ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF METHANOL EXTRACTS OF *Pistacia aethiopica* (Kokwaro) and *Warbugia ugandensis* (Sprague) IN MICE MODELS

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DECLARATION

I, IRERI MOSES MUNENE, duly 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.

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DEDICATION

This thesis is dedicated to my mum, Rose Mary Njeru, for her love, relentless prayers and immense support towards my education.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

COX	Cyclooxygenase
ANOVA	Analysis of Variance
DMSO	Dimethylsulphoxide
WHO	World Health Organization
NSAIDs	Non-steroidal anti-inflammatory drugs
bw	Body weight
Ip	Intraperitoneal
iNOS	Inducible Nitric oxide synthase
NO	Nitric oxide
GABA	Gamma- Aminobutyric acid
NMDA	N-methyl-D-aspartate
IL-1	Interleukin-1
TNF-α	Transcription Nuclear Factor-alpha
PAF	Platelet Activating Factor
5-HT5	Hydroxytryptamine
^{o}C	Degrees celcius

ABSTRACT

Inflammation and pain are symptoms associated with many pathological conditions. These symptoms cause distress to the victims. Management of pain and inflammation is done by conventional drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) which may be expensive, not easily available and cause adverse effects. Traditional medicines provide viable alternatives in the management of pain and inflammation. Traditional medicines are easily accessible, cheap with minimal side effects. *Warbugia ugandensis* Sprague and *Pistacia aethiopica* Kokwaro are medicinal plants, which have for long been used by people in Embu County as analgesic and anti-inflammatory drugs. In spite being in use for long, no scientific study has validated their use. This study was therefore designed to establish the claimed analgesic and anti-inflammatory effects of these plants. Fresh leaf samples of *Warbugia ugandensis* and fresh bark samples of *Pistacia aethiopica* were collected in Embu County, Kenya. The plant samples were then air dried after which they were transported to the Department of Biochemistry, Microbiology, and Biotechnology, Kenyatta University. The dry plant materials were then pulverized by electric mill. Crude methanol extracts were prepared using 1 litre methanol per 200g powder. Male albino mice were divided into six groups of 5 animals each; normal control, negative control, positive control and three experimental groups for extract dose levels of 50, 100 and 150mg/kg body weight. Analgesic studies used formalin model while anti-inflammation studies used carrageenan-induced acute edema model. Diclofenac was used as the positive control in analgesic and anti-inflammatory studies. Stem bark extracts of *P. aethiopica* inhibited paw licking in mice by between 47.24-55.13% in the early phase and by between 30.69-52.12% in the late phase. *W. ugandensis* leaf extracts inhibited paw licking by between 38.45-51.85% in the early phase and by between 43.48-65.61% in the late phase. Diclofenac inhibited paw licking by between 30.33-30.36% in the early phase and by between 62.93-77.08% in the late phase. For anti-inflammatory effects *P. aethiopica* extract suppressed carrageenan induced paw edema by between 4.6-7.6% while *W. ugandensis* suppressed paw edema by between 6.08-7.59%. Diclofenac suppressed carrageenan induced paw edema by between 8.86-9.57%. Qualitative phytoconstituents screening revealed presence of phenols, saponins, flavonoids alkaloids and terpenoids which have been previously linked to analgesic and anti-inflammatory effects. Therefore the current study has validated folkloric use of *P. aethiopica* and *W. ugandensis* as remedies for pain and inflammation.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Pain refers to an unpleasant sensation, or a feeling of discomfort resulting from stimulation of pain receptors in the body when tissue damage occurs or is about to occur (Prabhu *et al*., 2011). Pain is a valuable symptom of an underlying pathology and may be vital in the diagnosis of diseases. As an essential body's defense mechanism, pain serves as a warning of a problem particularly when it is acute (Guyton and Hall, 2006). However excessive pain is extremely noxious and can lead to other side effects such as sweating, apprehension, nausea and palpation. Pain is a common and distressing feature of many medical cases such as cancer, surgical procedures, physical trauma and noxious chemical stimulation (Ezeja *et al*., 2011).Pain is a ubiquitous human experience and one of the most common symptoms among individuals who seek medical treatment since most pathological conditions are manifested by pain. However, pain is unique to the individual experiencing it.

Management of pain is one of the most important therapeutic priorities (Rang *et al*., 2012). Effective pain management exerts economic and social burden to the society (Arzi*et al.,* 2013). Failure to treat pain adequately and expeditiously may affect later responses and behavior to pain. Negative experiences with painful procedures may cause an individual to delay or even avoid seeking medical care, creating delays in diagnosis and treatment with worse and even more costly outcomes. Pain aggravates distress and morbidity and if unchecked, it results in a vicious cycle of associated pathological conditions (Owolabi and Anaka, 2013).

Inflammation, also called phlogosis, refers to a pathophysiological reaction of a tissue to injury that results in accumulation of plasmatic fluid and blood cells at the injury site (Panda *et al.*, 2009). Inflammation is a defense mechanism against injurious stimuli. Despite the importance of inflammation to the body's well-being, inflammation subjects a person to distress. Appropriate inflammation can be irritating to healthy tissues while exaggerated inflammatory response has deleterious effects on the victim (Ahmed, 2011). Inflammation is regarded as a silent killer responsible for the onset of numerous acute and chronic diseases such as septic shock, cancer, diabetes, atherosclerosis and obesity (Guzik *et al*., 2006). Moreover, if inflammation is left undeterred, it would serve as an etiological factor for various chronic diseases such as cancers, colitis, arthritis and atherosclerosis (Kumar *et al*., 2004).

Numerous scientific researches have given a lot of attention to inflammation as inflammatory diseases such as chronic asthma, psoriasis, rheumatoid arthritis, multiple sclerosis and colitis are on the rise (Gautum and Jachack, 2009). Inflammation has been implicated in almost all human and animal diseases (Nazia *et al*., 2011). It is associated with different types of tissue injury and is considered as a major health concern (Zaman *et al*., 2009). Initially, inflammation was considered as a single disease caused by disturbances of body fluids. However, inflammation is considered as a healthy process resulting from some disturbances or diseases (Alamgeer *et al*., 2015).

The presently available synthetic analgesic and anti-inflammatory agents pose several health problems during their clinical use. Therefore, development of new

and more effective drugs with fewer or no adverse effects is necessary (Uma *et al.*, 2012). Despite availability of various analgesic and anti-inflammatory drugs, the search for novel agents to complement the existing therapies continues.

Over 80% of the world's population in underdeveloped and developing countries depend on herbal therapy for their primary health care needs. Moreover, medicinal plants provide the best source of variety of drugs (Karani *et al*., 2013). Demand for herbal therapy is on the rise given the increasing recognition of natural remedies being thought to be potent, easily available, affordable, and having less or no adverse effects when used in management of various medical conditions (Dubey *et al*., 2004).

The continued research in novel analgesic and anti-inflammatory agents is mainly prompted by occurrence of adverse effects and low potency of some existing drugs (Amaral*et al*., 2007). In addition, herbal medicines remain popular among people, especially in many countries, due to social and economic situations (Aburjai *et al*., 2007). Many people in rural set-up use herbal remedies regularly due to their cultural convictions (Rai *et al*., 2000). Natural products are key sources in the development of novel therapeutic analgesic and anti-inflammatory agents (Harvey, 2000).

Plants often produce secondary metabolites under stressful ecological conditions. These metabolites exhibit various pharmacological effects associated with medicinal plants (Wink, 2015). *Warbugia ugandensis* and *Pistacia aethiopica* have for long been used in management of pain and inflammation related pathological conditions

by the Embu community, Kenya (Kareru *et al*., 2007). However, no scientific study has been undertaken to confirm and/or validate their use. It is against this background that this study was designed to bioscreen methanolic extracts of *Pistacia aethiopica* and *Warbugia ugandensis* for analgesic and anti-inflammatory effects in Swiss albino mice. The findings of this study provided preliminary information on the potency of *Pistacia aethiopica* and *Warbugia ugandensis* in management of pain and inflammation, as a preliminary step towards development of a pure plant derived analgesic and anti-inflammatory agents.

1.2 Problem statement and justification

Pain and inflammation are symptoms manifested in various ailments (Rang *et al*., 2011). These symptoms exert a heavy economic and social burden on the victims as they are associated with distress, unpleasant feeling and cause high morbidity. Studies show many people suffer from mild to severe pain. Management of pain is a daily challenge in modern medicine, despite the currently available wide range of analgesics (Nkomo*et al.*, 2010). Moreover, inflammation is closely associated with pain and distress (Yan *et al*., 2008). Therefore, there is need for appropriate measures in management of pain and inflammation.

In spite of the recent advancement in pain and inflammation therapies, safe, effective and potent drugs are still required for the treatment of various painful and inflammatory conditions (Pires *at al*., 2009). The current therapeutic approaches available for management of inflammation and pain often trigger potentially serious adverse effects (Khan *et al*., 2011). It is therefore, imperative to explore potential agents for generation of novel drugs.

Usually, the management of pain and inflammation is done using conventional drugs such as NSAIDs, steroidal drugs, immune-suppressants and opioids (Shulan *et al*., 2011). These drugs may not be easily accessible to local populace, particularly in remote places of developing nations (Mukherjee *et al*., 2010). Moreover, these conventional drugs are relatively unaffordable and are associated with adverse effects such as gastrointestinal irritation and peptic ulcers, kidney, liver and heart disorders (Corley *et al*., 2003). Given these limitations of conventional drugs in management of pain and inflammation, it is imperative to find alternative novel agents that are equally potent, affordable, accessible and comparatively free of adverse effects. This alternative therapy may be obtained from herbal extracts.

Herbal extracts have been used as remedies for various disorders including pain and inflammation hence, they are viable alternatives to conventional synthetic drugs. Herbal medicines, as alternative therapies, are thought to be effective, easily accessible, affordable, and, arguably, have limited or no side effects (Sheir *et al*., 2001). *Pistacia aethiopica* and *Warbugia ugandensis* are medicinal plants used as anti-inflammatory and analgesic agents among the Embu and Mbeere communities in Kenya. Despite long history of use, limited scientific research has been undertaken to determine their effectiveness in management of pain and inflammation.

This study will explore and provide important information on ethno-medicinal importance of *Pistacia aethiopica* and *Warbugia ugandensis* as alternative medical intervention in the management of pain and inflammation. Information from this study may be used to support the folkloric use of *Pistacia aethiopica* and *Warbugia*

ugandensis as viable therapies against pain and inflammation. Moreover, information from this study may serve as a preliminary step in the development of safe and efficacious standardized herbal formulation for treatment of inflammation and pain.

1.3 Null Hypotheses

i) Methanol extracts of *Pistacia aethiopica* and *Warbugia ugandensis* do not have *in vivo* analgesic activity in Swiss albino mice.

ii) Methanol extracts of *Pistacia aethiopica* and *Warbugia ugandensis* do not have *in vivo* anti-inflammatory activity in Swiss albino mice.

iii) Methanol extracts of *Pistacia aethiopica* and *Warbugia ugandensis* do not contain phytochemicals associated with analgesic and anti-inflammatory activities.

1.4 Objectives

1.4.1 General objective

To determine *in vivo* analgesic and anti-inflammatory potentials of methanolic extracts of *Pistacia aethiopica* and *Warbugia ugandensis* in mice.

1.4.2 Specific objectives

i) To determine *in vivo* analgesic potential of methanolic extracts of *Pistacia aethiopica* and *Warbugia ugandensis* in mice.

ii) To determine *in vivo* anti-inflammatory potential of methanolic extracts of *Pistacia aethiopica* and *Warbugia ugandensis* in mice.

iii) To determine the qualitative phytochemical composition of methanolic extracts of *Pistacia aethiopica* and *Warbugia ugandensis*.

CHAPTER TWO

LITERATURE REVIEW

2.1 Physiological and biochemical basis of pain and inflammation

2.1.1 Pain

Pain is an essential defense mechanism in the body and serves as a warning to an anomaly in the physiology of the body (Guyton and Hall, 2006). Pain, particularly when acute, is primarily protective and mobilizes the individual to take immediate action to relieve it (Wall and Melzack, 1994). It is categorized into two; acute and chronic. Acute pain is usually short lived while chronic pain is persistent and occurs over a prolonged duration of time (Hooten *et al.,* 2013).

The pain stimuli are caused by infection(s), tissue injury and inflammation (Barbara, 2006). When body tissues and cells receive a noxious stimulation or disturbance due to injury or infection(s), nociception mediators such as protons (H^+), prostaglandin E_2 (PGE_2), serotonin (5-HT), among others are released. Subsequently, nociceptors are sensitized resulting in pain sensation (Bannerman *et al*., 1986).

Pain stimuli are received by pain receptors, which are free nerve endings of sensory nerves, present in virtually all body tissues (Barbara, 2006). The pain receptors are referred to nociceptors since they are involved in detecting noxious stimuli (Goci *et al*., 2013). The nociceptors detect noxious stimuli and initiate nociception, which is a mechanism through which noxious peripheral stimuli are transmitted to the central nervous system (Basbaum and Jessell, 2000; Rang *et al*., 2012).

Nociceptors may be stimulated by a variety of physical, chemical and thermal means. The physical means are mainly due to pressure changes, the thermal means due to temperature variations while chemical means are due to exogenous agents such as acid or endogenous substances such as biochemical mediators. The chemical mediators of pain include prostaglandins E_2 (PGE_2), leukotrienes, platelet-activating factor, substance P, serotonin (5-HT) and bradykinin (Bannerman *et al*., 1986). These chemical mediators sensitize the first-order afferent receptors to the stimuli and may be involved in the conduction of stimuli (Goci *et al*., 2013).

The activated nociceptors initiate a sequence of events that bring about the subjective experience of pain (Barbara, 2006). The stimulated afferent nociceptors initiate pain impulses that are transmitted along myelinated A delta (Aδ) and the unmyelinated C fibers to the spinal cord via the dorsal horn synapses (Rang *et al.*, 2012).The myelinated Aδ fibers transmit impulses rapidly and are majorly involved in transmission of acute pain impulses from the skin and mucous membranes (Basbaum and Jessell, 2000).

It is suggested that glutamate is the neurotransmitter of the Aδ fibers and is involved in transmission of fast pain since upon its secretion in the spinal cord at the terminals of Aδ fibers, it acts instantaneously and only lasts for a few milliseconds (Guyton and Hall, 2006). The non-myelinated C fibers transmit impulses slowly and, therefore, transmit chronic pain that is usually experienced as dull, diffuse and aching sensation. Substance P is the neurotransmitter of C fibers, which is released slowly, accumulates for a duration of upto a few minutes and is involved in transmission of chronic pain (Guyton and Hall, 2006).

The rapidly and slow- conducting nerve fibers terminate in the dorsal horn of the spinal cord (Meyer *et al*., 2008). A network of cells at the dorsal horn of the spinal cord, which includes the substantia gelatinosa regulates transmission of impulses between the nociceptive neurons and those at the spinothalamic tract. The A delta and C nociceptive fibers form synaptic connection with transmission neurons which are the second order neurons (Rang *et al*., 2012). The second-order neurons then transmit the impulses from the spinal cord to the thalamus in the lateral spinothalamic tracts. The thalamus, which is part of the forebrain, is responsible for integration of pain impulses (Dubin and Patapoutian, 2010). From the thalamus, third-order neurons convey pain impulses to the post-central gyri of the cerebral cortex. The cerebral cortex further integrates the pain impulses to bring about precise and meaningful subjective interpretation of pain. Consequently, appropriate motor response is initiated in response to a painful stimulus (Vadivelu *et al*., 2009).

The pain pathway has three natural mechanisms of pain modulation. These mechanisms are; the descending pain modulatory system, the central modulatory mechanism and the peripheral modulatory system. The descending fibers from the higher centers inhibit impulse transmission to the thalamus and cerebral cortex. This forms the descending pain modulatory mechanism (Ossipov *et al*., 2010). The descending inhibitory system is mediated by noradrenalin and serotonin and modulates pain by reducing the amount of signal passing through the spinothalamic tract. The second modulatory system is coordinated by endogenous opioids such as endorphins and enkephalins, which bind to the opioid receptors in the central nervous system thus preventing the release of neurotransmitters through blockage of calcium channels (Lantero *et al*., 2014). Moreover, endogenous opioids

hyperpolarize the nerve fibers inhibiting conduction of action potential in the spinothalamic tract. Endogenous opioids act both centrally and peripherally. Pain may also be modulated by a peripheral system that is based on activation of mechanoreceptors, which exert an inhibitory effect on transmission neuron thus regulating the amount of signal passing into spinothalamic tract through the dorsal horn ganglia (Basbaum and Jessell, 2000). Moreover, monoamines modulate nociception transmission by acting on the peripheral nociceptive fibres, sympathetic terminals, processing of afferent nociceptive signals in the spinal cord, thalamic integration of these signals and in the higher limbic and cortical integration structures. The modulation of nociceptive afferent signals by monoaminergic inhibitory system in the spinal cord forms the first step in the processing of the pain signal (Pinardi *et al*., 2002).

Response to pain usually involves a stress response as well as emotional response such as crying, moaning or anger. There may be a physical response perhaps rigidity, splinting or guarding of the area of the body. The thalamus processes many types of sensory stimuli as they enter the brain and is important in the emotional response to pain through the limbic system (Woolf, 2004).

In other instances, pain results from inflammatory reactions (Orlandi *et al*., 2011). Inflammatory pain is attributed to peripheral and spinal sensitizations that are usually mediated by biochemical mediators released during inflammation. These inflammatory mediators include; substance P, serotonin (5-HT), histamine, bradykinin and eicosanoids such as lipoxins, leukotrienes, prostaglandins (PGE_2) and thromboxanes (Santos and Calixto, 1997; Bennet and Brown, 2003). These

mediators affect the transmission properties of the free nerve endings by either activating or sensitizing the neurons (Rang *et al*., 2012). For instance, bradykinin and related kinins are released in the extracellular fluid whenever tissue injury occurs and they contribute to vascular and extravasation alterations that occur in inflammatory reactions (Kidd and Urban, 2001). Moreover, bradykinins sensitize and stimulate nerve endings thereby causing pain and also stimulate prostaglandin release. Prostaglandins enhance the nociceptive effects of other mediators such as serotonin and bradykinin. Prostaglandins of E and F series are released during inflammation and tissue ischemia (Rao and Knaus, 2008). Prostaglandins sensitize free nerve endings to other agents partly by inhibiting potassium channels and partly through second messenger mediated phosphorylation reactions in the cation channels opened by noxious agents. Inflammatory pain also involves release of glutamate, which is a fast transmitter (Gottschal and Szewyki, 1998) and eventual increase in glutamate receptor expression, which contributes to pain sensation (Tverskoy *et al*., 1994).

2.1.2 Inflammation

Inflammation is considered a primary physiological defense mechanism and is associated with the protection of the body against harmful stimuli (Alamgeer *et al*., 2015). Inflammation is of two types; acute and chronic inflammation (Ezeugwu *et al*., 2004). Acute inflammation is the early response of a tissue to injury while chronic inflammation is a prolonged inflammatory response that leads to a progressive change in the type of cells present at the inflammatory site (Ullah *et al*., 2014). Chronic inflammation is depicted by simultaneous tissue destruction and repair (Ullah*et al*., 2014). In chronic inflammation cases, inflammation persists,

resulting in complications as seen in inflammatory diseases such as arthritis, asthma, tuberculosis, multiple sclerosis among others.

In the body, inflammation serves as a defense reaction in eliminating and/or limiting the spread of injurious agents (Agnel and Shobaha, 2012). In spite of inflammation being a defense mechanism, the intricate cascade of biochemical and cellular events comprising enzyme activation, mediator release, fluid exudation, cell migration and accumulation, tissue destruction and eventual healing, inflammatory reactions are associated with fair share of distress to the victim (Alamgeer *et al.*, 2015). Mediators involved in inflammatory responses may initiate, support or exacerbate numerous pathological conditions (Panda *et al*., 2009). The inflammatory response occurs in about the same way irrespective of the cause. Thus, it is considered a non-specific response (Huether and McCance, 2004).

Inflammation is caused by tissue damage or infection (Ahmed, 2011). Tissue injury may be due to physical trauma, ischemia and necrosis, allergic reactions, autoimmune reactions and infections (Young *et al*., 2002). Inflammation occurs in different phases; the initial phase is due to increased vascular permeability, followed by leukocyte infiltration in the second phase and granulation in the third phase (Panda *et al*., 2009). Inflammation represents an intricate sequence of events that permit injured cells to respond against injury or infection (Babu *et al*., 2009). It entails the participation of various cell types that produce and respond to diverse mediators along a very precise series of events (Sowemimo *et al*., 2013).

The inflammatory response is nonspecific with redness, swelling (edema), warmth and pain and perhaps loss of function being its key symptoms (Valdelania *et al.*, 2013). Edema is due to increased passage of fluid from dilated and permeable blood vessels into surrounding tissues, infiltration of cells into the damaged area and, in prolonged inflammatory responses, deposition of connective tissue. Redness is due to increased blood flow at the inflamed site. The increased numbers of erythrocytes traversing the inflammatory site enhance the redness of the site. The feeling of heat is caused by the increased movement of warm blood from the core of the body through dilated vessels of the inflamed site.

The inflammatory pain is due to irritation of the nerves by chemical mediators and increased fluid pressure on nerves particularly in confined regions such as joints (Barbara, 2006). Pain may also be attributed to hyperalgesia, a condition brought about by chemical mediators. In hyperalgesia, the inflammatory mediators increase the sensitivity of the nociceptors (Maina *et al*., 2015b). Increased sensitivity makes it easier to generate pain impulses by reducing the polarization threshold, thereby creating an area of hyperalgesia. The loss of function occurs due to nutrient deprivation, mechanical interference of action due to swelling and substitution of functional tissue with scar tissue.

Inflammatory response is initiated by occurrence of an injury to the tissue. The injured cells release chemical mediators such as histamine, platelet activating factor, NO, thromboxanes, leukotrienes, prostaglandins, serotonin, kinins and cytokines such as interleukin 1(IL-1) and tumor necrosis factor (TNF-α) (Bennet and Brown, 2003; Ullah*et al*., 2014). The mast cells are the main initiators of an inflammatory

response. They initiate inflammation by releasing biochemical mediators into the tissue fluid and blood (Huether and McCance, 2004). These chemical mediators work collectively to cause vasodilation and increased blood flow at the injured site. This is followed by increased capillary permeability, exudation of proteins and fluids, and leucocytes infiltration in to the injury site (Ullah*et al*., 2014). Phagocytosis then occurs in preparation for healing (Vane and Botting, 1995; Perianayagam *et al*., 2006).

Serotonin and histamine cause constriction of endothelial muscles, dilation of capillaries and retraction of capillaries lining resulting in elevated capillary membrane permeability (Barbara, 2006). Increased permeability as a result of endothelial retraction allows leukocytes to immigrate into extra-vascular space (Huether and McCance, 2004). Increased vascular permeability continues throughout acute inflammation, allowing blood cells and plasma proteins to infiltrate inflamed tissues. The cells and proteins stimulate and regulate subsequent inflammatory reactions while interacting with immune system components such as the cytokines.

The TNF-α, a cytokine, mediates inflammation by stimulating release of other mediators and cytokines (Verma *et al*., 2010). Elevated TNF-α production is associated with various inflammatory complications (Williams *et al*., 2007). Interleukin (IL)-10, a cytokine with potent anti-inflammatory effect, is released from activated immune cells, mainly macrophages (Clark *et al*., 2013). IL-10 modulates various immunological mechanisms such as down-regulation of inflammatory mediator production and inhibition of antigen presentation (Sabat *et al*., 2010).

Inflammation is usually accompanied by elevated levels of arachidonic acid (AA) metabolites through the lipooxygenase and cyclooxygenase-2 enzyme pathways (Rao and Knaus, 2008). The arachidonic acid metabolites, particularly prostaglandins in the E series, act on the endothelial walls to bring about vasodilation, retraction and permeability (Randy, 2005). Nitric oxide (NO) released by endothelial walls through the L-arginine NO cGMP pathway, catalyzed by inducible nitric oxide synthase (iNOS), also stimulates vasodilation of blood vessels. High NO concentration, exerts oxidative damage on tissues (Nijveldt *et al*., 2001). Therefore, NO is an important mediator in inflammatory reactions (Sarkar *et al*., 2005).

Leukocytes such as neutrophils, macrophages, oesinophils, basophils and lymphocytes immigrate into the extravascular space as a result of endothelial retraction (Huether and McCance, 2004). Emigration involves margination, rolling, activation, adhesion and transmigration (Kumar *et al*., 2004). The neutrophils arrive first at the inflamed site. Neutrophils containing lipoxygenase metabolise Arachidonic Acid (AA) producing chemotactic metabolites. Moreover, neutrophils also produce cytokines (Nijveldt *et al*., 2001). They destroy dead cells and bacteria through phagocytosis. Consequently, neutrophils die and are eliminated as pus via the lymphatic system or epithelium (Actor, 2012).

Macrophages, which are derivatives of monocytes, enter the extravascular space following elimination of neutrophils. Macrophages are endowed with lytic enzymes which eliminate cellular debris and bacteria through phagocytosis. Unlike neutrophils, macrophages stay in the inflamed site for a longer time and are involved

in subsequent inflammatory reactions through production of cytokines, NO, prostaglandins, leukotrienes and thrombocyte activating factors (Basbaum *et al.*, 2009). The cytokines released by macrophages include IL-1, IL-6, IL-8and TNF-α. These cytokines exert both local and systemic effects while coordinating the healing process (Actor, 2012). Locally, IL-1, IL-8 and TNF-α increase capillary permeability. This allows more immune cells to infiltrate the inflammation site. Macrophages then recruit lymphocytes into the inflammatory site. The lymphocytes institute the immune response through production of cytokines such as IL-1 and TNF-α that activate the macrophages which eliminate pathogens and debris present at the inflammatory site (Rang *et al*., 2012).

The presence of lymphocytes in inflamed tissue is attributed to presence of specific pathogens. Other leukocytes present in the inflamed tissues include basophils and oesinophils. Eosinophils act directly against pathogens and modulate the inflammatory response. Moreover, oesinophils are usually present in chronic inflammation and are more prominent in allergic reactions. Basophils produce inflammatory mediators and perform similar function to mast cells.

Thrombocytes stop bleeding if vascular injury had occurred and compartmentalize the injured site to prevent the spread of infection to other unaffected cells. The various leukocytes and platelets with the help of kinin, complement and immunoglobulins systems along with the various mediators produced during inflammation act to kill pathogens and eliminate dead tissue at the injured site in readiness to tissue reconstruction, repair and healing (Barbara, 2006).

2.2 Conventional management of pain and inflammation

To alleviate pain medications such as NSAIDs, immunosuppressants, opiates and steroidal drugs are often used (Willich *et al*., 2010). NSAIDs prescriptions are among the most common worldwide for painful conditions (Wolfe *et al*., 1999). Pain management approaches vary with pain intensity. Mild pain is usually managed with, aspirin or ibuprofen, which act primarily at the peripheral site (Hinz *et al*., 2008). Aspirin is particularly useful when inflammation is present. Aspirin has for long been used in management of pain, fever and inflammation. Its mode of action is by reducing the production of prostaglandins. Aspirin causes allergy in many individuals. In some individuals it causes stomach irritation leading to ulcers, nausea and bleeding and delays blood clotting (Vane and Botting, 1995; Rang *et al*., 2012). NSAIDs such as naproxen and ibuprofen are widely used to treat both acute and chronic pain particularly when inflammation is present (Warden, 2010). The mode of action of NSAIDs, the commonly used analgesics, is mainly through inhibition of COX enzymes and eventual reduction in prostaglandins and thromboxanes synthesis (Payne *et al*., 2000). The COX enzymes catalyze conversion of arachidonic acid to prostaglandins (Shukla and Mehta, 2015). Reduction in prostaglandins synthesis is essential to amelioration of pain since prostaglandins are key mediators of inflammatory pain.

Moderate pain is commonly managed by codeine alone or in combination with aspirin or acetaminophen. Codeine is a narcotic, a morphine derivative, acting at the opiate receptors in the central nervous system. Management of severe pain usually involves the use of opiates such as morphine. Opiates block the pain pathways in the spinal cord and brain inhibiting central and peripheral pain mechanisms (Pinardi *et*

al., 2002). Opioids also enhance the natural endorphins response which is vital in pain modulation. Endorphins are the endogenous neuropeptides that inhibit transmission of pain impulses in the central nervous system. Endorphins are used in natural modulation of pain in the body. However opioid analgesics do not inhibit inflammation. Prolonged administration opioid analgesics in chronic pain conditions results in drug tolerance to the analgesic effects thus limiting their therapeutic potential (Esmaeili *et al*., 2007). Abuse of opioids can cause serious damage including addiction, overdose and even death. At worst they can cause respiratory failure and comma. In situations when addiction develops as a result of opioid use, drug tolerance develops hence an individual requires higher doses for the desired therapeutic effect.

To ameliorate inflammation and associated pain, NSAIDs are commonly prescribed. NSAIDs such as ibuprofen, porixicam or diclofenac sodium are extensively used to treat many types of inflammatory conditions (Warden, 2010). Besides, NSAIDs have been used to treat inflammation in the musculoskeletal system, both acute and long-term problems such as rheumatoid arthritis and osteoarthritis (Rao and Knaus, 2008). They are also used in many dental procedures when analgesic and anti-inflammatory agents are needed. The mechanism of action of NSAIDs is by inhibiting the conversion of arachidonic acid (AA) into prostaglandin PGE_2. The reaction is catalyzed by cyclo-oxygenase (COX) enzymes. NSAIDs are basically cyclo-oxygenase inhibitors that act through inhibition of prostaglandins production. Relief from inflammation and associated pain is achieved when prostaglandins and thromboxanes production, which are mediators in pain and inflammation pathways, is suppressed (Vane, 1971).

Despite their effectiveness in ameliorating painful and inflammatory conditions, NSAIDs possess several adverse effects (Bennet and Brown, 2003). Adverse effects associated with NSAIDs include delays in blood clotting, nausea, allergy, stomach ulceration and bleeding (Sachs, 2005). Liver damage, kidney failure and heart conditions may also occur due to extended NSAIDs use (Nalamachu and Wortmann, 2014). In addition, acetaminophen has been reported to induce liver damage after prolonged use. Diclofenac, a commonly used NSAID, has been reported to increase the risk to heart conditions, stroke and liver damage (Rao and Knaus, 2008). Nonetheless, diclofenac use may erode the stomach mucosa predisposing one to peptic ulcers. NSAIDs that particularly inhibit the COX-2 pathway increases risk to cerebrovascular and cardiovascular conditions such as stroke and myocardial infarction (Munir *et al*., 2007).

In light of the aforementioned undesirable impacts of conventional medications it is necessary to search for new analgesic and anti-inflammatory drugs that are equally potent and with minimal or no adverse effects (Ezeja *et al*., 2011). Steroids are also used in management of inflammation and associated pain. However, their use is associated with Cushing's syndrome (Rang *et al*., 2012)

2.3 Herbal management of pain and inflammation

Medicinal plants present an important source of novel agents with desired pharmacological activity against inflammation and pain (Nkomo*et al*., 2010). Majority of plant derived natural therapeutic products are effective and safe remedies for various ailments among them inflammation and pain (Sheir *et al*., 2001). Moreover, herbal medicines are endowed with analgesic and anti-

inflammatory potentials attributed to the presence of various phytocompounds that are effective and safe when used within therapeutic limits (Posadzki *et al*., 2013). In the past, search for novel pharmacologically active analgesic and anti-inflammatory agents from plants led to the discovery of some clinically useful drugs such as aspirin and morphine (Calixto *et al*., 2000, Gilani and Atta-ur-Rahman, 2005). Numerous medicinal plants and the associated phytochemical components have been shown to exhibit anti-inflammatory and analgesic effects in animal models (Dhiman *et al*., 2012).

Dichloromethane: Methanol leaf extracts of *Caesalpinia volkensii* and *Maytenus obscura* were found to have anti-inflammatory activities in mice (Maina*et al*., 2015a). According to Mworia *et al*. (2015a) acetone leaf extracts of *Caesalpinia volkensii* and *Carissa spinarum* have potent antinocipeptive activities in mice. *Solanum incanum* was found to possess potent analgesic and anti-inflammatory activities in mice (Mwonjoria *et al*., 2014). Analgesic studies of *Caryophyllus aromaticus* revealed that this plant exhibits potent analgesic principles (Sateesh *et al*., 2013). Koech *et al.* (2017), demonstrated *Clutia abyssinica* exhibited potent anti-inflammatory effects in mice. Stem bark extracts of *Byrsonima intermedia* A. Juss reduced pain and inflammation in mice (Orlandi *et al.*, 2011). Similarly, Nkomo *et al*., (2010), while working on *Gunnera perpensa* extracts revealed that the plant exhibited analgesic and anti-inflammatory effects in mice. Moreover, *L. Buchananii* and *H. abyssinica* had potent analgesic and anti-inflammatory effects in mice models (Nthiga *et al*., 2016). In another study by Couto *et al*., (2011), *Emilia sonchifolia* extracts ameliorated pain in mice. Likewise, *Azandirachta indica*, a

popular herbal plant, was shown to possess active principles against pain and inflammation (Dinda *et al.,* 2013).

2.4 Models used in pain and inflammation studies

2.4.1 Models used in pain studies

2.4.1.1 Models using chemical stimulus

Pain induction by administration of algogenic substances into an organism closely mimics clinical pain. The stimulus due to the presence of an algogenic agent produces a slow, gradually increasing and irreversible form of stimulation hence is closest in nature to clinical pain (Parle and Yadav, 2013). Chemical stimulation lasts longer than thermal and mechanical stimulation. The chemical models of pain are distinct from other methods since measurement involves scoring pain behavior as opposed to threshold. This makes them the methods of choice as they are more reliable in mimicking clinical pain.

2.4.1.1.1 Acetic acid writhing test

This is a non-selective analgesic model (Yu *et al*., 2012). Pain is often induced in mice or rats through intraperitoneal injection of chemical irritants such as acetic acid (Rahman *et al*., 2011). Following the injection of the acetic acid into the peritoneal cavity, endogenous substances are released (Couto *et al*., 2011). These substances excite the pain nerve endings and the animal responds with characteristic stretching of the abdominal region (Gyires and Torna, 1984). This behavior is called writhing, hence the term writhing test. The intraperitoneal injection of an algogenic substance irritates the serous membranes in the abdominal region. Irritation of serous membranes initiates twisting of dorsal-abdominal muscles and motor in-

coordination eliciting abdominal constrictions. This behavior is indicative of pain of peripheral origin (Gyires and Torna, 1984).

The measurement of nociception involves counting the number of writhes in a thirty minutes duration following irritant injection. The frequency and intensity of writhing decreases spontaneously as time lapses, this necessitates use of more than one animal to enhance evaluation of an analgesic agent. Analgesic behavior is indicated by reduction in the number of writhes (Parle and Yadav, 2013).

2.4.1.1.2 Formalin test

The formalin test in mice or rats is considered as a chronic pain model. Formalin induces neurogenic, inflammatory and tonic pain that closely mimics clinical pain (Zhao *et al*., 2003). Besides, formalin model is a highly specific and reliable mode for chronic pain (Meunier *et al*., 1998). Formalin is injected in the plantar region of the hind paw of the animal. Formalin induces two distinct phases with quantifiable nociceptive behaviour that is characterized by lifting, and licking biting of the injected paw. A formalin solution, 0.5 to 15%, is usually used (Dubuisson and Dennis, 1977; Tjolsen *et al*., 1992).

The response to formalin injection is shown in two phases, early phase and late phase. The early phase is mainly due to direct stimulation of nociceptors particularly the C-afferent and A delta fibres by the peripheral stimulus (Mahdi and Rasta, 2008). The late phase is as a result of inflammatory responses in the peripheral tissue and functional alterations in the dorsal horn of the spinal cord (Tjolsen *et al*., 1992). The functional alterations are initiated by the C-fibre barrage during the early

phase resulting in alterations in central processing of stimuli (Tjolsen *et al*., 1992; Mahdi and Rasta, 2008). Bradykinin and substance P are the mediators in the early phase while nitrous oxide, prostaglandins, serotonin and histamine participate in the late phase (Safari *et al*., 2016). The formalin model is a selective nociceptive model that allows discrimination between centrally and peripherally acting analgesics (Couto *et al*., 2011). The analgesic behavior or protection is indicated by the reduction in paw licking, biting and lifting (Parle and Yadav, 2013).

2.4.1.2 Models using thermal stimuli

2.4.1.2.1 Hotplate

The hot plate test is an acute pain model of thermal nature (Savage and Ma, 2014). The hot plate model is a frequently used approach in anti-nociception studies particularly for central analgesic tests (Orlandi *et al*., 2011). Hot plate test is mainly a spinal reflex or behavioral reaction and is used to test supra-spinal analgesia of compounds (Couto *et al*., 2011). The model is a supra-spinally integrated test and involves placing the animal on a heated metallic surface (Mwonjoria *et al*., 2011). The metallic platform is often heated to a temperature of about 55-66 degrees celcius.

Upon placement on the hot platform, the animal responds in distinct quantifiable nociceptive flinching behaviour (paw licking and jumping) that can be scored in terms of their reaction times (Menendez *et al*., 2002). The hot plate method is selective for centrally acting analgesics like morphine since the nociceptive responses are supra-spinally integrated (Ullah *et al*., 2014). Peripheral anti-inflammatory antinociceptive agents are found to be inactive on thermal stimuli

(Couto *et al*., 2011). The nociceptive behavior is monitored by observing jumping or licking of the hind paw (Mwonjoria *et al*., 2011).

2.4.1.2.2 Tail flick test

Tail flick test is an acute pain model that measures pain threshold by scoring the animal's response to thermal stimuli (D'Amour and Smith, 1941). Is a spinally mediated nociceptive model that is often used to elucidate analgesic mechanism(s). The mice show quantifiable behavior when tested using this model (Mwonjoria *et al*., 2011). The method can be used to measure effectiveness of an analgesic by monitoring how mice respond to heat (D'Amour and Smith, 1941). The animal's tail is placed on a hot spot and the nociceptive behavior that entails withdrawing the tail from a hot spot is monitored and recorded (Savage and Ma, 2014). However, in tail flick test the animal's skin temperatures influence its response to thermal stimuli thus the effectiveness of this model in scoring pain threshold is limited (Berge *et al*., 1988). Moreover, tail flick model measures response to spontaneous pain, thus unsuitable model for clinical pain that is usually persistent. This method is selective to screening effectiveness of centrally acting analgesics such as morphine (Ullah *et al*., 2014).

2.4.2 Models used in inflammation studies

2.4.2.1 Carrageenan induced paw edema

Carrageenan is a sulphated polysaccharide obtained from red sea weeds. Carrageenan induces paw edema in 2 phases, the early phase and the late phase (Di Rosa *et al*., 1971; Vinegar *et al*., 1987). The early phase is commonly a non-phagocytic inflammation and occurs within one hour of carrageenan injection. The

early phase is attributed to the release of mediators such as serotonin, histamine particularly 5-hydroxytryptamine (5-HT), cytoplasmic enzymes and platelet activating factor (PAF) from the mast cell (Bose, 2013). The late phase that occurs after one hour is associated with the increased production of inducible cyclooxygenase (COX) and thereby increased prostaglandins synthesis which is mediated by leukotrienes, prostaglandins, bradykinin and polymorphonuclear cells in the inflammatory site (Brito and Antonio, 1998; Perez-Guerrero *at al*., 2001; Gupta *et al*., 2006). The second phase edema is more sensitive to NSAIDs and steroidal anti-inflammatory agents (Di Rosa *et al*., 1971; Ullah *et al*., 2014). Carrageenan induced paw edema may involve L-arginine NO pathway whereby carrageenan injection results in release of NO this is attributed to elevated expression of inducible isoform of NO synthase. NO is a key mediator of inflammation (Chang *et al*., 2009). TNF-α, a key mediator of carrageenan induced edema, causes inflammatory incapacitation and stimulates production of kinins and leukotrienes which are responsible for long lasting nociceptive response (Chang *et al*., 2009).

2.4.2.1 Egg albumin induced edema

Freshly prepared egg albumin is used in *in vivo* studies to assay for anti-inflammatory agents. Egg albumin being, a phlogistic agent, initiates acute inflammation by inducing release of serotonin, histamine, prostanoids and kinins (Saad *et al*., 2009).

2.5 Plants used in this study

2.5.*1 Warbugia ugandensis* Sprague

2.5.1.1Classification and distribution

Warbugia ugandensis is a plant species in the family Canellaceae. It is native in Africa particularly in central, eastern and southern regions. The plant is exotic in India. It occurs in lowland rainforest, upland dry evergreen forest and also relicts in secondary bushland and grassland (Orwa *et al.,* 2012).

2.5.1.2 Plant description

Warbugia ugandensis is an evergreen plant that can grow to 4.5-30metres tall with 70 cm in diameter. The tree has a smooth or scaly barkwith pale green to brown colour (Figure 2.1). The leaves are alternate, simple, dotted with glands, without stipules. The leaf petioles are 1-5mm long. The leaf blade is oblong-lanceolate, elliptic or oblong-elliptic, 3-15×1.4-5cm, apex and base and dull bellow, midrib frequently slightly off-centre (Orwa *et al.,* 2012). The fruits are berries that are first green when young that later turn purplish with a size of about 3-5 cm in diameter as they mature. The seeds are two or more with oily endosperms that are yellow-brown in colour and about 1.0-1.5 cm long.

Figure 2.1: A photograph of *Warbugia ugandensis* plan (Munene, 2017)

2.5.1.3 Medicinal use

Traditional medical practitioners in Africa consider *Warbugia ugandensis* an important medicinal plant (Olila, *et al.*, 2001). The barks, leaves and roots of *Warbugia ugandensis* are used as remedy for various ailments. The leaves, barks and the roots have active principles that facilitate their ethno-pharmacological use. Most herbal preparations of *Warbugia ugandensis* commonly use the bark. Dried bark is commonly chewed and the juice swallowed as a remedy for stomach-ache, constipation, toothache, cough, fever, muscle pains, weak joints and general body pains (Orwa *et al*., 2012). Among Embu community, it is used for treatment of asthma and dental pains. Dried root is ground into a powder and used as a remedy for toothache while a decoction of stem barks and leaves of *Warbugia ugandensis* are folklore medicine for asthma and bronchitis (Kareru *et al*., 2007). Extracts of *Warbugia ugandensis* have been reported to show anti-malarial, antifungal and antibacterial properties in vitro (Olila *et al*., 2001). According to Kokwaro (1993)

Warbugia ugandensis is used among the Maasai community in Kenya as a remedy for respiratory ailments. The Maasai herders of Kenya, soak the bark of *Warburgia ugandensis* in water or milk and drink the decoction to treat tuberculosis (Kokwaro, 1993). A study by Maobe and Nyarango (2013) revealed *Warbugia ugandensis* is a phytomedicine for pneumonia, malaria and diabetes among the Kisii people in south west Nyanza, Kenya.

2.5.2 *Pistacia aethiopica* Kokwaro

2.5.2.1 Classification and distribution

Pistacia aethiopica is plant species in the Anacardiaceae family native to Africa and Arabian coast Peninsula. It is found in the eastern Africa countries and Yemen. Common names include: musaa (Kamba), muhehete, mucherere (Kikuyu), mugegeti (Kiembu), chepkorokwet (kipsigis), oldangudwa, oltanguota (Maasai).

2.5.2.2 Plant description

It is a dioecious genus, growing up to about 20m tall and is adapted to dry environment. The plant is an evergreen, slow growing tree that emits a very intense smell, bitter, resinous or similar to medication (Figure 2.2). The tree reaches 5-15m tall and is rarely a shrub, often with multiple stems. The trunk is about 0.6m in diameter and its bark colour is brown-black and fissured. It has glabrous leathery leaves with a thick cuticle. The leaves are aromatic, 4-16-18 foliate and glossy bright green. The leaves have rachis 10cm long.

Figure 2.2: A photograph of *Pistacia aethiopica* plant (Munene, 2017)

2.5.2.3 Medicinal use

According to Kareru *et al.* (2007) *Pistacia aethiopica* is used among the Embu and Mbeere communities as remedy for malaria and toothache. A root or bark infusion is drunk as herbal tea. The Maasai herders chew sticks of *Pistacia aethiopica* since its trunk yields a high-quality gum (Kiringe, 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection and preparation of plant materials

Fresh plant samples were collected from Kevote village, Embu west sub-county in Embu County, Kenya with the assistance of traditional medical practitioners. Information on the local names of the plants, parts used, mode of preparation and the season when their curative potency is maximal was provided by the herbalists. Nonetheless bioconservation measures were considered during sample collection. For *Warbugia ugandensis,* fresh leaf samples were collected whereas stem barks were collected for *Pistacia aethiopica.* Sample collection was done during the dry season in January; the time that the herbalists believed the plant had optimal medicinal properties. The samples were then air dried under a shade after which they were transported to Kenyatta University, Department of Biochemistry, Microbiology and Biotechnology laboratories for further processing. The plant samples were authenticated by a taxonomist and samples deposited with Department of Plant Sciences, Kenyatta University. Dried plant materials were pulverized into a fine homogeneous powder using a laboratory mill and then put in air tight polythene bags ready for extraction.

3.2 Extraction

For each sample, 200 g of powder was soaked separately in one litre of cold methanol for 48 hours with regular agitation to extract the phytocompounds. The extracts were then filtered using Whitman’s filter paper No.1 and the filtrate concentrated under reduced pressure using rotary evaporator at a temperature of

65ºC. The concentrates were then put in an air tight container and stored at 4ºC until used in bioassay studies.

3.3 Experimental design

3.3.1 Laboratory animals

Male Swiss albino mice (*Mus musculus*), aged between 2-3 months old and weighing 18-35 grams were used to bio-screen for analgesic and anti-inflammatory activities of the two medicinal plants. The animals were acquired from Kenya Medical Research Institute and kept in the animal breeding facility in the Department of Biochemistry, Microbiology and Biotechnology at Kenyatta University where experiments were carried out. A 48 hours acclimatization period was allowed prior to experimentation. The mice were kept in the standard cages and maintained under the standard laboratory conditions of ambient temperature (25ºC) and with 12-hour day light. The animals were fed on mouse cubes and supplied with water *ad libitum* (Vogel *et al*., 2002). The animals were fasted 12 hours before conducting the bioassays. Bioscreening was conducted in accordance with the internationally accepted procedures and ethical guidelines for evaluation of efficacy and safety of herbal medicines in animal models (WHO, 2000; Lilienblum *et al*., 2008).

3.4 Bioscreening

3.4.1 Determination of analgesic activities

The analgesic activities of the methanolic extracts of *Pistacia aethiopica* and *Warbugia ugandensis* were determined using formalin-induced paw licking model in mice as described by Hunskaar and Hole (1987). Thirty male Swiss albino mice

were randomly categorized into six groups of five animals each (n=5). The animals were first weighed and their respective weights recorded. Group I was the normal control in which the mice were intraperitoneally administered with 10 % DMSO solution without subsequent pain induction. Group II was the negative control in which pain was induced and no treatment was administered. Group III was the positive control, in which, diclofenac sodium was intraperitoneally administered at a dose of 15 mg/kg body weight 30 minutes prior to pain induction. Treatment for Group IV involved intraperitoneal administration of 0.1ml of the methanolic extracts at 50 mg/kg body weight dose level. Similarly, 0.1 ml of the methanolic extracts at 100 and 150 mg/kg body weight dose levels were intraperitoneally administered into the mice in groups V and VI, respectively (Table 3.1).

Thirty minutes after the administration of the methanolic extracts, pain was induced by injecting 0.05 ml of 2.5 % formalin into the sub-plantar region of the hind paw and the pain behavior observed and recorded. The pain behaviour of the mice was then individually observed and recorded. Nonetheless, in all groups the mice were individually placed in a transparent plexiglass observation chamber with mirrors placed on two sides of the chamber to enhance observation of the nociceptive behaviours from all angles. All injections were done using 30-gauge needles. The treatment protocol for analgesic assays was as follows:

Table 3.1: Treatment protocol for evaluation of analgesic activities of methanolic extracts of *Warbugia ugandensis* and *Pistacia aethiopica* in mice

Group	Status	Treatment
I	Normal control	DMSO (10%)
II	Negative control	Formalin (2.5%)
III	Positive control	Formalin+ Diclofenac (15mg/kg body weight)
IV	Experimental group A	Formalin+Extract (50mg/kg body weight)
V	Experimental group B	Formalin+ Extract (100mg/kg body weight)
VI	Experimental group C	Formalin+ Extract (150mg/kg body weight).

Nociceptive behavior was indicated and assessed through paw licking and biting (Heapy *et al*., 1987; Tjolsen *et al*., 1992). Formalin administration into plantar region of the left hind paw ensured a more specific nociceptive response, since during grooming the animals most frequently use their forelegs (Tjolsen *et al.*, 1992). The time, in seconds, that the mouse spent lifting, licking or biting the injected paw was measured using a stop watch. The nociceptive behavior was monitored and recorded according to response pattern described by Tjolsen *et al*. (1992), whereby two distinct periods of intensive licking, lifting and biting activity were identified and scored separately. The early phase, which was due to direct chemical stimulation of nociceptors, was recorded 1-5 minutes after formalin injection. The late phase, occasioned by release of inflammatory mediators, was recorded 15-30 minutes following formalin injection. The duration between the fifth and the fifteenth minute is the remission period with minimal nociceptive behaviour (Couto *et al*., 2011). The percentage paw licking inhibition was determined using the formula described by Mohan *et al*., (2011).

$$\% \text{ Paw licking/lifting inhibition} = \frac{N-T}{N} X100$$

Where;

N-The negative control group value for the each phase

T-The treated group value for each phase

3.4.2 Determination of anti-inflammatory activities

The anti-inflammatory effects of the methanolic extracts of *P. aethiopica* and *W. ugandensis* were determined using carrageenan-induced acute edema model in mice as described by Winter *et al*. (1962). Thirty male Swiss albino mice were randomly

divided into six groups of five mice each. The animals were first weighed and their respective weights recorded. Inflammation was induced by administration of 0.01 ml of 1 % carrageenan solution into the sub-plantar region of the hind paw half an hour prior to the treatment. Inflammation was induced in all the groups except the Group I which was the normal control.

The mice in Group I were treated with intraperitoneal administration of 0.01ml of 10% DSMO solution only. However, no inflammation was induced before the treatment in this group. The mice in Group II (negative control) were administered with 0.01 ml of 1 % carrageenan solution to induce inflammation then treated with 0.01 ml of 10 % DMSO solution that was intraperitoneally administered. In Group III (positive control), inflammation was induced in all the mice in the left hind paw thirty minutes after intraperitoneal administration of 0.01 ml of diclofenac sodium (reference drug) at a dose of 15 mg/kg body weight. Similarly, the mice in groups IV, V and VI were treated with 0.01 ml of 50, 100 and 150 mg/kg body weight extract dose levels respectively,30 minutes before inflammation was induced.

Table 3.2: Treatment protocol for evaluation of anti-inflammatory activities of methanol extracts of *Warbugia ugandensis* and *Pistacia aethiopica* in Swiss albino mice

Group	Status	Treatment
I	Control	DMSO (10%)
II	Negative control	Carrageenan (2.5%)
III	Positive control	Carrageenan+Diclofenac (15 mg/kg body weight)
IV	Experimental group A	Carrageenan +Extract (50 mg/kg body weight)
V	Experimental group B	Carrageenan + Extract (100 mg/kg body weight)
VI	Experimental group C	Carrageenan + Extract (150 mg/kg body weight)

Prior to carrageenan injection paw diameter readings were measured and recorded as the baseline. Subcutaneous administration of 0.01 ml of 1 % carrageenan solution into sub-plantar tissue of the left hind paw induced biphasic acute edema (Winter *et al*., 1962). Subsequently, the paw diameter readings were measured at hourly intervals for four hours following carrageenan injection (Bamgbose and Noamesi, 1981). All paw diameter readings, in millimeters, were determined using a digital Vernier calipers. The baseline readings were then compared with the diameter readings of the same paw after carrageenan injection to assess edema. The percentage paw edema inhibition was determined using the formula described by Kamau *et al*. (2016).

$$\% \text{ Paw edema inhibition} = \frac{Ct - Tt}{Ct} \times 100$$

Where;

Ct is the paw diameter at 1 hour following carrageenan injection.

Tt is the paw diameter after treatment

3.5 Qualitative Phytochemical Screening

The methanolic extracts of *P. aethiopica* and *W. ugandensis* were subjected to qualitative phytochemical screening for presence or absence of phytochemicals using methods of analysis as described by Harbone (1998) and Kotake (2000). Secondary metabolites tested included: flavonoids, phenols, saponins, alkaloids, cardiac glycosides, steroids, terpenoids and tannins. These phytochemicals have been demonstrated to exhibit analgesic and anti-inflammatory activities.

3.5.1 Test for saponins

A few drops of 1 M sodium hydrogen carbonate solution were added into 2 ml of the extract in a test tube and shaken vigorously. The mixture was then allowed to stand for 10 minutes. Froth formation indicated presence of saponins (Shammy *et al*., 2010).

3.5.2 Test for alkaloids

The extracts solution was first acidified by adding 3 drops of 1 M hydrochloric acid solution into 3ml extract solution in a test tube. The acidified extract solution was heated and 3 drops of Dragendorff's reagent added. Formation of orange precipitate revealed presence of alkaloids (Shammy *et al*., 2010).

3.5.3Test for terpenoids

A mixture of 1 ml of ethyl acetate and 2 ml of chloroform was first prepared, then 0.5 g of plant extract was added and shaken vigorously. After shaking the mixture, 3 ml of concentrated sulphuric (VI) acid was added along the walls of the test tube. A

red brown color at the interface indicated presence of terpenoids (Shammy *et al*., 2010).

3.5.4 Test for flavonoids

Two milliliters of the plant extract were mixed with 2 ml of 1Msodium hydroxide solution. Yellow precipitate indicated presence of flavonoids (Shammy *et al*., 2010).

3.5.5 Test for cardiac glycosides

Five milligrammes of extract were dissolved in 2 ml glacial acetic acid containing 3 drops of 10 % iron (III) chloride solution. The solution was then under-layered with 1 ml of concentrated sulphuric (VI) acid. A brown, violet or greenish ring at the interphase indicated presence of deoxysugar, which is characteristic of cardenolides (Shammy *et al*., 2010).

3.5.6 Test for steroids

To test for steroids, 0.5 g of each of the extract was dissolved in 2 ml of chloroform followed by addition of 3 ml concentrated H_2SO_4 along the walls of the test tube. Appearance of a red-brown interface confirmed presence of steroids (Shammy *et al*., 2010).

3.5.7 Test for phenols

Presence of phenols was assayed by adding 1 ml of 1 % iron (III) chloride solution to 2 ml of plant extract. Presence of phenols was indicated by formation of blue-green precipitate (Shammy *et al*., 2010).

3.5.8 Test for tannins

To test for tannins, 0.5 g of plant extract was added to 10ml distilled waterin a test tube, the mixture was stirred and filtered. A few drops of 1 % iron (III) chloride solution were then added to the filtrate. Occurrence of a blue black, green or blue green precipitate indicated presence of tannins (Evans, 2002).

3.6 Data Management and Statistical Analysis

Qualitative data obtained from qualitative phytochemical screening was recorded. Quantitative experimental data on paw licking time in seconds and paw edema diameter in millimeters obtained from all the animals in various treatment groups were recorded and tabulated on excel spreadsheet using Microsoft Excel program. Analysis of the data was done using Minitab® Version 17 software. The results were expressed as Mean and Standard Error of Mean (SEM). Statistical significance of difference among groups was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc. Unpaired Student t-test was used to compare the activities of *P. Aethiopica* and *W. ugandensis* extracts against pain and inflammation. The values of $p < 0.05$ were considered significant. Results were presented in tables and figures.

CHAPTER FOUR

RESULTS

4.1 Analgesic activities of methanolic extracts of *Pistacia aethiopica* and *Warbugia ugandensis*

Results of the analgesic activities of the methanolic extracts of *P. aethiopica* and *W. ugandensis* are represented in Tables 4.1, 4.2 and Figures 4.1, 4.2. Generally, the methanolic stem bark extracts of *Pistacia aethiopica* reduced paw licking time significantly in a dose dependent manner compared to the negative control group in both early and late phases of formalin-induced pain in mice ($p < 0.05$). The analgesic effects were indicated by increased percent paw licking inhibition or reduced paw licking time on formalin-induced nociception in mice.

In the early phase, the methanolic stem bark extracts of *P. aethiopica* had significant pain inhibition activity indicated by percent paw licking inhibition of between 47.24 and 55.13 % by the three extract doses (50, 100 and 150 mg/kg body weight). Diclofenac sodium, which was the positive control, elicited a percent paw licking inhibition of 30.33% in the early phase. The percent paw licking inhibitions by the three methanolic stem barks extracts doses were 47.24, 52.29 and 55.13 %, respectively. In this phase, the analgesic effects of the three dose levels of 50, 100 and 150 mg/kg body weight were statistically similar ($p > 0.05$). The pain inhibitory effects of the three extract dose levels were significantly higher than the effect of the reference drug in the early phase.

In the late phase, the methanolic stem bark extracts of *P. aethiopica* reduced pain significantly compared to the negative control ($p < 0.05$). The percent paw licking

inhibitions by the three doses of *P. aethiopica* extract ranged between 30.69 and 52.12 % while that of the diclofenac (positive control) was 62.93%.

The analgesic activities of the three extract dose levels (50, 100 and 150 mg/kg body weight) of *P. aethiopica* were significantly lower than that exhibited by the positive control in the late phase ($p < 0.05$). The analgesic effects of the methanolic stem bark extracts, at dose levels of 50 and 100 mg/kg body weight, were statistically similar ($p > 0.05$). However, the dose level of 150 mg/kg body weight caused significantly larger pain reduction than the other two extract dose levels ($p < 0.05$).

Table 4.1: Analgesic effects of methanol leaf extracts of *Pistacia aethiopica* in Swiss albino mice

Group	**Treatment**	Early phase	Late phase
Normal control	DMSO only	00.00 ± 0.00 (100.00±0.00)[a]	00.00 ± 0.00 (100.00±0.00)[a]
Negative control	Formalin	126.00±3.9 (0.00±0.00)[e]	103.60± 1.75 (0.00±0.00)[e]
Positive control	15mg/kg bw Diclofenac+Formalin	88.20± 3.76 (30.33±1.44)[d]	38.40± 1.94 (62.93±1.87)[b]
Experimental groups	50 mg/kg bw *P. aethiopica*+ Formalin	66.80 ± 2.08 (47.24±1.65)[c]	71.80 ± 1.53 (30.69±1.48)[d]
	100 mg/kg bw+ *P. aethiopica*+Formalin	60.40 ± 2.20 (52.29±1.74)[bc]	67.60± 1.54 (34.75±1.48)[d]
	150 mg/kg bw + *P. aethiopica*+ Formalin	56.80± 2.29 (55.13±1.81)[b]	49.60± 6.30 (52.12±1.57)[c]

Values are expressed as Mean ± SEM. Values with the same superscript are not significantly different. Values in parenthesis indicate percent mean paw licking inhibition, bw is an abbreviation for body weight

The methanolic leaf extracts of *Warbugia ugandensis* also remarkably reduced paw licking time in the early and late phases of nociception a tall the three dose levels. In the early phase, intraperitoneal administration of the methanolic leaf extracts of *W. ugandensis*, at dose levels of 50, 100 and 150 mg/kg body weight, reduced pain appreciably compared to negative and normal controls. The percent inhibitions of paw licking during the early phase by the three experimental doses (50, 100 and 150 mg/kg body weight) were 38.45, 51.82 and 43.07 %, respectively. The reference drug (Diclofenac sodium) caused a percent paw licking inhibition of 30.36%. The analgesic effect of the extract dose level of 100 mg/kg body weight was significantly higher than those of dose levels 50 and 150 mg/kg body weight ($p < 0.05$). However, the analgesic activities of the three tested dose levels were significantly higher than that of diclofenac (positive control) in the early phase.

In the late phase, the methanolic leaf extract of *W. ugandensis*, at dose levels 50, 100 and 150 mg/kg body weight, significantly reduced paw licking ($p < 0.05$) compared to the negative control. The percent inhibitions in paw licking time by the three extract dose levels (50, 100 and 150 mg/kg body weight) were 43.48, 63.80 and 60.67 %, respectively. The percent paw licking inhibition by the 150 and 100 mg/kg body weight extract dose levels were statistically similar to that of the reference drug. However, the analgesic activity of 50 mg/kg body weight extract dose level was significantly lower than those of the 100 and 150 mg/kg body weight extract dose levels ($p < 0.05$).

Table 4.4: Analgesic effects of methanol leaf extracts of *Warbugia ugandensis* in Swiss albino mice

Group	Treatment	Early phase	Late phase
Normal control	DMSO only	00.00 ± 0.00 (100.00±0.00)^{e}	00.00 ± 0.00 (100.00±0.00)^{a}
Negative control	Formalin	121.20±3.06 (0.00±0.00)^{a}	101.20± 2.92 (00.00±0.00)^{e}
Positive control	15mg/kg bw Diclofenac+Formalin	55.40 ± 4.72 (30.36±2.10)^{d}	23.20± 3.68 (77.08±3.64)^{d}
Experimental groups	50 mg/kg bw *W. ugandensis* + Formalin	74.60 ± 2.27 (38.45±1.87)^{b}	57.20 ± 4.78 (43.48±4.72)^{b}
	100 mg/kg bw *W. ugandensis*+ Formalin	58.40 ± 3.08 (51.82±2.54)^{c}	34.80± 2.75 (63.80±2.71)^{cd}
	150 mg/kg bw *W. ugandensis*+ Formalin	69.00 ± 2.00 (43.07±1.65)^{b}	39.80± 2.11 (60.67±2.07)^{c}

Values are expressed as Mean ± SEM. Values with the same superscript are not significantly different. Values in parenthesis indicate percent mean paw licking inhibition, bw is an abbreviation for body weight

The analgesic effects of the two studied medicinal plants on formalin-induced pain in mice models were compared. In the early phase, the percentage paw licking time inhibitions of *P. aethiopica,* at the dose levels of 50 and 150 mg/kg body weight, were significantly higher than those of *W. ugandensis* at the two dose levels ($p < 0.05$). However, at the dose level 100 mg/kg body weight, the two medicinalplants were equally effective ($p > 0.05$).

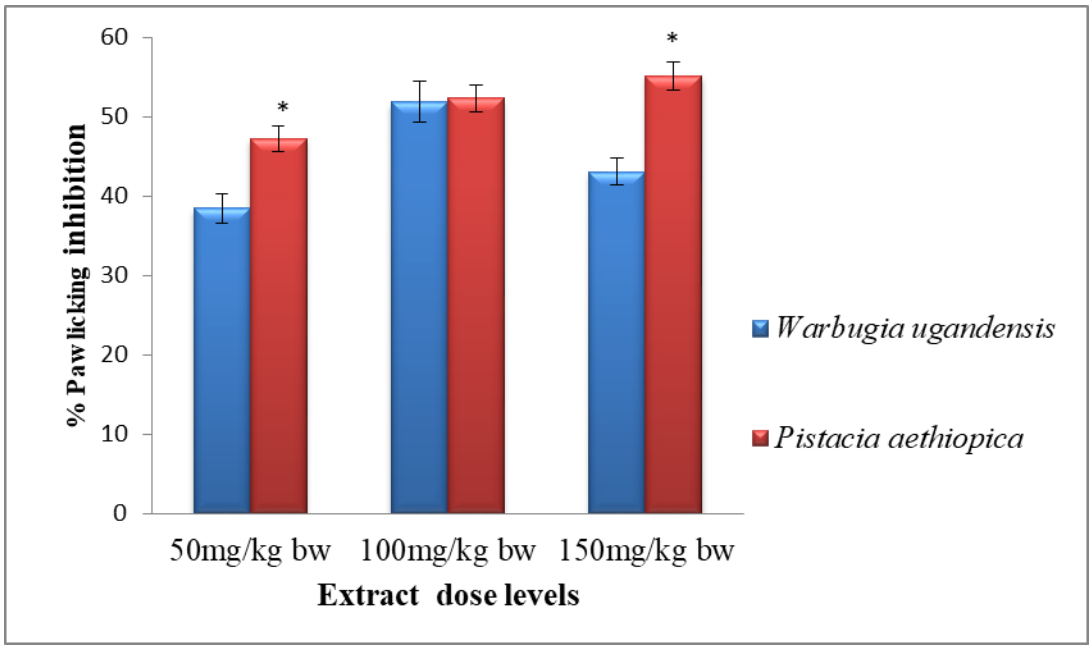

Figure 4.1: Comparison of paw licking inhibition of methanol extracts of *P. Aethiopica* and *W. ugandensis* in the early phase of formalin-induced nociception. * indicates variation in the analgesic effect between the two plants under study (Unpaired t-test; $p < 0.05$).

On the other hand, in the late phase, the methanolic leaf extracts of *W. ugandensis*, at the three dose levels (50, 100 and 150 mg/kg body weight), was significantly more effective than the methanolic stem bark extracts of *P. aethiopica* ($p < 0.05$).

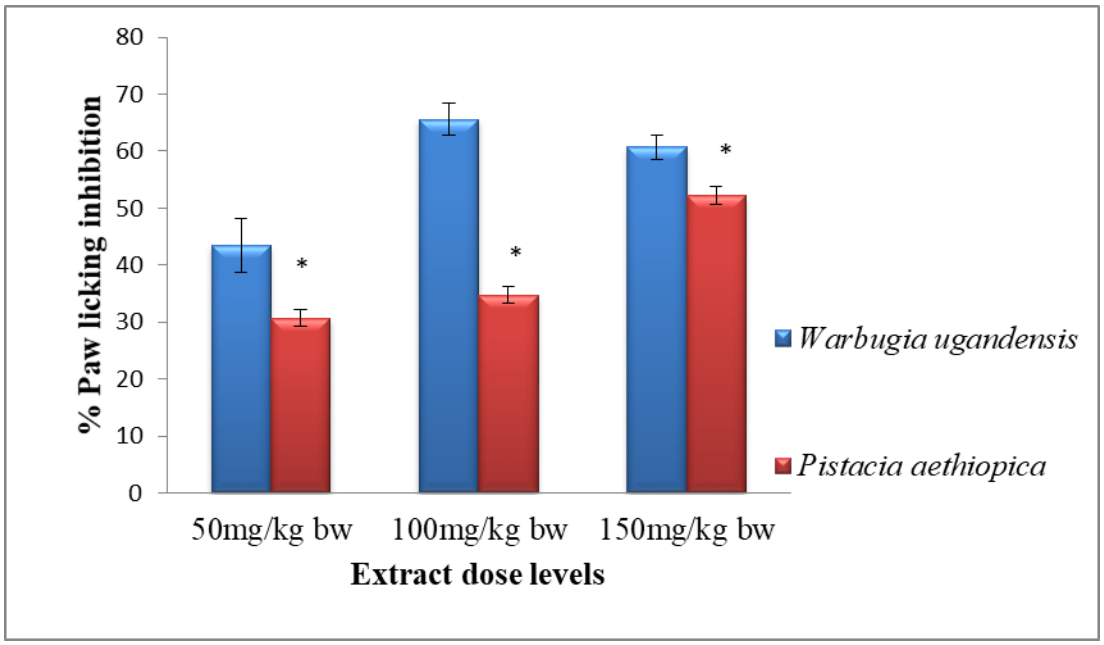

Figure 4.2: Comparison of paw licking inhibition by the methanol extracts of *P. aethiopica* and *W. ugandensis* during the late phase of formalin induced nociception. * denotes statistical variation in the effects of the methanolic extracts of the two plants (Unpaired t-test; $p < 0.05$).

4.2 Anti-inflammatory activities of methanolic extracts of *P. aethiopica* and *W. ugandensis*

The results of anti-inflammatory activities of methanolic extracts of *P. aethiopica* and *W. ugandensis* are represented in Tables 4.3, 4.4 and Figures 4.3, 4.4, 4.5. The methanolic stem bark extract of *P. aethiopica* significantly suppressed paw edema in mice models, at all the three extract dose levels, in a dose dependent manner. In the first hour, the percent paw edema inhibitions by the three extract dose levels (50, 100 and 150 mg/kg body weight) were 0.90, 0.91 and 2.07 %, respectively. The reference drug had a percent paw edema inhibition of 1.99%. The anti-inflammatory effects of the three *P. aethiopica* extract doses were equally effective to that of the reference drug, at this hour ($p > 0.05$).

In the second hour, the methanolic extracts of *P. aethiopica* inhibited paw edema by 2.36, 2.03, and 4.09 % at the dose levels of 50, 100 and 150 mg/kg body weight, respectively. Comparison of the three extract dose levels to the negative control revealed significant differences in the anti-inflammatory activities ($p < 0.05$). The 50 and 100 mg/kg body weight extract dose levels revealed significantly lower percent paw edema inhibitions than that caused by the 150 mg/kg body weight extract dose level ($p < 0.05$). However, the anti-inflammatory effects of the diclofenac and that of the extract dose level of 150 mg/kg body weight were similar ($p > 0.05$).

In the third hour, the methanolic stem bark extracts of *P. aethiopica* inhibited inflammation significantly when compared to the negative control ($p < 0.05$). The percent edema inhibitions by the three extract dose levels (50, 100 and 150 mg/kg body weight) were 3.23, 3.39 and 5.91 %, respectively. The 50 and 100 mg/kg body weight extract doses demonstrated similar percent paw edema inhibitions ($p > 0.05$), which were significantly lower to that of the 150 mg/kg body weight extract dose level ($p < 0.05$). The edema suppression activity of the 150 mg/kg body weight extract dose level was comparable to that of the diclofenac, which inhibited the paw edema by 7.11% ($p > 0.05$).

In the fourth hour, intraperitoneal administration of the methanolic extracts, at dose levels 50 and 100 mg/kg body weight, demonstrated statistically similar percent paw edema inhibitions, which were significantly different from that of the 150 mg/kg body weight extract dose level. However, the 150 mg/kg body weight extract dose level

demonstrated similar percent paw inhibition to that of the positive control drug, Diclofenac ($p > 0.05$).

Table 4.3: Anti-inflammatory activity of the methanolic stem bark extracts of *Pistacia aethiopica* in Swiss albino mice

Group	Treatment	Mean percent change in paw diameter(mm) after treatment				
		0hr	**1hr**	**2hr**	**3hr**	**4hr**
Normal Control	DMSO	100±0.00 (0.00±0.00)^{Aa}	100.00±0.00 (0.00±0.00)^{Ba}	100.00±0.00 (0.00±0.00)^{Ca}	100.00±0.00 (0.00±0.00)^{Ca}	100.00±0.00 (0.0±0.00)^{Ca}
Negative Control	Carrageenan	100±0.00 (0.00±0.00)^{Aa}	102.12±0.24 (-2.12±1.62)^{Ca}	104.96±0.30 (-.96±0.99)^{Db}	106.33±0.61 (-6.34±1.37)^{Dbc}	107.51±0.50 (-7.51±1.26)^{Dc}
Positive Control	Carrageenan + 15mg/kg bw Diclofenac	100±0.00 (0.00±0.00)^{Ae}	98.01±0.34 (1.99±0.76)^{Ad}	95.38±0.31 (4.62±0.69)^{Ac}	92.89±0.31 (7.11±0.69)^{Ab}	91.14±0.21 (8.86±0.47)^{Aa}
Experimental groups	Carrageenan +50mg/kg bw *P. aethiopica*	100±0.00 (0.00±0.00)^{Ac}	99.10±0.12 (0.90±0.39)^{ABc}	97.64±0.30 (2.36±0.68)^{Bb}	96.77±0.43 (3.23±0.95)^{Bb}	95.40±0.38 (4.60±0.84)^{Ba}
	Carrageenan+100mg/kg bw *P. aethiopica*	100±0.00 (0.00±0.00)^{Ad}	99.09±0.12 (0.91±.27)^{ABd}	97.97±0.20 (2.03±0.44)^{Bc}	96.61±0.43 (3.39±0.96)^{Bb}	94.43±0.33 (5.57±0.74)^{Ba}
	Carrageenan + 150mg/kg bw *P. aethiopica*	100±0.00 (0.00±0.00)^{Ae}	97.93±0.25 (2.07±0.56)^{Ad}	95.91±0.17 (4.09±0.37)^{Ac}	94.09±0.18 (5.91±0.41)^{Ab}	92.40±0.12 (7.60±0.27)^{Aa}

Values expressed as Mean ± SEM Values with the same capital letter down the column and small letters across the rows are not significantly different from one another. Percentage reduction is shown in edema in brackets, bw is an abbreviation for body weight.

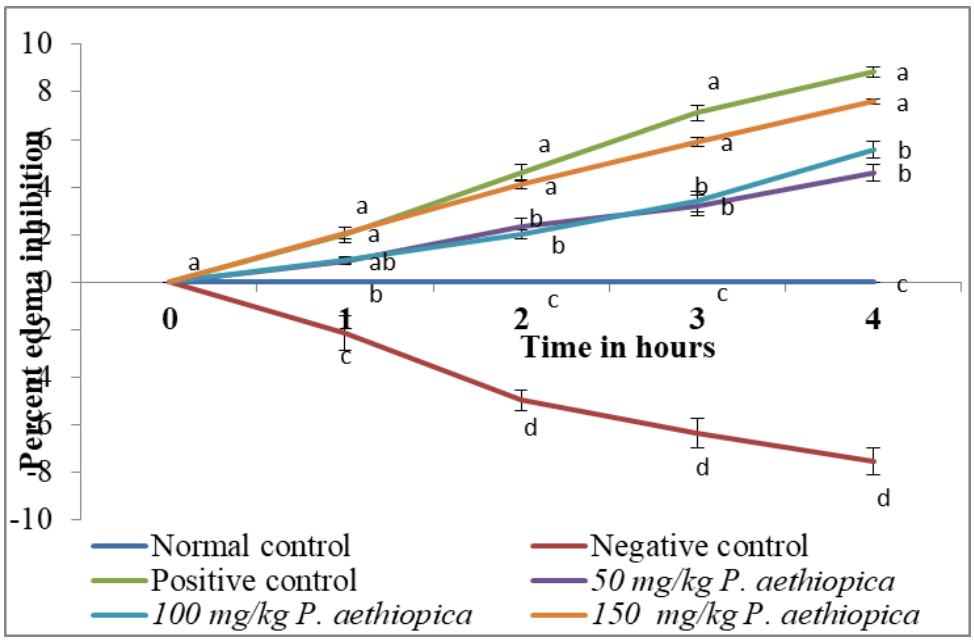

Figure 4.3: Percent paw edema inhibition following intraperitoneal administration of methanol extracts of *P. aethiopica*. The letters are the indicators of statistical variation.

On the other hand, the methanolic leaf extract of *W. ugandensis* generally reduced phlogosis in mice, at all the three dose levels (50, 100 and 150 mg/kg body weight). In the first hour, the positive control (diclofenac) inhibited inflammation by 0.76% while the methanolic extracts, at dose levels (50, 100 and 150mg/kg body weight), inhibited inflammation by 1.35, 1.09 and 0.62 %, respectively. There were no significant differences in the anti-inflammatory activities of the reference drug and the three extract dose levels at this hour ($p > 0.05$). In the second hour, the percent inhibition of paw edema by the three extract dose levels (50, 100 and 150 mg/kg body weight) were 2.92, 3.47 and 2.43 %, respectively. The reference drug inhibited paw edema by 4.58 %. At this hour, the positive control and three extract dose levels demonstrated similar percent paw edema inhibition ($p > 0.05$).

Significant anti-inflammatory activities were observed in the third hour with the percent inflammatory inhibition by the three extract dose levels ranging between

5.54 and 4.35 %. The anti-inflammatory activity of the positive control was statistically similar to those of the three extract dose levels at this hour ($p > 0.05$).

In the fourth hour, the percent paw edema inhibitions by the three extract dose levels were 6.08, 7.41 and 7.59 %, respectively. The effects of the extracts at dose levels of 100 and 150 mg/kg body weight dose levels were significantly higher than that of the 50 mg/kg body weight extract dose level ($p < 0.05$). At this hour, the highest percent paw edema inhibition (9.57%) was caused by diclofenac, which was significantly higher than those of the three extract dose levels ($p < 0.05$).

Table 4.4: Anti-inflammatory activity of the methanolic leaf extracts of *W. ugandensis* in Swiss albino mice

Group	Treatment	Mean percent change in paw diameter(mm) after treatment				
		0hr	**1hr**	**2hr**	**3hr**	**4hr**
Normal Control	DMSO	100.00±0.00 (0.00±0.00)^{Aa}	100.00±0.00 (0.00±0.00)^{Ba}	100.00±0.00 (0.00±0.00)^{Ba}	100.00±0.00 (0.00±0.00)^{Ca}	100.00±0.00 (0.00±0.00)^{Da}
Negative Control	Carrageenan	100.00±0.00 (0.00±0.00)^{Aa}	101.74±0.37 (-1.74±0.37)^{Cab}	103.01±0.72 (-3.01±0.72)^{Cb}	103.77±0.30 (-3.77±0.68)^{Dbc}	105.17±0.48 (-5.17±0.39)^{Ec}
Positive Control	Carrageenan +15mg/kg bw diclofenac	100.00±0.00 (0.00±0.00)^{Ad}	99.24±0.14 (0.76±0.14)^{ABd}	95.42±0.59 (4.58±0.06)^{Ac}	93.05±0.42 (6.95±0.42)^{Ab}	95.57±0.47 (9.57±0.04)^{Aa}
Experimental groups	Carrageenan + 50mg/kg bw *W. ugandensis*	100.00±0.00 (0.00±0.00)^{Ad}	98.65±0.20 (1.35±0.20)^{Acd}	97.08±0.57 (2.92±0.57)^{Abc}	95.67±0.18 (4.35±0.81)^{Bab}	93.92±0.52 (6.08±0.52)^{Ca}
	Carrageenan+100mg/kg bw *W. ugandensis*	100.00±0.00 (0.00±0.00)Ad	98.91±0.29 (1.09±0.29)Ad	96.53±0.61 (3.47±0.61)Ac	94.46±0.49 (5.54±0.49)ABb	92.59±0.16 (7.41±0.16)Ba
	Carrageenan + 150mg/kg bw *W. ugandensis*	100.00±0.00 0.00±0.00)^{Ad}	99.39±0.16 (0.61±0.16)^{ABd}	97.58±0.62 (2.43±0.62)^{Ac}	95.34±0.33 (4.66±0.33)^{Bb}	92.41±0.12 (7.59±0.12)^{Ba}

Values expressed as Mean±SEM. Values with the same capital letter down the column and small letters across the rows are not significantly different from one another Percentage reduction in edema is shown in brackets, bw is an abbreviation for body weight.

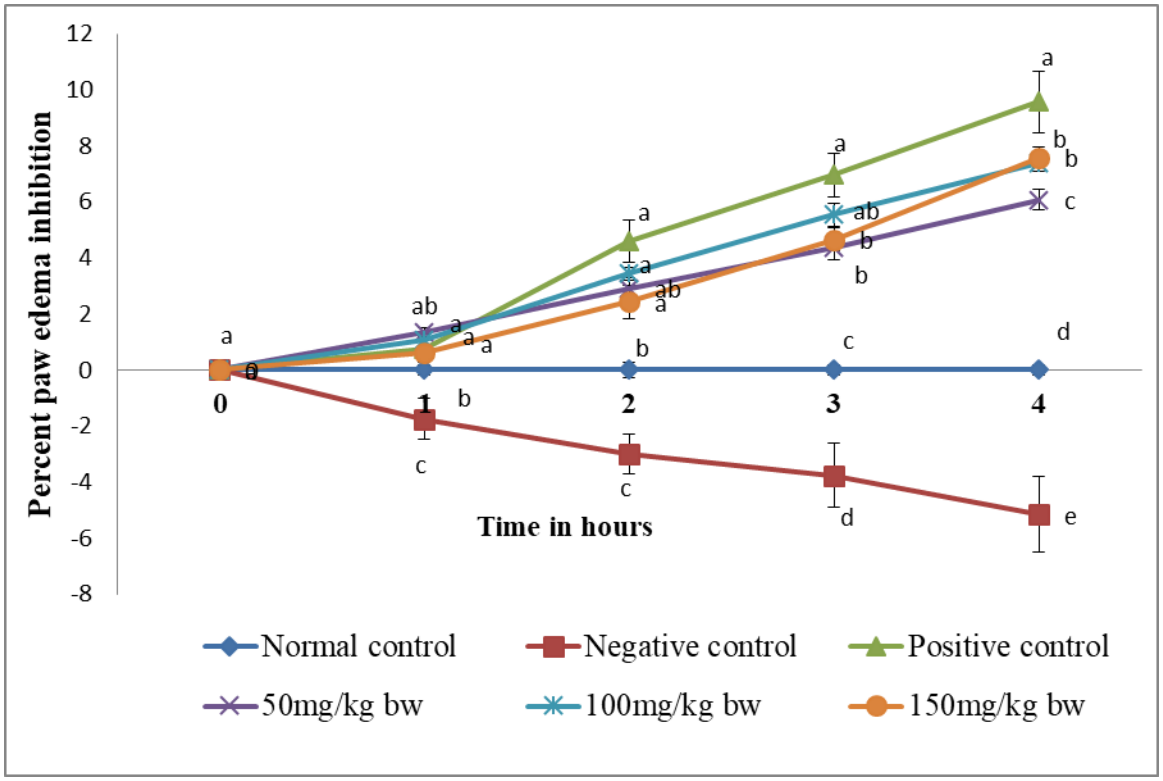

Figure 4.4: Percent paw edema inhibition following intraperitoneal administration of methanol extracts of *W. ugandensis.* The letters are the indicators of statistical variation.

Pairwise comparison of anti-inflammatory effects of the methanolic extracts of *P. aethiopica* and *W. ugandensis* at 50 mg/kg body weight dose level, indicated no significant differences during the four hours of the test period ($p > 0.05$; Figure 4.5a). At the dose level of 100 mg/kg body weight, the anti-inflammatory activities of both extracts were statistically similar in the first and the second hour ($p > 0.05$). However, comparison of the anti-inflammatory activities at 100 mg/kg body weight extract dose level, during the third and fourth hours, revealed that *W. ugandensis* had significantly higher activity ($p < 0.05$). At the dose level of 150 mg/kg body weight, the anti-inflammatory effects of *P. aethiopica* were significantly higher than those of *W. ugandensis* in the first and the third hour ($p < 0.05$). However, the anti-inflammatory effects of both plants' extracts were statistically similar at the dose level of 150 mg/kg body weight in the second and fourth hours ($p > 0.05$).

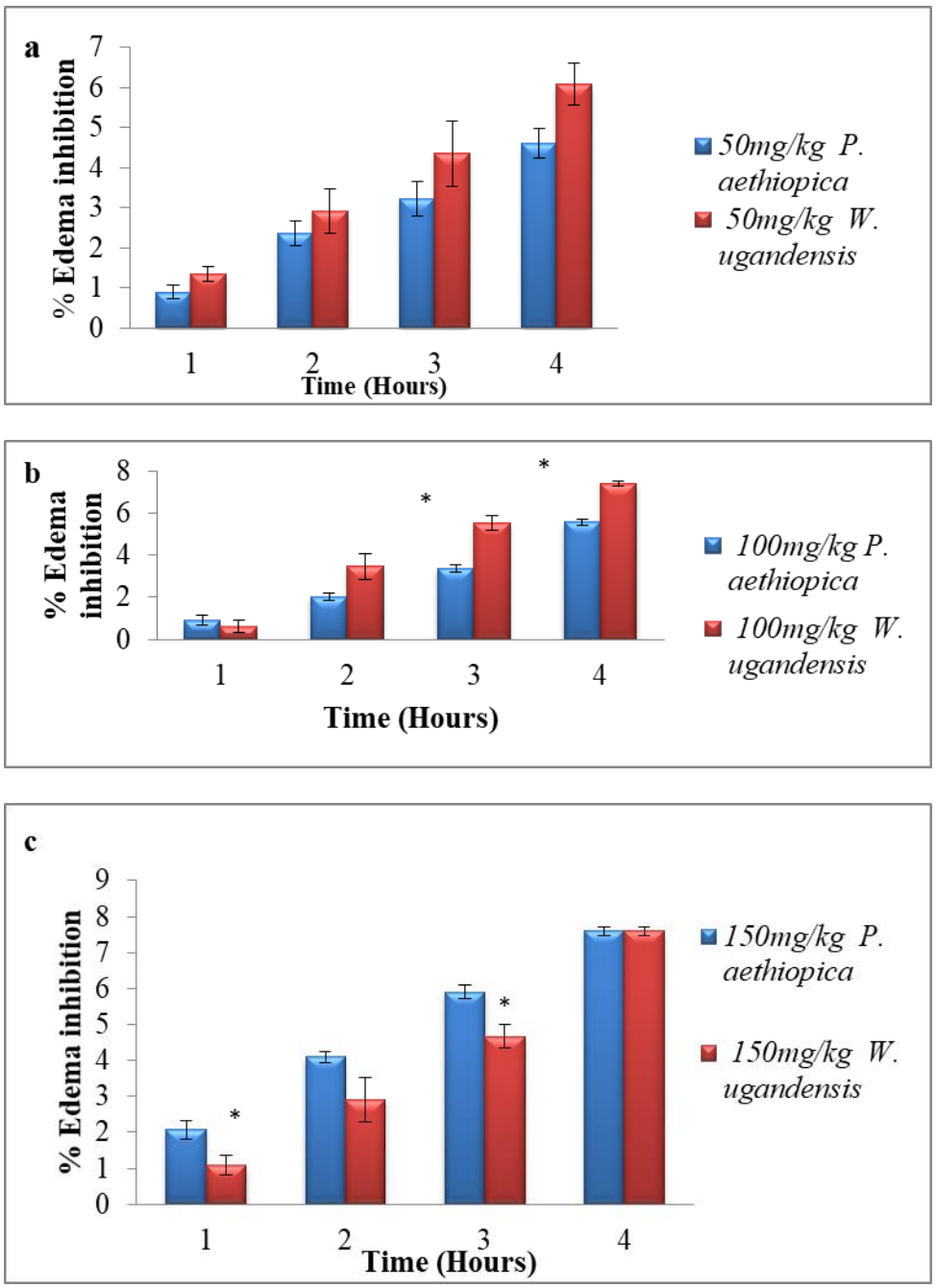

Figure 4.5: Comparison of the percent edema inhibition of *P. aethiopica* and *W. ugandensis* at a (50), b (100) and c (150) mg/kg body weight dose levels. The asterisk (*) denotes statistical variation following comparison of the effects of the methanolic extracts of the two plants at respective doses (unpaired t-test; $p < 0.05$).

4.3 Qualitative phytochemical profiles of the methanolic extracts of *P. aethiopica* and *W. ugandensis*

Qualitative phytochemical analysis of the methanolic leaf extracts of *Warbugia ugandensis* and stem bark extracts of *Pistacia aethiopica* indicated presence of

saponins, terpenoids, flavonoids, cardiac glycosides, steroids and phenols in *Warbugia ugandensis* and presence of saponins, alkaloids, terpenoids, flavonoids, cardiac glycosides, steroids, phenols and tannins in *Pistacia aethiopica*.

Table 4.5: Phytochemical constitution of methanol extracts of *Warbugia ugandensis* leaves and *Pistacia aethiopica* stem bark

Phytochemical	***Warbugia ugandensis***	***Pistacia aethiopica***
Saponins	+	+
Alkaloids	-	+
Terpenoids	+	+
Flavonoids	+	+
Cardiac glycosides	+	+
Steroids	+	+
Phenols	+	+
Tannins	-	+

The positive sign (+) indicates presence while the negative sign (-) indicates absence.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The methanolic extracts of *P. aethiopica* and *W. ugandensis* significantly suppressed formalin-induced pain in mice in both phases of formalin-induced nociception (Tables 4.1 and 4.2). The analgesic activities were indicated by reduction in paw licking time, which was expressed as percent paw licking inhibition. The inhibition of formalin-induced pain in both phases, by the methanolic extracts of *P. Aethiopica* and *W. ugandensis,* suggests that the analgesic effects elicited by the phyto-constituents, present in the methanolic extracts of the two plants, involved both central and peripheral mechanisms of analgesia in mice.

Nevertheless, the methanolic extracts of *P. aethiopica* and *W. ugandensis* did not equally inhibit pain in both of phases (Tables 4.1 and 4.2). This suggests that analgesic activities were not purely due to centrally acting analgesics but a combination of the central and peripheral analgesic acting active principles. The peripheral analgesic activities may be similar to that of NSAIDs that act through inhibition of prostaglandins synthesis (Ong *et al.,* 2011). In addition, the inhibition of both phases of formalin-induced pain implied that the methanolic extracts of both plants contained not only analgesic but also anti-inflammatory active principles given the inhibition of the early phase and late phase pain nociception of model (Ong *et al.,* 2011).

The results of this study are consistent with previous studies on analgesic activities of medicinal plants that observed analgesic activities in both phases of formalin-

induced nociception. Gitahi *et al*. (2015a) demonstrated that DCM: Methanolic leaf and root bark extracts of *Carissa edulis* inhibited pain in both phases of formalin-induced pain. Similarly, the methanolic stem bark extracts of *Harrisonia abyssinica* Oliv. And *Landolphia buchanani* Hallier F. reduced paw licking in both phases of formalin induced nociception in mice (Nthiga *et al*., 2016). Studies by Ullah *et al*. (2014) revealed that ethanolic extracts of *Curcuma zedoaria* rhizome ameliorated pain in both early and late phases of formalin-induced nociception. Ethylacetate fraction of *Cassia fistula* L. exhibited analgesic activities in early and late phases of formalin-induced nociception in rats (Kalaiyarasi *et al*., 2014). Moreover, *Allium sativum* powder suppressed formalin-induced nociception in mice in both phases of formalin-induced nociception (Jayanthi and Jyoti, 2012).

The mean percent paw licking inhibitions by the three *P. aethiopica* extract dose levels indicate that the methanolic extracts had higher analgesic effect in the early phase than in the late phase (Table 4.1). This may be due to fast diffusion of the active principles across the cell membranes into peritoneal cavity. Therefore, there was fast onset of analgesic effects. Additionally, the phytoconstituents present in the methanolic extracts of *P. aethiopica* might have exerted analgesic effects directly without undergoing any biotransformation. However, there is a possibility that the methanolic extracts of *P. aethiopica* may have had high concentrations of opioidlike analgesic phyto-constituents that exerted a central analgesic effect that was observed as heightened anti-nociceptive effect in the early phase.

On the other hand, the analgesic effects of the methanolic extracts of *W. ugandensis* were higher in the late phase than in the early phase (Table 4.2). This could have

been due to slow or delayed diffusion and absorption of the active principles into the peritoneal cavity which in turn caused delayed analgesic effects. The slow diffusion of bioactive compounds may also explain why analgesic effect increased with time leading to a higher effect in the late phase. Furthermore, the higher analgesic effect in the late phase may imply that the phytoconstituents in the methanolic extracts of *W. ugandensis* needed biotransformation into more active analgesic agents (Mahdi, 2014). Nonetheless, the possibility of higher concentration of peripherally acting active principles cannot be ignored.

The analgesic effects elicited by100 and 150 mg/kg body weight extract dose levels of *P. aethiopica* were higher than that of the 50 mg/kg body weight (Figure 4.1 and Table 4.1). This indicates the analgesic activities of *P. aethiopica* were dose dependent in both early and late phases of the formalin-induced nociception. The more pronounced analgesic activities at higher dose levels may have been due to higher concentration of bio-active compounds that exerted greater analgesic effects. Such a dose dependent effect was reported earlier by Nthiga *et al*. (2016) while working on antinociceptive activity of *Harrisonia abyssinica* and *Landolphia buchanii* in mice models, in which the extracts exhibited dose dependent analgesic activities in the early phase.

The results obtained in this study show that the methanolic extracts of *W. ugandensis* caused a non-dose dependent analgesic activity whereby the highest analgesic effect was observed at 100 mg/kg body weight dose level in both phases (Table 4.2). This suggests that the 100 mg/kg body weight extract dose level was a better analgesic agent compared to the 150 mg/kg body weight. Therefore, a higher

dose level (150 mg/kg body weight) produced less analgesic effect than the 100 mg/kg body weight dose level (Table 4.2). This scenario may be attributed to the saturation of the active principles at the binding sites of the enzymes or proteins involved in analgesic mechanisms. It implies that any dose beyond 100 mg/kg body weight could not yield any significant increase in analgesic effect. On the contrary, a dose beyond 100 mg/kg body weight led to a decline in the analgesic activity. Koech *et al.* (2017) observed a similar scenario whereby a higher extract dose level produced a diminished effect compared to a lower dose. The decline in pharmacological effect, beyond optimal dose, can also be ascribed to a natural pharmacokinetic mechanism within the animal that initiates excretion and clearance of the drug in order to avoid toxic effects by limiting their biological action (Andersen, 1981).

The analgesic activities of the methanolic extracts of *P. aethiopica* and *W. ugandensis* at 50mg/kg body weight dose level were less potent than higher doses in both phases (Tables 4.1 and 4.2). Probably, at lower dose levels the active principles in the extracts might have been metabolized, cleared or inactivated at a faster rate. Besides, the concentration of the active principles, at 50 mg/kg body weight dose level, may have had limited the pharmacological effects (Safari *et al*., 2016).

The results of this study suggest that the methanolic extracts of *P. aethiopica* and *W. ugandensis* exerted considerable analgesic effects in formalin-induced pain in mice (Table 4.1 and 4.2). The methanolic extracts might have acted through metabolic inhibition of the COX pathway in a similar version to that of NSAIDs. The analgesic activities observed in this study may be attributed to phytocompounds present in the

methanolic extracts of *P. aethiopica* and *W. ugandensis* (Table 4.5). Flavonoids have been found to inhibit lipoxygenase and cyclooxygenase pathways that are responsible for peripheral nociception (Velazquez-Gonzalez *et al*., 2014). Moreover, flavonoids inhibit prostaglandin biosynthesis by inhibiting the function of prostaglandin synthase (Panda *et al.*, 2009). Flavonoids have been shown to target synthesis of prostaglandins which are involved in pain perception through opioidergic mechanism (Chakraborty *et al*., 2004; Panda *et al*., 2009). Additionally, flavonoids are one of the nitric oxide synthase inhibitors that derail production of nitric oxide, which is a key agent of inflammatory nociception (Nijveldt *et al*., 2001). Flavonoids prevent activation of N-methyl-D-aspartate (NMDA) receptors and lower the levels of intracellular calcium. These cause a decrease in the activity of nitric oxide synthase enzyme and phospholipase $A_2$with eventual decline in NO and prostaglandin production (Mokhtar *et al*., 2011).

Alkaloids and tannins may also have been responsible for the observed analgesic activities. Previous studies have demonstrated that analgesic activities may be linked to tannins (Picq *et al*., 1991; Zulfiker *et al*., 2010), alkaloids (Hayfaa *et al*., 2013) and steroidal compounds (Ara *et al*., 2010). Terpenoids have also been associated with antinociceptive activity through inhibition of thrombocyte aggregation and interference with signal transduction of pain mechanisms (Mworia *et al*., 2015a).

Saponins have also been associated with analgesic activities by modulation of the $GABA_A$, NMDA and non-NMDA receptors for central nociception (Orlandi *et al*., 2011). Suh *et al*. (1996) linked analgesic activities of saponins to non-opioid

mediated activity through activation of the descending serotonin and α_2-adrenergic pathways.

Therefore, this study postulates that synergistic activities of flavonoids, tannins, terpenoids, saponins and steroids were responsible for the observed analgesic activities. However, the precise mechanism of these active principles is still obscure.

The anti-inflammatory assays of the current study used carrageenan. Carrageenan is often used as a phlogistic agent in *in vivo* assays for anti-edema agents (Kangralkar *et al*., 2010). Carrageenan-induced edema is a classical pharmacological model in the study of NSAIDs or any other agents that act against mediators of acute inflammation (Chang *et al*., 2012; Dinda *et al*., 2013). Carrageenan induced paw edema model is widely used in bioscreening of anti-inflammatory agents (Winter *et al*., 1962). It was therefore, selected for this study.

After 4 hours of the observation period, the methanolic extracts of *P. aethiopica* and *W. ugandensis* demonstrated considerable inhibitory effects on paw edema. The anti-inflammatory activities were indicated by reduction in paw edema. The methanolic extracts of both plants progressively inhibited paw edema at the three dose levels with optimal anti-edema effects being observed in the fourth hour (Tables 4.3 and 4.4).

These results are comparable to previous studies that investigated anti-inflammatory activities of other medicinal plants. An earlier study revealed that *Alocasia indica* exhibited considerable anti-edema activities in animal models (Rahman *et al*., 2011).

Similarly, methanolic and aqueous extracts of *Vitex altissima* demonstrated potent anti-inflammatory activities in mice (Bose, 2013). Other studies revealed that *C. volkensii*, *M. obscura* and *T. brownii* significantly inhibited paw edema in Carrageenan-induced inflammation in rat models (Maina *et al*., 2015a; Wanja *et al*., 2016). Likewise, cassava (*Manihot esculenta* Crantz) leaf extracts significantly inhibited phlogosis in laboratory animals (Okechukwu *et al*., 2013). Another related study by Sowemimo *et al*. (2013) demonstrated anti-edema activities of *B. maderaspatensis* in Carrageenan-induced pedal edema.

The extract dose levels used in the current study are within the dose ranges used in earlier anti-inflammatory studies involving herbal extracts. Wanja *et al*. (2016) used 50, 100 and 150 mg/kg body weight dose level while evaluating anti-inflammatory activity of *T. brownii*. A study evaluating anti-inflammatory activity of stem bark extract of *Stachytarpheta indica* used 50,100 and 200mg/kg body weight dose levels (Akuodor *et al*., 2015). A related study by Maina *et al*. (2015a) used 50, 100 and 150 mg/kg body weight dose levels.

The methanolic extracts of *P. aethiopica* and *W. ugandensis* produced dose dependent paw edema inhibitions (Tables 4.3 and 4.4). The highest paw edema inhibitions by *P. aethiopica* and *W. ugandensis* were observed at the extract dose level of 150 mg/kg body weight (Table 4.3 and 4.4). Higher extract dose levels (100 and 150 mg/kg body weight) progressively suppressed paw edema in a much greater magnitude compared to the 50 mg/kg body weight (Figures 4.3 and 4.4; Tables 4.3 and 4.4). This phenomenon may be ascribed to higher concentrations of active principles. The 50 mg/kg body weight extract dose level elicited the least anti-

inflammatory activity in both plant extracts. This may be due limited concentration of active principle (s), metabolic inactivation or clearance of some active principle(s) hence reduced anti-inflammatory effect (Koech *et al*., 2017).

Similarly, Sayyah *et al*. (2004) observed dose dependent anti-inflammatory activities in a study involving *Lactuca sativa* seed extracts in laboratory animals. In another related study, aqueous and methanolic extracts of *Vitex altissima* exhibited dose dependent anti-edema activities in animal models (Bose, 2013). Additionally, the methanolic extracts of *Jatropha gossypifolia* elicited a dose dependent anti-inflammatory activity in animal models (Panda *et al*., 2009).

The methanolic extracts, of both plants, at the three extract dose levels, achieved optimal anti-inflammatory effects in the fourth hour. The anti-inflammatory effects gradually increased from the first to the fourth hour. This may be attributed to the fact that long duration may have been required for the absorption of the active principles present in the methanolic extracts. The delayed anti-inflammatory effects suggested a gradual but steady passive diffusion of the active principles across the cell membrane into the peritoneal cavity (Wanja *et al*., 2016). Therefore, by the fourth hour, most of the active principles had been completely absorbed into the peritoneal cavity, thereby exerting higher anti-inflammatory effects.

Moreover, the higher anti-inflammatory effects observed in the third and the fourth hour than in the first and the second hour may imply that the active principles in the methanolic extracts underwent biotransformation prior to exerting a more pronounced effect. Mahdi (2014), observed a similar scenario whereby β-D-salicin

(a pro-inflammatory compound) was metabolized into salicylic acid, a more active analgesic and anti-inflammatory compound.

Common interventions for management of inflammation and associated pain involve use of NSAIDs such as diclofenac (Vane and Botting, 1995). The mode of action of NSAIDs is through inhibition of COX enzymes that convert arachidonic acid to prostaglandins (Wanja *et al*., 2016). Truncating the prostaglandin synthesis results in edema reduction (Rang *et al*., 2012). NSAIDs also inhibit the lipooxygenase and L-arginine nitric oxide pathways, thereby reducing the concentration of pro-inflammatory mediators such as nitric oxide.

Qualitative phytochemical screening revealed that the methanolic extracts of *P. aethiopica* and *W. ugandensis* contained terpenoids, flavonoids, phenols and steroids (Table 4.5). Flavonoids and triterpenes possess anti-edema activities (Orlandi *et al*., 2011). Flavonoids can inhibit the cyclooxygenase, lipoxygenase and L-arginine nitric oxide pathways. These pathways have all been associated with inflammatory and nociceptive reactions (Mechelska *et al*., 1997; Robak *et al*., 1998; Kim *et al*., 2004; Meotti *et al*., 2006). Flavonoids derail inflammation by scavenging NO molecules in inflamed cells and tissues. Silibin, a flavonoid, has been shown to inhibit NO concentration in cells (Nijveldt *et al*., 2001). Moreover, flavonoids inhibit leucocytes aggregation inhibiting inflammatory reactions. Additionally, flavonoids and triterpenes inhibit the nuclear factor-kappa B (NF-κB) that is usually active and elevated in inflammatory disorders (Nam, 2006). Huang *et al.* (2011) revealed that plant phenolics are endowed with protective agents against excessive production of NO in chronic inflammatory disorders. Therefore, phenols may have

played a key role in the observed anti-inflammatory activities through inhibition of NO generation. The anti-inflammatory activity of *Phyllanthus simplex* was linked to the inhibition prostaglandins synthesis (Chouhan and Singh, 2011).

Alkaloids with pyridine ring have been reported to have anti-inflammatory effects. Moreover, terpenoids have been shown to inhibit the development of chronic joint swelling (Okechukwu *et al*., 2013). Steroids have also been reported to reduce inflammation. Steroids reduce edema through inhibition of phospholipase A_2 that hydrolyze arachidonic acid from phospholipids in the cell membranes. Therefore, steroids truncate the pathway that leads to formation of prostanoids and leukotrienes which are inflammation mediators (Wanja *et al*., 2016).

The aforementioned phytoconstituents were present in the methanolic extracts of *P. aethiopica* and *W. ugandensis* (Table 4.5). These phyto-components may have acted individually or synergistically to bring about edema reduction. This study hypothesizes that the observed anti-inflammatory activities of the phytoconstituents present in the methanol extracts of *Pistacia aethiopica* and *Warbugia ugandensis* are similar to those of NSAIDs. Therefore, the active principle (s) present in the extracts maybe responsible for alleviating inflammation through inhibition of prostaglandin synthesis. The analgesic and anti-inflammatory potencies of *P. aethiopica* and *W. ugandensis* demonstrated in this study supports, at least in part, the folkloric uses of these plants in the managements of painful and inflammatory related ailments. The null hypotheses are thus rejected.

5.2 Conclusions

This study concludes that:

i) The methanolic extracts of Pistacia *aethiopica* and *Warbugia ugandensis* have analgesic effects in mice.

ii) The methanolic extracts of *Pistacia aethiopica* and *Warbugia ugandensis* have anti-inflammatory effects in mice.

iii) The observed analgesic and anti-inflammatory activities of the methanolic extracts of *Pistacia aethiopica* and *Warbugia ugandensis* are due to phytochemicals present in the extracts such as flavonoids, terpenoids, tannins, phenols and saponins

5.3 Recommendations

The study recommends use of *Pistacia aethiopica* and *Warbugia ugandensis* as analgesic and anti-inflammatory agents once safety has been established.

5.4 Recommendations for further research

i) Elucidation of the mode action of analgesic and anti-inflammatory activities of *Pistacia aethiopica* and *Warbugia ugandensis.*

ii) Evaluation of analgesic and anti-inflammatory activities of *Pistacia aethiopica* and *Warbugia ugandensis* when administered through other routes of administration particularly oral route.

iii) Isolation and identification of active analgesic and anti-inflammatory compounds. These will serve as potential leads in the development of more efficacious novel agents for management of painful and inflammatory conditions.

iv) Research on *in vivo* safety of *Pistacia aethiopica* and *Warbugia ugandensis.*

v) Evaluation of combinational therapy of different extracts of *Pistacia aethiopica* and *Warbugia ugandensis*

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APPENDICES

Appendix I: Descriptive statistics of percentage inhibition of the early and late phases of formalin induced pain by the methanolic extracts of *P. aethiopica*.

Descriptive Statistics: Raw Data: Early phase, Late phase – *P. aethiopica*

Variable	C1	Mean	SE Mean
Early phase raw	100mg/kg bw	60.40	2.20
	150mg/kg bw	56.80	2.29
	50mg/kg bw	66.80	2.08
	Negative control	126.60	3.91
	Normal control	0.000000	0.000000
	Positive control	36.20	1.83
Late phase raw	100mg/kg bw	67.60	1.54
	150mg/kg bw	49.60	1.63
	50mg/kg bw	71.80	1.53
	Negative control	103.60	1.75
	Normal control	0.000000	0.000000
	Positive control	38.40	1.94

Descriptive Statistics: % inhibition Early phase*P. aethiopica*

Variable	C1	Mean	SE Mean	StDev
% inhibition Early phase	100mg/kg bw	52.29	1.74	3.89
	150mg/kg bw	55.13	1.81	4.04
	50mg/kg bw	47.24	1.65	3.68
	Negative control	0.000000	0.000000	0.000000
	Normal control	100.00	0.000000	0.000000
	Positive control	30.33	2.97	6.64

Descriptive Statistics: % inhibition Late phase *P. aethiopica*

Variable	C1	Mean	SE Mean	StDev
% inhibition Late phase	100mg/kg bw	34.75	1.48	3.32
	150mg/kg bw	52.12	1.57	3.52
	50mg/kg bw	30.69	1.48	3.30
	Negative control	0.000000	0.000000	0.000000
	Normal control	100.00	0.000000	0.000000
	Positive control	62.93	1.87	4.19

One-way ANOVA: % inhibition *P. aethiopica* early phase versus C1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	5	26942.8	5388.56	362.60	0.000
Error	24	356.7	14.86		
Total	29	27299.4			

Means

C1	N	Mean	StDev	95% CI
100mg/kg bw	5	52.29	3.89	(48.73, 55.85)
150mg/kg bw	5	55.13	4.04	(51.58, 58.69)
50mg/kg bw	5	47.24	3.68	(43.68, 50.79)
Negative control	5	0.000000	0.000000	(-3.558159, 3.558159)
Normal control	5	100.0	0.0	(96.4, 103.6)
Positive control	5	30.33	6.64	(26.77, 33.89)

Pooled StDev = 3.85498

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

C1                N      Mean  Grouping
Normal control    5     100.0  A
150mg/kg bw       5     55.13    B
100mg/kg bw       5     52.29    B C
50mg/kg bw        5     47.24      C
Positive control  5     30.33        D
Negative control  5  0.000000          E

Means that do not share a letter are significantly different.
```

One-way ANOVA: % inhibition *P. aethiopica* late phase versus C1

```
Analysis of Variance

Source  DF   Adj SS   Adj MS  F-Value  P-Value
C1       5  28568.6  5713.72   661.78    0.000
Error   24    207.2     8.63
Total   29  28775.8

Means

C1                N      Mean     StDev          95% CI
100mg/kg bw       5     34.75      3.32  (    32.04,    37.46)
150mg/kg bw       5     52.12      3.52  (    49.41,    54.84)
50mg/kg bw        5     30.69      3.30  (    27.98,    33.41)
Negative control  5  0.000000  0.000000  (-2.712098, 2.712098)
Normal control    5     100.0       0.0  (     97.3,    102.7)
Positive control  5     62.93      4.19  (    60.22,    65.65)

Pooled StDev = 2.93834
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

C1                N      Mean  Grouping
Normal control    5     100.0  A
Positive control  5     62.93    B
150mg/kg bw       5     52.12      C
100mg/kg bw       5     34.75        D
50mg/kg bw        5     30.69        D
Negative control  5  0.000000          E

Means that do not share a letter are significantly different.
```

One-way ANOVA: Early phase % inhibition versus C1

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.

Factor Information
Factor  Levels  Values
C1             6  100mg/kg bw, 150mg/kg bw, 50mg/kg bw, Negative control,
Normal control,
                Positive control
```

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	5	29049.6	5809.92	399.74	0.000
Error	24	348.8	14.53		
Total	29	29398.4			

Means

C1	N	Mean	StDev	95% CI
100mg/kg bw	5	51.82	5.67	(48.30, 55.33)
150mg/kg bw	5	43.07	3.69	(39.55, 46.59)
50mg/kg bw	5	38.45	4.19	(34.93, 41.97)
Negative control	5	0.000000	0.000000	(-3.518841, 3.518841)
Normal control	5	100.0	0.0	(96.5, 103.5)
Positive control	5	75.25	4.88	(71.73, 78.77)

Pooled StDev = 3.81238

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C1	N	Mean	Grouping
Normal control	5	100.0	A
Positive control	5	75.25	B
100mg/kg bw	5	51.82	C
150mg/kg bw	5	43.07	D
50mg/kg bw	5	38.45	D
Negative control	5	0.000000	E

Means that do not share a letter are significantly different.

One-way ANOVA: Late phase % inhibition versus C1

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
C1	6	100mg/kg bw, 150mg/kg bw, 50mg/kg bw, Negative control, Normal control, Positive control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	5	28838.0	5767.61	146.57	0.000
Error	24	944.4	39.35		
Total	29	29782.4			

Means

C1	N	Mean	StDev	95% CI
100mg/kg bw	5	65.61	6.07	(59.82, 71.40)
150mg/kg bw	5	60.67	4.66	(54.88, 66.46)
50mg/kg bw	5	43.48	10.56	(37.69, 49.27)
Negative control	5	0.000000	0.000000	(-5.789957, 5.789957)
Normal control	5	100.0	0.0	(94.2, 105.8)
Positive control	5	77.08	8.13	(71.29, 82.87)

Pooled StDev = 6.27295

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C1	N	Mean	Grouping
Normal control	5	100.0	A
Positive control	5	77.08	B
100mg/kg bw	5	65.61	B C
150mg/kg bw	5	60.67	C
50mg/kg bw	5	43.48	D
Negative control	5	0.000000	E

Means that do not share a letter are significantly different.

Appendix II: Descriptive statistics of percentage inhibition of the early and late phases of formalin induced pain by the methanolic extracts of *W. ugandensis*.

Descriptive Statistics: RAW DATA EARLY PHASE W. ugandensis

Variable	C1	Mean	SE Mean	StDev
RAW DATA EARLY PHASE	100mg/kg bw	60.40	2.20	4.93
	150mg/kg bw	56.80	2.29	5.12
	50mg/kg bw	66.80	2.08	4.66
	Negative control	126.60	3.91	8.73
	Normal control	0.000000	0.000000	0.000000
	Positive control	88.20	3.76	8.41

Descriptive Statistics: RAW DATA LATE PHASE *W.ugandensis*

Variable	C1	Mean	SE Mean	StDev
RAW DATA LATE PHASE	100mg/kg bw	67.60	1.54	3.44
	150mg/kg bw	49.60	1.63	3.65
	50mg/kg bw	71.80	1.53	3.42
	Negative control	103.60	1.75	3.91
	Normal control	0.000000	0.000000	0.000000
	Positive control	38.40	1.94	4.34

Descriptive Statistics: Early phase % inhibition, Late phase % inhibition *W.ugandensis*

Variable	C1	Mean	SE Mean
Early phase % inhibition	100mg/kg bw	51.82	2.54
	150mg/kg bw	43.07	1.65
	50mg/kg bw	38.45	1.87
	Negative control	0.000000	0.000000
	Normal control	100.00	0.000000
	Positive control	75.25	2.18
Late phase % inhibition	100mg/kg bw	65.61	2.71
	150mg/kg bw	60.67	2.08
	50mg/kg bw	43.48	4.72
	Negative control	0.000000	0.000000
	Normal control	100.00	0.000000
	Positive control	77.08	3.64

One-way ANOVA: Early phase raw data versus C1

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
C1	6	100mg/kg bw, 150mg/kg bw, 50mg/kg bw, Negative control, Normal control, Positive control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	5	42672.3	8534.45	292.94	0.000
Error	24	699.2	29.13		
Total	29	43371.5			

Means

C1	N	Mean	StDev	95% CI
100mg/kg bw	5	58.40	6.88	(53.42, 63.38)
150mg/kg bw	5	69.00	4.47	(64.02, 73.98)
50mg/kg bw	5	74.60	5.08	(69.62, 79.58)
Negative control	5	121.20	6.83	(116.22, 126.18)
Normal control	5	0.000000	0.000000	(-4.981939, 4.981939)
Positive control	5	30.00	5.92	(25.02, 34.98)

Pooled StDev = 5.39753

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C1	N	Mean	Grouping
Negative control	5	121.20	A
50mg/kg bw	5	74.60	B
150mg/kg bw	5	69.00	B
100mg/kg bw	5	58.40	C
Positive control	5	30.00	D
Normal control	5	0.000000	E

Means that do not share a letter are significantly different.

One-way ANOVA: Late phase raw data versus C1

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
C1	6	100mg/kg bw, 150mg/kg bw, 50mg/kg bw, Negative control, Normal control, Positive control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	5	29534	5906.86	124.57	0.000
Error	24	1138	47.42		
Total	29	30672			

Means

C1	N	Mean	StDev	95% CI
100mg/kg bw	5	34.80	6.14	(28.44, 41.16)
150mg/kg bw	5	39.80	4.71	(33.44, 46.16)
50mg/kg bw	5	57.20	10.69	(50.84, 63.56)
Negative control	5	101.20	6.53	(94.84, 107.56)
Normal control	5	0.000000	0.000000	(-6.355780, 6.355780)
Positive control	5	23.20	8.23	(16.84, 29.56)

Pooled StDev = 6.88598

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C1	N	Mean	Grouping
Negative control	5	101.20	A
50mg/kg bw	5	57.20	B
150mg/kg bw	5	39.80	C
100mg/kg bw	5	34.80	C D
Positive control	5	23.20	D
Normal control	5	0.000000	E

Means that do not share a letter are significantly different.

T TEST

Two-Sample T-Test and CI: 50mg/kg bw AP, 50mg/kg bw W. ugandensis

Two-sample T for 50mg/kg bw AP vs 50mg/kg bw AWU

	N	Mean	StDev	SE Mean
50mg/kg bw AP	5	47.24	3.68	1.6
50mg/kg bw AWU	5	38.45	4.19	1.9

Difference = μ (50mg/kg bw AP) - μ (50mg/kg bw AWU)
Estimate for difference: 8.79
95% CI for difference: (2.89, 14.68)
T-Test of difference = 0 (vs ≠): T-Value = 3.52 P-Value = 0.010 DF = 7

Two-Sample T-Test and CI: 100mg/kg bw AP, 100mg/kg bw W. ugandensis

Two-sample T for 100mg/kg bw AP vs 100mg/kg bw AWU

	N	Mean	StDev	SE Mean
100mg/kg bw AP	5	52.29	3.89	1.7
100mg/kg bw AWU	5	48.02	1.84	0.83

Difference = μ (100mg/kg bw AP) - μ (100mg/kg bw AWU)
Estimate for difference: 4.27
95% CI for difference: (-0.68, 9.22)
T-Test of difference = 0 (vs ≠): T-Value = 2.22 P-Value = 0.077 DF = 5

Two-Sample T-Test and CI: 150mg/kg bw AP, 150mg/kg bw W. ugandensis

```
Two-sample T for 150mg/kg bw AP vs 150mg/kg bw AWU

                  N   Mean  StDev  SE Mean
150mg/kg bw AP    5  55.13   4.04      1.8
150mg/kg bw AWU   5  34.82   9.50      4.2


Difference = μ (150mg/kg bw AP) - μ (150mg/kg bw AWU)
Estimate for difference:  20.32
95% CI for difference:  (8.45, 32.18)
T-Test of difference = 0 (vs ≠): T-Value = 4.40  P-Value = 0.007  DF = 5
```

Two-Sample T-Test and CI: 50mg/kg bw LP, 50mg/kg bw late phase W. ugandensis

```
Two-sample T for 50mg/kg bw LP vs 50mg/kg bw LWU

                 N   Mean  StDev  SE Mean
50mg/kg bw LP    5  30.69   3.30      1.5
50mg/kg bw LWU   5  37.55   3.52      1.6

Difference = μ (50mg/kg bw LP) - μ (50mg/kg bw LWU)
Estimate for difference:  -6.85
95% CI for difference:  (-11.96, -1.75)
T-Test of difference = 0 (vs ≠): T-Value = -3.18  P-Value = 0.016  DF = 7
```

Two-Sample T-Test and CI: 100mg/kg bw LP, 100mg/kg bw Late W. ugandensis

```
Two-sample T for 100mg/kg bw LP vs 100mg/kg bw LWU

                  N   Mean  StDev  SE Mean
100mg/kg bw LP    5  34.75   3.32      1.5
100mg/kg bw LWU   5  63.83   2.27      1.0


Difference = μ (100mg/kg bw LP) - μ (100mg/kg bw LWU)
Estimate for difference:  -29.08
95% CI for difference:  (-33.34, -24.83)
T-Test of difference = 0 (vs ≠): T-Value = -16.17  P-Value = 0.000  DF = 7
```

Two-Sample T-Test and CI: 150mg/kg bw LP, 150mg/kg bw Late W. ugandensis

```
Two-sample T for 150mg/kg bw LP vs 150mg/kg bw LWU

                  N   Mean  StDev  SE Mean
150mg/kg bw LP    5  52.12   3.52      1.6
150mg/kg bw LWU   5  62.25   3.45      1.5


Difference = μ (150mg/kg bw LP) - μ (150mg/kg bw LWU)
Estimate for difference:  -10.13
95% CI for difference:  (-15.34, -4.92)
T-Test of difference = 0 (vs ≠): T-Value = -4.59  P-Value = 0.003  DF = 7
```

Appendix III: Descriptive statistics of percentage inhibition of Carrageenan induced paw edema by the methanolic extracts of *P. aethiopica*.

Descriptive Statistics: 0HR, 1HR, 2HR, 3HR, 4HR P. aethiopica

Variable	C1	Mean	SE Mean	StDev
0HR	100 MG/KG EXTRACT	0.000000	0.000000	0.000000
	150 MG/KG EXTRACT	0.000000	0.000000	0.000000
	50 MG/KG EXTRACT	0.000000	0.000000	0.000000
	NEGATIVE CONTROL	0.000000	0.000000	0.000000
	NORMAL CONTROL	0.000000	0.000000	0.000000
	POSITIVE CONTROL	0.000000	0.000000	0.000000
1HR	100 MG/KG EXTRACT	0.910	0.121	0.271
	150 MG/KG EXTRACT	2.066	0.249	0.556
	50 MG/KG EXTRACT	0.897	0.175	0.391
	NEGATIVE CONTROL	-2.115	0.724	1.618
	NORMAL CONTROL	0.000000	0.000000	0.000000
	POSITIVE CONTROL	1.988	0.337	0.755
2HR	100 MG/KG EXTRACT	2.028	0.198	0.442
	150 MG/KG EXTRACT	4.092	0.165	0.370
	50 MG/KG EXTRACT	2.360	0.303	0.678
	NEGATIVE CONTROL	-4.964	0.443	0.990
	NORMAL CONTROL	0.000000	0.000000	0.000000
	POSITIVE CONTROL	4.619	0.307	0.686
3HR	100 MG/KG EXTRACT	3.390	0.427	0.955
	150 MG/KG EXTRACT	5.908	0.182	0.408
	50 MG/KG EXTRACT	3.227	0.426	0.953
	NEGATIVE CONTROL	-6.335	0.614	1.372
	NORMAL CONTROL	0.000000	0.000000	0.000000
	POSITIVE CONTROL	7.112	0.308	0.689
4HR	100 MG/KG EXTRACT	5.566	0.330	0.739
	150 MG/KG EXTRACT	7.596	0.122	0.273
	50 MG/KG EXTRACT	4.603	0.375	0.840
	NEGATIVE CONTROL	-7.513	0.564	1.261
	NORMAL CONTROL	0.000000	0.000000	0.000000
	POSITIVE CONTROL	8.855	0.211	0.471

One-way ANOVA: 0HR versus C1

```
* ERROR * All complete, included rows have the same response.
```

One-way ANOVA: 1HR versus C1

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.
Factor Information

Factor  Levels  Values
C1             6  100 MG/KG EXTRACT, 150 MG/KG EXTRACT, 50 MG/KG EXTRACT,
NEGATIVE CONTROL,
                NORMAL CONTROL, POSITIVE CONTROL
```

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	5	59.95	11.9909	19.33	0.000
Error	24	14.89	0.6204		
Total	29	74.84			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.787674	80.10%	75.96%	68.91%

Means

C1	N	Mean	StDev	95% CI
100 MG/KG EXTRACT	5	0.910	0.271	(0.183, 1.637)
150 MG/KG EXTRACT	5	2.066	0.556	(1.339, 2.793)
50 MG/KG EXTRACT	5	0.897	0.391	(0.170, 1.624)
NEGATIVE CONTROL	5	-2.115	1.618	(-2.842, -1.388)
NORMAL CONTROL	5	0.000000	0.000000	(-0.727026, 0.727026)
POSITIVE CONTROL	5	1.988	0.755	(1.261, 2.716)

Pooled StDev = 0.787674

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C1	N	Mean	Grouping
150 MG/KG EXTRACT	5	2.066	A
POSITIVE CONTROL	5	1.988	A
100 MG/KG EXTRACT	5	0.910	A B
50 MG/KG EXTRACT	5	0.897	A B
NORMAL CONTROL	5	0.000000	B
NEGATIVE CONTROL	5	-2.115	C

Means that do not share a letter are significantly different.

One-way ANOVA: 2HR versus C1

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
C1	6	100 MG/KG EXTRACT, 150 MG/KG EXTRACT, 50 MG/KG EXTRACT, NEGATIVE CONTROL, NORMAL CONTROL, POSITIVE CONTROL

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value

```
C1       5  306.857  61.3713   164.09    0.000
Error   24    8.976   0.3740
Total   29  315.833


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.611559  97.16%     96.57%      95.56%


Means

C1                 N      Mean     StDev          95% CI
100 MG/KG EXTRACT  5     2.028     0.442  (    1.464,    2.593)
150 MG/KG EXTRACT  5     4.092     0.370  (    3.527,    4.656)
50 MG/KG EXTRACT   5     2.360     0.678  (    1.795,    2.924)
NEGATIVE CONTROL   5    -4.964     0.990  (   -5.528,   -4.399)
NORMAL CONTROL     5  0.000000  0.000000  (-0.564471, 0.564471)
POSITIVE CONTROL   5     4.619     0.686  (    4.054,    5.183)

Pooled StDev = 0.611559
```

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

```
C1                 N      Mean  Grouping
POSITIVE CONTROL   5     4.619  A
150 MG/KG EXTRACT  5     4.092  A
50 MG/KG EXTRACT   5     2.360    B
100 MG/KG EXTRACT  5     2.028    B
NORMAL CONTROL     5  0.000000      C
NEGATIVE CONTROL   5    -4.964        D

Means that do not share a letter are significantly different.
```

One-way ANOVA: 3HR versus C1

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
C1             6  100 MG/KG EXTRACT, 150 MG/KG EXTRACT, 50 MG/KG EXTRACT,
NEGATIVE CONTROL,
                NORMAL CONTROL, POSITIVE CONTROL
Analysis of Variance

Source  DF  Adj SS   Adj MS  F-Value  P-Value
C1       5  590.11  118.023   163.07    0.000
Error   24   17.37    0.724
Total   29  607.48
```

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.850734	97.14%	96.54%	95.53%

Means

C1	N	Mean	StDev	95% CI
100 MG/KG EXTRACT	5	3.390	0.955	(2.604, 4.175)
150 MG/KG EXTRACT	5	5.908	0.408	(5.122, 6.693)
50 MG/KG EXTRACT	5	3.227	0.953	(2.442, 4.013)
NEGATIVE CONTROL	5	-6.335	1.372	(-7.120, -5.549)
NORMAL CONTROL	5	0.000000	0.000000	(-0.785230, 0.785230)
POSITIVE CONTROL	5	7.112	0.689	(6.327, 7.897)

Pooled StDev = 0.850734

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C1	N	Mean	Grouping
POSITIVE CONTROL	5	7.112	A
150 MG/KG EXTRACT	5	5.908	A
100 MG/KG EXTRACT	5	3.390	B
50 MG/KG EXTRACT	5	3.227	B
NORMAL CONTROL	5	0.000000	C
NEGATIVE CONTROL	5	-6.335	D

Means that do not share a letter are significantly different.

One-way ANOVA: 4HR versus C1

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
C1	6	100 MG/KG EXTRACT, 150 MG/KG EXTRACT, 50 MG/KG EXTRACT, NEGATIVE CONTROL, NORMAL CONTROL, POSITIVE CONTROL

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	5	919.46	183.892	351.65	0.000
Error	24	12.55	0.523		
Total	29	932.01			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.723149	98.65%	98.37%	97.90%

Means

C1	N	Mean	StDev	95% CI
100 MG/KG EXTRACT	5	5.566	0.739	(4.898, 6.233)
150 MG/KG EXTRACT	5	7.596	0.273	(6.929, 8.264)
50 MG/KG EXTRACT	5	4.603	0.840	(3.936, 5.271)
NEGATIVE CONTROL	5	-7.513	1.261	(-8.181, -6.846)
NORMAL CONTROL	5	0.000000	0.000000	(-0.667469, 0.667469)
POSITIVE CONTROL	5	8.855	0.471	(8.188, 9.523)

Pooled StDev = 0.723149

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C1	N	Mean	Grouping
POSITIVE CONTROL	5	8.855	A
150 MG/KG EXTRACT	5	7.596	A
100 MG/KG EXTRACT	5	5.566	B
50 MG/KG EXTRACT	5	4.603	B
NORMAL CONTROL	5	0.000000	C
NEGATIVE CONTROL	5	-7.513	D

Means that do not share a letter are significantly different.

Appendix IV: Descriptive statistics of percentage inhibition of Carrageenan induced paw edema by the methanolic extracts of *P. aethiopica*.

Descriptive Statistics: Inflammation W. ugandensis: 0hr

Variable	C1	Mean	SE Mean
0hr_1	100mg/kg bw	100.00	0.000000
	150mg/kg bw	100.00	0.000000
	50mg/kg bw	100.00	0.000000
	Negative control	100.00	0.000000
	Normal control	100.00	0.000000
	Positive control	100.00	0.000000

Descriptive Statistics: 1st hr

Variable	C1	Mean	SE Mean
1st hr_1	100mg/kg bw	98.914	0.290
	150mg/kg bw	99.390	0.160
	50mg/kg bw	98.646	0.195
	Negative control	101.74	0.373
	Normal control	100.00	0.000000
	Positive control	99.244	0.143

Descriptive Statistics: 2nd hr

Variable	C1	Mean	SE Mean
2nd hr_1	100mg/kg bw	96.527	0.608
	150mg/kg bw	97.575	0.615
	50mg/kg bw	97.084	0.567
	Negative control	103.01	0.721
	Normal control	100.00	0.000000
	Positive control	95.416	0.0585

Descriptive Statistics: 3rd hr

Variable	C1	Mean	SE Mean
3rd hr_1	100mg/kg bw	94.462	0.494
	150mg/kg bw	95.339	0.327
	50mg/kg bw	95.655	0.813
	Negative control	103.77	0.678
	Normal control	100.00	0.000000
	Positive control	93.047	0.416

Descriptive Statistics: 4th hr

Variable	C1	Mean	SE Mean
4th hr_1	100mg/kg bw	92.594	0.155
	150mg/kg bw	92.409	0.123
	50mg/kg bw	93.921	0.523
	Negative control	105.17	0.392
	Normal control	100.00	0.000000
	Positive control	90.427	0.0438

Descriptive Statistics: 0hr

Variable	C1	Mean	SE Mean
0hr	100mg/kg bw	0.000000	0.000000
	150mg/kg bw	0.000000	0.000000
	50mg/kg bw	0.000000	0.000000
	Negative control	0.000000	0.000000
	Normal control	0.000000	0.000000
	Positive control	0.000000	0.000000

Descriptive Statistics: 1st hr

Variable	C1	Mean	SE Mean
1st hr	100mg/kg bw	1.086	0.290
	150mg/kg bw	0.610	0.160
	50mg/kg bw	1.354	0.195
	Negative control	-1.743	0.373
	Normal control	0.000000	0.000000
	Positive control	0.756	0.143

Descriptive Statistics: 2nd hr

Variable	C1	Mean	SE Mean
2nd hr	100mg/kg bw	3.473	0.608
	150mg/kg bw	2.425	0.615
	50mg/kg bw	2.916	0.567
	Negative control	-3.011	0.721
	Normal control	0.000000	0.000000
	Positive control	4.5841	0.0585

Descriptive Statistics: 3rd hr

Variable	C1	Mean	SE Mean
3rd hr	100mg/kg bw	5.538	0.494
	150mg/kg bw	4.661	0.327
	50mg/kg bw	4.345	0.813
	Negative control	-3.769	0.678
	Normal control	0.000000	0.000000
	Positive control	6.953	0.416

Descriptive Statistics: 4th hr

Variable	C1	Mean	SE Mean
4th hr_1	100mg/kg bw	92.594	0.155
	150mg/kg bw	92.409	0.123
	50mg/kg bw	93.921	0.523
	Negative control	105.17	0.392
	Normal control	100.00	0.000000
	Positive control	90.427	0.0

Descriptive Statistics: 4th hr

Variable	C1	Mean	SE Mean
4th hr_1	100mg/kg bw	92.594	0.155
	150mg/kg bw	92.409	0.123
	50mg/kg bw	93.921	0.523
	Negative control	105.17	0.392
	Normal control	100.00	0.000000
	Positive control	90.427	0.0438

Descriptive Statistics: 4th hr

Variable	C1	Mean	SE Mean
4th hr	100mg/kg bw	7.406	0.155
	150mg/kg bw	7.591	0.123
	50mg/kg bw	6.079	0.523
	Negative control	-5.171	0.392
	Normal control	0.000000	0.000000
	Positive control	9.5734	0.0438

One-way ANOVA: 0hr versus C1

* ERROR * All complete, included rows have the same response.

One-way ANOVA: 1st hr versus C1

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.505987	83.64%	80.24%	74.45%

Means

C1	N	Mean	StDev	95% CI
100mg/kg bw	5	1.086	0.647	(0.619, 1.553)
150mg/kg bw	5	0.610	0.358	(0.143, 1.077)
50mg/kg bw	5	1.354	0.435	(0.887, 1.821)
Negative control	5	-1.743	0.835	(-2.210, -1.276)
Normal control	5	0.000000	0.000000	(-0.467027, 0.467027)
Positive control	5	0.756	0.320	(0.289, 1.223)

Pooled StDev = 0.505987

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C1	N	Mean	Grouping
50mg/kg bw	5	1.354	A
100mg/kg bw	5	1.086	A
Positive control	5	0.756	A B
150mg/kg bw	5	0.610	A B
Normal control	5	0.000000	B
Negative control	5	-1.743	C

Means that do not share a letter are significantly different.

One-way ANOVA: 2nd hr versus C1

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.15214	85.81%	82.86%	77.83%

Means

C1	N	Mean	StDev	95% CI
100mg/kg bw	5	3.473	1.361	(2.410, 4.536)
150mg/kg bw	5	2.425	1.375	(1.362, 3.489)
50mg/kg bw	5	2.916	1.267	(1.852, 3.979)
Negative control	5	-3.011	1.612	(-4.074, -1.947)
Normal control	5	0.000000	0.000000	(-1.063432, 1.063432)
Positive control	5	4.5841	0.1309	(3.5207, 5.6476)

Pooled StDev = 1.15214

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C1	N	Mean	Grouping
Positive control	5	4.5841	A
100mg/kg bw	5	3.473	A
50mg/kg bw	5	2.916	A
150mg/kg bw	5	2.425	A
Normal control	5	0.000000	B
Negative control	5	-3.011	C

Means that do not share a letter are significantly different.

One-way ANOVA: 3rd hr versus C1

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.17108	92.52%	90.96%	88.32%

Means

C1	N	Mean	StDev	95% CI
100mg/kg bw	5	5.538	1.105	(4.457, 6.619)
150mg/kg bw	5	4.661	0.732	(3.580, 5.742)
50mg/kg bw	5	4.345	1.818	(3.264, 5.426)
Negative control	5	-3.769	1.517	(-4.850, -2.688)
Normal control	5	0.000000	0.000000	(-1.080916, 1.080916)
Positive control	5	6.953	0.931	(5.872, 8.034)

Pooled StDev = 1.17108

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C1	N	Mean	Grouping
Positive control	5	6.953	A
100mg/kg bw	5	5.538	A B
150mg/kg bw	5	4.661	B
50mg/kg bw	5	4.345	B
Normal control	5	0.000000	C
Negative control	5	-3.769	D

Means that do not share a letter are significantly different.

One-way ANOVA: 4th hr versus C1

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.624629	98.84%	98.60%	98.19%

Means

C1	N	Mean	StDev	95% CI
100mg/kg bw	5	7.406	0.347	(6.830, 7.983)
150mg/kg bw	5	7.591	0.275	(7.015, 8.168)
50mg/kg bw	5	6.079	1.169	(5.503, 6.656)
Negative control	5	-5.171	0.877	(-5.748, -4.595)
Normal control	5	0.000000	0.000000	(-0.576534, 0.576534)
Positive control	5	9.5734	0.0980	(8.9969, 10.1499)

Pooled StDev = 0.624629

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