanol Root Extract of *A. gayanus* on Carrageenan-induced Rat Paw Oedema

Values are presented as Mean ± SEM, * = $p< 0.05$, ** = $p< 0.01$, *** = $p< 0.001$ compared to Distilled water (D/Water) group; a, b, and c = $p< 0.05$, $p< 0.01$ and $p< 0.001$ respectively compared to time 3 hr – Repeated measures ANOVA followed by Bonferroni- test,. n = 6, MEAG = Methanol root extract of *Andropogon gayanus*.

4.5 Mechanistic Studies

4.5.1 Effects of naloxone co-administration with methanol root extract of *A. gayanus* on acetic acid-induced writhing test in mice

Injection of 10 ml/kg of 0.6% $^{v}/_{v}$ acetic acid intraperitoneally caused abdominal pain evident by writhes observed in all the groups with the negative control showing the highest number of writhes. Morphine and the extract were able to significantly ($p< 0.001$) decrease the number of writhes compared to control group. Naloxone when separately interacted with both morphine and the extract, also decreased the number of writhes as compared to the control group. There was a statistically significant ($p< 0.01$) difference between the number of writhes in morphine-alone group and in naloxone + morphine group. Similarly, a statistically significant ($p< 0.05$) difference was found between the number of writhes in the extract-alone group and naloxone + extract group (Fig. 4.3).

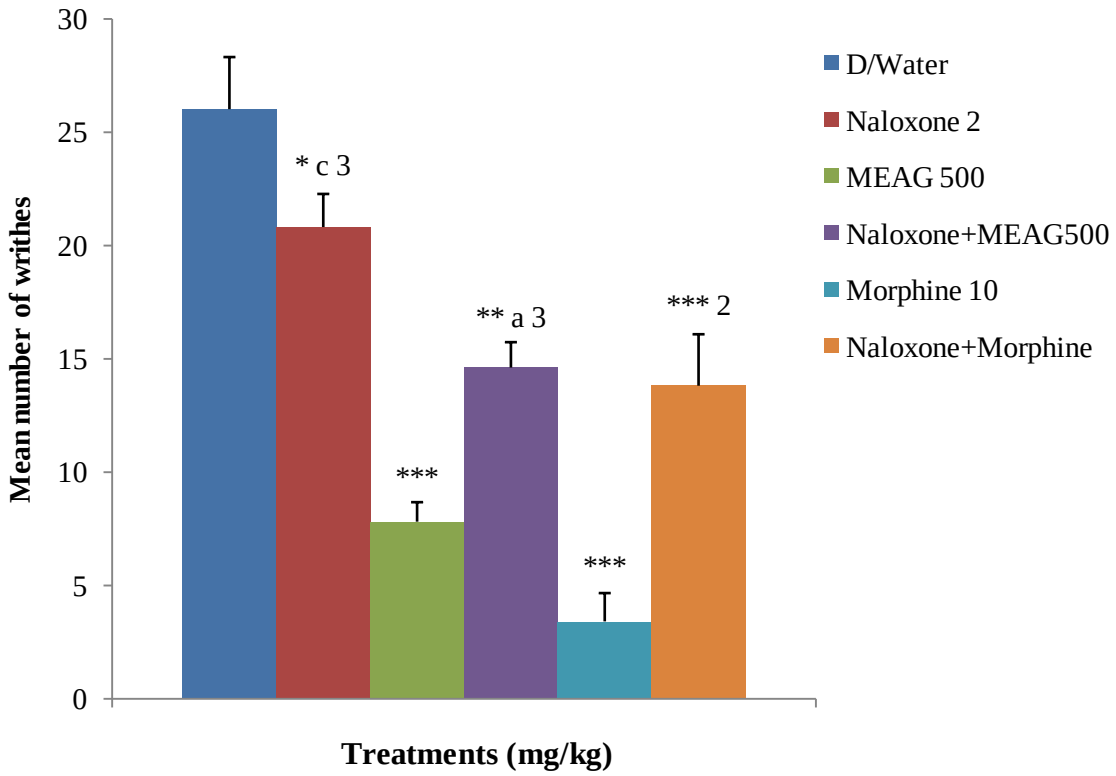


Fig. 4.3: Effects of Naloxone Co-administration with Methanol Root Extract of *A. gayanus* on Acetic Acid-Induced Writhing in Mice

Values are presented as Mean ± SEM, * = $p< 0.05$, ** = $p< 0.01$, *** = $p< 0.001$ compared to Distilled water (D/Water) group – One way ANOVA followed by Tukey *post hoc* test a, b and c = $p< 0.05$, $p< 0.01$ and $p< 0.001$ respectively compared to MEAG500 group and 1, 2 and 3 = $p< 0.05$, $p< 0.01$ and $p< 0.001$ respectively compared to morphine group. n = 5, MEAG = Methanol root extract of *Andropogon gayanus*

4.5.2 Effects of glibenclamide co-administration with methanol root extract of *A. gayanus* on acetic acid-induced writhing test in mice

Injection of 10 ml/kg of 0.6% $^{v}/_{v}$ acetic acid intraperitoneally caused abdominal pain evident by writhes observed in all the groups. The extract when given alone and in combination with the blocker (glibenclamide) was able to significantly ($p< 0.001$) decrease the number of writhes compared to the control group. The % inhibition of pain found in glibenclamide + extract was higher (77.15 %) than that of the extract alone (67.89 %). There was a statistically insignificant difference in number of writhes between the extract alone group and extract + glibenclamide group.

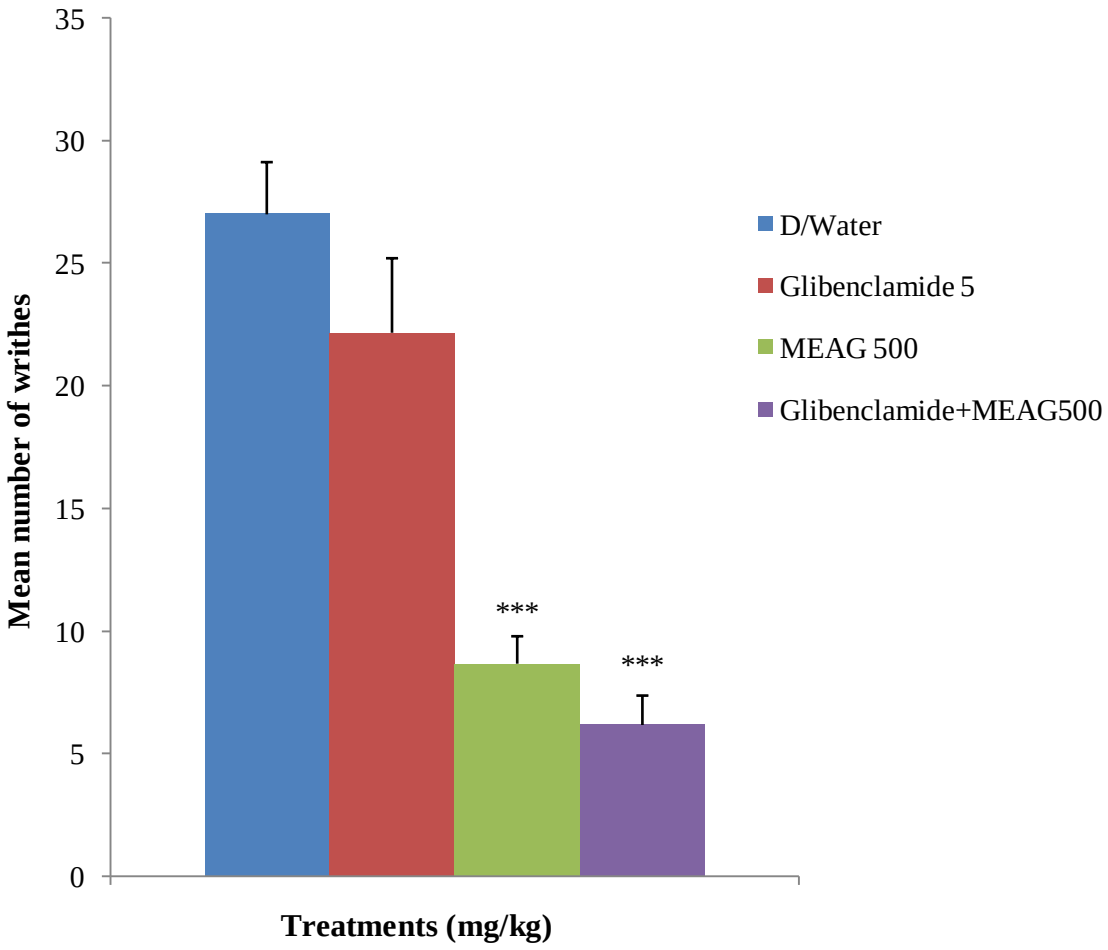


Fig. 4.4: Effects of Glibenclamide Co-administration with Methanol Root Extract of *A. gayanus* on Acetic Acid-Induced Writhing in Mice

Values are presented as Mean ± SEM, * = $p< 0.05$, ** = $p< 0.01$, *** = $p< 0.001$ compared to Distilled water (D/Water) group – One way ANOVA followed by Tukey *post hoc* n = 6, MEAG = Methanol root extract of *Andropogon gayanus.*

4.5.3 Effects of yohimbine co-administration with methanol root extract of *A. gayanus* on acetic acid-induced writhing test in mice

Injection of 10 ml/kg of 0.6% $^{v}/_{v}$ acetic acid intraperitoneally caused abdominal pain evident by writhes observed in all the groups. The extract when given alone and in combination with a blocker (yohimbine) was able to significantly at ($p< 0.001$) decrease the number of writhes compared to distilled water group. The % inhibition of pain found in yohimbine + extract was higher (74.70 %) than that of the extract alone (67.89 %). There was a statistically insignificant difference in number of writhes between the extract alone group and extract + yohimbine group.

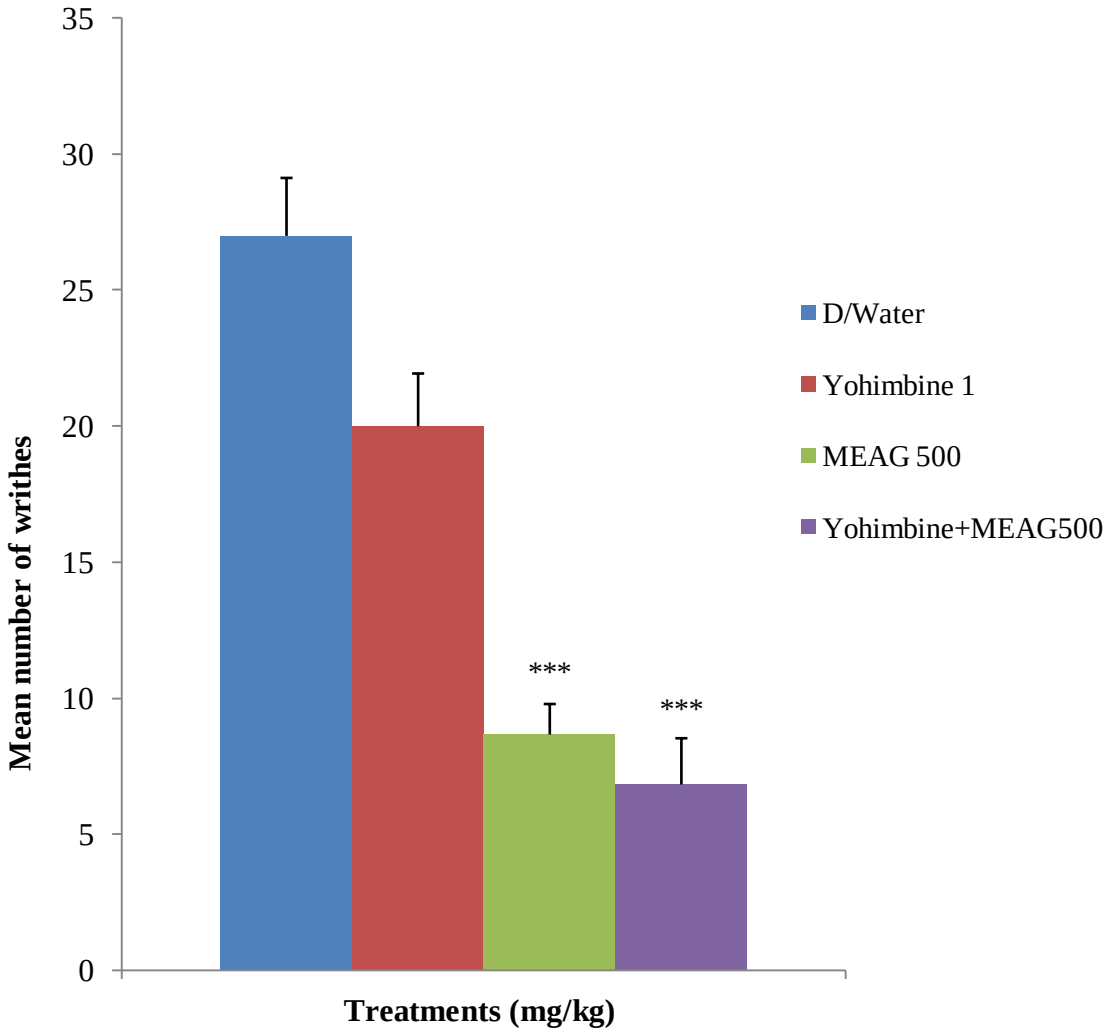


Fig. 4.5: Effects of Yohimbine Co-administration with Methanol Root Extract of *A. gayanus* on Acetic Acid-Induced Writhing in Mice

Values are presented as Mean ± SEM, *** = p< 0.001 compared to distilled water (D/Water) group – One way ANOVA followed by Tukey *post hoc* test n = 6, MEAG = Methanol root extract of *Andropogon gayanus*.

CHAPTER FIVE

5.0 DISCUSSION

The preliminary phytochemical screening and thin layer chromatography of the methanol root extract of *Andropogon gayanus* revealed the presence of some secondary metabolites including alkaloids, flavonoids, tannins, triterpenes, saponins, glycosides, cardiac glycosides, anthraquinones among others. These constituents are known to be responsible for several pharmacological activities. Saponins have been shown to possess anti-inflammatory and anti-allergic effects (Yassin *et al*., 2013), as well as antibacterial activity (Soetan *et al.,* 2006). Saponins and glycosides are also known to possess anti-inflammatory and anti-nociceptive activities (Akkol *et al*., 2007). Flavonoids were reported as prostaglandin synthetase inhibitors (Watanebe *et al.,* 2000). Prostaglandins are known to be involved in pain perception (Helms and Barone, 2008) which suggests that reduced availability of the prostaglandins by flavonoids might have been responsible for their analgesic activity. Also certain flavonoids possess potent inhibitory activity against many enzymes such as protein kinase C, protein tyrosine kinases, phospholipase A_2 and phosphodiesterases (Middleton, 1998). Alkaloids have been shown to exhibit anti-inflammatory, antioxidant, antidepressant, anticancer, anti-diarrheal, hepatoprotective and antimicrobial activities (Singh *et al.,* 2010). There are also reports on alkaloidal analgesic effects alone (Reanmongkol *et al.,* 2005), and in combination with saponins (Choi *et al.,* 2005, Arrau *et al*., 2010). Tannins are also used in the treatments of cuts and wounds, haemorrhoids, varicose veins and also in diarrhoea, catarrh, heavy menstrual flows and inflammatory conditions of the digestive tract (Evans, 1989). Thus, the antinociceptive and anti-inflammatory effects exhibited by the plant *Andropogon gayanus* may be due to the saponins, flavonoids, alkaloids, and tannins found present in the plant individually or in combination.

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 toxicological 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). Lorke (1983) postulated, classified chemical toxicity based on LD_{50} values as follows; LD_{50} < 1 mg/kg, substance is highly toxic, LD_{50} < 5 mg/kg, substance is toxic, LD_{50} < 100 mg/kg, substance is moderately toxic, LD_{50} < 1000 mg/kg, substance is slightly toxic while LD_{50} > 5000 mg/kg, substance is practically non-toxic. Acute toxicity studies of *Andropogon gayanus* using Lorke's method (1983) revealed LD_{50} (*p.o*) value of greater than 5000 mg/kg in both rats and mice which is non-toxic but slightly toxic intraperitoneally (1264 mg/kg) in both rats and mice. The extract was non-toxic in both species of animal orally but slightly toxic intraperitoneally probably due to bioavailability difference related to the routes of administration. This may be responsible for the non-toxic effect when the extract was administered orally.

Methanol root extract of *A. gayanus* significantly (p< 0.05) reduced the acetic acid induced writhing in mice in a non-dose dependent manner. The acetic acid induced writhing test was thought to be a peripheral pain model which is generally used for screening plants and new agents for analgesic properties (Gene *et al.,* 1998). However, it has also been shown that the acetic acid test is a non-specific nociceptive model (Bighetti *et al*., 1999) and is now thought to involve several pathways for nociception including the central and peripheral. The intra-abdominal injection of acetic acid leads to the release of pain mediators such as prostaglandin and cytokines which may be responsible for the induced pain (Ikeda *et al*., 2001). Thus, activity shown by the extract suggest that analgesic effects may be due to their inhibitory action on visceral receptors sensitive to

acetic acid, or production of algogenic substances, or at the central level of painful messages transmission or combination of all the three mentioned above. The data presented suggests that the methanol root extract of *A. gayanus* possess analgesic property which is mediated through central or peripheral pathways. The extract at all doses was shown to have analgesic property which is not a function of the doses as evidenced in the model used. 500 mg of the extract showed the best activity among the three doses tested, but the percentage inhibition of pain (59.54 %) was far less than that of Aspirin (77.40 %). This could be due to the fact that crude extract was used for the study. Probably when the constituent(s) responsible for the activity is/are isolated, lower doses might give better activity and the activity might even be dose dependent.

Hot plate method as described by Eddy and Liembach (1953) is the most common thermal nociception model used for evaluating central analgesic efficacy of drugs or compounds. This thermally induced pain model is utilized as a standard method for the evaluation of centrally mediated analgesia such as narcotic agents like morphine, pentazocine and codeine (Leonard *et al.*, 2006). The prolongation of the reaction time by the methanol root extract of *Andropogon gayanus* at all doses tested means the extract possesses analgesic property. The data presented suggests that the analgesic property shown by the extract was comparable to that of morphine, suggesting that the mechanism by which the extract and morphine exact their analgesic property might be similar. Morphine alleviates pain by acting on the pain receptors in the brain or by activating the opioid receptors in the central nervous system (Yousuf *et al.*, 2013).

Formalin test is a well established valid model for the study of central sensitization events at the spinal level and peripheral inflammatory pain (Diaz and Dickeson, 1997).

The two distinct phases in formalin test are due to direct effect of formalin on nociception (which are transmitted via C fibres and can be suppressed by opioids such as pentazocine) (Sayyah *et al*., 2004), and due to inflammation with the release of serotonin, histamine, bradykinin and prostaglandins and at least to some degree, the sensitization of central nociceptive neurons (thus sensitive to NSAIDs such as ketoprofen and which can also be blocked by opioids) (Dubuisson and Dennis, 1977; Huskaar and Hole, 1987; Tjølsen *et al.*, 1992). In the test, the extract showed a significant ($p< 0.05$) decrease in pain response in a non-dose dependent manner in both phases. However, the decrease in pain response is only statistically significant at the highest dose (1000 mg/kg) of the extract and morphine. This investigation reveals that, the extract has analgesic effect on both peripherally and centrally mediated pain in rats.

Carrageenan induced rat paw oedema is a well established animal model for evaluating the anti-oedematous effect of drugs or compounds (Sharma *et al.,* 2010). Formation of oedema caused by carrageenan is said to be in two phases; the first hour after carrageenan injection (first or early phase), involves the release of serotonin, histamine and bradykinin while the second or late phase (2–5 hours) with increased oedema formation that remains up to the fifth hour involves the release of prostaglandins (Khan *et al.,* 2009). The second phase of swelling not only involves the elevated production of prostaglandins, but has also been attributed to the induction of inducible cyclooxygenase (COX-2) in the hind paw (Nantel *et al.,* 1999). The result obtained from this study shows that oedema induced by carrageenan was inhibited by both the standard (aspirin) as well as the extract at all doses tested during the 5 hrs of the studies although not all inhibitions were statistically significant. Highest inhibition came up only at the fifth hour which was statistically significant ($p< 0.01$ and $p< 0.001$) for all doses of the extract tested and the

standard respectively. This indicates that the extract may not have activity on the early phase of inflammation (2-4 hours), thus it may act by inhibiting the release of histamine and other mediators as well as inhibiting the release of prostaglandins. Aspirin acts by inhibiting the activity of the enzyme cyclooxygenase (COX) which leads to the formation of prostaglandins (PGs) that cause inflammation, swelling, pain and fever (Vane and Botting, 2003).

The result obtained from the mechanistic studies using acetic acid induced writhing test indicates that both morphine and the extract have the ability to block the abdominal constriction caused by acetic acid evident by the statistically significant ($p< 0.001$) reduction in number of writhes compared to distilled water group and a percentage protection of 86.92 % and 70 % respectively. The studies also demonstrates that the anti-nociceptive effects of both morphine and the extract are related to their actions on the opioid receptors found in the central nervous system as seen in the reduction of their analgesic effects when interacted with an opioid receptor blocker (naloxone). There was a reduction in the analgesic effects of both morphine and the extract which are statistically significant ($p< 0.01$ and $p< 0.05$ respectively) when compared to the groups interacted with naloxone. The rats were pretreated with naloxone in groups 4 and 6 to confirm and verify the role of opioid receptors in the anti-nociceptive effects of morphine and the extract respectively. Naloxone is a classical non-selective opioid receptor antagonist (Rangel *et al.*, 2012) and it interferes with the anti-nociceptive effects of the extract suggesting that opioid receptors are involved in the analgesic effect of the extract.

Adenosine triphosphate-dependent potassium ion channels (K^+_{ATP}) are widely distributed in the central and peripheral nervous system (Yamada and Inagaki, 2005) and are involved in many different neuronal activities, such as nuero-protection, control of neurotransmitter release and regulation of membrane excitability (Miki and Seino, 2005). Interestingly, opening of K_{ATP} channels and the consequent cellular hyperpolarization are involved in the anti-nociceptive effects of drugs with dissimilar mechanism of action, such as ketorolac and baclofen (Lazaro-Ibanez *et al.,* 2001). In this study, glibenclamide (a K_{ATP} channel blocker) did not attenuate the anti-nociceptive effect of the extract significantly, suggesting that there is no involvement of the K_{ATP} pathway in the analgesic effect of the extract.

It has been documented that the activation of alpha-2 adrenergic receptor (α_2-AR) of both spinal and supraspinal nuclei is involved in the anti-nociceptive action of α_2-AR agonists e.g clonidine. Activation of α_2-ARs reduces the release of pronociceptive neurotransmitters, such as substance P and glutamate from primary afferent terminals (Millan, 2005) and hyperpolarizes spinal interneurons via G-protein-mediated activation of K^+ channels (Sonohata *et al.,* 2004). Blockage of α_2-AR will result in the inhibition of anti-nociceptive effects of any analgesic drug or compound that acts through this pathway. The result of the studies conducted revealed no statistically significant difference in the anti-nociceptive effect of the extract when interacted with yohimbine (an α_2 –AR blocker), suggesting there is little or no involvement of the α_2-AR in the anti-nociceptive effects of the extract.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Findings from this work showed that the methanol root extract of *Andropogon gayanus* Kunth possesses significant analgesic and anti-inflammatory activities, with the analgesic activity possibly being mediated via the central opioid receptors.

6.2 Recommendations

Based on the findings of this research work, the following recommendations for future work are proposed:

1. Detailed toxicological screening should be conducted to ascertain the complete safety profile of the plant.
2. Further studies should be carried out on the plant using other solvents (nonpolar) such as petroleum ether and hexane to explore the non-polar components of the plant.
3. Studies should be carried out to isolate, characterize and elucidate the structure of the bioactive constituents responsible for the observed pharmacological effects.
4. The exact mechanism of action and pharmacokinetic studies of the possible active compounds should be carried out.

REFERENCES

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.

Adjanohoun, E.M., Ahyi, R.A., Ake Assi, L.K., Dramane, J. A., Elewude, S.O., Fadoju, Z.O., Gbile, E., Goudote, C.L., Johnson, A.A., Keita, O., Morakinyo, J.A., Ojewole, A.O. and Olatunjia, E.A. (1991). Contribution to ethnobotanical and floristic studies in Western Nigeria. CSTR-OUA. *PHARMEL* 2(10) p. 420.

Akkol, E.K., Tatli, I.I. and Akdemir, Z.S., (2007). Antinociceptive and anti-inflammatory effects of saponin and iridoid glycosides from *Verbascum pterocalycinum* var. mutense Hub.-Mor. Zeitschrift fur Naturforschung - Section C. *Journal of Biosciences*, 62(11-12), 813–820.

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.

Badizadegan, D. (2003). Inflammation. *Principle and practice of Human Pathology*: HST: 035. Spring. Harvard MIT Division of Health Sciences and Technology.

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.

Bighetti E.B.J., Hiruma-Lima C.A., Gracioso, J.S., Arm, S.B. (1999). Anti-inflammatory and antinociceptive effects in rodents of the essential oil of *Croton cajucara* Benth. *Journal of Pharmacy and Pharmacology*, 51(12), 1447-1453.

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.

Brain, K.R. and Turner T. D. (1975). The Practical Evaluation of Phytopharmaceuticals. Wright Scientechnica, Bristol Britain. pp. 152 – 158.

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.

Chair, T. N., Allen S., Barker C., Clayton S., Day R., Henson C., Hester J., Knaggs R., Watson, P. and Williams A.C. (2010). Understanding and managing pain: Information for patients. *The British Society for Pain*. Churchill House 35 Red Lion Square London WC1R 4SG.

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), 122-145.

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.

Coulehan, J.L. (1980). "Navajo Indian Medicine: Implication for Healing." *The Journal of Family Practice*, 10(1), 55-61.

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

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.

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.

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.

Diaz, A. and Dickenson, A.H. (1997). Blockade of spinal N- and P-type but not L-type calcium channels inhibits the excitability of rat dorsal horn neurons produced by subcutaneous formalin inflammation. *Pain,* 69, 93-100.

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

Dray A. (1995). Inflammatory mediators of pain. *British Journal of Anaesthesia*; 75: 125-131.

Dubuisson, D. and Dennis, S.R. (1977). The formalin test: A quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain*, 4: 161-174.

Eddy N.B, and Leimback D. (1953). Synthetic analgesic. II. Dithienylbutenyl and dithienybutylamines. *Journal of Pharmacology and Experimental Therapeutics*, 107, 385-402.

Etuk, E.U., Ugwah, M.O., Ajagbonna, O.P. and Onyeyili, P.A. (2009). Ethnobotanical survey and preliminary evaluation of medicinal plants with antidiarrhoea properties in Sokoto state, Nigeria. *Journal of Medicinal Plants Research*. 3(10), pp. 763-766

Evans W.C. (1989). Trease and Evan's Pharmacognosy. 13th Edn. Bailliere Tindall, London.

Evans W.C. (2002). *Trease and Evans Pharmacognosy* ,15th edn. W.R Saunders, London. pp. 233-336.

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

Ezeonwumelu J.O.C., 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 Byarugaba, F. (2012). Phytochemical screening, acute toxicity, anti-inflammatory and antipyretic studies of aqueous extract of the root of *Flueggea virosa* (Roxb. ex Willd.) in rats. *Internationational Journalof Pharmaceutical and Biomedical Sciences*, 3(3), 128-135.

Feinberg, S., Leong, M., Bertagnolli, A., Keller, K., Pasero, C., Fong, A, and Feinberg, R. (2012). Resource Guide to Chronic Pain Medication and Treatment: Pain types and Chronic pain classification. *American Chronic Pain Association*. pp. 8-11.

Field, H.L., Rowbotham, M. and Baron, R. (1998). Post herpetic Neuralgia: irritable nociceptors and de-afferentation. *Neurobiology of Diseases*, 5 (4), 209-227.

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.

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 Anti-nociceptive 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.

Goldsberg, D.S. and McGee, S.J. (2011). Pain as a global public health priority. *BMC Public Health*, 11, 770.

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), 501-515.

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.

Hasselstrom, J., Liu-Palmgren, J. and Rasjo-Wraak, G. (2002). Prevalence of Pain in General Practice. *European Journal of Pain*, 6 (5), 375-385.

Haxaire C. (1979). Phytothérapie et Médecine Familiale chez les Gbaya-Kara (République Centrafricaine) Thèse de doctorat, Université de Paris, Fac. Pharmacie., p. 320.

Helms, J.E and Barone, C.P. (2008). Physiology and Treatment of Pain. *Journal of American Association of Critical Care Nurse*, 8, 6.

Hotamisligil, G.S. (2006) "Inflammation and metabolic disorders." *Nature*, 444, 860-867.

Hucho, T. and Levine, J.D. (2007). Signaling pathways in sensitization: towards a nociceptor cell biology. *Neuron*, 55(3), 365-376.

Huskaar, S. and Hole, K. (1987). The formalin test in mice: Dissociation between inflammatory and non inflammatory pain. *Pain*, 30, 103-114.

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.

Ikeda, Y., Ueno, A., Naraba, H., Oh-ishi, S., (2001). Involvement of vanilloid receptor VR1 and prostanoids in the acetic acid induced writhing responses in mice. *Life Science*, 69: 2911-2919.

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 and Lange. pp. 472-479.

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.

Katz P., Colwell J., Alguire P., and Preodor M. (2008). Chronic pain management: An appropriate use of opioid analgesics. *American college of Physicians.* Independence Mall West, 19106-1572. Philadelphia, PA.

Khan H., Saeed M., Gilani A.U.H., Khan M.A., Dar A., and Khan I. (2010). The antinociceptive activity of Polygonatumverticillatum rhizomes in pain models. *Journal of Ethnopharmacology,* 127(2), 521–527.

Khan I., Nisar M., Ebad F., Nadeem S., Saeed M., and Khan H. (2009). Anti-inflammatory activities of Sieboldogenin from *Smilax china* Linn.: Experimental and computational studies. *Journal of Ethnopharmacology*, 121(1), 175–177.

Khan M.A. and Solomon L.W. (2007). Acute Inflammation/Wound Healing and Repair. *Basic Human Pathology*: Parts I and II. pp. 1-36.

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., Anderson, M., Beer, E.J. (1959). Acetic acid for analgesic screening. *Federation Proceeds*, 18, 412–416.

Kumar B.S.A., Lakshman K., Jayaveera K.K.N., Shekar D.S, Muragan C.S.V, and Manoj, B. (2009). Antinociceptive and Antipyretic Activities of *Amaranthus viridis* Linn in Different Experimental Models. *Avicenna Journal of Medical Biotechnology*, 1(3), 167-171.

Kumar, V., Abbas, A.K., Fausto, N. and Mitchell, R. (2012). Robbins basic pathology. Philadelphia, PA: USA. Elsevier/Saunders.

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.

Lazaro-Ibanez, G.G., Torres-Lopez, J.E. and Granados-Soto V. (2001). Participation of the nitric oxide-cyclic GMP-ATP-sensitive K channel pathway in the antinociceptive action of ketorolac. *European Journal Pharmacology*, 426, 39-44.

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.

Li, L. (2000). Opportunity and challenge of traditional Chinese medicine in face of the entrance to WTO (World Trade Organization). *Chinese Information. Traditional Chinese Medicine*, 7, 7–8.

Lichtenstein, G.R., Hanaeur, S.B. and Sandborn, W.J. (2009). Management of Crohn's Disease in Adults. *American Journal of Gastroenterology*, 104(2), 465-483.

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 Practical Acute Toxicity Testing. *Archives of Toxicology*, 54, 275-287.

Macintyre P.E., Schug S.A., Scott D.A., Visser E.J, and Walker S.M. (2010). APM:SE working group of the Australian and New Zealand College of Anaesthetists and Faculty of Pain Medicine. *Acute Pain Management: Scientific Evidence*. (3rd edition), ANZCA & FPM, Melbourne.

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.

Malgras, D. (1992). Arbres et arbustes guérisseurs des savanes maliennes. Editions Karthala, 22 - 24, boulevard Arago, 75013 Paris, p. 480.

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.

Martin D. (2003). Traditional medicine in contemporary contexts: Protecting and Respecting Indigenous Knowledge and Medicine. *National Aboriginal Health Organization*.

Martin, C., (2013). Inflammation and repair. *Lecture on chronic inflammation*. Retrieved from: http://people.upei.ca/hanna/Inflam8/Inflam-L8-2013.pdf, May 15.

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.

McWhorten J. (1992). "American Indian Medicine." *Southern Medical Journal* 185(6), 625- 627.

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

Merskey H, and Bogduk N. (1994). Classification of Chronic Pain. Descriptions of Chronic Pain Syndromes and Definitions of Pain Terms. 2nd edition. Seattle, WA: IASP Press.

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

Miki, T. and Scino, S. (2005). Roles of K_{ATP} channels as metabolic sensors in acute metabolic changes *Journal of Mol. Cell Cardiol,* 38, 917-925.

Milind P. and Monu Y. (2013). Laboratory models for screening analgesics. *International Research Journal of Pharmacy*, 4(1), 1-19.

Millan, M.J., Dekeyne A. and Newman-Tancredi, A. (2005). A highly potent spiroimidazoline agonist at alpha(2) adrenoceptors: I. Receptor profile, antinociceptive and hypothermic actions in comparison with dexmedetonidine and clonidine. *Journal of Pharmacology and Experimental Therapeutics*, (295), 1192-1205.

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), 0974-2441.

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, R.D.H., Porreca, F. and Cowan, A. (1988). Methodological refinements in the mouse paw formalin test. New animal models of tonic pain. *J. Pharmacological Methods*, 20, 175-186.

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.

Nantel, F., Denis, D., Gordon, R., Northey, A., Cirino, M., Metters, K.M., and Chan ChCh. (1999). Distribution and regulation of cyclooxygenase-2 in carrageenan-induced inflammation. *British Journal of Pharmacology*, 28, 853–859.

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

Percival, M. (1999). Understanding the Natural Management of Pain and Inflammation. *Clinical Nutrition Insight*, 4(99), 1-5.

Perquin, C.W., Hezebroek-Kampschreur, A., Hunchfeld, J.A., Bohnen, A.M., Van Suijlekom-Smith, L.W., Passchier, J. and Vander Wouden, J.C. (2000). Pain in Children and Adolescents: a common experience. *Pain*, 87(1), 55-58.

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.

Prassas, I. and Diamandis, E.P. (2008). Novel Therapeutic Applications of Cardiac Glycosides. *Nature Reviews*, 7, 926-935.

Punchard, N.A., Whelan, C.J. and Adcock, I. (2004). *The Journal of Inflammation*, 1(1), 1-4.

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.

Rajagopal, M.R. (2006). Pain: Basic Considerations. *Indian Journal of Anesthesia*, 50(5): 331-334.

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.

Rangel, R.A., Marinho, B.G., Fernandes P.D., de Moura, R.S. and Lessa, M.A. (2012). Pharmacological mechanisms involved in the antinociceptive effects of dexmedetomidine in mice. *Fundamental and Clinical Pharmacology*, 28, 104-113.

Ray, A. (2001). Physiology and management of acute pain. Hospital Consultants Meeting; New Orleans.

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.

Saadou, M. (1979). Les plantes médicinales du Niger: premier supplément à l'enquête ethnobotanique de. *Rev. Méd. Pharm. Afr*., 3(7), pp. 11 – 24.

Salawu, O.A., Chindo, B.A., Tijani, A.Y., Obidike, I.C., Salawu, T.A. and Akingbasote, A.J. (2009). Acute and sub-acute toxicological evaluation of the methanolic stem bark extract of *Crossopteryx febrifuga* in rats. *African Journal of Pharmacy and Pharmacology*, 3(12), 621-626.

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.

Sayyah, M., Hadidi, N., Kamalinejad M (2004). Analgesic and anti-inflammatory activity of *Lactuca sativa* seed extract in rats. *Journal of Ethnopharmacology*, 92, 325-329.

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.

Singh, A., Duggal, S., Kaur, N. and Singh J. (2010). Berberine: Alkaloid with wide spectrum of pharmacological activities. *Journal of Natural Product*, 3, 64–75.

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

Sodano, W.L. and Grisanti, R. (2010). Functional Medicine University's Functional Diagnostic Medicine Training Program; *Biology and Physiology of inflammation*. Module 5, FDMT 541C.

Soetan, K.O., Oyekunle, M.A., Aiyelaagbe, O.O. and Fafunsho, M.A. (2006). Evaluation of the antimicrobial activity of saponins extract of *Sorghum bicolor* L. Moench. *African Journal of Biotechnology*, 5(23), 2405–2407.

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

Sonohata, M., Furue, H. and Katafuchi, T. (2004). Actions of noradrenaline on substantia gelatinosa neurons in the rat spinal cord revealed by in vivo patch recording. *Journal Physiology*, 555, 515-526.

Spanswick, C.C. and Main, C.J. (2000). Pain Management: Am interdisciplinary approach. Edinburgh: Churchill Livingstone, p. 93.

Stannard C., Collett B., Myles J., Roberts K., Stevenson B and Williams A.C. (2007). Pain and problem drug use: Information for patients. *The British Society for pain.* Churchill House 35 Red Lion Square London WC1R 4SG.

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.

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.

Thomas, L.V. (1972). De l'ethnobotanique à la médecine: l'exemple Diola. *Notes Africaines*, 134, 48-52.

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

Tjølsen, A., Berge, O., Hunskaar, S., Rosland, J.H. and Hole, K. (1992). The formalin test: An evaluation of the method. *Pain,* 52, 5-17.

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.

Vane, J.R. and Botting, R.M. (2003). The mechanism of action of aspirin. *Thrombosis Research*, 110, 255–258.

Verger, P.F. (1995). The use of plants in Yoruba society. Editoria Schwarcz, Sao Paulo. p. 744.

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.

Vogel, G.H. (2008). Ananlgesic, Anti-inflammatory and Antipyretic Activity In: Vogel, G. H. (Ed) *Drug discovery and Evaluation: Pharmacological Assays*, Springer-Verlag Berlin Heidelberg New York, pp. 982-1034.

Vogel, H.G. and Vogel, W.H. (1997). Drug Discovery and Evaluation: Pharmacological assays, springer-verlaag, Berlin 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.

Waldram J. (1990). "Access to Traditional Medicine in a Western Canadian City." *Medical Anthropology*, 12, 325-348.

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

Wassung K. (2012). The role of Inflammation in the Healing process. Planet Chiropractic. pp.2-7

Watanebe T., Takano A., Bista M.S., Saiju H.K. (2000). Intellectual Heritage on folk medicine in Nepal: Proceedings of Nepal- Japan Joint Symposium, Kathmandu, Nepal, 43-49.

WHO. (1996). *Annex II. Guidelines for the Assessment of Herbal Medicines* (WHO Technical Report Series, Geneva, No. 863.

WHO. (1999). *WHO Monographs on Selected Medicinal Plants*, Vol. 1, Geneva.

WHO. (2001). Legal Status of Traditional Medicine and Complementary/Alternative Medicine: A Worldwide Review; Geneva, Switzerland, p. 4.

WHO. (2005). *Traditional Medicine Strategy*, Geneva.

Winter, E.A., Risley, E.A. and Nuss G.W. (1963) *Journal of Pharmacology and Experimental Therapeutics,* 141, 369-373.

Woolf, C.J. (2004). Pain: Moving from Symptom Control toward Mechanism-Specific Pharmacologic management. *Annals of internal medicine*, 140, 441-451.

Yamada, K. and Inagaki, N. (2005). Neuroprotection by K_{ATP} channels. *Journal of Mol. Cell Cardiol.* 38, 945- 949.

Yassin, N.Z., Melek, F.R, Selim, M.A. and Kassem, I.A.A. (2013). Pharmacological activities of saponin-containing fraction derived from *Gleditsia caspica* Desf. Methanolic fruit extract. *Der Pharmacia Lettre*, 5(2), 247-253.

Yousuf, P.M.H., Noba, N.Y., Shohel, M., Bhattacherjee, R. and Das, B.K. (2013). Analgesic Anti-Inflammatory and Antipyretic Effect of *Mentha spicata* (Spearmint). *British Journal of Pharmaceutical Research*, 3(4), 854-864.

APPENDICES

Appendix A

Table: Effect of Methanol Root Extract of *Andropogon gayanus* on Hot Plate-induced-Pain in Mice

Treatment (mg/kg)	RT at 0 min	RT at 60 min	RT at 90 min	RT at 120 min	RT at 150 min
D/Water	1.33 ± 0.21	1.83±0.17	1.67 ± 0.21	1.67 ± 0.21	2.17 ± 0.17
MEAG 250	1.17±0.17	3.50±0.43*^{c}	3.67 ± 0.42^{b}	3.67±0.33***^{c}	3.83±0.79^{c}
MEAG 500	1.00 ± 0.00	3.17 ± 0.17^{b}	4.17±0.79*^{c}	3.17 ± 0.31**^{c}	3.17 ± 0.17^{c}
MEAG1000	1.50±0.22	2.67 ±0.49	3.00 ± 0.37	3.33 ± 0.21**^{c}	3.83 ± 0.60^{c}
Morphine10	1.00 ± 0.00	3.50±0.72*^{c}	4.00±0.89*^{c}	3.00±0.37*^{c}	3.00 ± 0.26^{b}

Values are presented as Mean ± SEM, * = p< 0.05, ** = p< 0.01, *** = p< 0.001 compared to Distilled water (D/Water) group – Repeated measures ANOVA followed by Dunnett's t- test; a, b, and c = p< 0.05, p< 0.01 and p< 0.001 respectively compared to reaction time 0. n = 6, MEAG = Methanol root extract of *Andropogon gayanus*, RT = Reaction time.

Appendix B

Table: Effect of Methanol Root Extract of *Andropogon gayanus* on Carrageenan-induced Rat Paw Oedema

Treatment (mg/kg)	O.I. at 1 hour	O.I. at 2 hours	O.I. at 3 hours	O.I. at 4 hours	O.I. at 5 hours
D/Water 10ml	1.14 ±0.11[b]	1.65 ± 0.18	2.02 ± 0.15	1.96 ± 0.19	2.18 ± 0.14
MEAG 250	0.97 ±0.10[b]	1.59 ± 0.28	1.77 ± 0.17	1.53 ± 0.15	1.58 ±0.14**
MEAG 500	0.83 ±0.02[c]	1.33 ±0.17[a]	1.92 ± 0.15	1.70 ± 0.10	1.53 ± 0.91**
MEAG 1000	0.73 ±0.05*	1.51 ± 0.11	1.92 ± 0.27	1.64 ± 0.13	1.51 ± 0.96**
ASA 300	0.77 ±0.09*	1.09 ± 0.16	1.28 ± 0.23	1.45 ± 0.13	1.15±0.13***

Values are presented as Mean ± SEM, * = p< 0.05, ** = p< 0.01, *** = p< 0.001 compared to Distilled water (D/Water) group; a, b, and c = p< 0.05, p< 0.01 and p< 0.001 respectively compared to time 3 hr – Repeated measures ANOVA followed by Bonferroni- test,. n = 6, ASA = Acetylsalicylic acid, MEAG = Methanol root extract of *Andropogon gayanus*, O.I. = Oedema Index

Appendix C

Table: Effects of naloxone co-administration with methanol root extract of *A. gayanus* on acetic acid-induced writhing in mice

Treatment (mg/kg)	Average number of writhes	Percentage Inhibition (%)
D/Water	26.00 ± 2.30$^{c\ 3}$	-
Naloxone 2	20.80 ± 1.46*$^{c\ 3}$	20.00
MEAG 500	7.80 ± 0.86***	70.00
Naloxone+MEAG500	14.60 ± 1.12**$^{a\ 3}$	43.85
Morphine 10	3.40 ± 1.25***	86.92
Naloxone+Morphine	13.80 ± 2.27***^{2}	46.92

Values are presented as Mean ± SEM, * = p< 0.05, ** = p< 0.01, *** = p< 0.001 compared to Distilled water (D/Water) group – One way ANOVA followed by Tukey post hoc test a, b and c = p< 0.05, p< 0.01 and p< 0.001 respectively compared to MEAG500 group and 1, 2 and 3 = p< 0.05, p< 0.01 and p< 0.001 respectively compared to Morphine group. n = 5, MEAG = Methanol root extract of *Andropogon gayanus*

Appendix D

Table: Effects of glibenclamide co-administration with methanol root extract of *A. gayanus* on acetic acid-induced writhing in mice

Treatment (mg/kg)	Average number of writhes	Percentage Inhibition %
D/Water	27.00 ± 2.13	-
Glibenclamide 5	22.17 ± 3.04	17.89
MEAG 500	8.67 ± 1.12***	67.89
Glibenclamide+MEAG500	6.17 ± 1.20***	77.15

Values are presented as Mean ± SEM, * = p< 0.05, ** = p< 0.01, *** = p< 0.001 compared to Distilled water (D/Water) group – One way ANOVA followed by Tukey post hoc n = 6, MEAG = Methanol root extract of *Andropogon gayanus*

Appendix E

Table; Effects of yohimbine co-administration with methanol root extract of *A. gayanus* on acetic acid-induced writhing in mice

Treatment (mg/kg)	Average number of writhes	Percentage Inhibition (%)
D/Water	27.00 ± 2.13	-
Yohimbine 1	20.00 ± 1.94	25.93
MEAG 500	8.67 ± 1.12***	67.89
Yohimbine+MEAG500	6.83 ± 1.70***	74.70

Values are presented as Mean ± SEM, * = p< 0.05, ** = p< 0.01, *** = p< 0.001 compared to Distilled water (D/Water) group – One way ANOVA followed by Tukey post hoc n = 6, MEAG = Methanol root extract of *Andropogon gayanus*