ANTIPYRETIC, ANTIINFLAMMATORY AND ANTINOCICEPTIVE ACTIVITIES OF FOUR SELECTED KENYAN MEDICINAL PLANTS IN MICE MODELS

SAFARI ZAWADI VICTORIA (B.Sc Biochemistry)

I56/CTY/PT/24941/2013

A Thesis Submitted in Partial Fulfilment of the Requirements for the Award of the Degree of Master of Science (Medical Biochemistry) in the School of Pure and Applied Sciences of Kenyatta University

November, 2016
DECLARATION

I, Victoria Zawadi Safari, declare that this thesis is my original work and has not been presented for a degree in any other university or for any other award.

Signature: [Signature] Date: 7/11/2016

Safari Zawadi Victoria (B.Sc Biochemistry)

I56/CTY/PT/24941/2013

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

SUPERVISORS

Signature: [Signature] Date: 7/11/2016

Prof. Eliud NM Njagi

Department of Biochemistry and Biotechnology,

Nairobi

Signature: [Signature] Date: 7/11/2016

Dr. George Orinda

Department of Biochemistry and Biotechnology,

Nairobi
DEDICATION

This thesis is dedicated to my lovely husband Richard Njuguna and my daughters Jasmine Wanjiru and Jahzara Kanze.
ACKNOWLEDGEMENTS

This research work would not have been possible were it not for the immense contribution of the following people to whom I am very grateful and hereby acknowledge; I recognize with deep appreciation, my supervisors, Prof. Eliud N.M Njagi and Dr. George O. Orinda for their support and tremendous guidance from the start of this work to its conclusive end. They were all available to me for consultations and guidance.

To the staff of animal house, may God bless you immensely for being available to provide me with the necessary laboratory support and equipment.

Sincere thanks go to my husband Richard Njuguna. This is one man whose goodness I need more time to figure out. Together with our daughters, Jasmine Wanjiru and Jahzara Kanze who have been a source of inspiration, may the dear Lord bless you children!

Most importantly I thank God for His great favour upon my life and for allowing me to grow as a holistic individual.
# TABLE OF CONTENTS

DECLARATION .......................................................... Error! Bookmark not defined.
DEDICATION ................................................................... ii
ACKNOWLEDGEMENTS ....................................................... iv
TABLE OF CONTENTS ......................................................... v
LIST OF FIGURES ................................................................ viii
LIST OF TABLES .................................................................... ix
LIST OF APPENDICES .......................................................... xi
LIST OF ABBREVIATIONS AND ACRONYMS ........................................... xii
ABSTRACT ............................................................................ xiii

CHAPTER ONE ......................................................................... 1
INTRODUCTION ........................................................................ 1
1.1 Background Information ...................................................... 1
1.2 Problem Statement ............................................................. 3
1.3 Hypothesis ........................................................................ 4
1.4 Objectives ........................................................................ 4
1.4.1 General objective .............................................................. 4
1.4.2 Specific objectives ............................................................ 4
1.5 Justification ..................................................................... 5

CHAPTER TWO ......................................................................... 6
LITERATURE REVIEW ............................................................. 6
2.1 Pain ................................................................................. 6
2.1.1 Management of Pain .......................................................... 9
2.1.2 Shortcomings of conventional management .......................... 10
2.1.3 Medicinal Plants as Alternative Management ...................... 11
2.2 Inflammation .................................................................... 14
2.2.1 Management of inflammation ............................................. 16
2.2.2 Shortcomings of conventional management ...................... 18
2.2.3 Medicinal Plants as Alternative Management .................................................. 19
2.3 Fever .................................................................................................................. 24
2.3.1 Management of Fever ..................................................................................... 26
2.3.2 Shortcomings of Conventional Management ...................................................... 27
2.3.6 Medicinal plants as Alternative Management ................................................... 27
2.4 Plants used in this Study ..................................................................................... 31
2.4.1 Acacia nilotica .............................................................................................. 31
2.4.2 Urtica dioica ................................................................................................ 32
2.4.3 Aloe volkensii ................................................................................................ 34
2.4.4 Cynanchum vimenale ..................................................................................... 35

CHAPTER THREE ........................................................................................................ 37
MATERIALS AND METHODS .................................................................................... 37
3.1 Collection and Preparation of Plant Materials ...................................................... 37
3.2 Extraction ............................................................................................................ 37
3.3 Preparation of Reagents and Extracts for Bioassay .............................................. 39
3.4 Animal Models .................................................................................................. 39
3.5 Experimental Design ......................................................................................... 39
3.5.1 Determination of antinociceptive Activity ...................................................... 39
3.5.2 Determination of anti-inflammatory activity ................................................... 41
3.5.3 Determination of antipyretic activity ............................................................... 42
3.6 Data management and analysis ......................................................................... 43

CHAPTER FOUR ......................................................................................................... 44
RESULTS .................................................................................................................. 44
4.1 Antinociceptive effects of aqueous bark extract of Acacia nilotica on formalin-induced pain in mice models ................................................................. 44
4.2 Antinociceptive effects of aqueous leaf extract of Aloe volkensii on formalin-induced pain in mice models ................................................................. 46
4.3 Antinociceptive effects of aqueous stem extract of Cynanchum vimenale on
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.4</td>
<td>Antinociceptive effects of aqueous leaf extract of <em>Urtica dioica</em> on formalin-induced pain in mice models</td>
<td>47</td>
</tr>
<tr>
<td>4.5</td>
<td>Anti-inflammatory effects of aqueous bark extract of <em>Acacia nilotica</em> on formalin-induced paw oedema in mice models</td>
<td>51</td>
</tr>
<tr>
<td>4.6</td>
<td>Anti-inflammatory effects of leaf extract of <em>Aloe volkensii</em> on formalin-induced paw oedema in mice models</td>
<td>54</td>
</tr>
<tr>
<td>4.7</td>
<td>Anti-inflammatory effects of stem extract of <em>Cynanchum viminalis</em> on formalin-induced paw oedema in mice models</td>
<td>57</td>
</tr>
<tr>
<td>4.8</td>
<td>Anti-inflammatory effects of aqueous stem extract of <em>Urtica dioica</em> on formalin-induced paw oedema in mice models</td>
<td>60</td>
</tr>
<tr>
<td>4.9</td>
<td>Antipyretic effects of bark extracts of <em>Acacia nilotica</em> on Brewer’s yeast-induced pyrexia in mice models</td>
<td>63</td>
</tr>
<tr>
<td>4.10</td>
<td>Antipyretic effects of aqueous leaf extract of <em>Aloe volkensii</em> on Brewer’s yeast-induced pyrexia in mice models</td>
<td>66</td>
</tr>
<tr>
<td>4.11</td>
<td>Antipyretic effects of stem extract of <em>Cynanchum viminalis</em> on Brewer’s yeast-induced pyrexia in mice models</td>
<td>69</td>
</tr>
<tr>
<td>4.12</td>
<td>Antipyretic effects of leaf extract of <em>Urtica dioica</em> on Brewer’s yeast induced pyrexia in mice models</td>
<td>72</td>
</tr>
</tbody>
</table>

CHAPTER FIVE ..............................................................................................................75
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS........................................75
5.1 Discussion ..............................................................................................................75
5.2 Conclusions ..........................................................................................................76
5.3 Recommendations .................................................................................................86
5.4 Suggestions for further studies ...............................................................................87

REFERENCES .............................................................................................................89
APPENDICES ............................................................................................................104
LIST OF FIGURES

Figure 2.4.1: Photograph showing a plant specimen of *A. nilotica* taken in July 2012 at Naikara, Narok County by Mukundi (2015) .................................................32

Figure 2.4.2: Photograph showing a plant specimen of *U. dioica* plant taken in July 2012 at Naikara, Narok County by Mukundi (2015) .................................33

Figure 2.4.3: Photograph showing a plant specimen of *A. volkensii* taken in July 2012 at Naikara, Narok County by Mukundi (2015) .................................................35

Figure 2.4.4: Photograph showing plant specimen of *Cynanchum viminal* taken in July 2012, at Naikara, Narok County by Mukundi (2015) ............................36
LIST OF TABLES

Table 3.5.1: Treatment protocol for the determination of antinociceptive activity for the aqueous extracts of the four medicinal plants .................................40

Table 3.5.2: Treatment protocol for the determination of anti-inflammatory activity for the aqueous extracts of the four medicinal plants .............................41

Table 3.5.3: Treatment protocol for the determination of antipyretic activity for the aqueous extracts of the four medicinal plants .........................................42

Table 4.1: Antinociceptive effects of aqueous bark extract of Acacia nilotica on formalin-induced pain in mice models ......................................................46

Table 4.2: Antinociceptive effects of aqueous leaf extract of Aloe volkensii on formalin-induced pain in mice models .........................................................47

Table 4.3: Antinociceptive effects of aqueous stem extract of Cynanchum viminale on formalin-induced pain in mice models ..............................................49

Table 4.4: Antinociceptive effects of aqueous leaf extract of Urtica dioica on formalin-induced pain in mice models .........................................................51

Table 4.5: Anti-inflammatory effects of aqueous bark extract of Acacia nilotica on formalin-induced paw oedema in mice models ........................................53

Table 4.6: Anti-inflammatory effects of aqueous leaf extract of Aloe volkensii on formalin-induced paw oedema in mice models ........................................56

Table 4.7: Anti-inflammatory effects of aqueous stem extract of Cynanchum viminale on formalin-induced paw oedema in mice models .............................59

Table 4.8: Anti-inflammatory effects of aqueous leaf extract of Urtica dioica on formalin-induced paw oedema in mice models ............................................63

Table 4.9: Antipyretic effects of aqueous bark extract of Acacia nilotica on Brewer’s yeast-induced pyrexia in mice models ................................................65

Table 4.10: Antipyretic effect of aqueous leaf extract of Aloe volkensii on Brewer’s yeast-induced pyrexia in mice models .................................................68

Table 4.11: Antipyretic effect of aqueous stem extract of Cynanchum viminale on Brewer’s yeast-induced pyrexia in mice models .................................71
Table 4.12: Antipyretic effect of aqueous leaf extract of *Urtica dioica* on Brewer’s yeast induced pyrexia in mice models ..........................................................74
LIST OF APPENDICES

Appendix I: Anti-inflammatory effects of aqueous bark extract of *Acacia nilotica* on formalin–induced paw oedema in mice models.........................................................104

Appendix II: Anti-inflammatory effects of aqueous leaf extract of *Aloe volkensii* on formalin–induced paw oedema in mice models.........................................................105

Appendix III: Anti-inflammatory effects of aqueous stem extract of *Cynanchum viminale* on formalin–induced paw oedema in mice models.................................106

Appendix IV: Anti-inflammatory effect of aqueous leaf extract of *Urtica dioica* on formalin–induced paw oedema in mice models......................................................107

Appendix V: Antipyretic effect of aqueous bark extract of *Acacia nilotica* on Brewer’s yeast-induced pyrexia in mice models.........................................................108

Appendix VI: Antipyretic effect of aqueous leaf extract of *Aloe volkensii* on Brewer’s yeast-induced pyrexia in mice models.........................................................109

Appendix VII: Antipyretic effects of aqueous stem extract of *Cynanchum viminale* on Brewer’s yeast-induced pyrexia in mice models.................................110

Appendix VIII: Antipyretic effect of aqueous leaf extract of *Urtica dioica* on Brewer’s yeast-induced pyrexia in mice models.........................................................111
## LIST OF ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>COX-1</td>
<td>Cyclooxygenase enzyme-1</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclooxygenase enzyme-2</td>
</tr>
<tr>
<td>COX-3</td>
<td>Cyclooxygenase enzyme-3</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>GI</td>
<td>Gastro intestinal</td>
</tr>
<tr>
<td>IL-1a</td>
<td>Interleukin-1a</td>
</tr>
<tr>
<td>IL-1b</td>
<td>Interleukin-1b</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>MS excel</td>
<td>Microsoft excel</td>
</tr>
<tr>
<td>NF-kB</td>
<td>Necrosis factor kappaB</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>Non-steroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>PGs</td>
<td>Prostaglandins</td>
</tr>
<tr>
<td>PGE₂</td>
<td>Prostaglandin E₂</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factors alpha</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
ABSTRACT

Acacia nilotica, Urtica dioica, Aloe volkensii and Cynanchum viminale have been used to manage several diseases including pain, inflammation and fever. However, their efficacy has not been scientifically validated. The aim of this study therefore is to investigate the antinociceptive, antipyretic and anti-inflammatory activities of their aqueous extracts. The plant materials were collected from Loita division, Narok County in Kenya. A total of 240 albino mice with an average weight of 20g were used for this study. Antinociceptive activity was determined by use of formalin–induced writhing test. A writhes was recorded by a stopwatch following the stretching of the abdomen and/or stretching of at least one hind limb. A total of 15 groups with 5 mice per group were considered for determination of antinociceptive activity. Diclofenac was administered as the reference drug. Anti-inflammatory activity was established by a formalin induced inflammation test. Hourly changes in paw sizes and reduction of edema around the paw was determined using a vernier calipers for five hours after extract and drug administration. A total of 15 groups with 5 mice per group were considered for determination of anti-inflammatory activity. Diclofenac was used as the reference drug. Antipyretic activity was carried out using Brewer’s yeast induced pyrexia. Temperatures of each mouse was then determined by thermal probe thermometer rectally at hourly interval for three hours after extract and drug administration. A total of 15 groups with 6 mice per group were considered for determination of antipyretic activity. The positive control group was treated with paracetamol at a dose of 150 mg/kg body weight. The aqueous extracts of Acacia nilotica, Aloe volkensii, Cynanchum viminale and Urtica dioica reduced pain, inflammation and fever mostly at dose 150 mg/kg body weight. Based on these findings it was concluded that the present study has demonstrated the antinociceptive, anti-inflammatory and antipyretic potential of aqueous extracts of Acacia nilotica, Aloe volkensii, Cynanchum viminale and Urtica dioica in albino mice. It will therefore serve as a good bio-resource for generating readily available herbal formulations that are more effective in the treatment of pain, inflammation and fever.
CHAPTER ONE

INTRODUCTION

1.1 Background information

Synthetic drugs have for many years, been effectively used for the treatment of many illnesses (Vasanthi et al., 2012). Traditional plant–derived compounds have also been used as medicine since antiquity, playing an important role in health care, especially in the rural settings where access to modern medicine is limited (Kiringe, 2006; Mahesh and Satish, 2008; Soetan and Aiyelaagbe, 2009; Recio et al., 2012; Vasanthi et al., 2012). Plants have been shown to contain phytochemicals that act as defense systems to combat various diseases (Vasanthi et al., 2012). These bioactive compounds include polyphenols, anti-inflammatory flavonoids and catechins and others that have been shown to reduce risks of major ailments such as cardiovascular disease (Vasanthi et al., 2012), rheumatoid arthritis and inflammatory bowel disease (Recio et al., 2012). These plant derived drugs also have an advantage of minimal side effects compared to synthetic chemicals in both human and livestock therapeutics (van Wyk and Gericke, 2000).

Numerous studies have reported success in validation of medicinal plants towards treatment of various diseases (Elisabetsky et al., 1995; Hosseinzadeh and Younesi, 2002; Yodsaoue et al., 2010; Shilpi et al., 2012). For instance, Mahesh and Satish (2008) demonstrated that methanolic leaf, root and bark extracts from A. nilotica among other selected plant species were effective against various strains of phytopathogenic bacteria
and fungi. Other plant species such as *Acanthus hirsutus* (Harput *et al*., 2011), *Aegiceras corniculatum* (Roome *et al*., 2011) and *Pongamia pinnata* (Srinivasan *et al*., 2003) have also been reported for their antinociceptive, anti-inflammatory and antipyretic activities respectively.

The basic steps in the development and use of plant-derived compounds as medicine entails the identification of plants with medicinal attributes, extraction of the bioactive compounds and validation of the effects of these compounds on the treatment of various illnesses *in vitro* using model animals (Cechinel-Filho, 2012). Further, various solvents including methanol (Harput *et al*., 2011), hexane and ethanol (Roome *et al*., 2011) have been employed in extraction of these phytochemicals from plants.

The final step in preliminary validation of medicinal drugs involves induction of disease or disease-like conditions in suitable model animals and use of the extracted compounds to ascertain their effect on treatment (Shilpi *et al*., 2012). Various methods for induction of pain, fever and inflammation have been extensively investigated. For instance, writhing tests (using a hot plate, formalin or acetic acid) have been used to study antinociception of plant-derived medicinal compounds in mice in various experiments (Hosseinzadeh and Younesi, 2002; Harput *et al*., 2011; Roome *et al*., 2011). Further, formalin-induced rat paw edema and Freund's adjuvant-induced arthritis (Sumithra *et al*.,...
are the commonly used test methods to study anti-inflammatory activities of medicinal plants. To validate the antipyretic effects of plant derived medicinal compounds, the brewers yeast induced pyrexia has been extensively used (Srinivasan et al., 2003). The test animals are then monitored for response to the administered herbal compounds and the data compared to reference groups administered with a well-known drug (as a positive control) for correlation.

1.2 Problem statement

Pain, fever and inflammation are common conditions among human populations. These conditions which are mainly symptoms of major illness have made life unbearable. Studies have implicated the use of modern medicine in adverse side effects that result in chronic and acute inflammation associated with diseases such as arthritis, cancer, diabetes and vascular complications. Furthermore, modern medicine is not capable of providing a cure-all solution against a vast array of human diseases since most of them comprise a single active constituent. Due to these limitations of modern medicine, herbal medicine serves as an alternative. Studies have shown the potential of these four plants in treatment of pain, fever and inflammation hence the need for further research in albino mice.
1.3 Hypothesis

The aqueous extracts of *Acacia nilotica*, *Urtica dioica*, *Aloe volkensii* and *Cynanchum viminale* have no antinociceptive, anti-inflammatory and antipyretic activities in albino mice.

1.4 Objectives

1.4.1 General objective

To determine the antinociceptive, anti-inflammatory and antipyretic activities of *Acacia nilotica*, *Urtica dioica*, *Aloe volkensii* and *Cynanchum viminale* in albino mice.

1.4.2 Specific objectives

i. To determine the anti-nociceptive activity of *Acacia nilotica*, *Urtica dioica*, *Aloe volkensii* and *Cynanchum viminale* in albino mice.

ii. To determine the anti-inflammatory activity of *Acacia nilotica*, *Urtica dioica*, *Aloe volkensii* and *Cynanchum viminale* in albino mice.

iii. To determine the antipyretic activity of *Acacia nilotica*, *Urtica dioica*, *Aloe volkensii* and *Cynanchum viminale* in albino mice.
1.5 Justification

Determination of the efficacy of herbal medicinal extracts against the basic symptoms of disease is imperative. Ascertaining the correct dosage using experimental animals will go a long way in helping avoid overexposure to the active ingredients and hence prevent overdose. Various techniques for extraction of compounds of medicinal importance from plants using suitable solvents exist. This will go a long way in ensuring recovery of anti-inflammatory, antipyretic and anti-nociceptive extracts for use in this study. Furthermore, the use of albino mice as test animals in numerous pharmacological therapeutics validates their use in the experiments herein.
CHAPTER TWO
LITERATURE REVIEW

2.1 Pain

Pain is not easily or satisfactorily defined and therefore is often interpreted as a suffering that results from the perception of painful stimuli. It is a common symptom and it indicates that something is wrong in the body and may give a clue to the nature of disease. Hence, pain is a specific sensation with its own peripheral and central mechanisms independent of other five senses. Pain itself is not a disease; it is by far the most common medical complaint. It is usually perceived as an indication of ill health and most diseases have a component of pain. Pain is a common and distressing feature of many diseases such as tumor, surgical procedures, physical trauma, noxious chemical stimulation (Aliu, 2007). It is mostly a warning signal and primarily protective but excessive pain can lead to other side effects such as sweating, apprehension, nausea and palpitation.

Certain noxious stimuli are painful and reflex movements or behaviors resulting from such stimuli are indicative of a pain threshold (Shetty and Anika, 1982). The stimulus may be thermal, electrical, mechanical or chemical (George et al., 2009). The distribution of pain therefore depends largely on the type of noxious stimuli. For example acetic acid induced writhing reflex is sensitive method for screening peripherally acting analgesics and the response is thought to involve local peritoneal cells and mediated through the prostaglandin pathway (Ronaldo et al., 2000). Others like hot plate and tail flick models
are used to test pains mediated by central activity. In these models the sensory nerves sensitize the nociceptors and there is minimized involvement of endogenous substances such as prostaglandins (Bachlev et al., 2009).

The action of an analgesic may be mediated through both peripheral and central mechanisms. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic descending noradrenergic and serotonergic system (Bensreiti and Sewell, 1983; Headley and Shaughnessy, 1985; Wigdor and Wilcox, 1987). The analgesic effect produced by the drugs may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes, and other endogenous substances that are key players in pain.

Various techniques have been used to measure pain. These techniques employ the induction of pain first then the analgesic agents or activity is screened in animal models. They include, pain-state models using thermal stimuli where heat is a suitable stimulus for activating cutaneous receptors. The source of nociceptive stimulation can be distant from its target or in direct contact with the skin, for example, the tail-flick model using radiant heat/Immersion of the tail in hot water. Others pain-state models use cold-stimuli which are very rarely used though common in animal models of neuropathies (George et al., 2009)
The preferred sites of applying nociceptive mechanical stimuli are the hind paw and the tail. A pressure of increasing intensity is applied to a punctiform area on the hind paw or, far less commonly on the tail. The devices used here permit the application of increasing measurable pressures and the interruption of the test when the threshold is reached. The measured parameter is the threshold (weight in grams) for the appearance of a given behavior. When the pressure increases, the reflex withdrawal of the paw, or a complex movement of the animal to release its trapped limb and finally a vocal reaction is observed (Ronaldo et al., 2000).

Such models include strain gauges, Von-Frey filaments and the inclined-plane test. Such models are sometimes difficult to measure the intensity of the stimulus with precision. Repetition of the mechanical stimulus can produce a diminution or conversely an increase in the sensitivity of the stimulated part of the body, in the latter case, this carries the risk that the tissues may be altered by inflammatory reactions that could call into question the validity of repeated tests. The necessity of applying relatively high pressures, which explains the weak sensitivity of the method and the relatively small number of substances that have been shown to be active by these tests (George et al., 2009).

Other techniques employ the use of an electrical stimuli and chemical stimuli with formalin test being the mostly used. Formalin is injected into the front paw and reaction is recorded as excessive licking and biting of the paw. The term formalin usually means a 37% solution of formaldehyde. A solution of 0.5 to 15% formalin when injected into the
dorsal surface of the rat or mouse forepaw provokes a painful behavior that can be assessed on a four-level scale related to posture: 0 denotes normal posture; 1 denotes the injected paw remains on the ground but not supporting the animal; 2 denotes the injected paw clearly raised; 3 denotes the injected paw being licked, nibbled or shaken. This behavior is recorded with a stopwatch as time in seconds with the duration of pain indicative of intensity of the pain (Bachlev et al., 2009).

2.1.1 Management of pain

Pain itself is not any disease. It is manifested in certain disease or pathological conditions. Use of natural products in the management of pain goes back to thousands of years. Use of poppy seed by various civilizations or the use of willow bark to cure fever led to the isolation of morphine and salicylic acid, respectively (Lorenzo, 2000). These two drugs are still used extensively in modern medical practice. Analgesics are agents which selectively relieve pain by acting in the CNS or by peripheral pain mechanisms without significantly altering consciousness. Analgesics may be narcotic or non-narcotic (Milind et al., 2013).

Non-narcotic analgesics such as aspirin, ibuprofen and paracetamol possess not only anti-inflammatory properties but also antipyretic activity besides analgesic activity. For many of them the mode of action has been elucidated as an inhibition of cyclooxygenase in the prostaglandin pathway (Milind et al., 2013).
Non-steroidal anti-inflammatory drugs (NSAIDs) reduce pain and edema by suppressing the formation of prostaglandins, by inhibiting the activity of the enzyme Cyclooxygenase (COX-1 and COX-2). However, prostaglandins are key mediators of several components of GI mucosal defense, so suppression of synthesis of prostaglandins (PGs) by NSAIDs greatly reduces the resistance of the mucosa to injury as well as interfering with repair processes. Selective COX-2 inhibitors were thought to be the solution to this conundrum as it is required that NSAIDs suppress prostaglandin synthesis at sites of inflammation, and not in the GI tract (Milind et al., 2013).

2.1.2 Shortcomings of conventional management

The control of pain is one of the most important uses to which drugs are put. Pain can be defined as the effect produced in consciousness by the arrival of nerve impulses generated by noxious stimuli in the brain. Drugs, which alter the pain sensitivity or remove pain, are known as painkillers or analgesics.

Non-steroidal anti-inflammatory drugs (NSAID) are used worldwide for the treatment of inflammation, pain and fever, as well as for cardiovascular protection as in the case with aspirin. However, they often produce significant side-effects, which include gastric ulcer, renal damage, bronchospasm and cardiac abnormalities, thus limiting their use (Burke et al., 2006).
Due to having adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap and have little side effects (Burke et al., 2006).

2.1.3 Medicinal plants as alternative management

Drugs of natural origin are an important source for the treatment of many diseases worldwide (Pandima et al., 2003). Traditional medicinal practice, both ancient and modern, has been considered as the key treatment options for the treatment of various human ailments over the centuries (Arora and Kaur, 2007). Medicines from plants origin comprise the major constituents of most indigenous medicines and a large number of modern medicinal preparations contain one or more ingredients of plant origin (Bannerman, 1982).

The use of commonly prescribed analgesic drugs exemplified as opiates and NSAIDs are criticized due to their adverse effects, more over they are not useful in all cases. That is
why new compounds with improved pain management potential with minimum side effects are being sought with urgency (Burke et al., 2006).

The number of chemical compounds, called phytochemicals, found within the plant kingdom is truly vast and their range of activity is equally as great. Some of the phytochemicals found in certain herbs and plants are reported to demonstrate pain and inflammation-reducing properties. Like aspirin, many are presumed to work by blocking the cyclooxygenase and lipoxygenase pathways and possibly by other mechanisms as well (Bannerman, 1982).

Bioflavonoids are a broad class of phytochemicals found largely in citrus fruits, tea, and wine. Examples of medicinal plants that have been used to treat pain include seed extract of *Buchholzia coriacea* commonly known as “wonderful kola”. It belongs to the family *Capparaceae*. The plant was named after R.W Buchholz who worked with some plants in Cameroon in late 19th century (Keay, 1989). The leaves and seeds of *B. coriacea* have been reported to have anthelmintic properties (Keay, 1989; Nweze and Asuzu, 2006). Also the seeds have been shown to have antimicrobial properties (Ezekial and Onyeoziri, 2009). In Nigeria traditional medicine, the seeds have been used for the treatment of cough, irregular menstruation, toothache, waist pain, malaria etc. Also the seeds are grinded and small quantity placed on the fore head for instant relief of headache (Keay, 1989).
Securinega virosa is a low branching, dioecious shrub, or a small tree, distributed throughout Tropical Africa (Dalziel, 1936). The plant has enjoyed wide patronage among the people of Tropical Africa and its efficacy is widely acclaimed (Neuwinger, 1996). The decoction of the leaves and roots is used for abdominal pain in Tanzania while the leave decoction is drunk for fever by the Yorubas of South western Nigeria. The decoction of the leaves with other herbs is used in Northern Nigeria for treatment of painful swellings (Neuwinger, 1996).

Another plant with antinociceptive value is Ruta graveolens L. Ruta graveolens belongs to the Rutaceae family. Native to Europe and commonly grown in South Africa, it is locally known as Rue or the Herb of Grace in English, and “wynruit” or “binnewortel” in Afrikaans. R. graveolens is a woody, evergreen shrub of up to a meter in height with a characteristic aromatic scent. The plant has yellow flowers that are made up of 4 petals each. The leaves are irregularly divided into hairless leaflets that have translucent glands (van Wyk et al., 1997). The leaves can be used fresh or dried for medicinal purposes. Leaf infusions are taken for fever, epilepsy and hysteria. Alcoholic tinctures have been used for respiratory problems and heart diseases. Bruised leaves are used for toothache and earache, while decoctions have been used to ease childbirth (van Wyk et al., 1997; Watt and Breyer-Brandwijk, 1962).

Another plant of great importance is Solanum nigrum. Solanum nigrum (black nightshade) is a medicinal plant member of the Solanaceae family of plants. This family
comprises many genera, well known for their therapeutic properties. In addition to *S. nigrum*, this family includes fruits and vegetables such as potato (*Solanum tuberosum*), tomato, and peppers, ornamental plants such as petunia, and other medicinal plants such as *Atropa belladonna* L. (deadly nightshade), *Datura stramonium* L. (Jimson weed), and *Hyoscyamus niger* L. (black henbane).

*S. nigrum* has been extensively used traditionally to treat various ailments such as pain, inflammation and fever (Acharya and Pokhrel, 2006; Zakaria *et al*., 2006). The plant is also used in the Oriental systems of medicine for various purposes; as an antitumorigenic, antioxidant (Lee and Lim, 2003), anti-inflammatory (Zakaria *et al*., 2006), hepatoprotective (Raju *et al*., 2003), diuretic (Zakaria *et al*., 2006) and antipyretic agent (Zakaria *et al*., 2006). Various compounds have been identified which are responsible for diverse activities.

Other natural antinociceptive agents include ginger, garlic, capsaicin (chili pepper) among others which are widely used in homes for cooking and mainly as spices.

### 2.2 Inflammation

Inflammation is a severe response by living tissue to any kind of injury. There can be four primary indicators of inflammation: pain, redness, heat or warmthness and swelling. When there is injury to any part of the human body, the arterioles in the encircling tissue dilate. This gives a raised blood circulation towards the area (redness) (Burke *et al*.,
Vasoactive chemicals also increase the permeability (increase pore size) of these arterioles which allows blood cells, chemical substance, blood proteins and fluid to accumulate in that region. This fluid accumulation causes swelling and may compress nerves in the area resulting in pain. In addition, prostaglandins, that might also result in ‘irritation’ of the nerves and further contribute to pain.

Prostaglandins act as short-lived localized hormones that can be released by any cell of the body during tissue, chemical, or traumatic injury, and can induce fever, inflammation and pain, once they are present in the intercellular space. Thromboxanes, which are also hormone activators, can regulate blood vessel tone, platelet aggregation, and clot formation to increase the inflammatory response (Nelson et al., 1998; Rehman and Sack, 1999). The inflammatory pathway is a complex biochemical pathway which, once stimulated by injury, leads to the production of these and other inflammatory mediators whose initial effect is pain and tissue destruction, followed by healing and recovery (Fitzgerald, 2004; Harris and von Schacky, 2004). A major component of the inflammatory pathway is called the arachidonic acid pathway because arachidonic acid is immediately released from traumatized cellular membranes. Membrane-based arachidonic acid is transformed into prostaglandins and thromboxanes partly through the enzymatic action of cyclooxygenase (COX) (Hostanska et al., 2002; Fitzgerald, 2004). There are two types of COX enzymes, COX-1 and COX-2. Both the enzymes act similarly, but selective inhibition (as accomplished by selective COX-2 inhibiting NSAIDs) can make a difference in terms of side effects.
Pain, heat, redness, and swelling (dolor, calor, rubor, and tumor) are the classic manifestations of the inflammatory process. Abnormalities of the joints of the spine, associated muscles, tendons, ligaments and bone structural abnormalities can all result in pain and need for neurosurgical consultations. Typically, patients will not require immediate surgical intervention, and therefore require treatments to reduce pain and enhance quality of life activities (Marienfeld et al., 1997).

In animal models, inflammation is diagnosed as paw oedema. In both carrageenan-induced paw oedema and formalin-induced paw oedema, the thickness of the injected paw is measured before and after inflammation occurs and the difference is the value of paw oedema. This thickness is measured with vernier calipers or sometimes with a string which is later transferred on a ruler to obtain the diameter of the paw (Burke et al., 2007).

2.2.1 Management of inflammation

Body defense mechanism, commonly known as inflammation, is a response to many physiological conditions such as infection and thermal and/or physical injuries. Inflammatory response is necessary for the survival against environmental pathogens and harms (Das and Kanodia, 2011). Inflammation is categorized into five cardinal signs which are known as redness, swelling, heat, pain and loss of function (Purnima et al., 2010).
Prostaglandins are produced by the cells which are involved in the production of pain, fever and inflammation. Several enzymes such as cyclooxygenase including COX-1, COX-2, and COX-3 are responsible for the production of prostaglandin. COX-2 is responsible for promoting pain, inflammation and fever by producing the prostaglandin. Hence by inhibiting the cyclooxygenase enzyme, prostaglandin production can be blocked. Non-steroidal anti-inflammatory drugs (NSAIDs) are usually indicated in order to relieve the symptoms. Use of NSAIDs and other opioids, many side effects can occur such as gastric lesion; so the uses of these drugs are not successful.

The use of non-steroidal anti-inflammatory drug (NSAID) medication is still the mainstay of most classically taught clinicians for joint and spine related inflammatory pain, despite their commonly known side effects. Non-steroidal anti-inflammatory drug mechanisms are primarily through interaction with pro-inflammatory cytokines interleukin (IL)-1a, IL-1b, IL-6 and tumor necrosis factor (TNF-α). Increased concentrations of TNF-α is believed to cause the cardinal signs of inflammation to occur (Ghosh et al., 1988). These pro-inflammatory cytokines result in chemo-attractant for neutrophils and help them to stick to the endothelial cells for migration. They also stimulate white cell phagocytosis and the production of inflammatory lipid prostaglandin E2 (PGE₂). NSAID ability to interfere with the production of prostaglandin during the inflammatory cascade is the major mechanism cited for the anti-inflammatory success of these medications (Talalay and Talalay, 2001).
2.2.2 Shortcomings of conventional management

Inflammation is characterized by redness, pain and swelling. Anti-inflammatory drugs are agents that reduce inflammation. It has been found that conventional synthetic NSAIDs accelerate damage and erosion of joint cartilage, advancing the osteoarthritis process. These NSAIDs are also known to cause liver and kidney damage with long-term use (Burke et al., 2006).

The advancement of allopathic medication shifted scientific and general people’s interest from conventional medicinal preparations. However, in recent years, a significant paradigm change has taken place. Attraction has re-focused in traditional medicine, simply because of the higher cost of modern drugs, time and expenditure which is essential to bring a drug to market after proper clinical tests, severe side-effects of a variety of modern drugs, and drug-resistance developing in both microorganisms and parasites. Experimental research have shown that the use of proven natural anti-inflammatory herbal agents have not been shown to cause erosion injury to the intestinal tract, acceleration of cartilage destruction or production of liver and kidney toxicities (Burke et al., 2006). This enables practitioners to use these substances in a safe and responsible way.
The NSAIDs are also known to have adverse effects on kidney function (Farquhar and Kenney, 1997). Dehydration or preexisting chronic renal failure or disease, resulting in stimulation of the renin–angiotensin system, may predispose certain populations to acute renal failure through inhibition of prostaglandin synthesis, which can occur when taking NSAIDs. The National Kidney Foundation asserts that approximately 10% of kidney failures per year are directly correlated to substantial overuse of NSAIDs.

2.2.3 Medicinal plants as alternative management

Despite the progresses in modern medicine, it has been reported that more than 70% of the developing world's population still depends on complementary and alternative systems of medicine, otherwise known as traditional medicine (Shaikh et al., 2005). Some herbs possess anti-inflammatory properties and have the ability to reduce both internal and external swelling and inflammation. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. In Bangladesh there are several indigenous medicinal plants available that have anti-inflammatory capabilities (Shaikh et al., 2005).

Traditional medicinal practice, both ancient and modern, has been considered as the key treatment options for the treatment of various human ailments over the centuries (Arora and Kaur, 2007). Medicines from plants origin comprise the major constituents of most indigenous medicines and a large number of modern medicinal preparations contain one
or more ingredients of plant origin (Bannerman, 1982). The use of commonly prescribed analgesic drugs exemplified as opiates and NSAIDs are criticized due to their adverse effects, more over they are not useful in all cases. That’s why new compounds with improved pain management potential with minimum side effects are being sought with urgency.

*Glinus oppositifolius* (Family Molluginaceae) which is locally known as ‘Gima shak’ in Bangladesh and has been reported in favor of various traditional uses (Burkhill, 1985). Dried stems with leaves of *G. oppositifolius* are used for treating abdominal pain and jaundice, while decoction of fine powder of the aerial parts is used in the treatment of malaria (Diallo *et al.*, 1999). *G. oppositifolius* is also reported to possess wound healing remedy (Debes, 1998) and used as folk medicine by traditional healers for treating joint pain, inflammation, diarrhea, intestinal parasites, fever, boils and skin disorders (Diallo, 2000). A bioactive pectic polysaccharide had been isolated from *G. oppositifolius* which has immunomodulating property (Inngerdingen, 2005). As the plant is traditionally used in the treatment of inflammation and various pains, an attempt has been taken to evaluate the anti-inflammatory and analgesic activity of this plant as there is no scientific and methodical investigation so far been documented in literature concerning its analgesic and anti-inflammatory properties.

*Alafia barteri* (olive), in the family Apocynaceae, is a climbing shrub distributed widely in the tropics. It is valued for its efficacy in the traditional medicine system in Nigeria.
and other African countries, as an anti-inflammatory and fever remedy. The infusion of the leaves and twining stem are used for the treatment of inflammation and fever (Burkill, 1985; Iwu, 1993). The decoction of root and leaves of the plant is also taken internally or applied externally to treat rheumatic pain, toothache and eye infections (Odugbemi, 2008). The extracts of the leaves were found to have antibacterial and antifungal activities (Adekunle and Okoli, 2002; Hamid and Aiyelaagbe, 2011). The aqueous leaf extract was reported to display potent antiplasmodial activity (Lasisi et al., 2012).

In Malaysia, Labisia pumila (Blume) F. Vill-Naves is one of the most popular traditional medicinal plants known to have good effect on women’s health. It is a small sub-herbaceous plant with creeping stems from the genus of Labisia that belongs to the family Myrsinaceae. L. pumila is commonly found in shady areas at 80 to 100 m elevations above mean sea level (Burkill, 1935) and its natural distribution covers most areas of the South East Asian region including Malaysia, Thailand and Indochina. It is locally known as “Kacip Fatimah” and sometimes also referred as “Selusoh Fatimah”, “Rumput Siti Fatimah”, “Akar Fatimah”, “Tadah matabhari”, “Bunga belangkas hutan” and “Pokok pinggang”. The species has three varieties in Malaysia, namely, L. pumila var. alata, L. pumila var. pumila and L. pumila var. lanceolata (Stone, 1989). L. pumila has been commonly linked and acknowledged to be effective in curing many ailments such as post-partum treatment, anti-flatulence, rheumatism and preventing osteoporosis caused by post menopause (Jamia et al., 2003; Nadia et al., 2012). The values of this
species as protection from disease are largely influenced by its phytoestrogen, anti-inflammatory and antioxidative properties (Nadia et al., 2012).

Caesalpinia decapetala (C. decapetala) is commonly known as Roth (Haq et al., 2011). It is a pantropical genus which belongs to the family of Caesalpiniaceae having 120-150 species of trees, shrubs, and lianas (Rehman et al., 2012). The genus consists of several members of species that are used traditionally for the treatment of inflammation, hepatotoxicity as well as diabetes (Shi-Jin, 2004; Narkhede, 2011). C. decapetala is widely spread in subcontinent regions. It is thorny climber up to 25 m in height and its leaves are 11-37.5 cm long. Flowers are yellow in color and 1.2-1.8 cm long. Its branches are hairy with hooked or straight prickles. Traditionally, C. decapetala has had many medicinal properties. A bath with decoction of C. decapetala is valuable for the treatment of jaundice (Bhadoriya et al., 2012). Leaves are used for the treatment of burns, biliousness and stomach disorders. Leaves and roots are also used as a purgative and emmenagogue. Other uses of C. decapetala are as laxative, tonic, anti-pyretic and carminative (Pawar and Surana, 2010). The anti-oxidant, anti-tumor and anti-fertility activities of C. decapetala have been reported (Keshri, 1988; Pawar, 2010; Bhadoriya, 2012; Xiao et al., 2013).

Resveratrol is a plant-based polyphenol molecule that has anti-inflammatory molecule is present in many plants including weed or Polygonum cuspidatum. In plants, resveratrol is generally found in the plant skin and acts as a phytoalexin to protect the plant from
infection and excessive UV radiation. Resveratrol has also been found to have significant anti-mutation, anti-inflammatory, antioxidant and DNA protective actions, when consumed by animals and humans. Most of the active research with resveratrol has been done in neuro and cardio protection, but several studies are being reported on resveratrol’s use for arthritic joint pain. Elmali et al. (2007) on using animals treated with intra-articular injection of resveratrol protects cartilage and reduces the inflammatory reaction in simulated knee osteoarthritis. The anti-inflammatory properties of resveratrol have also been observed in experimental animal models with paw edema, which is attributed to suppression of inflammatory prostaglandin synthesis (Elmali et al., 2007).

Resveratrol is also a potent and specific inhibitor of TNF-α- and IL-1β-induced NF-κB activation. Resveratrol shows the anti-inflammatory properties as it suppresses COX-2 by blocking NF-κB activation.

Curcumin is a naturally occurring yellow pigment derived from turmeric (Curcuma longa), a flowering plant of the ginger family. It has traditionally been used as a coloring and flavoring spice in food products. Curcumin has long been used in both Ayurvedic and Chinese medicines as an anti-inflammatory agent, a treatment for digestive disorders, and to enhance wound healing. Several clinical trials have demonstrated curcumin’s antioxidant, anti-inflammatory, and antineoplastic effects. Results of a study by Zandi and Karin suggested that curcumin might be efficacious in the treatment of cystic fibrosis because of its anti-inflammatory effect (Yang and Wang, 1993). Curcumin is known to inhibit inflammation by suppressing NF-κB, restricting various activators of NF-κB as
well as stemming its expression. Curcumin has also been suggested as a treatment for colitis, chronic neurodegenerative diseases, arthritis, and cancer. In addition, it regulates the activity of several enzymes and cytokines by inhibiting both COX-1 and COX-2. Most studies to date have been performed in animals, but given the centuries of use of curcumin, as well as its now demonstrated activity in the NF-κB, COX-1, and COX-2 inflammatory pathways, it may be considered a viable natural alternative to non-steroidal agents for the treatment of inflammation (Yang and Wang, 1993).

2.3 Fever

Fever is a rising of the body temperature above the normal level and can be caused by many different things. While there are no strict rules, fever is generally considered to be temperature above 37.2°C in the morning or 37.8°C at other times of the day. When someone is suffering from fever they are sometimes said to be 'febrile'. When the body temperature rises above the normal level of 37°C if measured in the mouth, or 37.7°C if measured in the rectum. Fever, also known as pyrexia (Axelrod and Diringer, 2008) is a common medical sign characterized by an elevation of temperature above the normal range of 36.5–37.5°C due to an increase in the body temperature regulatory set-point (Karakitsos and Karabinis, 2008). This increase in set-point triggers increased muscle tone and shivering. As a person's temperature increases, there is, in general, a feeling of cold despite an increasing body temperature. Once the new temperature is reached, there is a feeling of warmth. A fever can be caused by many different conditions ranging from benign to potentially serious. There are arguments for and against the usefulness of fever,
and the issue is controversial. With the exception of very high temperatures, treatment to reduce fever is often not necessary; however, antipyretic medications can be effective at lowering the temperature, which may improve the affected person's comfort. A wide range for normal temperatures has been found (Mayer et al., 2004).

The secondary impact of infection, malignancy or other diseased states leads to pyrexia or fever. It is the body’s natural function to create an environment where infectious agents or damaged tissues cannot survive. Normally, the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediators (cytokines, such as interleukin 1β, α, β, and TNF-α), which increase the synthesis of prostaglandin E2 near hypothalamic area and thereby trigger the hypothalamus to elevate the body temperature. When body temperature becomes high, the temperature regulatory system, which is governed by a nervous feedback mechanism, dilates the blood vessels and increases sweating to reduce the temperature. When the body temperature becomes low, hypothalamus protects the internal temperature by vasoconstriction. High fever often increases faster disease progression by increasing tissue catabolism, dehydration, and existing complaints, as found in HIV. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE2 biosynthesis. These synthetic agents irreversibly inhibit COX-2 with a high selectivity and are toxic to the hepatic cells, glomeruli, cortex of brain, and heart muscles (Spacer and Breder, 1994; Chattopadhyay et al., 2005).
Fever is generally agreed to be present if the elevated temperature is caused by a raised set point and: temperature in the anus (rectum/rectal) is at or over 37.5–38.3°C, (Turner, 1965; Axelrod and Diringer, 2008); temperature in the mouth (oral) is at or over 37.7°C, (Oliveira-Filho et al., 2001); temperature under the arm (axillary) or in the ear (otic) is at or over 37.2°C. Fever is measured with a thermometer. There are different types of thermometers which are used to measure fever.

2.3.1 Management of fever

Drugs have been developed to reduce elevated body temperature, of which some mostly inhibit COX-2 expression to reduce PGE2 biosynthesis which is mostly produced in increased or elevated temperature (Cheng et al., 2005; Sharma et al., 2010). There are other therapeutic agents that are employed such as NSAIDs, opioid etc.

Anti-pyretic categorized drugs or compounds which have an inhibitory effect on prostaglandin biosynthesis and do not influence body temperature when it is elevated by factors such as exercise or increases in ambient temperature are used to treat fever. Regulation of body temperature requires a delicate balance between production and loss of heat. The hypothalamus regulates the set point at which body temperature is maintained. This set point is elevated in fever (Vane, 1987; Goodman and Gilman, 1996).
2.3.2 Shortcomings of conventional management

Pyrexia or fever is a disease caused as a result of secondary impact of other diseased states due to the resetting of the hypothalamic set-point. From scientific discovery, antipyretic drugs such as aspirin, NSAIDs, opioids have been developed for use and of which mostly produces side effects including gastrointestinal bleeding, renal and hepatic effect, central nervous system and dermatological effects (Sharma et al., 2010; Chaudhary, 2001).

Though plants have been used as a source of antipyretic agent from time immemorial to treat fever, due to discovery of chemical antipyretic agents they were neglected. Fortunately, due to their low cost, easy access and reduced side effects, there is therefore revisititation to herbal medicines (Graz et al., 2011). Herbal medicines tend to look primitive and unscientific when compared to synthetic (conventional) drugs, which are thought to be more reliable than those made from plants. Yet, herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries for primary health care (Kamboj, 2000).

2.3.6 Medicinal plants as alternative management

Herbal medicines are assumed to be of great importance in the primary health care of individual and communities (Sheldon et al., 1997). The World Health Organization has estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for the primary health care needs. The high degree of
efficacy and safety with herbal medicines make them more acceptable compared to other therapeutic invention (Chaturvedi et al., 2007). Plant-based traditional knowledge has become a recognized tool in search for new sources of drugs and neutraceuticals (Ghosh, 2003; Sharma and Mujundar, 2003).

*Pedalium murex* L (*Pedaliaceae*) is a diffuse, more or less succulent herb found near the coastal area of South India (Nadkarani, 1982). Mucilage obtained from leaves, stem as well as fruits is used to treat gonorrhea (Mhaskar et al., 2000). An infusion or extract prepared from leaves have diuretic and demulcent properties and also useful in treating disorders of the urinary system such as odour urine, dysuria, spermatorrhoea and incontinence of urine. As an emmenagogue, the juice is used in puerperal diseases and also to promote lochial discharge (Chopra et al., 1996). The mucilage from leaves and young shoots is used as an aphrodisiac in seminal debility (Shukla and Khanuja, 2004). The aqueous extract of the whole plant has been found to possess analgesic and anti-inflammatory properties (Muralidharan and Balamurugan, 2008). The principle rationale behind the use of this plant for the study of antipyretic effects is that the tribal community of Kumaragiri hills of Salem district of Tamil Nadu has been using the leaf extract for fever (Alagesboobathy, 2009). Research done on this plant by Siva et al. (2012) at the Research Department of Botany in India has shown that the plant extract of *Pedalium murex* possesses a significant antipyretic effect in brewer’s yeast induced elevation of body temperature in rats. These results support the traditional use of this plant in fever remedies.
*Fagonia cretica* L., a member of the family Zygophyllaceae, is a small spiny undershrub. It is reputed to be a medicinal plant in scientific and folkloric literature, and its medicinal values are well documented (Hooker and Thomson, 1881; Saeed and Hamdard, 1969; Chopra *et al.*, 1982). *Fagonia cretica* is astringent, febrifuge and prophylactic against small-pox. The plant is bitter and used for the treatment of fever, thirst, vomiting, dysentery, asthma, urinary discharges, liver trouble, typhoid, toothache, stomach troubles and skin diseases (Meyer *et al.*, 1982; Baquar, 1989). Boiled residue of the plant in water is used to induce abortion. It is externally applied as a paste on tumors and other swellings of the neck. An aqueous decoction of the considering medicinal activity of *Fagonia cretica* based on traditional information, a study was conducted to evaluate the aqueous extract of whole plant of *Fagonia cretica* L. for its antipyretic potential by Brewer’s yeast induced pyrexia. This plant is a popular remedy for fever in the indigenous system of medicine and results from the study investigations concluded that the aqueous extract of whole plant of *Fagonia cretica* L. had antipyretic activity (Hooker and Thomson, 1881; Saeed and Hamdard, 1969; Chopra *et al.*, 1982).

Another plant that has been used to treat fever is *Ocimum gratissimum*. *Ocimum gratissimum* is a shrub commonly found in Africa and has been used to treat bacterial fevers locally. An experiment done on this plant showed that the antipyretic effect of *ocimum gratissimum* is dose dependent and the effect is as a result of the flavonoid component of the extract. These data therefore suggest that methanolic extract of
*Ocimum gratissimum* leaves possesses significant antipyretic activity and its mechanism could be by inhibition/release of inflammatory mediators (Gege-Adebayo *et al.*, 2013).

*Lepidagathis cristata* L. has been used to treat fever because like other plants it is also believed to have antipyretic activities. *Lepidagathis cristata*, family *Acanthaceae*, is a perennial herb, with almost no stem. Branches 20 cm long, arise out of a globose head on the ground, and spread out. Flowers also arise stalkless from this globose head. Flowers are pale-pink, 2-lipped. The upper lip is notched, and the lower lip is divided into 3 lobes. In Chattisgarh, they use this herb in treatment of fever particularly in treatment of malarial fever. The decoction of leaves is used internally for this purpose. Its utility in treatment of fever has given it the name Bukhar Jadi in reference literatures; the use of this herb in treatment of itchy affections of skin has been mentioned. The traditional healers of Chhattisgarh Plains are aware of this use. In many parts of Chhattisgarh, the cattle owners use the decoction of this herb to wash the cattle in rainy season in order to keep it free from flies (Madhava, 2005). Research has proved that the petroleum ether extracts of *Lepidagathis cristata* L. possesses a significant antipyretic effect in the yeast-provoked elevation of body temperature in rats, and its effect is comparable to that of paracetamol (standard drug).
2.4 Plants used in this study

2.4.1 Acacia nilotica

*Acacia nilotica* L (family Mimosaceae) is a multipurpose tree that grows to a height of 20 m (Kaur *et al*., 2005). The tree is a subtropical species spreading throughout Asia, Africa and America and is an integral part of rural and agro pastoral systems in these regions (Shittu, 2010). It has been reported that different parts of the plant contain various compounds that can be harnessed to provide therapeutic ingredients against diseases (Meena *et al*., 2006; Banso and Adeyemo, 2007). Rural populations have used the barks of the plant as an anthelmintic, aphrodisiac and antiuretic agent. Furthermore, illnesses such as wounds, leprosy and skin diseases have also been shown to be treated by the bark exudates of *A. nilotica* (Del, 2009; Singh *et al*., 2009). *A. nilotica* roots have been effective in treating tumors, cancer and tuberculosis as reported by Kalaivani and Mathew (2010). Leaves and the gum from this tree have also been shown to be effective antibacterial agents and success has been achieved in their use against diarrhea, dressing of wounds and inflammation (Kalaivani and Mathew, 2010; Shittu, 2010; Kalaivani *et al*., 2010). The medicinal properties of this tree are attributed to the presence and abundance of chemical substances such as alkaloids, phenols, flavonoids and steroids that aid in the healing processes (Banso and Adeyemo, 2007; Jigam *et al*., 2010). Despite these numerous reports on the effectiveness of *A. nilotica* against a vast array of diseases, data on specific disease symptoms such as inflammation, pyrexia and nociception is scarce.
2.4.2 *Urtica dioica*

*Urtica dioica* L (family Urticaceae) commonly known as stinging nettle has, for a long time, been used as herbal remedy to a vast array of diseases (Bisht *et al*., 2012). The most common ailments treated by this species are bites and stings from insects and burns. The plant is an annual shrub that grows to 0.6m tall and commonly occurs in the temperate zones of Asia, America, Europe and parts of Africa (Bisht *et al*., 2012). The shrub is covered with stinging trichomes that contain histamine, acetylcholine and formic acid that cause irritation and blistering of the skin. For this reason, it has been undervalued by most communities (Bisht *et al*., 2012).
Despite this limitation, the stinging nettle has been extensively used for medicinal purposes (Ziyyat et al., 1997; Randall, 1999; Alford, 2007). For instance, fresh juice from the plant has been reported to stimulate the digestive system and flow of milk in nursing mothers, provide temporary relief from pain as well as treating fever and diabetes (Randall, 1999; Alford, 2007). It has also been shown to counter symptoms of allergies (Roscek et al., 2009) and increase thyroid function (Bisht et al., 2012). Although this shrub has been used from the ancient to modern times with great success achieve, the mode of preparation of the medicinal compounds and administration (popularly cooked and orally taken) could lead to less efficiency. It is therefore important to scientifically determine the right dosage as well as the time of action in vitro through extraction of active compounds and testing on experimental animals.

Figure 2.4.2: Photograph showing a plant specimen of *U. dioica* plant taken in July 2012 at Naikara, Narok County by Mukundi (2015)
2.4.3 *Aloe volkensii*

*Aloe volkensii* (family Asphodeteceae) is of great utility and bio-cultural value worldwide occurring as a succulent-leaved shrub (Grace *et al*., 2009). The plant is mainly found in Africa, western India and the Arabian Peninsula (Newton, 2001). The cultivated species in this family such as *A. vera* are mainly used as a source of natural products for industries (Grace *et al*., 2009). The Aloe specie has been extensively documented for its medicinal value against infectious parasites (van Damme and van den Eynden, 2000), digestion problems (Steen and Stewart, 2007), treatment of injuries (Rood, 2008) and skin diseases (Jia *et al*., 2008). Particularly, *Aloe volkensii* has been shown to be effective against whooping cough in children (Olembo *et al*., 1995). This species is however no commonly used for treatment of illnesses due to the nature of its exudates that are considered unsuitable. For instance, Olembo *et al*., 1995 reported the use of *A. volkensii* in preparation of rodentiles used to kill moles. Similarly, Rood *et al*., (2008) reported that the exudates from this shrub were painted on children’s fingers to discourage nail biting and also painted on structures to discourage gnawing by animals. Despite these, the plant has shown medicinal attributes which if exploited could be of great importance to the therapeutics industry.
2.4.4 *Cynanchum viminalis*

*Cynanchum viminalis* (family asclepiadaceae) is a perennial shrub that originated from Madagascar and grows in the Acacia savanna in semi-arid habitats of Kenya, Uganda, Tanzania and Somalia where it is comparatively widespread (Liede and Meve, 2005). *Cynanchum viminalis* subspecies include *crassicaule*, another sub-specific taxon in the *C. viminalis* complex, is described as new, and based on morphological, ecological and molecular evidence. *Cynanchum viminalis* subspecies *crassicaule* occurs at altitudes of around sea level close to shore up to the higher foot of Mt. Kilimanjaro. Typically it grows in Acacia savanna and scrub of semi-arid to arid habitats in Tanzania, Kenya,
Uganda, and Somalia. There is less information on the antinociceptive, anti-inflammatory and antipyretic properties of this plant and that’s why this research was believed to cover the gap on studies about its use as a herbal medicine to manage these ailments (Liede and Meve, 2005).

Figure 2.4.4: Photograph showing plant specimen of *Cynanchum viminal* taken in July 2012, at Naikara, Narok County by Mukundi (2015)
CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection and preparation of plant materials

Fresh plant materials were collected from Loita division, Narok county Kenya. Four (4) indigenous plants, *U. dioica*, *A. nilotica*, *A. volkensii* and *C. viminale*, were used in this study. These plants are believed by the locals to have medicinal value against wounds and diabetes. The plant materials were identified by Mukundi (2015) and authenticated with help from the Department of Botany, Kenyatta University. Leaf tissues were collected from *U. dioica* and *A. volkensii* and dried under shade for one and two months respectively. Stems and barks from *C. viminale* and *A. nilotica* respectively were also collected and dried under shade for two and three months respectively. Preparation of plant extracts was carried out using a protocol as described by Nostro *et al.* (2000). Briefly, the collected plant material was first washed 2–3 times using tap water, to remove adherent particles, and then dried. The dried material was ground to a fine powder using a grinder and passed through a mesh sieve. The powdered materials were kept at room temperature away from direct sunlight in closed dry khaki paper bags.

3.2 Extraction

The powdered material was separately extracted with single distilled water at 125 g/L on a 60°C water bath for 6 hours. The solvent extract was then freeze-dried and the residue preserved at 4°C for future use. About 400g of *Acacia nilotica* were therefore dissolved in 3.2 L of single distilled water in a conical flask and the mixture put on the water bath.
Decantation and filtration processes through a No.1 Whatman filter paper were repeated until the sample became clear. The filtrate was freeze-dried, weighed and stored in an airtight plastic bag and refrigerated until it was used for bioassay. This procedure yielded 20 g of freeze-dried *Acacia nilotica*.

Powdered material of about 375 g of *Urtica dioica* was dissolved in 3 L of single distilled water. Decantation and filtration processes through a No.1 Whatman filter paper were repeated until the sample became clear. The filtrate was freeze-dried, weighed and stored in an airtight plastic bag and refrigerated until it was used for bioassay. This procedure yielded 76 g of freeze-dried *Urtica dioica*.

About 400 g of *Aloe volkensii* was dissolved in 3.2 L of single distilled water. Decantation and filtration processes through a No.1 Whatman filter paper were repeated until the sample became clear. The filtrate was freeze-dried, weighed and stored in an airtight plastic bag and refrigerated until it was used for bioassay. A yield of 60 g of freeze-dried *Aloe volkensii* was obtained.

About 375 g of *Cynanchum viminale* was dissolved in 3 L of single distilled water. Decantation and filtration processes through a No.1 Whatman filter paper were repeated until the sample became clear. The filtrate was freeze-dried, weighed and stored in an airtight plastic bag and refrigerated until it was used for bioassay. A yield of about 44 g of freeze-dried *Cynanchum viminale* was obtained.
3.3 Preparation of reagents and extracts for bioassay

A volume of 0.1 ml of normal saline was used as a control on each laboratory animal and also for preparation and dissolving of reagents, standard drug and each plant extract. The dosages of 50 mg/kg, 100 mg/kg and 150 mg/kg body weight of plant extracts were prepared by dissolving freeze-dried plant material in normal saline. A dose of 10 ml/kg of 15% w/v yeast was subcutaneously injected to induce pyrexia. Paracetamol was used as a reference drug for fever and was orally administered. Diclofenac sodium was the reference drug administered to the standard group to treat inflammation and pain.

3.4 Animal models

Female Swiss albino mice of about three weeks old and average weight of 20 g were used in this study. These animals were maintained in the experimental room at the Animal House, Department of Biochemistry and Biotechnology, Kenyatta University. The mice were kept in a cage and fed with standard laboratory food and water.

3.5 Experimental design

3.5.1 Determination of antinociceptive activity

To determine the antinociceptive activity of the plant extracts, a formalin–induced writhing test was carried out using a method described by Wheeler–Aceto et al. (1989). A total of 15 groups of 5 mice per group were used as test and control specimen.
Individual extracts from the four plants were separately administered intraperitoneally to the mice at concentrations of 50 mg/kg, 100 mg/kg and 150 mg/kg body weight and the animals left to rest for 30 minutes. Then, the mice were given an intraperitoneal injection of 0.05ml of 2.5% formalin in the left hind paw. The mice were then individually placed in a glass beaker and observed for writhing. The number of stretches per animal was recorded for the following 30 minutes with the first 5 minutes as the early phase and the last 15 minutes as the late phase of pain. A writhe was recorded following the stretching of the abdomen and/or stretching of at least one hind limb according to Hosseinzadeh and Younesi (2002). The control groups were intraperitoneally administered with 75 mg/3ml Diclofenac sodium (for positive) and 0.1ml normal saline (for negative control) prior to administration of formalin.

Table 3.5.1: Treatment protocol for the determination of antinociceptive activity for the aqueous extracts of the four medicinal plants

<table>
<thead>
<tr>
<th>Group</th>
<th>Status</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>Normal saline (0.1 ml) + Formalin (0.05 ml of 2.5% formalin)</td>
</tr>
<tr>
<td>II</td>
<td>Standard</td>
<td>Diclofenac (12 µl of 75 mg/3 ml diclofenac sodium) + Formalin</td>
</tr>
<tr>
<td>III</td>
<td>Test-1</td>
<td>50 mg/kg extract (0.001 g) + Formalin (0.05 ml of 2.5% formalin)</td>
</tr>
<tr>
<td>IV</td>
<td>Test-2</td>
<td>100 mg/kg extract (0.002 g) + Formalin (0.05 ml of 2.5% formalin)</td>
</tr>
<tr>
<td>V</td>
<td>Test-3</td>
<td>150 mg/kg extract (0.003 g) + Formalin (0.05 ml of 2.5% formalin)</td>
</tr>
</tbody>
</table>
3.5.2 Determination of anti-inflammatory activity

To determine the anti-inflammatory effect of the extracts in mice, a formalin induced inflammation test was carried out as described by Hosseinzadeh and Younesi (2002). Briefly, 15 groups of 5 mice per group were used. Inflammation was induced by intraperitoneal injection of 0.05ml of 2.5% formalin into the left hind paw of each mouse. This was done 30 minutes after administration of individual extracts (for test experiments) and Diclofenac sodium and normal saline for positive and negative controls respectively. Hourly changes in paw sizes and reduction of edema around the paw was determined using a vernier caliper. Paw diameter before and after treatment was recorded and the percentage change in paw diameter was calculated.

**Table 3.5.2: Treatment protocol for the determination of anti-inflammatory activity for the aqueous extracts of the four medicinal plants**

<table>
<thead>
<tr>
<th>Group</th>
<th>Status</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>Normal saline (0.1 ml) + Formalin (0.05 ml of 2.5% formalin)</td>
</tr>
<tr>
<td>II</td>
<td>Standard</td>
<td>Diclofenac (10 µl of 75 mg/3 ml diclofenac sodium) + Formalin</td>
</tr>
<tr>
<td>III</td>
<td>Test-1</td>
<td>50 mg/kg extract (0.001 g) + Formalin (0.05 ml of 2.5% formalin)</td>
</tr>
<tr>
<td>IV</td>
<td>Test-2</td>
<td>100 mg/kg extract (0.002 g) + Formalin (0.05 ml of 2.5% formalin)</td>
</tr>
<tr>
<td>V</td>
<td>Test-3</td>
<td>150 mg/kg extract (0.003 g) + Formalin (0.05 ml of 2.5% formalin)</td>
</tr>
</tbody>
</table>
3.5.3 Determination of antipyretic activity

The antipyretic activity of the plant extracts was evaluated using Brewer’s yeast induced pyrexia as described by Loux et al. (1972). According to the protocol, 15% aqueous suspension of Brewer’s yeast was first prepared using normal saline. 10 ml/kg of the suspension was administered subcutaneously to each test mouse to induce fever. A total of 15 groups of 6 mice per group were screened.

After induction of fever, various concentrations of the plant extracts were orally administered to each mouse. The reference groups were administered with paracetamol and normal saline for positive and negative controls respectively. Temperatures of each mouse were then determined rectally by thermal probe thermometer at hourly interval for three hours after treatment.

Table 3.5.3: Treatment protocol for the determination of antipyretic activity for the aqueous extracts of the four medicinal plants

<table>
<thead>
<tr>
<th>Group</th>
<th>Status</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>Yeast (0.03 g of 10 ml/kg of 15% w/v yeast) + Normal saline (0.1 ml)</td>
</tr>
<tr>
<td>II</td>
<td>Standard</td>
<td>Yeast (0.03 g of 10 ml/kg of 15% w/v yeast) + Paracetamol (0.286 mg)</td>
</tr>
<tr>
<td>III</td>
<td>Test-1</td>
<td>Yeast (0.03 g of 10 ml/kg of 15% w/v yeast) + 50 mg/kg extract (0.001 g)</td>
</tr>
<tr>
<td>IV</td>
<td>Test-2</td>
<td>Yeast (0.03 g of 10 ml/kg of 15% w/v yeast) + 100 mg/kg extract (0.002 g)</td>
</tr>
<tr>
<td>V</td>
<td>Test-3</td>
<td>Yeast (0.03 g of 10 ml/kg of 15% w/v yeast) + 150 mg/kg extract (0.003 g)</td>
</tr>
</tbody>
</table>
Rectal temperature before and after treatment was recorded and the percentage change in rectal temperature was calculated by the following formula described by Hukkeri et al. (2006) and Ray (2006):

\[
\frac{B - C_n}{B} \times 100
\]

Where,

B - Rectal temperature at 19 hrs after yeast administration

C_n - Rectal temperature after drug administration

3.6 Data management and analysis

The duration of paw-licking behavior produced by each mouse was recorded after injection with the extracts and formalin and expressed as mean with its respective standard deviation (Mean ± SD). Data was presented in table format. Paw sizes were also recorded (in millimeters) and the data was tabulated and expressed as mean and standard deviation (Mean ± SD). The reaction time on the effect of the extracts on fever was expressed as mean and standard deviation (Mean ± SD) and presented in table format. The analysis was done using one way analysis of variance (ANOVA) and the difference between the means tested using Tukey’s HSD test. The results obtained were compared with the vehicle control group. The value of \( p < 0.05 \) was considered statistically significant. Analysis of variance was done using a statistical analysis software (IBM SPSS) version 20.
CHAPTER FOUR

RESULTS

4.1 Antinociceptive effects of aqueous bark extract of *Acacia nilotica* on formalin-induced pain in mice models

The results showed that the aqueous bark extract of *Acacia nilotica* had some antinociceptive activity against formalin-induced nociception, which was indicated by reduction in paw-licking time compared to the control (Table 4.1).

In early phase, the baseline was non-significantly lowered compared to the control ($p > 0.05$) but significantly elevated compared to the reference drug, plant extract at dose level of 50 mg/kg, 100 mg/kg and 150 mg/kg body weight ($p < 0.05$). Diclofenac sodium (reference drug) was significantly lowered compared to the control ($p < 0.05$) but non-significantly lowered compared to the plant extract at dose of 50 mg/kg body weight ($p > 0.05$). Plant extract at dose of 50 mg/kg was significantly lowered compared to the control ($p < 0.05$). Plant extract at dose of 100 mg/kg body weight was significantly lowered compared to the control ($p < 0.05$). Plant extract at dose of 150 mg/kg body weight was significantly lowered compared to the control ($p < 0.05$).

The administration of diclofenac sodium before injection of formalin significantly reduced pain in the acute phase and was not statistically different from plant extract at dose 50 mg/kg body weight ($p < 0.05$; Table 4.1). Results of this study showed that the
dose of 50 mg/kg aqueous bark extract of *Acacia nilotica* exerts significant antinociceptive effect (*p* < 0.05) characterized by decreasing duration of licking and biting time in the acute or first phase (0-5 min) of formalin induced pain. This analgesic effect was statistically different from the control (*p* < 0.05; Table 4.1). However, at a dose of 100 mg/kg plant extract, the duration of pain response increased significantly and it was statistically different from the control (*p* < 0.05; Table 4.1). Dose of 150 mg/kg plant extract significantly reduced paw licking time compared to the control (*p* < 0.05; Table 4.1).

In late phase, aqueous bark extract of *Acacia nilotica* showed a significant antinociceptive activity on the late phase of formalin induced pain though not in a dose dependent manner (Table 4.1). The baseline was significantly elevated compared to the control (*p* < 0.05). Diclofenac sodium (reference drug) was significantly lowered compared to the control (*p* < 0.05). Plant extract at dose of 50 mg/kg was significantly lowered compared to the control and the rest (*p* < 0.05). Plant extract at dose of 100 mg/kg body weight was significantly lowered compared to the control (*p* < 0.05) but non-significantly lowered compared to plant extract at dose of 150 mg/kg body weight (*p* > 0.05). Plant extract at dose of 150 mg/kg body weight was significantly lowered compared to the control (*p* < 0.05). Diclofenac sodium (reference drug) did lower chronic pain significantly compared to the control (*p* < 0.05; Table 4.1). The plant extract at dose
of 50 mg/kg body weight had a significant decrease in paw licking time compared to the control ($p<0.05$; Table 4.1).

**Table 4.1: Antinociceptive effects of aqueous bark extract of *Acacia nilotica* on formalin-induced pain in mice models**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean paw-licking time (sec) ± SD</th>
<th>Early Phase</th>
<th>Late phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control</td>
<td>Normal saline (0.1 ml)</td>
<td>262.4 ± 3.84$^d$</td>
<td>347.4 ± 4.39$^d$</td>
<td></td>
</tr>
<tr>
<td>2 Baseline</td>
<td>Formalin (0.05 ml of 2.5 %)</td>
<td>248.0 ± 12.20$^d$</td>
<td>444.4 ± 8.50$^c$</td>
<td></td>
</tr>
<tr>
<td>3 Standard</td>
<td>Diclofenac (75 mg/3 ml)</td>
<td>96.4 ± 10.35$^a$</td>
<td>203.6 ± 9.86$^a$</td>
<td></td>
</tr>
<tr>
<td>4 Test-1</td>
<td>50 mg/kg bw</td>
<td>97.4 ± 8.96$^a$</td>
<td>237.0 ± 17.88$^b$</td>
<td></td>
</tr>
<tr>
<td>5 Test-2</td>
<td>100 mg/kg bw</td>
<td>201.8 ± 9.90$^c$</td>
<td>311.4 ± 5.36$^c$</td>
<td></td>
</tr>
<tr>
<td>6 Test-3</td>
<td>150 mg/kg bw</td>
<td>152.0 ± 9.27$^b$</td>
<td>317.6 ± 6.80$^c$</td>
<td></td>
</tr>
</tbody>
</table>

Mean values ± SD with the same letters are not statistically different from one another by ANOVA followed by Tukey’s post hoc test ($p>0.05$). n=5

**4.2 Antinociceptive effects of aqueous leaf extract of *Aloe volkensii* on formalin-induced pain in mice models**

In early phase, aqueous leaf extracts of *Aloe volkensii* reduced formalin induced pain in mice but not in a dose dependent manner (Table 4.2). The baseline was non-significantly lowered compared to the control ($p>0.05$) but significantly elevated compared to the rest ($p<0.05$). Diclofenac sodium (reference drug) was significantly lowered compared to the control and the rest ($p<0.05$). Plant extract at dose of 50 mg/kg was significantly lowered compared to the control ($p<0.05$). Plant extract at dose of 100 mg/kg body weight was significantly lowered compared to the control ($p<0.05$). Plant extract at dose of 150 mg/kg body weight was significantly lowered compared to the control ($p<0.05$). At the dose level of 50 mg/kg body weight, the aqueous leaf extract exhibited significant
antinociceptive effect compared with control and baseline groups ($p< 0.05$; Table 4.2). Diclofenac sodium (reference drug) showed a significant reduction in pain.

In the late phase, this study showed that the aqueous leaf extract of *Aloe volkensii* did not exert antinociceptive activity against formalin induced pain (Table 4.2). The baseline was significantly elevated compared to the control ($p< 0.05$). The reference drug was significantly lowered compared to the control ($p< 0.05$). The plant extract at all dose levels were significantly lowered compared to the control ($p< 0.05$). The drug of reference showed greater antinociceptive activity.

**Table 4.2: Antinociceptive effects of aqueous leaf extract of *Aloe volkensii* on formalin-induced pain in mice models**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean paw-licking time(sec) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Early phase</td>
</tr>
<tr>
<td>1 Control</td>
<td>Normal saline (0.1 ml)</td>
<td>262.4 ± 3.84&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 Baseline</td>
<td>Formalin (0.05 ml of 2.5 %)</td>
<td>248.0 ± 12.20&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 Standard</td>
<td>Diclofenac (75 mg/3 ml)</td>
<td>96.4 ± 10.35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 Test-1</td>
<td>50 mg/kg bw</td>
<td>195.0 ± 15.81&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 Test-2</td>
<td>100 mg/kg bw</td>
<td>204.8 ± 9.01&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 Test-3</td>
<td>150 mg/kg bw</td>
<td>223.8± 16.52&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values ± SD with the same letters are not statistically different from one another by ANOVA followed by Tukey’s post hoc test ($p > 0.05$). n=5

4.3 Antinociceptive effects of aqueous stem extract of *Cynanchum viminale* on formalin-induced pain in mice models

This study showed that the aqueous stem extract of *Cynanchum viminale* exhibited an antinociceptive activity against the first phase of formalin induced pain in mice though not in a dose dependent manner (Table 4.3). The baseline was non-significantly lowered
compared to the control ($p>0.05$) but significantly elevated compared to the rest ($p<0.05$). Diclofenac sodium (reference drug) was significantly lowered compared to the control and all the other treatments ($p<0.05$). Plant extract at dose of 50 mg/kg was significantly lowered compared to the control ($p<0.05$). Plant extract at dose of 100 mg/kg body weight was significantly lowered compared to the control ($p<0.05$). Plant extract at dose of 150 mg/kg body weight was significantly lowered compared to the control ($p<0.05$) but non-significantly elevated compared to plant extract at dose of 100 mg/kg body weight ($p>0.05$). There was a significant antinociceptive activity at dose of 100 mg/kg body weight as seen by decreased paw licking time. Diclofenac sodium lowered the pain significantly compared to the plant extract at all dose levels ($p<0.05$; Table 4.3).

In late phase, aqueous stem of *Cynanchum viminale* showed antinociceptive activity though not in a dose dependent manner (Table 4.3). The baseline was significantly elevated compared to the control ($p<0.05$). Diclofenac sodium (reference drug) was significantly lowered compared to the control and the rest ($p<0.05$). Plant extract at dose of 50 mg/kg body weight was significantly lowered compared to the control ($p<0.05$). Plant extract at dose of 100 mg/kg body weight was non-significantly elevated compared to the control ($p>0.05$). Plant extract at dose of 150 mg/kg body weight was significantly lowered compared to the control and the other treatments ($p<0.05$). Antinociceptive effectiveness of the stem extract at the dose level of 150 mg/kg body weight was better
compared to the other dose levels. All these dose levels however were not as effective as diclofenac sodium (reference drug).

Table 4.3: Antinociceptive effects of aqueous stem extract of *Cynanchum viminale* on formalin-induced pain in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean paw-licking time (sec) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Early phase</td>
</tr>
<tr>
<td>1 Control</td>
<td>Normal saline (0.1 ml)</td>
<td>262.4 ± 3.84&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 Baseline</td>
<td>Formalin (0.05 ml of 2.5 %)</td>
<td>248.0 ± 12.20&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 Standard</td>
<td>Diclofenac (75 mg/3 ml)</td>
<td>96.4 ± 10.35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 Test-1</td>
<td>50 mg/kg bw</td>
<td>198.4 ± 12.34&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 Test-2</td>
<td>100 mg/kg bw</td>
<td>175.8 ± 10.54&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 Test-3</td>
<td>150 mg/kg bw</td>
<td>192.2 ± 14.0&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values ± SD with the same letters are not statistically different from one another by ANOVA followed by Tukey’s post hoc test (*p* > 0.05). n=5

**4.4 Antinociceptive effects of aqueous leaf extract of *Urtica dioica* on formalin-induced pain in mice models**

Aqueous leaf extract of *Urtica dioica* reduced acute pain significantly in a dose dependent manner (Table 4.4). The baseline was non-significantly lowered compared to the control (*p* > 0.05) but significantly elevated compared to the reference drug and plant extract at dose levels of 100 mg/kg and 150 mg/kg body weight (*p* < 0.05). Diclofenac sodium (reference drug) was significantly lowered compared to the control and all the other treatment (*p* < 0.05). Plant extract at dose of 50 mg/kg body weight was significantly elevated compared to the control (*p* < 0.05). Plant extract at dose of 100 mg/kg body weight was significantly lowered compared to the control (*p* < 0.05) but non-significantly elevated compared to plant extract at dose of 150 mg/kg body weight (*p* > 0.05). Plant extract at dose of 150 mg/kg body weight was significantly lowered
compared to the control ($p<0.05$). At the dose level of 150 mg/kg body weight, the aqueous leaf extract exhibited significant antinociceptive effect compared to the control and baseline ($p<0.05$; Table 4.4). Diclofenac sodium reduced pain significantly compared to the control and baseline groups ($p<0.05$; Table 4.4). Dose level of 100 mg/kg body weight was as effective as that at dose 150 mg/kg body weight.

In late phase, aqueous leaf extract of *Urtica dioica* showed antinociceptive activity against formalin induced pain but not in a dose dependent manner (Table 4.4). The baseline was significantly elevated compared to the control and all the other treatments ($p<0.05$). Diclofenac sodium (reference drug) was significantly lowered compared to the control and the rest ($p<0.05$). Plant extract at dose of 50 mg/kg was non-significantly lowered compared to the control ($p>0.05$). Plant extract at dose of 100 mg/kg body weight was significantly lowered compared to the control and plant extract at dose 50 mg/kg and 150 mg/kg body weight ($p<0.05$). Plant extract at dose of 150 mg/kg body weight was significantly lowered compared to the control ($p<0.05$). The plant extract at dose level of 100 mg/kg body weight showed decreased paw licking time and was significantly different from the rest ($p<0.05$; Table 4.4). Diclofenac sodium did reduce pain significantly compared to the control ($p<0.05$; Table 4.4).
Table 4.4: Antinociceptive effects of aqueous leaf extract of *Urtica dioica* on formalin-induced pain in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean paw-licking time(sec) ± SD</th>
<th>Early phase</th>
<th>Late phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control</td>
<td>Normal saline (0.1 ml)</td>
<td>262.4 ± 3.84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>347.4 ± 4.39&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>2 Baseline</td>
<td>Formalin (0.05 ml of 2.5%)</td>
<td>248.0 ± 12.20&lt;sup&gt;e&lt;/sup&gt;</td>
<td>444.4 ± 8.50&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3 Standard</td>
<td>Diclofenac 75 mg/3 ml</td>
<td>96.4 ± 10.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>203.6 ± 9.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>4 Test-1</td>
<td>50 mg/kg bw</td>
<td>290.0 ± 4.18&lt;sup&gt;d&lt;/sup&gt;</td>
<td>342.8 ± 3.56&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>5 Test-2</td>
<td>100 mg/kg bw</td>
<td>169.6 ± 8.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>282.0 ± 8.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>6 Test-3</td>
<td>150 mg/kg bw</td>
<td>154.8 ± 8.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>298.4 ± 7.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Mean values ± SD with the same letters are not statistically different from one another by ANOVA followed by Tukey’s post hoc test (*p* > 0.05). n=5

4.5 Anti-inflammatory effects of aqueous bark extract of *Acacia nilotica* on formalin-induced paw oedema in mice models

Treatment of mice with bark extracts of *Acacia nilotica* showed some anti-inflammatory activity against formalin-induced oedema, which was indicated by reduction in paw oedema (Table 4.5). Diclofenac sodium was significantly lowered compared to the control (*p* < 0.05; Table 4.5). The plant extract at dose of 50 mg/kg body weight was non-significantly lowered compared to the control (*p* > 0.05). The plant extract at dose 100 mg/kg body weight was significantly lowered compared to the control (*p* < 0.05) but non-significantly lowered compared diclofenac sodium and plant extract at dose of 150 mg/kg body weight (*p* > 0.05).

In the first hour, plant extract at dose of 100 mg/kg body weight showed greater inhibition of inflammation and this was indicated by a reduction in paw diameter to 81.73% compared to the other dose levels and the reference drug (Table 4.5). Aqueous
bark extract of *A. nilotica* caused anti-inflammatory compared to the control (*p* > 0.05; Table 4.5).

In the second hour, all mice treated with the bark extracts of *A. nilotica* at doses of 50, 100 and 150 mg/kg body weight recorded a reduction of inflammation to 83.88%, 76.46% and 80.07% respectively (Table 4.5). Although, the anti-inflammatory effectiveness was not in a dose dependent manner, the extract at dose level of 100 mg/kg body weight was significantly lowered compared to the control (*p* > 0.05; Table 4.5).

In the third hour, the anti-inflammatory activity of the plant extract was in a dose dependent manner. The anti-inflammatory properties of aqueous leaf extracts of *A. nilotica* at dose of 50 mg/kg and 100 mg/kg body weight was comparable to reference drug. At this hour, the herbal extract at dose of 150 mg/kg body weight exhibited the highest anti-inflammatory effect.

Four hours after drug administration, *A. nilotica* at all dose levels (50, 100 and 150 mg/kg body weight) was found to lower the formalin-induced inflammation (Table 4.5). The plant extract at dose of 150 mg/kg body weight inhibited inflammation to 57.16%.
Table 4.5: Anti-inflammatory effects of aqueous bark extract of *Acacia nilotica* on formalin-induced paw oedema in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>100.00 ± 0.00</td>
<td>98.87 ± 1.54</td>
<td>86.24 ± 2.06</td>
<td>72.44 ± 1.98</td>
<td>68.42 ± 3.04</td>
</tr>
<tr>
<td>Baseline</td>
<td>Formalin</td>
<td>100.00 ± 0.00</td>
<td>88.98 ± 2.28</td>
<td>75.01 ± 1.32</td>
<td>76.00 ± 1.14</td>
<td>73.54 ± 3.32</td>
</tr>
<tr>
<td>Standard</td>
<td>Diclofenac</td>
<td>100.00 ± 0.00</td>
<td>93.96 ± 7.23</td>
<td>84.45 ± 4.35</td>
<td>70.38 ± 3.62</td>
<td>56.30 ± 2.90</td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>100.00 ± 0.00</td>
<td>97.29 ± 3.51</td>
<td>83.88 ± 2.94</td>
<td>72.08 ± 1.36</td>
<td>58.10 ± 1.17</td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>100.00 ± 0.00</td>
<td>81.73 ± 2.53</td>
<td>76.46 ± 2.71</td>
<td>70.61 ± 1.71</td>
<td>65.40 ± 1.87</td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>100.00 ± 0.00</td>
<td>87.48 ± 4.14</td>
<td>80.07 ± 4.20</td>
<td>69.15 ± 2.18</td>
<td>57.16 ± 1.15</td>
</tr>
</tbody>
</table>

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another by ANOVA followed by Tukey’s post hoc test (\(p>0.05\)). n=5
4.6 Anti-inflammatory effects of leaf extract of *Aloe volkensii* on formalin-induced paw oedema in mice models

Treatment of mice with leaf extract of *Aloe volkensii* showed some anti-inflammatory activity against formalin-induced oedema, which was indicated by reduction in paw oedema (Table 4.6).

In the first hour, the plant extract at dose of 50 mg/kg body showed greater inhibition of inflammation and this was indicated by a reduction to 88.54% (Table 4.6). Aqueous leaf extract of *A. volkensii* at dose of 50 mg/kg body weight caused anti-inflammatory effect inhibiting inflammation significantly compared to the control (*p* < 0.05; Table 4.6) but non-significantly low compared to plant extract at dose of 100 mg/kg and 150 mg/kg body weight (*p* > 0.05).

In the second hour, the leaf extracts of *A. volkensii* at doses of 50, 100 and 150 mg/kg body weight recorded a reduction in paw diameter (Table 4.6). The anti-inflammatory effectiveness at dose level of 50 mg/kg body weight had greater inhibition of inflammation by 76.49% compared to diclofenac sodium, which reduced inflammation by 84.45% (Table 4.6).

In the third hour, *A. volkensii* at all dose levels (50, 100 and 150 mg/kg body weight) was found to lower the elevated paw diameter (Table 4.6). At this hour, the mice treated with
100 mg/kg of the herbal extract exhibited the highest anti-inflammatory effect of 66.75% (Table 4.6).

Four hours after drug administration, A. volkensii at all dose levels (50, 100 and 150 mg/kg body weight) was found to lower the formalin-induced inflammation (Table 4.6). The plant extract at dose of 100 mg/kg body weight was even better than the other dose levels for it lowered the inflammatory activity to 57.14%.
Table 4.6: Anti-inflammatory effects of aqueous leaf extract of *Aloe volkensii* on formalin-induced paw oedema in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>100.00 ± 0.00&lt;sup&gt;E&lt;/sup&gt;&lt;sub&gt;c&lt;/sub&gt;</td>
<td>98.87 ± 1.54&lt;sup&gt;D&lt;/sup&gt;&lt;sub&gt;c&lt;/sub&gt;</td>
<td>86.24 ± 2.06&lt;sup&gt;C&lt;/sup&gt;&lt;sub&gt;c&lt;/sub&gt;</td>
<td>72.44 ± 1.98&lt;sup&gt;Re&lt;/sup&gt;</td>
<td>68.42 ± 3.04&lt;sup&gt;A&lt;/sup&gt;&lt;sub&gt;c&lt;/sub&gt;</td>
</tr>
<tr>
<td>Baseline</td>
<td>Formalin</td>
<td>100.00 ± 0.00&lt;sup&gt;E&lt;/sup&gt;&lt;sub&gt;bc&lt;/sub&gt;</td>
<td>88.98 ± 2.28&lt;sup&gt;D&lt;/sup&gt;&lt;sub&gt;bc&lt;/sub&gt;</td>
<td>75.01 ± 1.32&lt;sup&gt;C&lt;/sup&gt;&lt;sub&gt;bc&lt;/sub&gt;</td>
<td>76.00 ± 1.14&lt;sup&gt;Dbc&lt;/sup&gt;</td>
<td>73.54 ± 3.32&lt;sup&gt;Abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Standard</td>
<td>Diclofenac</td>
<td>100.00 ± 0.00&lt;sup&gt;E&lt;/sup&gt;&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>93.96 ± 7.23&lt;sup&gt;D&lt;/sup&gt;&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>84.45 ± 4.35&lt;sup&gt;C&lt;/sup&gt;&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>70.38 ± 3.62&lt;sup&gt;Bab&lt;/sup&gt;</td>
<td>56.30 ± 2.9&lt;sup&gt;Ab&lt;/sup&gt;&lt;sub&gt;ab&lt;/sub&gt;</td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>100.00 ± 0.00&lt;sup&gt;E&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt;</td>
<td>88.54 ± 4.21&lt;sup&gt;D&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt;</td>
<td>76.49 ± 6.52&lt;sup&gt;C&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt;</td>
<td>69.06 ± 2.83&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>60.24 ± 4.67&lt;sup&gt;Aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>100.00 ± 0.00&lt;sup&gt;E&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt;</td>
<td>90.43 ± 3.21&lt;sup&gt;D&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt;</td>
<td>78.83 ± 4.99&lt;sup&gt;C&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt;</td>
<td>66.75 ± 7.07&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>57.14 ± 6.39&lt;sup&gt;Aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>100.00 ± 0.00&lt;sup&gt;E&lt;/sup&gt;&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>92.04 ± 6.42&lt;sup&gt;D&lt;/sup&gt;&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>80.26 ± 8.59&lt;sup&gt;C&lt;/sup&gt;&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>69.63 ± 9.65&lt;sup&gt;Bab&lt;/sup&gt;</td>
<td>60.71 ± 7.29&lt;sup&gt;Ab&lt;/sup&gt;&lt;sub&gt;ab&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another by ANOVA followed by Tukey’s post hoc test (*p* > 0.05). n=5
4.7 Anti-inflammatory effects of stem extract of *Cynanchum viminale* on formalin-induced paw oedema in mice models

For the aqueous stem extract of *Cynanchum viminale*, diclofenac sodium was significantly lowered compared to the control and plant extract at dose 50 mg/kg ($p < 0.05$; Table 4.7). The plant extract at dose of 50 mg/kg body weight was significantly lowered compared to the control ($p < 0.05$; Table 4.7). The plant extract at dose 100 mg/kg body weight was significantly lowered compared to the control ($p < 0.05$; Table 4.7). The plant extract at dose of 150 mg/kg body weight was significantly lowered compared to the control ($p < 0.05$; Table 4.7) but non-significantly lowered compared to the rest ($p > 0.05$; Table 4.7)

In the first hour after drug administration, plant extract at dose of 100 mg/kg body weight showed the highest inhibition of inflammation by 87.42% among the extract dosages and diclofenac (Table 4.7). Aqueous extract of *C. viminale* exhibited anti-inflammatory activities but not in a dose dependent manner (Table 4.7).

In the second hour, plant extract at dose of 50 mg/kg body weight showed the highest inhibition of inflammation by 75.09% among all the treatments. *C. viminale* had anti-inflammatory effect though not in a dose dependent way (Table 4.7).

In the third hour, plant extract at dose 50 mg/kg body weight was more effective by reduction of paw diameter by 66.84% compared to the other treatment (Table 4.7).
In the fourth hour, the plant extract at dose 100 mg/kg body weight was found to reduce inflammation better by 53.13% (Table 4.7).
Table 4.7: Anti-inflammatory effects of aqueous stem extract of *Cynanchum viminale* on formalin-induced paw oedema in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>100.00 ± 0.00&lt;sup&gt;Eh&lt;/sup&gt;</td>
<td>98.87 ± 1.54&lt;sup&gt;Dh&lt;/sup&gt;</td>
<td>86.24 ± 2.06&lt;sup&gt;Cd&lt;/sup&gt;</td>
<td>72.44 ± 1.98&lt;sup&gt;Bd&lt;/sup&gt;</td>
<td>68.42 ± 3.04&lt;sup&gt;Ad&lt;/sup&gt;</td>
</tr>
<tr>
<td>Baseline</td>
<td>Formalin</td>
<td>100.00 ± 0.00&lt;sup&gt;Ecd&lt;/sup&gt;</td>
<td>88.98 ± 2.28&lt;sup&gt;Dcd&lt;/sup&gt;</td>
<td>75.01 ± 1.32&lt;sup&gt;Ccd&lt;/sup&gt;</td>
<td>76.00 ± 1.14&lt;sup&gt;Bcd&lt;/sup&gt;</td>
<td>73.54 ± 3.32&lt;sup&gt;Ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>Standard</td>
<td>Diclofenac</td>
<td>100.00 ± 0.00&lt;sup&gt;Ebc&lt;/sup&gt;</td>
<td>93.96 ± 7.23&lt;sup&gt;Dbc&lt;/sup&gt;</td>
<td>84.45 ± 4.35&lt;sup&gt;Cbc&lt;/sup&gt;</td>
<td>70.38 ± 3.62&lt;sup&gt;Bbc&lt;/sup&gt;</td>
<td>56.30 ± 2.9&lt;sup&gt;Abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>100.00 ± 0.00&lt;sup&gt;Ea&lt;/sup&gt;</td>
<td>88.57 ± 2.44&lt;sup&gt;Da&lt;/sup&gt;</td>
<td>75.09 ± 2.97&lt;sup&gt;Ca&lt;/sup&gt;</td>
<td>66.84 ± 4.31&lt;sup&gt;Ha&lt;/sup&gt;</td>
<td>59.11 ± 3.46&lt;sup&gt;Aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>100.00 ± 0.00&lt;sup&gt;Fab&lt;/sup&gt;</td>
<td>87.42 ± 7.64&lt;sup&gt;Dab&lt;/sup&gt;</td>
<td>80.79 ± 6.33&lt;sup&gt;Cab&lt;/sup&gt;</td>
<td>69.65 ± 5.55&lt;sup&gt;Hab&lt;/sup&gt;</td>
<td>53.13 ± 5.70&lt;sup&gt;Aab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>100.00 ± 0.00&lt;sup&gt;Eabc&lt;/sup&gt;</td>
<td>90.20 ± 6.06&lt;sup&gt;Dabc&lt;/sup&gt;</td>
<td>82.21 ± 4.98&lt;sup&gt;Cabc&lt;/sup&gt;</td>
<td>70.01 ± 2.44&lt;sup&gt;Habc&lt;/sup&gt;</td>
<td>60.07 ± 3.86&lt;sup&gt;Aabc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another by ANOVA followed by Tukey’s post hoc test (p> 0.05). n=5
4.8 Anti-inflammatory effects of aqueous stem extract of *Urtica dioica* on formalin-induced paw oedema in mice models

Treatment of mice with leaf extract of *Urtica dioica* showed some anti-inflammatory activity against formalin-induced oedema, which was indicated by reduction in paw oedema (Table 4.8). Diclofenac sodium was significantly lowered compared to the control (*p* < 0.05; Table 4.8). The plant extract at dose of 50 mg/kg body weight was non-significantly lowered compared to the (*p* > 0.05; Table 4.8). The plant extract at dose 100 mg/kg body weight was non-significantly lowered compared to the control and plant extract at dose 50 mg/kg (*p* > 0.05; Table 4.8). The plant extract at dose of 150 mg/kg body weight was significantly lowered compared to the control (*p* < 0.05; Table 4.8).

In the first hour, leaf extracts of *U. dioica* at doses of 50, 100 and 150 mg/kg body weight recorded slightly lower paw diameters (Table 4.8). The anti-inflammatory effects at dose level of 150 mg/kg reduced inflammation to 88.94% (Table 4.8).

In the second hour, the anti-inflammatory activity of *U. dioica* at all dose level (50, 100 and 150 mg/kg body weight) was found to lower the elevated paw diameter (Table 4.8). At this hour, the leaf extract at dose 150 mg/kg body weight exhibited greater anti-inflammatory effect by 75.38% (Table 4.8).

Three hours after drug administration, *U. dioica* at all dose levels (50, 100 and 150 mg/kg body weight) was found to lower the formalin-induced inflammation (Table 4.8). The
plant extract at dose of 100 mg/kg showed better anti-inflammatory effect than the other dose levels by reducing paw oedema to 72.88%. However, diclofenac sodium had greater anti-inflammatory activities for it lowered the paw diameter to 70.38% (Table 4.8).

In the fourth hour, *U. dioica* exhibited anti-inflammatory activity with plant extract at dose of 150 mg/kg body weight reducing paw diameter to 61.82%.
Table 4.8: Anti-inflammatory effects of aqueous leaf extract of *Urtica dioica* on formalin-induced paw oedema in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>100.00 ± 0.00&lt;sup&gt;Ecd&lt;/sup&gt;</td>
<td>98.87 ± 1.54&lt;sup&gt;Dcd&lt;/sup&gt;</td>
<td>86.24 ± 2.06&lt;sup&gt;Ccd&lt;/sup&gt;</td>
<td>72.44 ± 1.98&lt;sup&gt;Bcd&lt;/sup&gt;</td>
<td>68.42 ± 3.04&lt;sup&gt;Acd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Baseline</td>
<td>Formalin</td>
<td>100.00 ± 0.00&lt;sup&gt;Ebc&lt;/sup&gt;</td>
<td>88.98 ± 2.28&lt;sup&gt;Dbc&lt;/sup&gt;</td>
<td>75.01 ± 1.32&lt;sup&gt;Cbc&lt;/sup&gt;</td>
<td>76.00 ± 1.14&lt;sup&gt;Bbc&lt;/sup&gt;</td>
<td>73.54 ± 3.32&lt;sup&gt;Abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Standard</td>
<td>Diclofenac</td>
<td>100.00 ± 0.00&lt;sup&gt;Eab&lt;/sup&gt;</td>
<td>93.96 ± 7.23&lt;sup&gt;Dab&lt;/sup&gt;</td>
<td>84.45 ± 4.35&lt;sup&gt;Cab&lt;/sup&gt;</td>
<td>70.38 ± 3.62&lt;sup&gt; Bab&lt;/sup&gt;</td>
<td>56.30 ± 2.9&lt;sup&gt;Aab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>100.00 ± 0.00&lt;sup&gt;Ecd&lt;/sup&gt;</td>
<td>92.97 ± 6.05&lt;sup&gt;Dbcd&lt;/sup&gt;</td>
<td>80.80 ± 7.54&lt;sup&gt;Cbcd&lt;/sup&gt;</td>
<td>75.66 ± 1.83&lt;sup&gt;Bbcd&lt;/sup&gt;</td>
<td>69.61 ± 4.89&lt;sup&gt;Abcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>100.00 ± 0.00&lt;sup&gt;Ecd&lt;/sup&gt;</td>
<td>96.71 ± 4.50&lt;sup&gt;Dcd&lt;/sup&gt;</td>
<td>84.81 ± 2.71&lt;sup&gt;Cd&lt;/sup&gt;</td>
<td>82.54 ± 4.18&lt;sup&gt;Bd&lt;/sup&gt;</td>
<td>67.40 ± 7.94&lt;sup&gt;Ad&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>100.00 ± 0.00&lt;sup&gt;Ea&lt;/sup&gt;</td>
<td>88.94 ± 1.32&lt;sup&gt;Da&lt;/sup&gt;</td>
<td>75.38 ± 0.85&lt;sup&gt;Ca&lt;/sup&gt;</td>
<td>72.88 ± 2.50&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>61.82 ± 1.78&lt;sup&gt;Aa&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another by ANOVA followed by Tukey’s post hoc test (*p* > 0.05). n=5
4.9 Antipyretic effects of bark extracts of *Acacia nilotica* on Brewer’s yeast-induced pyrexia in mice models

Treatment of mice with bark extract of *Acacia nilotica* showed some antipyretic activity against brewer’s yeast induced pyrexia, which was indicated by reduction in rectal temperature (Table 4.9). There was no statistical difference between the baseline, reference drug, plant extract at dose 100 mg/kg and 150 mg/kg (*p* < 0.05; Table 4.9) but a significant difference among the control and plant extract at dose 50 mg/kg body weight (*p* < 0.05; Table 4.9). There was a significant difference between the zero hour rectal temperatures and temperature taken 1hr, 2hr and 3hr after treatment (*p* < 0.05; Table 4.9).

In the first hour after treatment, the plant extract at dose of 50 mg/kg body weight showed the highest antipyretic activity among the extract dosages by reducing fever to 99.07% (Table 4.9). Aqueous extract of *A. nilotica* exhibited antipyretic activities but not in a dose dependent manner (Table 4.9).

In the second hour, the plant extract at dose of 150 mg/kg body weight showed the highest effectiveness in reducing the rectal temperature to 98.89% compared to the reference drug. The Plant extract at dose 50 mg/kg showed a pyretic effect instead as the rectal temperature was increased at this hour to 101.10% (Table 4.9).
In the third hour, the plant extract at dose 150 mg/kg was more effective as shown by a reduction of fever to 98.23% compared to the plant extract at the other dosages and reference drug, diclofenac.
Table 4.9: Antipyretic effects of aqueous bark extract of *Acacia nilotica* on Brewer’s yeast-induced pyrexia in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Percent change in rectal temperature (°C) after drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0hr</td>
</tr>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>100.0 ± 0.00&lt;sup&gt;Ba&lt;/sup&gt;</td>
</tr>
<tr>
<td>Baseline</td>
<td>Yeast</td>
<td>100.0 ± 0.00&lt;sup&gt;Bb&lt;/sup&gt;</td>
</tr>
<tr>
<td>Standard</td>
<td>Paracetamol</td>
<td>100.0 ± 0.00&lt;sup&gt;Bb&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>100.0 ± 0.00&lt;sup&gt;Bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>100.0 ± 0.00&lt;sup&gt;Bh&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>100.0 ± 0.00&lt;sup&gt;Bh&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another by ANOVA followed by Tukey’s post hoc test (*p* > 0.05). n=6
4.10 Antipyretic effects of aqueous leaf extract of *Aloe volkensii* on Brewer’s yeast-induced pyrexia in mice models

Treatment of mice with leaf extracts of *Aloe volkensii* showed some antipyretic activity against brewer’s yeast induced pyrexia, which was indicated by reduction in rectal temperature (Table 4.10). The paracetamol was non-significantly lowered compared to the control to all the other treatment groups but significantly different from the baseline \((p>0.05; \text{Table 4.10})\). The plant extract at dose 50 mg/kg body weight was significantly lowered compared to the control \((p<0.05; \text{Table 4.10})\). The plant extract at doses 100 mg/kg and 150 mg/kg body weight were significantly lowered compared to the control \((p<0.05; \text{Table 4.10})\). There was a significant difference between the zero hour temperature and the temperature taken 1hr, 2hrs and 3hrs after treatment \((p<0.05; \text{Table 4.10})\). Aqueous extract of *A. volkensii* exhibited antipyretic activities in a non-dose dependent manner (Table 4.10).

In the first hour after treatment, plant extract at dose of 150 mg/kg body weight caused the highest antipyretic activity by reducing fever to 97.83% among the extract dosages and the reference drug (Table 4.10).

In the second hour, plant extract at dose of 50 mg/kg body weight showed better effectiveness in reducing the rectal temperature to 96.05% (Table 4.10).
In the third hour, plant extract at dose of 150 mg/kg showed a greater antipyretic activity as the rectal temperature reduced to 97.55% compared to the reference drug.
Table 4.10: Antipyretic effect of aqueous leaf extract of *Aloe volkensii* on Brewer’s yeast-induced pyrexia in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Percent change in rectal temperature (°C) after drug administration</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>100.0 ± 0.00&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>94.49 ± 0.55&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>93.70 ± 0.84&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>95.28 ± 0.85&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>Yeast</td>
<td>100.0 ± 0.00&lt;sup&gt;Bc&lt;/sup&gt;</td>
<td>98.10 ± 0.01&lt;sup&gt;Ac&lt;/sup&gt;</td>
<td>99.09 ± 0.21&lt;sup&gt;Ac&lt;/sup&gt;</td>
<td>99.91 ± 0.70&lt;sup&gt;Ac&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>Paracetamol</td>
<td>100.0 ± 0.00&lt;sup&gt;Bar&lt;/sup&gt;</td>
<td>98.50 ± 0.61&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>98.96 ± 2.04&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>99.03 ± 2.20&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>100.0 ± 0.00&lt;sup&gt;Bra&lt;/sup&gt;</td>
<td>98.20 ± 0.51&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>96.05 ± 0.68&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>97.94 ± 0.67&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>100.0 ± 0.00&lt;sup&gt;Brc&lt;/sup&gt;</td>
<td>98.39 ± 0.00&lt;sup&gt;Abc&lt;/sup&gt;</td>
<td>97.86 ± 0.00&lt;sup&gt;Abc&lt;/sup&gt;</td>
<td>99.33 ± 0.65&lt;sup&gt;Abc&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>100.0 ± 0.00&lt;sup&gt;Brc&lt;/sup&gt;</td>
<td>97.83 ± 5.26&lt;sup&gt;Abc&lt;/sup&gt;</td>
<td>100.48 ± 1.73&lt;sup&gt;Abc&lt;/sup&gt;</td>
<td>97.55 ± 2.32&lt;sup&gt;Abc&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another by ANOVA followed by Tukey’s post hoc test (p > 0.05). n=6
4.11 Antipyretic effects of stem extract of *Cynanchum viminale* on Brewer’s yeast-induced pyrexia in mice models

Treatment of mice with stem extract of *Cynanchum viminale* showed some antipyretic activity against brewer’s yeast induced pyrexia, which was indicated by reduction in rectal temperature (Table 4.11). Rectal temperatures at zero hour were not comparable to the rectal temperatures recorded 1hr, 2hrs and 3hrs after treatment ($p<0.05$; Table 4.11). The standard drug (Paracetamol) was significantly lowered compared to the control ($p<0.05$; Table 4.11) but non-significantly lowered compared to the baseline and plant extract at dose 50 mg/kg and 100 mg/kg body weight ($p>0.05$; Table 4.11). Plant extract at dose 50 mg/kg was significantly lowered compared to the control ($p<0.05$; Table 4.11). Plant extract at dose 100 mg/kg was significantly lowered compared to the control ($p<0.05$; Table 4.11). Plant extract at dose 150 mg/kg body weight was significantly lowered compared to the control ($p<0.05$; Table 4.11). *C. viminale* exhibited an antipyretic effect in a non-dose dependent way (Table 4.11).

In the first hour after treatment, plant extract at dose of 150 mg/kg body weight caused the highest antipyretic activity among the extract dosages by reducing rectal temperatures to 94.08% (Table 4.11). Aqueous extract of *C. viminale* exhibited antipyretic activities but not in a dose dependent manner and was seen to be better in reducing fever at dose 50 mg/kg body weight than the reference drug (Table 4.11).
In the second hour, plant extract at dose of 100 mg/kg body weight caused the highest effectiveness in reducing the rectal temperature to 96.91% compared to the reference drug. Plant extract at dose 50 mg/kg and 150 mg/kg body weight caused a pyretic effect instead as the rectal temperature was increased to 100.52% (Table 4.11).

In the third hour, plant extract at dose 150 mg/kg was more effective compared to the reference drug as fever was reduced to 96.29% (Table 4.11).
Table 4.11: Antipyretic effect of aqueous stem extract of *Cynanchum viminale* on Brewer’s yeast-induced pyrexia in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>100.0 ± 0.00&lt;sup&gt;BA&lt;/sup&gt;</td>
<td>94.49 ± 0.55&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>93.70 ± 0.84&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>95.28 ± 0.85&lt;sup&gt;Aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>Baseline</td>
<td>Yeast</td>
<td>100.0 ± 0.00&lt;sup&gt;BEd&lt;/sup&gt;</td>
<td>98.10 ± 0.01&lt;sup&gt;Ac&lt;/sup&gt;d</td>
<td>99.09 ± 0.21&lt;sup&gt;Acd&lt;/sup&gt;</td>
<td>99.91 ± 0.70&lt;sup&gt;Ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>Standard</td>
<td>Paracetamol</td>
<td>100.0 ± 0.00&lt;sup&gt;BEd&lt;/sup&gt;</td>
<td>98.50 ± 0.61&lt;sup&gt;Acd&lt;/sup&gt;</td>
<td>98.96 ± 2.04&lt;sup&gt;Acd&lt;/sup&gt;</td>
<td>99.03 ± 2.20&lt;sup&gt;Acd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>100.0 ± 0.00&lt;sup&gt;Bd&lt;/sup&gt;</td>
<td>99.20 ± 0.79&lt;sup&gt;Ad&lt;/sup&gt;d</td>
<td>100.52 ± 2.10&lt;sup&gt;Ad&lt;/sup&gt;d</td>
<td>98.06 ± 2.15&lt;sup&gt;Ad&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>100.0 ± 0.00&lt;sup&gt;Bc&lt;/sup&gt;</td>
<td>98.98 ± 0.25&lt;sup&gt;Ac&lt;/sup&gt;c</td>
<td>96.91 ± 0.61&lt;sup&gt;Ac&lt;/sup&gt;</td>
<td>98.01 ± 0.75&lt;sup&gt;Ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>100.0 ± 0.00&lt;sup&gt;Bh&lt;/sup&gt;</td>
<td>94.08 ± 0.21&lt;sup&gt;Ab&lt;/sup&gt;b</td>
<td>97.12 ± 0.25&lt;sup&gt;Ab&lt;/sup&gt;b</td>
<td>96.29 ± 0.52&lt;sup&gt;Ab&lt;/sup&gt;b</td>
</tr>
</tbody>
</table>

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another by ANOVA followed by Tukey’s post hoc test (p > 0.05). n=6
4.12 Antipyretic effects of leaf extract of *Urtica dioica* on Brewer’s yeast induced pyrexia in mice models

Treatment of mice with leaf extract of *Urtica dioica* showed some antipyretic activity against brewer’s yeast induced pyrexia, which was indicated by reduction in rectal temperature (Table 4.12). The standard drug (paracetamol) was significantly lowered compared to the control ($p< 0.05$; Table 4.12). Plant extract at dose 50 mg/kg was significantly lowered compared to the control but non-significantly lowered compared to plant extract at dose of 150 mg/kg body weight ($p > 0.05$; Table 4.12). Plant extract at dose 100 mg/kg was significantly lowered compared to the control ($p < 0.05$; Table 4.12). Plant extract at dose 150 mg/kg body weight was significantly reduced compared to all the other treatment ($p < 0.05$; Table 4.12). *U. dioica* exhibited an antipyretic effect though not in a dose dependent way (Table 4.12).

In the first hour after treatment, plant extract at dose of 100 mg/kg body weight caused the highest antipyretic activity among the extract dosages as it reduced fever to 97.89% (Table 4.12). Aqueous extract of *U. dioica* at dose of 100 mg/kg body weight was found to be better in reducing fever than the reference drug weight (Table 4.12).

In the second hour, plant extract at dose of 100 mg/kg body weight caused the highest effectiveness in reducing the rectal temperature to 93.95% (Table 4.12). Plant extract at dose 50 mg/kg showed a pyretic effect instead as the rectal temperature was increased to 101.44% at this hour compared with the value at the first hour after treatment (Table...
4.12). However the rectal temperature of the group treated with herbal medicine at dose 150 mg/kg showed a slight decrease in temperature to 97.75% compared to the first hour after treatment (Table 4.12).

In the third hour, plant extract at dose 100 mg/kg was more effective compared to the reference drug and plant extract at the other dosages as it lowered fever to 95.26%, the antipyretic effect of the herbal medicine on fever was not in a dose dependent manner (Table 4.12).
Table 4.12: Antipyretic effect of aqueous leaf extract of *Urtica dioica* on Brewer’s yeast induced pyrexia in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>100.0 ± 0.00&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>94.49 ± 0.55&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>93.70 ± 0.84&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>95.28 ± 0.85&lt;sup&gt;Aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>Baseline</td>
<td>Yeast</td>
<td>100.0 ± 0.00&lt;sup&gt;Be&lt;/sup&gt;</td>
<td>98.10 ± 0.01&lt;sup&gt;Ade&lt;/sup&gt;</td>
<td>99.09 ± 0.21&lt;sup&gt;Ade&lt;/sup&gt;</td>
<td>99.91 ± 0.70&lt;sup&gt;Ade&lt;/sup&gt;</td>
</tr>
<tr>
<td>Standard</td>
<td>Paracetamol</td>
<td>100.0 ± 0.00&lt;sup&gt;Bd&lt;/sup&gt;</td>
<td>98.50 ± 0.61&lt;sup&gt;Ad&lt;/sup&gt;</td>
<td>98.96 ± 2.04&lt;sup&gt;Ad&lt;/sup&gt;</td>
<td>99.03 ± 2.20&lt;sup&gt;Ad&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>100.0 ± 0.00&lt;sup&gt;Be&lt;/sup&gt;</td>
<td>99.19 ± 0.01&lt;sup&gt;Ae&lt;/sup&gt;</td>
<td>101.44 ± 0.82&lt;sup&gt;Ae&lt;/sup&gt;</td>
<td>98.75 ± 0.77&lt;sup&gt;Ae&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>100.0 ± 0.00&lt;sup&gt;Bh&lt;/sup&gt;</td>
<td>97.89 ± 0.01&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>93.95 ± 0.02&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>95.26 ± 0.02&lt;sup&gt;Ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>100.0 ± 0.00&lt;sup&gt;Bc&lt;/sup&gt;</td>
<td>98.72 ± 0.10&lt;sup&gt;Ac&lt;/sup&gt;</td>
<td>97.75 ± 0.21&lt;sup&gt;Ac&lt;/sup&gt;</td>
<td>96.43 ± 0.21&lt;sup&gt;Ac&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another by ANOVA followed by Tukey’s post hoc test (*p* > 0.05). n=6
CHAPTER FIVE
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The search for bioactive components which can be used as non-conventional analgesics, NSAIDs and antipyretics has received considerable attention in recent times because of the increasing worldwide development of lasting solutions to pain, inflammation and fever, which are safe to human and with no side effects as seen with modern medicine. Thus, this study was designed to evaluate the curative capacity of aqueous extract of Acacia nilotica, Aloe volkensii, Cynanchum viminale and Urtica dioica against pain, inflammation and fever.

The evaluation of antinociceptive, anti-inflammatory and antipyretic properties of the extracts was done by formalin induced pain and inflammation and brewer’s yeast induced pyrexia in Swiss albino mice. Subcutaneous injection of a dilute aqueous formalin (formaldehyde) solution into the dorsal surface of the rat or mouse hind paw elicits two distinct quantifiable nociceptive behaviors; flinching / shaking and licking / biting of the injected paw (Dubuisson and Dennis, 1977; Tjolsen et al., 1992). This formalin-induced nociceptive behavior shows an early and a late phase. The early phase, which starts immediately following injection of formalin, only lasts approximately 5 min and is probably due to direct chemical stimulation of nociceptors (acute pain). The second phase, which lasts 20 to 40 min, starts approximately 15 to 30 min following formalin injection and experimental data suggest that peripheral, inflammatory processes are
involved (Haley et al., 1989). The formalin test differs from most other nociceptive tests, such as the hot plate, tail flick and tail pinch tests, in that it enables evaluation of analgesic activity towards moderate, continuous pain generated by injured tissue.

The two distinct phases in formalin test are due to direct effect of formalin on nociception 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 (Dubuisson and Dennis, 1977; Huskaar and Hole, 1987; Tjøsen et al., 1992). Stimulation of opioid receptors has also been suggested as a possible mechanism of action against neurogenic pain (Gaertner et al., 1999).

In this study, aqueous bark extract of Acacia nilotica showed a significant antinociceptive effect by reducing the formalin-induced paw licking time in both phases. The highest analgesic effect was at 50 mg/kg dose level for both acute and chronic pain. Aqueous leaf extract of Aloe volkensii also showed the highest analgesic effect at dose 50 mg/kg dose level for both early and late phases. However, for the aqueous stem extract of Cynanchum viminale, analgesic effect was observed at dose 100 mg/kg and also 50 mg/kg for acute pain but a dose of 150 mg/kg had the least paw licking time, indicating better analgesic effect. Aqueous leaf extract of Urtica dioica showed the highest analgesic effect at dose of 150 mg/kg for acute pain and dose 100 mg/kg and 150 mg/kg body weight for chronic pain. These findings suggest both direct analgesic effects on the nociceptor blockage and
an inhibition of the synthesis and/or release of inflammatory pain mediators such as prostaglandins.

These results are similar to other previous studies on evaluation of antinociceptive activities of medicinal plant extracts. That the aqueous extract of *Acacia nilotica, Aloe volkensii, Cynanchum viminale* and *Urtica dioica* demonstrated a reduction in the formalin-induced paw licking time in both phases is consistent with Mahnaz *et al.* (2012), who observed antinociceptive activity of hydro-alcoholic extract of *Marrubium parviflorum* against formalin-induced pain in mice. Similarly, the methanolic leaf extract of *Securinega virosa* demonstrated related antinociceptive effect in acetic acid induced writhing test and formalin test models (Yerima *et al.*, 2009).

That the aqueous extracts of *Acacia nilotica, Aloe volkensii* and *Cynanchum viminale* produced non-dose dependent analgesic activity is related to studies by Zarei *et al.* (2015), who observed the antinociceptive activities of *Melissa officinalis* leaf extracts in laboratory animals. The dose ranges used in this study were within the dose ranges used by Norma *et al.* (2013), Santanu *et al.* (2013) and Ishola *et al.* (2014). That the aqueous leaf extract of *Urtica dioica* produced a dose dependent analgesic activity is related to a study by Ezeja *et al.* (2011), who observed the analgesic effect of *B. coriacea* extract on acetic acid-induced writhing reflex method and showed that the extract at the doses used reduced the mean number of abdominal constrictions or writhing in a dose dependent manner when compared to the negative control group.
The aqueous extract of *Acacia nilotica, Aloe volkensii* and *Cynanchum viminale* showed the highest analgesic effect at lower dose of 50 mg/kg body weight in early and late phases. This may be due to the fact that the high dose takes longer to be absorbed across the peritoneum cavity (Gitahi *et al.*, 2015).

The aqueous leaf extract of *Urtica dioica* at the lower dose level of 50 mg/kg body weight was not as effective as the two higher doses (100 and 150 mg/kg body weight) in both phases. These findings may be explained by the fast metabolism, clearance and inactivation of the lower concentration of the active principle. It is also likely that, at the lower dose, there is simply not a sufficient concentration of the active principle(s) (Gitahi *et al.*, 2015).

The antinociceptive effect of *Acacia nilotica, Aloe volkensii, Cynanchum viminale* and *Urtica dioica* can be attributed to one or more groups of the phytoconstituents observed in the extracts. Several studies have shown the antinociceptive activity of such compounds. Phytochemical screening of methanolic leaf extract of *Securinaga viroso* revealed the presence of flavonoids, saponins, tannins, glycosides, alkaloids and steroids (Yerima *et al.*, 2009). A study on the phytochemical composition of *Acacia nilotica, Aloe volkensii, Cynanchum viminale* and *Urtica dioica* has revealed presence of saponins, tannins, flavonoids, alkaloids and phenols (Mukundi *et al.*, 2015). Analgesic and anti-inflammatory effects have been observed in flavonoids as well as tannins (Ahmadiani *et al.*
Flavonoids such as quercetin are known to be effective in acute inflammation (Rajnarayana et al., 2001). There are also reports on the analgesic effects of alkaloids, essential oils and saponins (Choi et al., 2005; de Araujo et al., 2005; Reanmongkol et al., 2005). The analgesic and anti-inflammatory effect of the extracts in this study may, therefore, be due to the presence of flavonoids, tannins, alkaloids or saponins. Flavonoids are widely shown to target prostaglandins which are involved in the pain perception through moderating opioidergic mechanism.

These findings strongly recommend that these medicinal plants have peripheral analgesic activity and their mechanisms of action may be mediated through inhibition of local peritoneal receptors which may be the involvement of cyclooxygenase inhibition potential. The profound analgesic activity of these medicinal plants may be due to the interference of their active principle(s) with the release of pain mediators (Gitahi et al., 2015).

Tissue damage and injury are always associated with pain and inflammation. In this formalin test, the mice used were treated with several treatments to reduce inflammation. Formalin test is a biphasic response where first phase is the direct effect of formalin which involves neurogenic pain. The pain is usually initiated when harmful mechanical, thermal or chemical stimuli agitate the peripheral terminals of particular main afferent neuron named nociceptors (Tominaga et al., 2004). The second phase is involved in the inflammatory reactions. In this study, it was noticed that exposure of formalin induced
inflammation to various treatments resulted in a significant inhibition of inflammation. The aqueous extracts of *Acacia nilotica*, *Aloe volkensii*, *Cynanchum viminale* and *Urtica dioica* were found to significantly suppress the inflammation when treated with different concentrations.

After five hours of the test period, the aqueous bark extract of *Acacia nilotica* produced appreciable anti-inflammatory activity against formalin induced inflammation in albino mice. A dose level of 100 mg/kg and 150 mg/kg body weight showed the highest anti-inflammatory activity. Lower dose of 50 mg/kg was not as effective and may be explained by the fast metabolism, clearance and inactivation of the lower concentration of the active principles or the lower dose was an insufficient concentration of the active principles (Gitahi *et al.*, 2015).

The aqueous leaf extract of *Aloe volkensii* showed the highest effectiveness against inflammation at dose 50 mg/kg indicating a possibility that maybe the high dose takes longer to be absorbed across the peritoneum cavity. This was as effective as the reference drug, diclofenac.

The aqueous extracts of *Cynanchum viminale* and *Urtica dioica* exhibited greater anti-inflammatory activity at dose 150 mg/kg. Lower dose of 50 mg/kg was not as effective and may be explained by the fast metabolism, clearance and inactivation of the lower
concentration of the active principles or the lower dose was an insufficient concentration of the active principles (Gitahi et al., 2015).

The association of both antinociceptive activity and moderate anti-inflammatory effect observed with the extracts has also been shown with non-steroidal anti-inflammatory drugs (NSAIDs). It is a well-established fact that NSAIDs exert their analgesic and anti-inflammatory activity by the inhibition of cyclooxygenase activity (Vane, 1971).

The anti-inflammatory effects of the extracts may be due to their content of flavonoids, tannins, alkaloids and saponins. Several studies have shown the antinociceptive activity of such compounds. A study by Muhammad et al. (2013) showed that the Viola betonicifolia methanolic extract was found to contain alkaloids, saponins, flavonoids, tannins, proteins, and phenolic compounds where the anti-inflammatory activity of V. betonicifolia was attributed to these groups of chemical compounds.

The anti-inflammatory effect of the four medicinal plants extracts was not evident in every concentration of the extracts as early as the first hour of formalin injection but maximum inhibition was during the fifth hour. They did not maintain the suppression of the inhibition throughout the duration of the study. These findings could have been due to the fact that the active principles in the extracts required biotransformation so as to have an anti-inflammatory effect (Gitahi et al., 2015).
Brewer’s yeast was used to induce fever in albino mice. Fever was recorded 19hrs after yeast injection since yeast takes about 19hrs to cause the elevation of body temperature (Turner, 1965). Subcutaneous injection of Brewer’s yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered as a useful test for the screening of plants materials as well as synthetic drugs for their antipyretic effect (Devi et al., 2003; Khan et al., 2009).

Yeast induced pyrexia is called pathogenic fever and its etiology could be the production of prostaglandins (Moltz, 1993). The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclooxygenase enzyme activity. There are several mediators for pyrexia and the inhibitions of these mediators are responsible for the antipyretic effect (Rawlins, 1973).

The oral administration of Acacia nilotica, Aloe volkensii, Cynanchum viminale and Urtica dioica significantly attenuated rectal temperature of yeast induced albino mice. Thus it can be postulated that Acacia nilotica, Aloe volkensii, Cynanchum viminale and Urtica dioica contained pharmacologically active principle(s) that interfere with the release of prostaglandins.

After three hours of the test period, the aqueous extracts of Acacia nilotica, Aloe volkensii, Cynanchum viminale and Urtica dioica produced appreciable antipyretic
activity against brewer’s yeast induced pyrexia in albino mice. Dose of 150 mg/kg body weight demonstrated the greatest rectal temperature lowering activity for all medicinal plants. These findings were in agreement with the effects of other medicinal plants in laboratory animals. Similar work carried out by Bhavani et al. (2012) showed that the hydro alcoholic extract of *Rosa alba* possessed a significant antipyretic effect in yeast induced elevation of body temperature in experimental rats. It was revealed that the extract showed dose dependent antipyretic activity. At a dose of 200mg/kg body weight, it caused a significant antipyretic activity.

Non-steroidal anti-inflammatory drugs produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus. Work done by Bhavani et al. (2012) showed that the antipyretic activity of hydro alcoholic extract of *Rosa alba* is probably by inhibition of prostaglandin synthesis in hypothalamus. Therefore, it is possible that the antipyretic action of aqueous extracts of *Acacia nilotica, Aloe volkensii, Cynanchum viminale* and *Urtica dioica* was related to the inhibition of prostaglandin synthesis in hypothalamus. However, other alternative mechanisms for blocking fever cannot be ruled out.

Further hydro alcoholic extract of *Rosa alba* was found to contain carbohydrates, alkaloids, glycosides, flavonoids and tannins, through preliminary photochemical screening. Qualitative phytochemical screening in this study revealed that the aqueous extracts of *Acacia nilotica, Aloe volkensii, Cynanchum viminale* and *Urtica dioica*
contain tannins, saponins, phenolics, alkaloids and flavonoids. A number of these phytochemicals have been shown to exhibit inhibitory action on cyclooxygenase enzyme and, as a result, produce antipyretic activity by preventing the formation of prostaglandins or by increasing the concentration of body’s own antipyretic components (Okokon and Nwafor, 2010).

Flavonoids are known to target prostaglandins which are involved in the pyrexia. Hence the presence of flavonoids in the aqueous extract of *Acacia nilotica*, *Aloe volkensii*, *Cynanchum viminalae* and *Urtica dioica* plant may be contributory to its antipyretic activity.

The presence of alkaloids in these extracts could also be responsible for the antipyretic activity. For instance, according to Reanmongkol *et al.* (1994), while evaluating on antipyretic effects of alkaloids extracted from the stem bark of *Hunteria zeylanica*, reported that alkaloids also possesses antipyretic effects.

The antipyretic activity of the aqueous extracts of *Acacia nilotica*, *Aloe volkensii*, *Cynanchum viminalae* and *Urtica dioica* may also be attributed to the presence of saponins, which are involved in inhibition of prostaglandin synthesis. According to the study of Zakaria *et al.* (2007) saponins are suggested to act synergistically to exert antipyretic activity. In a related study, the antipyretic effect of ethanolic root extracts of
Asparagus racemosus on yeast-induced hyperthermia in rats was attributed to the saponins in the extracts (Vasundra and Divya, 2013).

It was observed that the aqueous extracts of *Acacia nilotica*, *Aloe volkensii*, *Cynanchum viminale* and *Urtica dioica* at lower dose levels of 50 and 100 mg/kg body weight were not as effective as the higher dose of 150 mg/kg body weight. Thus may be explained by the fast metabolism, clearance and inactivation of the lower concentration of the active principles. It is also likely that at the lower dose there is simply not a sufficient concentration of the active principle(s) (Gitahi *et al*., 2015).

The aqueous extracts of *Acacia nilotica*, *Aloe volkensii*, *Cynanchum viminale* and *Urtica dioica* at all the dose levels, did not lower rectal temperature in the first and second hours as effectively as in the third hour. These findings could have been due to the fact that the active principles in the extracts required biotransformation so as to become antipyretic (Gitahi *et al*., 2015).

That the dose level of 150 mg/kg body weight of the aqueous extracts of *Acacia nilotica*, *Aloe volkensii*, *Cynanchum viminale* and *Urtica dioica* were marginally effective than paracetamol suggests a possibly better blockage of prostaglandins biosynthesis or mimicry of paracetamol action by the active principles in the extracts. It is also possible that the herbal extracts were efficiently inhibiting alternative mechanisms for blocking fever. The decline in rectal temperature in case of treatment with the medicinal plants
extracts was not as sudden as that of paracetamol administration. Therefore, the extracts offer some advantage over the standard drug (paracetamol).

5.2 Conclusions

In conclusion, the present study has demonstrated the antinociceptive, anti-inflammatory and antipyretic potential of aqueous extracts of *Acacia nilotica*, *Aloe volkensii*, *Cynanchum viminale* and *Urtica dioica* in albino mice.

The aqueous extracts of *Acacia nilotica*, *Aloe volkensii*, *Cynanchum viminale* and *Urtica dioica* were able to inhibit pain sensation of both phases. It is, therefore, possible to find opioid analgesics as well as analgesics in aqueous extracts of *Acacia nilotica*, *Aloe volkensii*, *Cynanchum viminale* and *Urtica dioica* that act by inhibition of inflammatory pathways responsible for pain.

This study has also indicated that the aqueous extracts of *Acacia nilotica*, *Aloe volkensii*, *Cynanchum viminale* and *Urtica dioica* have potent anti-inflammatory activity in mice in a dose dependent manner.

The significant reduction in pyrexia in mice when treated with standard drugs as well as different doses of extracts, reflect that aqueous extracts of *Acacia nilotica*, *Aloe volkensii*, *Cynanchum viminale* and *Urtica dioica* are endowed with potent antipyretic properties. It is also evident from the study that the antipyretic activity of aqueous extracts of *Acacia*
*nilotica, Aloe volkensii* and *Cynanchum viminalis* at 150 mg/kg body weight was more effective compared to other doses used in this study.

They may serve as good bio-resource for generating readily available herbal formulations that are more effective in the treatment of pain, inflammation and fever conditions. However, the modes of antinociceptive, anti-inflammatory and antipyretic actions of the studied extracts are still obscure. The present study, therefore, scientifically confirms and supports the traditional use of aqueous extracts of *Acacia nilotica, Aloe volkensii, Cynanchum viminalis* and *Urtica dioica* for management of fever, inflammation and painful conditions. In this study, the null hypothesis is hence rejected.

**5.3 Recommendations**

I. In antinociceptive studies using mice model, *A. nilotica* at a dose level of 50 mg/kg body weight is an effective positive control especially in both early and late phase pain.

II. In mice model investigating inflammation, *A. nilotica* and *C. viminalis* are appropriate anti-inflammatory control at a dose level of 150 mg/kg and 100 mg/kg body weight respectively.

III. In antipyretic studies using mice model, *U. dioica* at a dose level of 150 mg/kg body weight is an effective positive control.
Therefore, I recommend their traditional use in management of pain, fever and inflammation.

5.4 Suggestions for further studies

I. The promising results obtained from *Acacia nilotica*, *Aloe volkensii*, *Cynanchum viminale* and *Urtica dioica* encourages further investigations to establish the synergistic effects of the combined plant extracts.

II. This study should be replicated to establish the antinociceptive, anti-inflammatory and antipyretic activities of the organic extracts of the plant extracts.
REFERENCES


Diallo, D. (2000). Ethnopharmacological survey of medicinal plants in Mali and phytochemical study of four of them: Glinus oppositifolius (aizoaceae), Diospyros abyssinica (Ebenaceae), Entada Africana (Mimosacee), Trichilia emetica (meliaceae). These de Doctorate. Faculte de Science, University de Lausanne, Switzerland.


APPENDICES

Appendix I: Anti-inflammatory effects of aqueous bark extract of *Acacia nilotica* on formalin–induced paw oedema in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
<th>5hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>2.0 ± 0.00&lt;sup&gt;Ad&lt;/sup&gt;</td>
<td>3.48 ± 0.08&lt;sup&gt;Fd&lt;/sup&gt;</td>
<td>3.44 ± 0.05&lt;sup&gt;Ed&lt;/sup&gt;</td>
<td>3.0 ± 0.00&lt;sup&gt;Bd&lt;/sup&gt;</td>
<td>2.52 ± 0.04&lt;sup&gt;Cd&lt;/sup&gt;</td>
<td>2.38 ± 0.83&lt;sup&gt;Bd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Baseline</td>
<td>Formalin</td>
<td>2.0 ± 0.00&lt;sup&gt;Ac&lt;/sup&gt;</td>
<td>4.0 ± 0.07&lt;sup&gt;Fc&lt;/sup&gt;</td>
<td>3.56 ± 0.13&lt;sup&gt;Ec&lt;/sup&gt;</td>
<td>3.0 ± 0.00&lt;sup&gt;Dc&lt;/sup&gt;</td>
<td>3.04 ± 0.05&lt;sup&gt;Ec&lt;/sup&gt;</td>
<td>2.94 ± 0.08&lt;sup&gt;Ec&lt;/sup&gt;</td>
</tr>
<tr>
<td>Standard</td>
<td>Diclofenac</td>
<td>2.0 ± 0.00&lt;sup&gt;Ac&lt;/sup&gt;</td>
<td>3.56 ± 0.19&lt;sup&gt;Fc&lt;/sup&gt;</td>
<td>3.34 ± 0.23&lt;sup&gt;Dc&lt;/sup&gt;</td>
<td>3.0 ± 0.00&lt;sup&gt;Bc&lt;/sup&gt;</td>
<td>2.5 ± 0.00&lt;sup&gt;Cc&lt;/sup&gt;</td>
<td>2.0 ± 0.00&lt;sup&gt;Bcc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>2.0 ± 0.00&lt;sup&gt;Acd&lt;/sup&gt;</td>
<td>3.58 ± 0.13&lt;sup&gt;Fcd&lt;/sup&gt;</td>
<td>3.48 ± 0.04&lt;sup&gt;Ecd&lt;/sup&gt;</td>
<td>3.0 ± 0.00&lt;sup&gt;Dcd&lt;/sup&gt;</td>
<td>2.58 ± 0.08&lt;sup&gt;Ccd&lt;/sup&gt;</td>
<td>2.08 ± 0.08&lt;sup&gt;Ccd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>2.0 ± 0.00&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>3.06 ± 0.08&lt;sup&gt;Fa&lt;/sup&gt;</td>
<td>2.5 ± 0.07&lt;sup&gt;Ea&lt;/sup&gt;</td>
<td>2.34 ± 0.11&lt;sup&gt;Da&lt;/sup&gt;</td>
<td>2.16 ± 0.54&lt;sup&gt;Ca&lt;/sup&gt;</td>
<td>2.0 ± 0.00&lt;sup&gt;Ba&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>2.0 ± 0.00&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>3.5 ± 0.07&lt;sup&gt;Fb&lt;/sup&gt;</td>
<td>3.06 ± 0.08&lt;sup&gt;Eb&lt;/sup&gt;</td>
<td>2.8 ± 0.07&lt;sup&gt;Db&lt;/sup&gt;</td>
<td>2.42 ± 0.08&lt;sup&gt;Cb&lt;/sup&gt;</td>
<td>2.0 ± 0.00&lt;sup&gt;Bb&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another. n=5
Appendix II: Anti-inflammatory effects of aqueous leaf extract of *Aloe volkensii* on formalin–induced paw oedema in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean paw volume (mm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>1hr</td>
</tr>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>2.0 ± 0.00&lt;sup&gt;A&lt;/sup&gt;&lt;sub&gt;b&lt;/sub&gt;</td>
</tr>
<tr>
<td>Baseline</td>
<td>Formalin</td>
<td>2.0 ± 0.00&lt;sup&gt;A&lt;/sup&gt;&lt;sub&gt;d&lt;/sub&gt;</td>
</tr>
<tr>
<td>Standard</td>
<td>Diclofenac</td>
<td>2.0 ± 0.00&lt;sup&gt;A&lt;/sup&gt;&lt;sub&gt;b&lt;/sub&gt;</td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>2.0 ± 0.00&lt;sup&gt;A&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>2.0 ± 0.00&lt;sup&gt;A&lt;/sup&gt;&lt;sub&gt;c&lt;/sub&gt;</td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>2.0 ± 0.00&lt;sup&gt;A&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another. n=5
Appendix III: Anti-inflammatory effects of aqueous stem extract of *Cynanchum viminale* on formalin–induced paw oedema in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean paw volume (mm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial 1hr 2hr 3hr 4hr 5hr</td>
</tr>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>2.0 ± 0.00 Aa 3.48 ± 0.08 Ca 3.44 ± 0.05 BCa 3.0 ± 0.00 ABCa 2.52 ± 0.04 BCa 2.38 ± 0.83 ABa</td>
</tr>
<tr>
<td>Baseline</td>
<td>Formalin</td>
<td>2.0 ± 0.00 Aa 4.0 ± 0.07 Ca 3.56 ± 0.13 BCa 3.0 ± 0.00 ABCa 3.04 ± 0.05 BCa 2.94 ± 0.08 ABa</td>
</tr>
<tr>
<td>Standard</td>
<td>Diclofenac</td>
<td>2.0 ± 0.00 Aa 3.56 ± 0.19 Ca 3.34 ± 0.23 BCa 3.0 ± 0.00 ABCa 2.5 ± 0.00 BCa 2.0 ± 0.00 ABa</td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>2.0 ± 0.00 Aa 3.86 ± 0.23 Ca 3.42 ± 0.23 BCa 2.9 ± 0.22 ABCa 2.58 ± 0.22 BCa 2.28 ± 0.16 ABa</td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>2.0 ± 0.00 Aa 3.96 ± 0.08 Ca 3.46 ± 0.28 BCa 3.2 ± 0.27 ABCa 2.76 ± 0.25 BCa 2.1 ± 0.17 ABA</td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>2.0 ± 0.00 Aa 3.66 ± 0.23 Ca 3.3 ± 0.27 BCa 3.0 ± 0.00 ABCa 2.56 ± 0.13 BCa 2.2 ± 0.21 ABA</td>
</tr>
</tbody>
</table>

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another. n=5
Appendix IV: Anti-inflammatory effect of aqueous leaf extract of *Urtica dioica* on formalin–induced paw oedema in mice models

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another. n=5
Appendix V: Antipyretic effect of aqueous bark extract of *Acacia nilotica* on Brewer’s yeast-induced pyrexia in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial</th>
<th>Rectal Temperature In °C After 19hrs Of Yeast Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0hr</td>
<td>1hr</td>
</tr>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>35.9 ± 0.1\textsuperscript{Aa}</td>
<td>37.83 ± 0.3\textsuperscript{Ca}</td>
</tr>
<tr>
<td>Baseline</td>
<td>Yeast</td>
<td>36.25 ± 0.36\textsuperscript{Ab}</td>
<td>36.85 ± 0.36\textsuperscript{Cb}</td>
</tr>
<tr>
<td>Standard</td>
<td>Paracetamol</td>
<td>35.73 ± 0.5\textsuperscript{Aab}</td>
<td>36.91 ± 0.77\textsuperscript{Cab}</td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>35.93 ± 0.16\textsuperscript{Ac}</td>
<td>37.65 ± 0.17\textsuperscript{Cc}</td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>35.96 ± 0.05\textsuperscript{Ac}</td>
<td>37.8 ± 0.34\textsuperscript{Cc}</td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>35.98 ± 0.07\textsuperscript{Ac}</td>
<td>37.8 ± 0.16\textsuperscript{Cc}</td>
</tr>
</tbody>
</table>

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another. n=6
Appendix VI: Antipyretic effect of aqueous leaf extract of *Aloe volkensii* on Brewer’s yeast-induced pyrexia in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>35.9 ± 0.1\textsuperscript{Aa}</td>
<td>37.83 ± 0.3\textsuperscript{Ca}</td>
<td>35.75 ± 0.4\textsuperscript{Ba}</td>
<td>35.45 ± 0.4\textsuperscript{Ba}</td>
<td>36.05 ± 0.4\textsuperscript{Ba}</td>
</tr>
<tr>
<td>Baseline</td>
<td>Yeast</td>
<td>36.25 ± 0.36\textsuperscript{Aab}</td>
<td>36.85 ± 0.36\textsuperscript{Cab}</td>
<td>36.15 ± 0.36\textsuperscript{Bab}</td>
<td>36.51 ± 0.31\textsuperscript{Bab}</td>
<td>36.81 ± 0.21\textsuperscript{Bab}</td>
</tr>
<tr>
<td>Standard</td>
<td>Paracetamol</td>
<td>35.73 ± 0.5\textsuperscript{Ab}</td>
<td>36.91 ± 0.77\textsuperscript{Cb}</td>
<td>36.36 ± 0.93\textsuperscript{Bb}</td>
<td>36.53 ± 1.03\textsuperscript{Bb}</td>
<td>36.55 ± 0.46\textsuperscript{Bb}</td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>35.91 ± 0.00\textsuperscript{Ac}</td>
<td>38.00 ± 0.14\textsuperscript{Cc}</td>
<td>37.31 ± 0.29\textsuperscript{Cc}</td>
<td>36.5 ± 0.26\textsuperscript{Bc}</td>
<td>37.21 ± 0.17\textsuperscript{Bc}</td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>35.95 ± 0.05\textsuperscript{Abc}</td>
<td>37.35 ± 0.05\textsuperscript{Cbc}</td>
<td>36.75 ± 0.05\textsuperscript{Bbc}</td>
<td>36.55 ± 0.05\textsuperscript{Bbc}</td>
<td>37.16 ± 0.12\textsuperscript{Bbc}</td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>35.61 ± 0.51\textsuperscript{Ab}</td>
<td>37.25 ± 0.58\textsuperscript{Cb}</td>
<td>35.65 ± 0.58\textsuperscript{Bb}</td>
<td>37.16 ± 0.59\textsuperscript{Bb}</td>
<td>37.83 ± 0.31\textsuperscript{Bb}</td>
</tr>
</tbody>
</table>

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another. n=6
## Appendix VII: Antipyretic effects of aqueous stem extract of *Cynanchum viminale* on Brewer’s yeast-induced pyrexia in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>35.9 ± 0.1&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>37.83 ± 0.3&lt;sup&gt;Ca&lt;/sup&gt;</td>
<td>35.75 ± 0.4&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>35.45 ± 0.4&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>36.05 ± 0.4&lt;sup&gt;Ba&lt;/sup&gt;</td>
</tr>
<tr>
<td>Baseline</td>
<td>Yeast</td>
<td>36.25 ± 0.36&lt;sup&gt;Aab&lt;/sup&gt;</td>
<td>36.85 ± 0.36&lt;sup&gt;Cab&lt;/sup&gt;</td>
<td>36.15 ± 0.36&lt;sup&gt;Bab&lt;/sup&gt;</td>
<td>36.51 ± 0.31&lt;sup&gt;Bab&lt;/sup&gt;</td>
<td>36.81 ± 0.21&lt;sup&gt;Bab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Standard</td>
<td>Paracetamol</td>
<td>35.73 ± 0.5&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>36.91 ± 0.77&lt;sup&gt;Ca&lt;/sup&gt;</td>
<td>36.36 ± 0.93&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>36.53 ± 1.03&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>36.55 ± 0.46&lt;sup&gt;Ba&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>35.95 ± 0.15&lt;sup&gt;Abc&lt;/sup&gt;</td>
<td>37.26 ± 0.83&lt;sup&gt;Cbc&lt;/sup&gt;</td>
<td>36.96 ± 0.69&lt;sup&gt;Bbc&lt;/sup&gt;</td>
<td>37.45 ± 0.34&lt;sup&gt;Bbc&lt;/sup&gt;</td>
<td>36.53 ± 0.42&lt;sup&gt;Bbc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>35.91 ± 0.11&lt;sup&gt;Ac&lt;/sup&gt;</td>
<td>37.83 ± 0.13&lt;sup&gt;Cc&lt;/sup&gt;</td>
<td>37.45 ± 0.10&lt;sup&gt;Bc&lt;/sup&gt;</td>
<td>36.66 ± 0.18&lt;sup&gt;Bc&lt;/sup&gt;</td>
<td>37.08 ± 0.2&lt;sup&gt;Bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>35.95 ± 0.05&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>37.73 ± 0.15&lt;sup&gt;Ca&lt;/sup&gt;</td>
<td>35.5 ± 0.15&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>35.65 ± 0.16&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>36.33 ± 0.12&lt;sup&gt;Ba&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Rectal Temperature In °C After 19hrs Of Yeast Injection

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another. n=6
Appendix VIII: Antipyretic effect of aqueous leaf extract of *Urtica dioica* on Brewer’s yeast-induced pyrexia in mice models

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial</th>
<th>Rectal Temperature In °C After 19hrs Of Yeast Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0hr</td>
<td>1hr</td>
</tr>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>35.9 ± 0.1^Aa</td>
<td>37.83 ± 0.3^Ca</td>
</tr>
<tr>
<td>Baseline</td>
<td>Yeast</td>
<td>36.25 ± 0.36^Ab</td>
<td>36.85 ± 0.36^Cb</td>
</tr>
<tr>
<td>Standard</td>
<td>Paracetamol</td>
<td>35.73 ± 0.5^Aabc</td>
<td>36.91 ± 0.77^Cab</td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>35.93 ± 0.1^Ac</td>
<td>37.23 ± 0.52^Cc</td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>35.51 ± 0.16^Abc</td>
<td>38.05 ± 0.16^Cbc</td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>35.93 ± 0.05^Ac</td>
<td>37.85 ± 0.05^Cc</td>
</tr>
</tbody>
</table>

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another. n=6