ANTINOCICEPTIVE, ANTI-INFLAMMATORY AND ANTIPYRETIC EFFECTS OF *Solanum incanum* (Linnaeus), *Craterostigma pumilum* (Hochst) AND *Euclea divinorum* (Hiern) IN ANIMAL MODELS.

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (MEDICAL BIOCHEMISTRY) IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY

OCTOBER; 2016
DECLARATION

I declare that this is my original work and has not been presented for a degree in Kenyatta or any other university

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DEDICATION

This work is dedicated to my family;

My father Samwel Mwonjoria, my dear wife Mary Njeri, son Sam Mwonjoria,
daughters Brijo Wangeci & Jackie Njoki for their prayers, patience,
encouragement and material support during the course of this study.
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LIST OF ABBREVIATIONS AND ACRONYMS

DCM: Dichloromethane

DMSO: Dimethylsulfoxide

g: Grams

i.p: Intraperitoneal

LPS: Lipopolysaccharide

mg: Milligrams/kg body weight

NSAIDs: Non steroidal antiinflammatory drugs

s.c.: Subcutaneous

TNF: Tumor necrotic factor

IL: Interleukin

Ediv: *Euclea divinorum*
ABSTRACT

*Solanum incanum, Craterostigma pumilum* and *Euclea divinorum* have been used for generations as folklore medicine for various ailments associated with pain and inflammation in humans in Kenya. However, there is scarcity of data on scientific studies done on their effectiveness, modes of action, toxicity and their phytochemical composition. The aims of this study was to evaluate the antinociceptive, anti-inflammatory and antipyretic potential of these plants crude extracts, to determine the antinociceptive and anti-inflammatory mechanisms of action, toxicity as well as the phytochemical composition of alkaloid rich fractions of these plants. Antinociceptive and anti-inflammatory effect assays were carried out using formalin test and formalin induced paw edema in rats. Pyrexia was induced in rats using lipopolysaccharid and rectal temperature taken using a digital thermometer. Alkaloid rich fractions of *S. incanum* and *E. divinorum* were screened using formalin pain and inflammation tests in mice while antipyretic effect of *S. incanum* alkaloids was tested on rats. The antinociceptive mode of action assays involved injection of various receptor agonists and antagonists which included atropine an antagonist for M2 muscarinic receptors, and ketamine an N-methyl D-aspartate receptor blocker. Evaluation of anti-inflammatory mode of actions involved carrageenan leukocyte migration assay and histamine induced pedal edema. Phytochemical assay was carried out using standard procedures while LC-QToF MS was used in identification of the metabolites in alkaloid rich fractions. Extracts from the three plants caused significant (p < 0.05) anti-inflammatory effects while only the *S. incanum* and *E. divinorum* extract exhibited significant (p < 0.05) antinociceptive effect. The alkaloids rich fraction of *S. incanum* exhibited significant antipyretic effect. Antinociception was significantly attenuated by atropine and ketamine in *S. incanum* and *E. divinorum* alkaloids treated animals respectively. Alkaloids from *S. incanum* showed no toxic effect unlike those from *E. divinorum*. The extracts contained several types of metabolites of varying quantities. QToF-MS results for *S. incanum* showed presence of tri- and tetra glycosides identified as solamargine and its derivatives *E. divinorum* contained an unidentified xylose containing glycosidic alkaloids. Hence *S. incanum* and *E. divinorum* contains alkaloids and perhaps other metabolites with analgesic and anti-inflammatory effects. The analgesic and anti-inflammatory of *S. incanum* alkaloids involved inhibition of M2 receptors and leukocyte migration respectively. *E. divinorum* alkaloids inhibited pain via NMDA receptors and inflammation via either or both H1 and H4 receptors. The finding lends support to traditional use of these plant parts for relief of pain pain and inflammation. Further investigation may lead to development of novel drugs for management of these conditions.
CHAPTER ONE: INTRODUCTION

1.1 Background

Plant extracts have been used as remedy for various ailments since time immemorial. Paleontological findings show that even *Homo sapiens neanderthalensis*, the now extinct *Homo sapiens*, may have had the knowledge and used herbal remedies over 60,000 years ago (Lietava, 1992). The earliest archaeological records on use of herbs remedy is from Mesopotamia and ancient Egypt (Balick *et al.*, 1995). The Assyrians and the Egyptians were aware of the analgesic effects of a decoction of myrtle or willow leaves for joint pain while Hippocrates, the father of modern medicine, recommended chewing willow leaves for analgesia in childbirth (Levesque & Lafont, 2000). The plant parts and extracts are made in concoctions, infusions, crush and use, chewed, sniffed or even rubbed on aching parts of the body. The use of plants parts for medicinal purposes range from simple antidotes to management of serious life threatening ailments like cancer.

Many plants are used for treatment of myriad conditions that include microbial infections, *Solanum incanum* and *Euclea divinorum* (Kokwaro, 1993) parasitic infestations, Cinchona bark (Achan *et al.*, 2011), fungal infections, *S. incanum* and *Cassia fistularis* (Bhalodi & Shukla, 2011), tumours, *S. incanum* (Kokwaro, 1993), mental diseases, St John's wort, *Rhodiola rosea*, *Crocus*
*sativus* and *Passiflora incarnata* (Sarris, 2007), alleviation of pain, fever and inflammation *Solanum nigrum* (Saleem *et al*., 2009) among many other reports.

The World Health Organization (WHO) estimates show that about 80% of the people living in Africa rely on folklore herbal medicine for their primary health care (Ekor, 2014). This is primarily due to scarcity or exorbitant prices of modern medicines and the belief that herbal remedies exhibit less toxic effects than their synthetic counterparts.

Inflammation, fever and pain are associated with various pathological processes in the body. There are two major types of pain nociceptive that occur following tissue injury and activation of specific nociceptors and neuropathic pain arising from structural damage as well as nerve cell dysfunction (WHO 2012). Pain is a major health problem especially in the U.S.A (Berdine, 2002), while chronic inflammation is associated with cancer development (Rakoff-Nahoum, 2006). Fever or regulated rise in body temperature (Atkin, 1982; Blatteis, 1986) is a cardinal signs of disease which enhances the inflammatory response, function of the immune system and limits microbial and tumor cells proliferation. However it comes with with substantial cost to the host such as metabolic, elevated level of oxygen and fuel demands, as well as actual or potential lesions of neurologic tissues (Dinarello *et al*., 1988).
In spite of all these, many patients may go untreated due to high cost of drugs among other issues in poor developing world. In East Africa, there are several folklore remedies for the three conditions which include herbs such as *Euclea divinorum*, *Solanum incanum* and *Craterostigma spp.* However, there is scarcity of published reports of scrutiny to validate their their effectiveness, modes of action, toxicity and their phytochemical composition. The aim of this study was to investigate whether extracts of *S. incanum*, *C. pumilum* and *E. divinorum* have antinociceptive, antipyretic and anti-inflammatory effects and to establish their respective modes of action and toxicity as well as determine the types of the secondary metabolites responsible for these activities.

**1.2 Statement of the problem**

Pain, Fever & inflammation are common signs and symptoms present in majority of the ailments affecting humanity. They are cause disability, poor quality of life, depressive illness and death and massive economic loss of income due loss of man hours and drug procurement as an aftermath. Management of pain, inflammatory conditions and fever an expensive affair that involve administration of several types of drugs which exhibit many undesirable side effects for instance aspirin causes gastric ulcers (Lanza, 1989; Chan *et al.*, 2005; Laine, 2006)) and paracetamol induces hepatotoxicity (James *et al.*, 2003; Mahadevan *et al.*, 2006). Use of herbal remedies, are cheap easily available and plays an important role in
primary health care in developing countries (Ekor, 2014). However, many of the herbs have not been subjected to scientific scrutiny in order to evaluate their efficacy, mechanism of action, composition and toxicity.

1.3 Justification

Pain, inflammation & fever are managed using conventional or synthetic drugs which are associated with various adverse side effects. However herbal remedy such as S. incanum, Craterostigma spp., & E. divinorum have been used with a certain degree efficacy. They are cheap, available and are believed to posses fewer side effects though there is scarcity of scientific data concerning their use, efficacy, mode of action, toxicity and phytochemical composition.

1.4 Research questions

i. Do the extracts of E. divinorum, C. pumilum, S. incanum have antinociceptive, anti-inflammatory and antipyretic effects in rats?

ii. Do the alkaloid rich fractions of E. divinorum and S. incanum have antinociceptive, anti-inflammatory and antipyretic effects in rats?

iii. What type of secondary metabolites are there in E. divinorum, C. pumilum and S. incanum and what are their quantities in the stem and root bark of E. divinorum and root of S. incanum?

iv. What are the major types of alkaloids found in the stem and root extracts of E. divinorum and root of S. incanum?
v. What are the antinociceptive and anti-inflammatory mechanisms of action of the alkaloid rich fractions of *S. incanum* and *E. divinorum*?

vi. Are the alkaloid rich fractions of *S. incanum* and *E. divinorum* toxic?

**1.5 Objective**

To evaluate the antinociceptive, anti-inflammatory and antipyretic effects of DCM and aqueous extract of *Solanum incanum, Craterostigma pumilum* and *Euclea divinorum* extracts in animal models

**1.5.1 Specific objectives**

i. To determine the antinociceptive, anti-inflammatory and antipyretic effects of dichloromethane and aqueous extracts of *S. incanum, C. pumilum* and *E. divinorum* in rats.

ii. To determine the effects of DCM and aqueous extracts of *E. divinorum* and *S. incanum* as well as alkaloid rich fraction on leukocytes migration

iii. To determine the effects of *E. divinorum* and *S. incanum* alkaloids on nociception, fever & inflammation

iv. To determine the antinociceptive and anti-inflammatory mechanisms of action of alkaloid rich fractions of *E. divinorum* and *S. incanum* in mice.

v. To determine the toxic effects of *E. divinorum* and *S. incanum* alkaloids in mice

vi. To determine the major type of alkaloids in *S. incanum* and *E. divinorum*
CHAPTER TWO: LITERATURE REVIEW

2.1 Inflammation

Inflammation refers to a generalized non-specific beneficial response of tissue to injury. Inflammation is the basic mechanism available for repair of tissue after an injury and consists of a cascade of cellular and micro-vascular reactions that serve to remove damaged tissue and generate new ones (Schmid-Schönbein, 2006). It also serves as a defense response of the body to invasion by infectious agent and antigen, or cell damage hence it is a fundamental biological process and the most frequent sign of disease (Kulinsky, 2007). The process is associated with increased capillary permeability, migration of several cell types, cell apoptosis, and growth of new tissue and blood vessels (Schmid-Schönbein, 2006).

Infections by microbes as well as presence of cytokines secreted by immune cells such as the macrophages, cause endothelial cells lining post capillary venules at the site of injury to rapidly up-regulate expression of surface proteins known as selectins (Thiemann & Baum, 2011) which bind to mucin like cellular adhesion molecules on leukocyte membrane making leukocytes to repetitively bind and detach and hence roll along the endothelial surface (Firrell, & Lipowsky, 1989).

Most of inflammatory responses are associated with system repair processes and involve increased capillary permeability, migration of several cell types, cell
apoptosis, and growth of new tissue and blood vessels (Schmid-Schönbein, 2006). Causes of inflammatory processes includes conditions such as hypoxia (Holger & Carmeliet, 2011), injury, hypersensitivity states, diet and infection among others (Basu et al., 2006).

Although controlled inflammatory responses are beneficial especially in the defence against infections they may be detrimental if poorly regulated as it happens in septic shock. The inflammatory process is involved in pathogenesis of various diseases and has both an acute and chronic phases. The acute phase is characterized by fever, pain and oedema (Barret et al., 2010) while in chronic phase there is cellular proliferation, action of complement fibrinolytic system and hyaluronidase (Armando & Massimo, 1994).

Several substances that serve as inflammatory mediators in the body can be classified according to their biochemical properties into seven groups namely the cytokines, lipid derivatives, vasoactive amines and peptides, chemokines, proteases, as well as the complement system (Medzhitov, 2008). Examples of these cell derived substances known to mediate inflammatory processes include histamine, serotonin, bradykinnin (Proud & Kaplan, 1988) nitric oxide (NO) (Giuseppe et al., 2006), Interleukin-1, 2,4,5,6, leukotriene B4, granulocyte monocytes colony stimulating factor (GMCSF), and tumour necrotic factor alpha
(TNF-α) (Martha, 1999), interferon γ and prostaglandins (Vane, 1976).

There are numerous etiological factors of inflammation that include products of bacterial degradation such as: - peptidoglycans, lipopeptides, formylmethionyl peptides, lipopolysaccharides, flagellin, microbial DNA, fungi (zymosans), viruses as well as products of damaged cells (Kulinsky, 2007) and diet (Basu et al., 2006). Conditions such as hypoxia where the oxygen-sensing prolyl hydroxylases that hydroxylate proline (O-SPH) in the alpha subunit of the hypoxia-inducible transcription factor (H-IT) play an important role as both (O-SPH) and (H-IT) affect inflammatory processes (Holger & Carmeliet, 2011).

Controlled inflammatory responses are beneficial especially in the host defence against microbial infections but detrimental if poorly regulate, e.g. in septic shock. Inflammatory process is involved in pathogenesis of various diseases and has two phases, an acute and chronic phase. The acute phase is characterized by fever, pain and oedema while in chronic phase there is cellular proliferation, action of complement fibrinolytic system and hyaluronidase. There are several substances that serve as inflammatory mediators in the body which can be classified according to their biochemical properties into seven groups namely; - cytokines, lipid mediator derivatives, vasoactive peptides and amines, chemokines, proteases, as well as fragments of complement system (Medzhitov, 2008).
Some of these cell derived substances known to mediate inflammatory process includes histamine, serotonin, bradykinin (Proud & Kaplan, 1988), nitric oxide (NO) (Giuseppe et al., 2006), Interleukin-1, 2,4,5,6, leukotriene B4, granulocyte monocytes colony stimulating factor (GMCSF), and tumour necrotic factor alpha (TNF-α) (Martha, 1999), interferon γ and prostaglandins (Vane, 1976).

Some inflammatory responses are associated with depressive illness (Maes et al., 2012) and the risk of development of depression increases with rise in the level of acute inflammations. It was also observed that administration of both endogenous and exogenous cytokines induces typical depressive like behaviour in healthy humans (Berk et al., 2013).

The phlogistic effect of formalin and carrageenan is a well established fact. Injections of either of the two substances induce similar local inflammatory reactions, though formalin induces more persistent reaction (Goulart et al., 2005). Administration of low doses of formalin induces edema mainly due to neurogenic inflammation mediated by peptides especially substance P. Nevertheless, at higher doses, the edema effect is mainly due to release of substance P, serotonin, prostanoids, and histamine. These substances increase capillary permeability resulting in exudation of fluid in the interstitial space thereby causing edema. Surprisingly bradykinin plays insignificant role in this process in spite of it supposedly stimulatory activity on the nociceptive afferent neurons. It is believed
that this phenomenon arises from the variation in the stimulation threshold of the nociceptive neurons and endothelium or perhaps due to formation of kinins in proximity to the neurons (Damas & Légois, 1999).

2.2 Pain

Pain is a noxious stimuli that occurs when tissues are being damaged (Woolf, 2004) while the international association of pain societies defines it as unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Sussman, 2008). Nociception or pain perception is associated with both patho-physiological as well as psychological components that complicate its interpretation (Marazziti et al., 2006). Although acute pain typically resolves on its own with little need for intervention, for some people pain persists past the point where it is considered an adaptive reaction to injury.

Pain that persists for longer than 3 months, that accompanies a disease process, or that is associated with a bodily injury that has not resolved over time may be referred to as chronic pain. It has been observed that loss of nociception for example in hereditary disorders such as congenital insensitivity to pain is associated with repeated injury and inadvertent self mutilation (Costigan et al., 2009). Therefore though nociceptive pain is undoubtedly a very unpleasant phenomenon it has a highly protective function.
2.2.1 Transduction of pain or noxious stimuli

In muscles, visceral, and cutaneous tissues, pain stimuli is transduced by pain receptors (nociceptors) which are distributed all over the body. They are usually free nerve endings of afferent nerves. Painful sensations results from activation of these nociceptors by their adequate stimuli which includes thermal, chemical and mechanical stimuli (Millan, 1999). Nociceptors are usually classified according to their adequate stimuli into thermonociceptor (heat sensitive), mechanonociceptors responds to tissue stretch or deformation and chemonociceptors responds to noxious chemicals (Guyton & Hall, 2006).

Heat sensitive nociceptors respond to hot and cold stimuli by increasing Na+, K+ or Ca$^{2+}$ ion channels flux causing membrane depolarization and transduction (Mckemy et al., 2002; Woof, 2004; Giordano, 2005). Chemical sensitive nociceptors such as the acid/proton-sensing receptors (PSR) respond to binding of certain substances that are usually released during tissue damage and or inflammation. These substances open the ligand gated cat-ion channels resulting in influx of cat-ions and hence depolarization of primary sensory nerves (Huang et al., 2007). All these changes results in sensitization of peripheral afferents. High threshold stimuli may release arachidonic acid from the membranes which is acted upon by cyclo-oxygenase-2 (COX-2) to form prostaglandin E-2 (PGE2).
2.2.2 Transmission of pain sensation

From the peripheral pain receptors (nociceptors), the sensation is transmitted to spinal cord through type A-delta and the small type C primary efferent nerve fibers. The type A-delta nerve fibers are small (1-5um) in diameter myelinated nerve fibers that conduct fast pain and have small receptive fields. There are two types of A-delta nerve fibers namely type I and type II. Type II are thermoresponsive A-delta fibers which respond to noxious heat of 40 – 45°C and are capsaicin sensitive as well as A-delta cold afferents that have their optimal responsiveness at about 8°C Celsius.

Type I A-delta nerve fibers are not capsaicin sensitive but respond to noxious heat ranging from 52 – 56°C. In contrast the type C-fibers are small unmyelinated polymodal nerve fibers that conduct slow pain and have large receptive field. They are stimulated by products of cell disruption, inflammation cascade and immunology mediators and have a large receptive area (Giordano, 2005).

Modulation of pain takes place at different levels in the central nervous system and the spinal cord. Other sites where pain modulation takes place are dorsal column by activation of A- delta mechano-stimulation brain stem and mid-brain by the sensory cortex (Giordano, 2005).
2.2.3 Nociceptive systems and neural transmitters

Several neural transmitters such as acetylcholine, opioids, serotonin, gamma aminobutyric acid (GABA), glutamate, neurokinins (substance P) etc. subserve the pain states. Acetylcholine exerts antinociceptive through central nicotinic and peripheral muscarinic cholinergic receptors (Decker et al., 2004). There are several endogenous opioids with antinociceptive effects in the body which can be classified into three groups namely endorphins, enkaphalins and dynorphins. These substances exert their analgesic effect through their respective G-protein linked opioids receptors classified as μ, δ, κ opioid receptors (Al-Hassani, 2011).

Serotonin has a ‘Janus faced’ effect on transmission nociceptive signals. The amine exerts pronociceptive effect in the periphery via 5-HT7 receptors (Rocha-González et al., 2005) and 5-HT2 receptors (Rahman et al., 2011) as well as antinociceptive action in the central nervous system via 5-HT3 receptors (Bardin et al., 2000). GABA usually exhibits inhibitory effects in the central nervous system via GABA-A and GABA-B receptor subtypes. The GABA-A subtypes are ionotropic receptors located in lamina II (substantia gelatinosa) of the dorsal horn of the spinal cord where they play an important role in nociception (Woolf & Salter, 2000).
2.2.4 Antinociceptive tests

There are several tests that have been developed in order to access behavioral nociception in experimental animals. Examples of these tests include the writhing or abdominal constriction test, formalin test, hot plate, tail flick and paw pressure tests.

2.2.4.1 Writhing test

Writhing test is mainly used in the study of visceral pain and it refers to induction of abdominal constriction by injection of irritant substances in the peritoneum of an animal (Collier et al., 1968; Gawade, 2012). The animals respond by arching their back, extending the hind limbs and contracting their abdominal muscles (Gawade, 2012). In mice and rats the response consists of a wave of constriction and elongation that passes caudally along the abdominal wall and twisting of the trunk followed by extension of the hind limbs (Collier et al., 1968). The nociceptive signals generated are transmitted to central nervous system causing release of mediators such as prostaglandins which contributes to the lowering of nociceptors threshold.

Substances exhibiting analgesic activity are expected to reduce the frequency of writhes. It is commonly used as a screening test due to its simplicity. Though it is a sensitive method for investigating the antinociceptive effects of drugs, it cannot be reproduced in clinical setting in humans hence sensations involved are
unknown (Gawade, 2012). The number of writhes in 20 second are counted and quantified as latency of nociception (Le Bars, 2001).

There are several substances that are known to induce abdominal constriction when injected in the peritoneal cavity, which include acetylcholine, bradykinin, hypertonic saline, phenylbenzoquinone. However, these substances with exception of acetic acid and phenylbenzoquinone induce constriction of short duration thereby making the latter two to be more preferred for nociceptive assays (Collier & Schneider, 1969).

2.2.4.2 Formalin test

This test is used to study the response of the animal to a continuous moderate pain stimuli arising from an injured tissue. It involves injection of low concentrations of formalin into the dorsal surface of the paw of the experimental animal. The behaviors observed after formalin administration include licking, biting and lifting or shaking (flinching) the injected paw. The total time spent in these behavioral responses is noted and quantified as the latency of nociception. During the course of the experiment, two phases of nociception are observed with the test, namely the early and late phase representing acute and chronic pain states respectively.
The early phase is observed between 0-5 minutes and is thought to be due to direct stimulation of nociceptors by the chemical (neurogenic) while the late phase start 15-30 minutes following formalin injection and it is due to inflammatory process as well as central sensitization (Hunskaar & Hole, 1987; Rosland et al., 1990; McNamara et al., 2007). This test is easy and cheap to perform. It is also very sensitive to mild analgesic drugs such as acetominorphen and acetyl salicylate, (Hunskaar et al., 1985; Hunskaar & Hole, 1987; Rosland et al., 1990), clinical pain, and disadvantages prolonged subjection of animal to pain, tissue damage or mutilation.

2.2.4.3 Tail Flick Test

There are two variants of tail flick tests namely the heat emersion and radiant heat test methods. In radiant heat test method, thermal radiation is directed onto the tail of the animal it provokes a brief but vigorous withdrawal of the tail. During the test, the reaction time of this movement is recorded and is often referred to as tail flick latency. Any substance or activity that lengthens the reaction time is said to exhibit analgesic activity. The mechanism involved in the flicking of the tail is integrated in the spinal cord hence it is a spinal reflex. Some of the advantages of this method include its simplicity and small inter-animal variations in reaction time measurements under a given set of controlled conditions (Le Bars, 2001). While the main disadvantage is that it requires expensive apparatus.


### 2.2.4.4 Hot Plate Method

In this test a rat or mouse is placed on a hotplate heated by electricity or hot boiling water. Animals placed on a plate heated at a constant temperature show behavioral changes such as; licking of paws and jumping that can be quantified in terms of their reaction times. These responses are supra-spinally integrated. The paw licking behavior is only prolonged by opioids while jumping reaction time is increased by some non-steroidal anti-inflammatory drugs such as aspirin as well as paracetamol. The latter effect is more pronounced when temperature is about 50° Celcius or less (Le Bars, 2001).

### 2.3 Fever

Fever or regulated rise in body temperature has been associated with infectious diseases since time immemorial (Atkin, 1982; Blatteis, 1986). It is one of the cardinal signs of disease and it is believed that it enhances the host ability to survive infection especially in lower vertebrates. Hyperthermia is known to cause enhancement of both the inflammatory response as well as function of the immune system besides reducing microbial and tumor cells proliferation. (Dinarello et al., 1988) The febrile condition is associated with substantial cost to the host such as metabolic, elevated level of oxygen and fuel demands, as well as actual or potential lesions of neurologic tissues (Dinarello et al., 1988).
The rise in body temperature observed during fever involves adjustment in the set-point of hypothalamic thermostat (Lipton & Fossler, 1974). The adjustment of the set point may be caused by microbial infections, immunological reactions, injury, inflammations and neoplasm (Kluger, 1999). The pyrogenic molecules increase production of hypothalamic arachidonate metabolites. Thermostat resetting and intact peripheral temperature production and regulating mechanisms are important factors in febrogenesis.

Drugs that inhibit vasoconstriction or muscle contraction can act as an antipyretic. Conversely low environmental heat load can suppress expression of fever. Intact CNS activity is of paramount importance in genesis of fever for example psychotropic drugs that interfere with either transmission in the neurons or across the synapses have profound effects on thermoregulation and can either suppress or exacerbate fever. Very severe febrile illness may be associated with hyperpyrexia or abnormally high fever of above 41.5°C Celsius. Such phenomenon may occur in certain conditions such as some infections, CNS bleeding, and intake of certain drugs (Dinarell et al., 1988) and by several other factors that include fever, environmental heat load, ultrasound, electromagnetic radiation as well as some drugs (Marshall, 2006). The condition may cause heat stroke and heat exhaustion (Guyton & Hall, 2006). It has also been suggested that it can also act as a teratogen (Marshall, 2006).
The release of pro-inflammatory cytokines by white blood cells such as Interleukin-1 beta, Interleukin-6 following stimulation by the exogenous pyrogen acts as the first step in the genesis of fever (Scapini et al., 2000). Harmful stimuli cause the tissues to liberate arachidonic acid from the cell membranes, which is converted to prostaglandin by the leukocytes. Synthesis of prostaglandin E-2 in the pre-optic area of the anterior hypothalamus that is evoked by both exogenous and endogenous pyrogen is the final step in the development of fever. Macrophage inflammatory protein-2 (MIP-2), a powerful chemotaxic cytokine for neutrophils is an important mediator in lipopolysaccharide initiated febrogenesis via the prostaglandin dependent pathway (Tavares, 2004). Prostaglandin E-2 acts through four Prostaglandin E series of receptors namely EP1, EP2, EP3 and EP4. The EP3 receptor subtype appears to play the most important role in fever and hyperalgesic effects of prostaglandin E2 (Caterina et al., 1999).

2.4 Conventional management of fever, pain and inflammation

Several drugs are used in inflammation, fever and pain management. They include non-steroidal anti-inflammatory drugs such as acetyl salicylic acid which inhibit cyclo-oxygenases and hence reduce the levels of prostaglandins (Vane, 1976; Giovanni & Luca, 2015). Prostaglandins sensitize the nociceptors to noxious stimuli (Woolf & Salter, 2000), acts as an inflammatory mediator and as
the final step in genesis of fever (Tavares, 2004). Drugs that inhibit leukocyte migration and activity such as corticosteroids induce significant reduction in the inflammatory process (Higgs et al., 1980).

Opioids analgesics have been used in clinical settings for a long time (Feng et al., 2012). They exert their pharmacological activity via µ, δ and κ opioid receptors. These receptors types belong to the seven transmembrane spanning (serpentine) receptors a G-protein coupled receptor super family (Waldhoer et al., 2004). They are stimulated by endogenous opioid peptides such as enkephalins, dynorphin and endorphin which are associated with δ, κ and µ receptors respectively as well as by exogenous opiate drugs like morphine, pethidine, codeine, heroin etc. these group of drugs are among the most potent analgesics known so far but have a weakness of causing tolerance, dependence and addiction and hence are liable to abuse. They cause analgesia in the central nervous system by reducing neuronal excitability (Diaz et al., 2000). These receptors are further classified into several subtypes depending on pharmacological activity. They are best known for their pain modulation action and addiction. However these receptors are ubiquitously involved in various physiological and pathophysiological activities, which include regulation of membrane ionic channels activity, proliferation cell, emotional response, epileptic seizures, immunity, appetite, obesity, cardiovascular, respiratory control as well as some neurodegenerative disorders (Feng et al., 2012).
2.5 Complimentary/alternative treatment for pain, inflammation and fever

There are several complimentary methods used in alleviation of suffering in complimentary /alternative medicine which include:- botanicals, nutritional supplements, health food, meditation, magnetic therapy, acupuncture, massage, reflexology, chiropractic and osteopathic manipulations, yoga, biofeedback, Tai Chi, homoeopathy, Alexander therapy, ayurveda, aromatherapy, stress induced analgesia, use of placebo (Smith et al., 2006).

2.5.1 Acupuncture and acupressure

This is the practice of inserting thin needles or application of pressure into specific body points to improve health and well-being. It originated in Far East more than 2,000 years ago it may have a role with reducing pain, besides alleviating other medical conditions and hence reducing the use of the pharmacological intervention (Smith et al., 2011).

2.5.2 Placebo

Some placebo responses, such as analgesia, are initiated and maintained by expectations of symptom change and changes in motivation/emotions. Such psychological factors modulate the earliest stages of the pain processing in the central nervous system (Eippert et al., 2009).
2.5.3 Stress Induced Analgesia

During stress induced analgesia the pain threshold is progressively increased with the repetition of the stress and hence attenuates perception of pain. Acute stress suppresses pain by activating brain pathways that engage opioid and non-opioid mechanisms such as cannabinoids (Butler, 2009), opioids (Feng et al., 2012).

2.5.4 Use of plants extracts extract in analgesia, fever and inflammation

Several plants extracts such as willows bark, poppy, cannabis, and Solanoceae are used in pain, inflammation or fever management. The alkaloid and flavonoids are the major secondary metabolites that are mostly reported to exhibit the analgesic and anti-inflammatory effects in plants. Examples of herbs used in East Africa for the above named conditions include E. divinorum, Craterostigma spp. and S. incanum among others.

2.5.4.1 Euclera divinorum (Elkinye; Maasai, Mukinyai; Kikuyu)

Euclera divinorum is a small tree that belongs to Ebenaceae family (Kokwaro, 1993) that grows up to about 6 m in height (Plate 2.1).
Plate 2. 1: *Euclea divinorum*

It is used as a purgative, folklore remedy for head ache, tooth ache, constipation, chest pain, pneumonia, abscess, anti-helmiths, snake bite, dental hygiene (Kokwaro, 1993), cancers, arthritis, jaundice, wounds, ulcers, miscarriage, leprosy and gonorrhea. Other uses include as a source of fast dyes that can be used with or without the addition of metallic salts as mordant, due to the mordanting effect of the tannins present in the plant (Njuguna, 2005).

Ethanol extract the root bark elevated the frequency of isolated rabbit uteri and augmented the effect of oxytocin on the same (Kaluwa *et al.*, 2012). The crude acetone root bark extract of E. undulate exhibit anti-diabetic activity in type 2 induced diabetic rats (Deutschländer *et al.*, 2012) while the methanol extracts of the root and stem exhibited antimicrobial effect on multi-drug resistant (MDR) *Streptococcus mutans* (Mbanga *et al.*, 2013).
Organic extracts of the root back exhibited significant antimicrobial effects to Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Lactobacillus acidophilus (Ngari et al., 2013).

Though qualitative and quantitative phytochemical screening has shown that the herb contains appreciable amount of alkaloids, so far there is no reported case on their molecular characterization. Phytochemical studies on Euclea divinorum resulted in the isolation of lupeol, lupene, betulin, 7-methyljuglone, isodiospyrin, shinalone, catechin and 3β-(5-hydroxyferuloyl) lup-20(30)-ene (Mebe et al., 1998) and naphthalene derivative, eucleanal (A & B) eucanal (1 & 2) (Ng’ang’a et al., 2012).

2.5.4.2 Craterostigma pumilum (Hochst)

Craterostigma pumilum (Hochst) or Nairobi violet is a small perennial herb that belongs to figwort family Scrophulariaceae (Plate 2.2).
Plate 2.2: *Craterostigma pumilum*

The leaves are arranged in a basal rosette, and are obovate, 2-4 cm × 1-2 cm. They are hairless and smooth above (rarely with a few hairs), densely pubescent underneath and the margins are closely ciliate. While the inflorescence arising from the centre of the rosette on a ± sessile peduncle with relatively long (2-3 cm) pedicels bearing individual flowers. They have purple, blue or pink corolla about 15 mm long (Hepper, 1990).

The herb is a member of a group referred to as resurrection plants that can tolerate loss of water up to 98% of its content and yet return to active metabolism and growth within hours after rehydration. It is found in seasonally water filled rock pools, inselbergs, and heavy metal containing soils (Bartels, 2001). The roots chewed by humans and tastes like a sweet carrot. It is used in treatment of tooth ache where it is chewed and retained in the mouth for some time (Kokwaro, 1993) and joint and muscle pains (Bussman *et al.*, 2006). *Craterostigma* *spp.* contains raffinose series oligosaccharides, 2-octulose, sucrose etc (Norwood *et al.*, 2000).

2.5.4.3 *Solanum incanum*

*Solanum incanum* in Solanoaceae family, (Sodom apple; English and *Entulele*; Maasai) is an erect or spreading perennial shrub with stellate hairs on the stems
and leaves and has pale yellow to brown prickles that are about 1 cm long (Plate 3). It has simple alternate leaves and bisexual flowers. Its fruit is are globular or occasionally ovoid-ellipsoid berries 2.5–3.5 cm × 2–3 cm which are yellowish, orange or brown in color when ripe (Matu, 2008).

**Plate 2. 3: Solanum incanum**

The herb has been used as folklore remedy for various ailments that include sore throat, angina, stomach ache, and tooth ache (Kokwaro, 1993; Matu, 2008), and ear inflammation and snake bite (Matu, 2008).

It also contains vitamin C, B₂ and E which have anti-oxidant (Auta & Ali, 2011). In addition the herb contains steroidal glycoalkaloids (Fukuhara & Kubo, 1991) with the highest concentration of alkaloids occurring in the smallest leaves while
the roots and stem were shown to have similar concentration (Eltayeb et al., 1997).

*Solanum incanum* is known to have toxic compounds among them dimethylnitrosamine, which is associated with high incidence of esophageal cancer (Matu, 2008). The unripe fruits of *S. incanum* were also found to exhibit toxic effects in goats (Thaiyah et al., 2010). Methanolic extracts of the seeds significantly increased erythrocytes, hemoglobin level, total white blood cells count, platelets (Muriithi et al., 2015), while the fruit sap exhibited anti-cholinesterase activity (Umar et al., 2015a) and insecticidal effects on green peach aphids (Umar et al., 2015b). However, oral administration of high doses of root extract to mice did not show signs of conventional toxicity (Assefa et al., 2006).

Most of the studies done using the herb extract or the alkaloid solamargine which is abundant in the herb have shown that the herb possesses significant anti-cancer or tumor effects (Mwonjoria et al., 2013).

Though the three herbs above continue to be used in folklore management of pain, inflammation and fever, minimal scientific studies have been done on these effects. This study attempted to provide scientific data on the antinociceptive, anti-inflammatory and antipyretic effects of the three herbs and also endeavored to elucidate their corresponding modes of action.
CHAPTER THREE: ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECT OF CRUDE EXTRACTS OF Solanum incanum, Craterostigma pumilum AND Eucla divinorum

3.1 Introduction

Pain and inflammatory conditions are two interrelated phenomenon experienced during the disease processes and they both run an acute and chronic course. The acute pain consists of fast pain conducted via type A delta nerve fibers that terminate in the brainstem and slow, dull and agonizing pain conducted via small unmyelinated type C nerve fibers that terminate in lamina II (lamina gelatinosa) of the spinal cord. Chronic pain consists of hyperalgesic and allodynia states which may be associated with nerve cell plasticity (Sandkühler, 2009; Bahari, 2015).

Development of hyperalgesic states usually starts with tissue injury causing inflammatory reaction which is accompanied by release of potassium ions, substance P, bradykinin, prostaglandins, and other substances. These substances cause sensitization of peripheral receptors and even activate the normally inactive (silent) nociceptors. Inflammatory reaction also induces expression of the genes coding for peripheral nociceptors in the dorsal root ganglion hence leading to the increased sensitivity of the nociceptors. Following prolonged stimulation, the nociceptors that are normally found in type C nerve fibers may start being synthesized in type Aβ- nerve fibers hence simulating a phenotype shift, whereby
Aβ- nerve fibers adopt type C-fiber characteristics and hence mediate primary hyperalgesia (Curatolo et al., 2006).

Nociception refers to the process of detection of noxious stimuli which results in generation of a reflex withdrawal from the potentially harmful stimulus as well as to complex behavioral changes aimed at avoiding further contact with the stimuli (Le Bars et al., 2001). There are several pain test models that can be used in the study of nociception in small laboratory animals that are grouped into chemical (formalin & writhing) tests, thermal (hotplate & tail flick) test and mechanical test (involves application of noxious pressure). Despite the multiplicity of these tests, formalin test still remain the most preferred test because it has several advantages that includes ease of quantification, and the fact that it presents both acute and chronic phases hence can be used in the study of clinical pain states (Dubuisson & Dennis, 1977; Tjølsen et al., 1992).

The acute phase occurs between 0-5 minutes after injection and represents stimulation of nociceptors by the formalin while the chronic phase occurs between 15 to 30 minutes and represents both central nervous system (CNS) sensitization (facilitation) of pain pathways and local inflammatory process (Hunskaar & Hole, 1987; McNamara et al., 2007). Analgesics can block pain at different levels namely; by blockade of the nociceptors (Tabata-Imai, 2014), inhibition of the inflammatory effect (Hunskaar, 2007) and finally by causing
modulation of the central nervous system pain transmission pathways such as opioids and cannabinoid systems (Welch, 2009).

Inflammation is an important component in pathogenesis of various disease states, for example, it plays a vital role in various stages of tumour development such as initiation, promotion, malignant conversion, invasion and metastasis, besides affecting immune surveillance and responses to therapy (Grivennikov et al., 2010). It is also associated with depressive illness (Maes et al., 2012) and the risk of development of depression increases with rise in the level of acute inflammations. It is also known that administration of both endogenous and exogenous cytokines induce typical depressive like behaviour in healthy humans (Berk et al., 2013).

Acute inflammatory process which is accompanied by edema can be induced by injection of dilute formalin (Hunskaar & Hole, 1987), histamine (Tamaddonfard et al., 2012), carrageenan and lipopolysaccharide (Xu et al., 2012) among other substances in the paws of laboratory animals. Various methods have been used in the study of acute inflammation which includes induction and measurement of paw edema (Hunskaar & Hole, 1987), lung edema (Oyebanji et al., 2014) and pleurisy (Boschi et al., 2008) changes in levels of cytokines (Xu et al., 1998) and migration of pro-inflammatory cells (Estevão-Silva et al., 2014). Several drugs are used in management of
inflammatory process which includes steroids and non-steroidal anti-inflammatory drugs (NSAIDs). The steroids inhibit migration and degranulation of leukocytes while the NSAIDs attenuate the conditions by mainly inhibiting the activity of the cyclo-oxygenase. Other alternative methods which include administration of plant extracts and decoctions are widely used in treatment of these conditions (Long et al., 2001). The latter method is relatively cheaper and is believed to be more devoid of deleterious side effects than the conventional drugs.

Some of the herbal extracts used as folklore remedy for these ailments include Solanum incanum, Euclea divinorum and Craterostigma spp. The aim of this study was to evaluate the antinociceptive and anti-inflammatory activity of both dichloromethane and aqueous extracts of S. incanum, C. pumilum and E. divinorum using animal models.

3.2 Materials and methods

3.2.1. Collection and preparation of plant materials

The fresh Solanum incanum roots were collected in 2012 in the Roysambu Kasarani Subcounty of Nairobi County while stem and root of Euclea divinorum was collected form Narok County during the day. Craterostigma pumilum (whole plant) was also collected during the day, in 2013 from Sengera region of Laikipia County. They were identified and specimens deposited in the University of
Nairobi herbarium. Specimen voucher numbers 2013/JM01, 2013/JM02 and 2013/JM03 were obtained for *S. incanum, E. divinorum* and *C. pumilum* respectively. The plant materials were air dried in a room away from direct sunlight for one and half months. They were then ground into a fine powder using a glinding mill.

### 3.2.2 Organic extraction

About 100 grams of *S. incanum* powder was weighed and extracted using dichloromethane (DCM). First it was soaked in DCM stirred and allowed to stand for two hours. It was then decanted and the residue soaked again in DCM and allowed to stand for 24 hours at room temperature before decanting. The procedure was repeated twice in the next 48 hours. The supernatant obtained was filtered using Whatman No.1 paper and placed in sealed specimen bottles with seal. The procedure was repeated for *C. pumilum* and *E. divinorum*. Each of the extract was then concentrated and evaporated to dryness using a rotor evaporator at reduced pressure. The *S. incanum* extracts weighed about 0.253 g, *C. pumilum* extract weighed 0.98 g while *E. divinorum* stem and root extract weighed 1.63 g and 1.96 g respectively. The extracts were placed in universal bottles and stored in a cool dry place in the laboratory at 20-25°C.
3.2.3 Aqueous extraction

About 200 grams of *S. incanum* powder was soaked in distilled water in a conical flask, stirred and placed in a water bath at 60° C for 4 hours. It was then removed and filtered using Whatman No. 1 filter paper. The procedure was repeated for *C. pumilum and E. Divinorum*. The filtrates were then freeze dried to obtain a powder. About 11.64 g of *S. incanum*, 41.36 g of *C. pumilum*, 21.26 g of *E. divinorum* stem and 12.94 g of *E. divinorum* extract were obtained. The extracts were put in air tight containers and stored in the refrigerator at 4° C until further use.

3.2.4 Experimental animals

White Wister rats weighing 150 to 200 grams and in groups of five were used for both antinociceptive and anti-inflammatory assays. All the animals were placed in cages in rooms maintained at between 20 to 25° C and allowed to acclimatize for seven days before the start of the experiments. Standard commercial diet and water were provided *ad libitum*. A 12 hour day light/dark cycle was maintained throughout the period. All the *vivo* experiments were carried out as per the guidelines for care and use of laboratory animals (Wolfensohn & Lloyd, 1998).
3.2.5 Drugs and chemicals

The following drugs and chemicals were used in the study; diclofenac sodium (CSPC Pharma Co. Ltd), formalin, carrageenan (Sigma Aldrich), petroleum ether, methanol diethyl ether, dimethyl sulfoxide (DMSO), chloroform, EDTA (Loba Chemie PYT Ltd), dexamethasone (Cadila healthcare Ltd), histamine (Gland pharma Ltd), atropine sulphate, ketamine hydrochloride (Sigma Aldrich) morphine.

3.2.6 Antinociceptive activity assay

Evaluation of antinociceptive effect of the herbal extract was carried out using formalin test. The pull test (a sensory motor test) was used to evaluate the muscle relaxing effect of the extracts. It separates muscle relaxation from sedation, catalepsy and catatonia (sensory motor impairment) (Deacon & Gardner, 1984). Only the animals that showed no sensory motor impairment were used for subsequent experiments.

The antinociceptive effects of the extracts were evaluated using the formalin test as described by Hunskaar & Hole, (1987); Rosland et al., (1990); and Tjølsen et al., (1992). The left hind paw that was to be injected with formalin was marked with permanent marker pen. Pain was induced by administration of 50µl of 5% formalin in sub-plantar region of the paw using a 30 gauge needle (Rosland
et al., 1990). The white Wister rats in groups of five weighing 150-200g received three doses of DCM extracts of S. incanum, C. pumilum and E. divinorum, 15mg of injectable diclofenac sodium, 5mg morphine and vehicle (30% DMSO in normal saline) intraperitoneally while the doses of the aqueous extracts of the three extracts, oral formulation of diclofenac and vehicle (normal saline) was administered orally. All the treatments were administered 30 minutes prior to formalin injection.

The animals were individually placed in transparent plexiglass cage observation chamber and two mirrors were placed behind and on the side of the cage for ease of visualizing the paws from all sides. The amount of time spent lifting, biting, flinching and licking the injected paw was considered as indicator of pain and was recorded for 30 minutes after the formalin injection. Early phase of nociception was measured between 0-5 minutes while late phase took place between 15-30 minutes after formalin injection. The early phase represents neurogenic pain while the late phase is due to inflammatory pain response as well as central sensitization (Hunskaar & Hole, 1987; Rosland et al., 1990; McNamara et al., 2007).

3.2.7 Anti-inflammatory activity assay

Formalin induced paw edema (Hunskaar & Hole, 1987; Rosland et al., 1990) with some modification was used as model for acute inflammation. This was used to assess the anti-inflammatory affects of S. incanum, C. pumilum and E.
divinorum. One set of five white Wister rats (150-200 grams) received intraperitoneal injection of 10, 25, and 50 mg/kg doses of DCM extracts of *S. incanum*, 15 mg/kg diclofenac sodium and the vehicle (30% DCM in normal saline), while another received 10, 25, and 50 mg doses of aqueous extracts, 15 mg diclofenac sodium and the vehicle (normal saline) orally. They were then allowed to rest for 30 minutes to allow for drug distribution. After 30 minutes, inflammation was induced in the rats by injecting 50 µg of 5% formalin in the sub-plantar region of the left hind paw and the diameter of the paw measured using a digital caliper. The initial paw diameter was taken before formalin injection and the rest every 30 minutes for four hours. The difference between these initial values and the readings at subsequent times was quantified as the hind paw edema in rats and they were compared with the vehicle treated groups.

### 3.2.8 Data analysis

The data obtained for each set of experiment was pooled as a mean and standard errors of the mean. It was analysed using one way ANOVA with Scheffé post hoc test. A value of $p < 0.05$ was considered significant.
3.3 Results and Discussion

3.3.1 Antinociceptive effects of *S. incanum*, *C. pumilum* and *E. divinorum* in rats

Administration of 25 mg of DCM extract of *S. incanum* showed significant antinociceptive activity (p < 0.05) in the second phase only, while the 50 mg showed a significant (p < 0.001) activity in both phases of nociception relatively to the vehicle that was comparable to the effect of diclofenac. The 10 mg dose apparently had no effect on the nociception. The nociception observed with the doses of the herb were not dose dependent but were significantly (p < 0.05) lower than that of morphine (Fig. 3.1; Table 3.4.5a- Appendix ix).

![Figure 3.1: Antinociceptive effect of dichloromethane extract of *Solanum incanum*. *and ** indicate a value of (p < 0.05) and (p < 0.001) relative to the vehicle respectively.](image-url)
Similarly, all the doses of aqueous extract exhibited significant pain inhibitory effect (p < 0.05) in both phases of nociception that was comparable to diclofenac (Fig. 3.2; Table 3.4.5b Appendix x). However the effects observed with the herbal doses were not significantly different. In this study, morphine exhibited significantly higher antinociception compared to all the treatments.

![Figure 3.2: Antinociceptive effect of aqueous extract of Solanum incanum. *and ** indicate value of (p < 0.05) and (p < 0.001) relative to the vehicle respectively.](image)

Previous studies have shown that the DCM methanolic extract of the plant has significant spinal and supra spinal antinociceptive effects in thermal pain test models (Mwonjoria et al., 2011). However, it was not established if the activity was in the polar or less polar fraction neither its effect on chemical and inflammatory pain. From the results, from the current study, it is evident that at a lower dose, the DCM extract was active in the 2nd phase of nociception, with the higher dose being highly active in both phases. The aqueous
fraction, however, was active in both phases of nociception.

The different doses of DCM and aqueous extracts of *C. pumilum* used in this study had no antinociceptive effects on the formalin induced pain (Fig. 3.3 & 3.4; Table 3.4.6a & b Appendix xi & xii).

![Figure 3.3: Effect of dichloromethane extract of *Craterostigma pumilum* on paw edem. *and ** indicate value of (p < 0.05) and (p < 0.001) relative to the vehicle respectively.]

Interestingly, the DCM extracts significantly increased the time spent on nociception in the inflammatory phase. The second phase of nociception is usually due to inflammation and central sensitization of nociceptive neurons in the dorsal horn of spinal cord (Hunskaar & Hole 1987; McNamara *et al.*, 2007). Hence this observed increase in sensitivity to pain stimuli is likely to be due to
lowering of threshold to pain stimuli in the spinal and or central pain pathways in the brain (central sensitization). Therefore, though the herb exhibited splendid anti-inflammatory properties, it is not likely to be a good source of novel chemicals to be used in development of analgesic remedies.

![Graph showing effect of aqueous extract of Craterostigma pumilum on nociception. *and ** indicate a value of (p < 0.05) and (p < 0.001) relative to the vehicle respectively.]

The 50 and 100 mg doses of the DCM extract of *E. divinorum* stem showed no effect on time spent in pain behavior as compared to the vehicle during the first phase of nociception. However they exhibited a very significant (p < 0.001) antinociceptive effect in the second phase of nociception (Fig. 3.5; Table 3.4.7a-Appendix xiii),

**Figure 3.4: Effect of aqueous extract of *Craterostigma pumilum* on nociception. *and ** indicate a value of (p < 0.05) and (p < 0.001) relative to the vehicle respectively.**

The 50 and 100 mg doses of the DCM extract of *E. divinorum* stem showed no effect on time spent in pain behavior as compared to the vehicle during the first phase of nociception. However they exhibited a very significant (p < 0.001) antinociceptive effect in the second phase of nociception (Fig. 3.5; Table 3.4.7a-Appendix xiii),
Figure 3. 5: Antinociceptive effect of dichloromethane extract of *Euclea divinorum* stem. * and ** indicate a value of (p < 0.05) and (p < 0.001) relative to the vehicle respectively.

This was in contrast to the observation in the aqueous extract which showed relatively more activity in the first phase where the 25 mg dose of the extract exhibited significant (p < 0.05) while the 50 mg showed a highly significant (p < 0.001) antinociceptive effect (Fig. 3.6). The 100 mg dose failed to show any pain reduction effect. In the second phase, the 25 mg dose of the aqueous extract showed no significant effect, while both the 50 & 100 mg doses exhibited significant antinociceptive (p < 0.05) effect (Fig. 3.6; Table 3.4.8b Appendix xiv).
Figure 3.6: Effect of aqueous extract of Euclea divinorum stem bark on formalin induced nociception in rats. * and ** indicate a value of ($p < 0.05$) and ($p < 0.001$) relative to the vehicle respectively.

The 50 and 100 mg doses of DCM extract of E. divinorum root had no significant effect in the first phase. However it exhibited significant ($p < 0.05$) analgesic effect which was not dose dependent in the 2$^{nd}$ phase (Fig. 3.7).
Figure 3.7: Antinociceptive effect of dichloromethane extract of *Euclea divinorum* root. * and ** indicate a value of (p < 0.05) and (p < 0.001) relative to the vehicle respectively.

These results contrasted sharply from that obtained using the aqueous extract which showed more robust activity, where the doses of the extracts caused significant (p < 0.05) antinociceptive effect in both phases of nociception (Fig. 3.8; Appendix xv).
This first phase represents direct action of formalin on nociceptors (Hunskaar & Hole, 1987) while the second is mainly due to inflammatory activity where pain cum inflammatory mediators such as substance P, prostanoids, 5-hydroxytryptamine and histamine are release (Damas & Liegeois, 1999). It is also associated with sensitization of nociceptive afferents in the spinal cord (Harvey et al., 2004). The second phase of formalin induced nociception associated with inflammatory process where the pro-inflammatory cytokines IL-1β and TNFα are involved (Vinicio et al., 2001). It is believed that IL-1β acts via COX-2 during the process of inflammation. Therefore, COX-2 is not involved in the acute inflammatory pain model such as the 1st phase of formalin test. Nevertheless, both IL-1β and TNFα play a key role in inflammatory pain induction in the second phase of formalin test (McNamara et al., 2007).
It is also likely that the extract exerted its analgesic effect via inhibition of inflammatory process or by inhibiting a single or many neural transmitters involved ‘gating control of pain’ in the dorsal horn of the spinal cord (Huskar & Hole, 1987; McNamara et al., 2007).

The phytochemicals isolated from *Euclera divinorum* include lupeol, lupene, betulin, 7-methyljuglone, isodospyrin, shinalone, catechin and 3β-(5-hydroxyferuloyl)lup-20(30)-ene (Mebe et al., 1998), myricitrin (Hattas et al., 2011), eucleanal A & B (Ng’ang’a et al., 2012) glycosides of aromadendrin, quercetin and myricetin (Njuguna, 2005). These metabolites include several flavonoids. However, studies have shown that the plant posses appreciable amount of alkaloids in both aqueous and methanolic extracts though none has been describe (Amusan et al., 2007; Ngari et al., 2013). It is possible that one or several of these metabolites may be responsible for the antinociceptive effects observed in this study. In whole study, all the doses of the various plant extract showed far less level of pain blocking activity as compared to 5 mg dose of morphine.

Injection of dilute formalin solution in a paw of the animal produces nociception with two phases, the early phase lasting the first 5 minutes and late phase lasting from 20 to 30 minutes. The two phases have different mechanism where the first phase involves direct action that stimulates nociceptors (Hunskaar et al.,
1985) and it is mainly mediated via TRPA1 receptors a member of the transient Receptor Potential family of cation channels that is highly expressed by some of C-fiber nociceptors that plays an important role in inflammatory pain (McNamara et al., 2007). Stimulation of these channels by formalin causes tremendous influx of calcium ions in to the cells expressing this type of ion channel (McNamara et al., 2007). Prostaglandins have minimal or no role to play during this process however nociception in this phase can be inhibited by centrally acting drugs such as the opioids, paracetamol, non-steroidal anti-inflammatory drug such as acetylsalicylic acid (aspirin) but not by indomethacin.

The second phase is of dual origin that is it is due to the inflammatory process hence it can be inhibited by steroids such as dexamethasone and non-steroid anti-inflammatory drugs. It is also caused by central sensitization where D-serine an endogenous co-agonist of N-methyl-D-aspartate receptor plays an important role in pain processing and hyperalgesia in substantia gelatinosa or (lamina I-II) of the dorsal horn of the spinal cord (Tabata-Imai et al., 2014). This may explain why many centrally acting drugs with or without anti-inflammatory activity such as opioids, paracetamol and aspirin are known to strongly inhibit nociception in this phase.
3.3.2 Anti-inflammatory effects of DCM and aqueous extracts of *S. incanum*, *C. pumilum* and *E. divinorum* in rats

There are several drugs used in alleviation of inflammatory conditions that include NSAIDS such as aspirin and diclofenac that inhibit prostaglandin synthesis (Vane, 2000), steroids that target migration and ultimately degranulation of leukocytes (Farsky *et al*., 1995). In the current study, edema was obliterated by concomitant administration 15 mg of diclofenac sodium 30 minutes prior to injection with formalin. In the subsequent, experiments various dose of both DCM and aqueous extract of *S. incanum*, *C. pumilum* and *E. divinorum* were administered. *Solanum incanum* doses exhibited significant edema reduction effect relative to their respective vehicles. In the experiments involving use of DCM extract of *S. incanum*, both 25 and 50 mg does exhibited significant anti-inflammatory effects (p < 0.05) with the 25 mg dose showing a significant effect (p < 0.001) against the vehicle and a significant difference from the 50 mg, hence it had higher activity than the former (Fig. 3.9; Table 3.4.1a-Appendix i.). This difference in activity between the doses of the herb may be increase in active metabolites with concentration. However at a higher concentration, the level of either the inhibitors and or enzymatic modulators may have increased in proportion which may explain the relative decrease in activity.
However, the 10 mg dose failed to exhibit significant edema reduction effect throughout the experiments. In the aqueous fraction, 25 mg showed a highly significant effects (p < 0.001) throughout the study as compared to the vehicle while the 50 mg exhibited significant (p < 0.05) effect after 3 hours with the 100 mg doing so after 1 hour (Fig. 3.10; Table 3.4.1b- Appendix ii).
Figure 3.10: Effect of the aqueous extract of *Solanum incanum* on edema. ‘a’ and ‘b’ value of (p < 0.001) and (p < 0.05) compared to the vehicle respectively.

In both of these studies the 25 mg does came out as the most effective dose. Perhaps lower doses had sub-optimal levels of active ingredients while the higher does had higher levels of antagonistic metabolites that competitively inhibited the effect of active metabolites. Administration of formalin at a low dose induces edema that is mainly due to neuro-peptides (substance P) mediated neurogenic inflammation. However, at higher doses, it induces edema that is mainly associated with release of substance P, prostanoids, 5-hydroxytryptamine and histamine (Damas & Liegeois, 1999). Since a relatively higher dose of formalin was used in this study, it may suffice to implicate the participation of the latter substances in the induction of the inflammation response observed.

The DCM extract represents the less polar fraction while the aqueous extract
contains the most polar fraction. Hence it is apparent that both the polar and non-polar portions of the herb extracts contained relatively active secondary metabolites with anti-inflammatory effects. The activity of these secondary metabolites was comparable to the effect of diclofenac (Fig. 3.9 & 3.10). *Solanum incanum* contains several metabolites that include alkaloids and flavonoids (Mwonjoria *et al.*, 2014), similar phytochemicals from other plant sources have been shown to exhibit significant anti-inflammatory effects in several previous studies. Flavonoids were shown to exert anti-inflammatory effects that involved inhibition of arachidonic acid metabolism (Ferrandiz & Alcaraz, 1991). The alkaloids, which comprise one of the largest single class of secondary plant metabolite, possess a significant range of pharmacological activity which includes anti-inflammatory activity (Barbosa-Filho *et al.*, 2006; Souto *et al.*, 2010). Therefore, it is highly probable that the anti-inflammatory effects observed may be due to these phytochemicals individually or combined with others.

The 25 mg dose of DCM extract of *C. pumilum* was observed to exhibit significant (p < 0.05) anti-edema effect at 3rd hour following formalin injection (Fig. 3.11; Table 3.4.2a- Appendix iii).
Figure 3. 11: Effect of dichloromethane extract of *Craterostigma pumilum* on paw edema. ‘a’ and ‘b’ value of (p < 0.001) and (p < 0.05) compared to the vehicle respectively.

The 50 mg dose exhibited a significant (p < 0.001) effect at 30, 60, 90, 180 and 240 minutes and significant (p < 0.05) effect at 120 minutes as compared to the vehicle. However the higher dose of 100 mg showed less activity than the 50 mg dose whereby significant activity (p < 0.05) was observed after 60 and 90 minutes and highly significant (p < 0.001) effect at 120 and 240 minutes. In this group, diclofenac which acted as the positive control induced significant anti-edema effect at 180 minutes. Therefore apparently, the herbal extract exerted higher level of activity than the positive control. This may be attributed to the fact that the diclofenac was dissolved in normal saline but not the vehicle DMSO which is known to exhibit anti-inflammatory effects on its own (*Smith et al.*, 1998).
In the aqueous extract, 25 mg dose showed no anti-inflammatory effects, while the 50 mg dose showed significant (p < 0.05) effects at 30, 60, 120, and 240 minutes. This is in contrast to the 100 mg dose that exhibited very significant (p < 0.001) effects at 30, 60 and 120 minutes and significant (p < 0.05) effects at 180 and 240 minutes. Nevertheless, the effect of the extract increased in a dose dependent manner (Fig. 3.12; Table 3.4.b- Appendix iv).

The diclofenac in this case exhibited significant (p < 0.05) effect and a significant (p < 0.001) effect at (60, 120, 180 and 240 minutes). It however showed significant (p < 0.05) effect at 30 and 90 minutes. From the results it is evident that the herb probably posses’ phytochemicals with strong anti-inflammatory effects though there is scanty literature on these effects as well as its composition.
The DCM extract of *E. divinorum* stem showed robust anti-inflammatory activity for all the doses. The 50 mg dose exhibited significant (*p* < 0.05) anti-edema effect at 60 minutes and a significant (*p* < 0.001) effect at 90, 120, 180, 240 minutes, while the 100 mg showed a significant effect (*p* < 0.001) throughout the study period. The positive control, diclofenac induced significant edema control from 90 to 240 minutes (Fig. 3.13; Table 3.4.3a- Appendix v).

![Figure 3.13: Effect of dichloromethane extract of Eucla divinorum stem paw edema. ‘a’ and ‘b’ value of (p < 0.001) and (p < 0.05) compared to the vehicle respectively.](image)

In the experiments involving aqueous extract of *E. divinorum*, the 25 mg showed more enhanced activity at 30 minutes where it exhibited highly significant (*p* < 0.001) edema reduction effect while the 50, 100 mg doses and diclofenac exhibited a significant (*p* < 0.05) effect. Similar observation were made at 60 minutes with exception of diclofenac which exhibited a significant (*p* < 0.001) effect compared to the vehicle. During the subsequent times, all the doses and
positive control showed a significant (p < 0.001) edema limiting effect (Fig. 3.14; Table 3.4.2b - Appendix vi).

The DCM extract of the *E. divinorum* root bark did not exhibit marked edema limiting effect with only the 100 mg dose showing activity. It exhibited a highly significant (p < 0.001) anti-edema effect with exception of its activity at 90 minutes, where it showed a significant (p < 0.05) effect. This effect was comparable to the effect of the diclofenac. (Fig. 3.15; Table 3.4.4a - Appendix vii).
Figure 3.15: Effect of dichloromethane extract of *Euclea divinorum* root on paw edema. ‘a’ and ‘b’ value of (p < 0.001) and (p < 0.05) compared to the vehicle respectively.

The effect of the DCM extract of *E. divinorum* stem contrasted sharply with the observation made with the aqueous extract where all the doses tested exhibited highly significant (p < 0.001) anti-inflammatory effects (Fig. 3.16; Table 3.4.4b-Appendix viii). This outcome may mean that the metabolites of this fraction with higher polarity have higher anti-inflammatory activity.
Figure 3. 16: Effect of aqueous extract of *Euclea divinorum* root on formalin induced paw edema diameter in rats as a function of time. a and b value of (p < 0.001) and (p < 0.05) compared to the vehicle respectively.

Some of the phytochemicals isolated from *Euclea divinorum* include lupeol, lupene, betulin, 7-methyljuglone, isodiospyrin, shinalone, catechin and 3β-(5-hydroxyferuloyl) lup-20(30)-ene (Mebe *et al.*, 1998) and myricitrin (Hattas *et al.*, 2011), eucalanal A & B (Ng’ang’a *et al.*, 2012) glycosides of aromadendrin, quercetin and myricetin (Njuguna, 2005). These metabolites include several flavonoids. Therefore, since flavonoids are known to exert anti-inflammatory effect (Ferrandiz & Alcaraz, 1991), it can be inferred that they may have contributed to a sizeable amount of the anti-inflammatory effect observed in these extract alongside other potential but unidentified phytochemicals. Qualitative analysis of the plant extracts had shown that they contain several of these metabolites that include alkaloids among others (Amusan *et al.*, 2007; Ngari *et al.*, 2013).
CHAPTER FOUR: ANTIPYRETIC EFFECTS OF *Craterostigma pumilum*,
AND *Euclia divinorum* EXTRACTS

4.1. Introduction

Fever or regulated rise in body temperature is a haul mark of disease. The febrile condition is associated with substantial cost to the host such as metabolic, elevated level of oxygen and fuel demands, as well as actual or potential lesions of neurologic tissues (Dinarello *et al.*, 1988). Very severe febrile illness may be associated with hyperpyrexia or abnormally high fever of above 41.5° (Dinarello *et al.*, 1988). The condition may cause heat stroke and heat exhaustion which are known to cause fatalities if not treated (Guyton & Hall, 2006). Studies have also indicated that febrile illness causes teratogenesis or congenital malformations (Edwards, 2006).

Though fever is beneficial in some cases, it suffices to say that management of febrile illness is associated with good prognosis. However many of the drugs used mainly inhibit prostaglandin synthesis which may also interfere with other multiple functions in the body. It is important to search for drugs utilizing other mechanisms or exhibiting more receptor specificity to minimize the side effects observed. Plant materials that have been used as traditional remedies for the condition which include *S. incanum* among others (Kokwaro, 1993). *Solanum incanum* had shown antipyretic effect (Mwonjoria *et al.*, 2011).
hence it was omitted from screening assay at this stage. This study evaluated the effects of *C. pumilum* and *E. divinorum* extract on lipopolysaccharide fever in the hope of providing data on an alternative source of antipyretic compounds.

### 4.2 Materials and Methods

#### 4.2.1 Experimental animals

White male Wister rats 150 to 200g and in groups of five were used for the antipyretic assay. All the animals were placed in cages room between 20 to 25 °C and allowed to acclimatize for seven days before the start of the experiments. Standard commercial diet and water was provided *ad libitum*. A 12 hour day light/dark cycle was maintained throughout the period.

#### 4.2.2 Drugs and chemicals

Drugs and chemicals used in the study included; diclofenac sodium, lipopolysaccharides from *Escherichia coli*, and dimethyl sulfoxide (DMSO).

#### 4.2.3 Antipyretic assays

Groups of non-febrile male white Wister rats weighing between 150 to 2000 grams were used in the study. The test groups (*n = 5*) received doses of herb extracts while the control groups (*n = 5*) received either diclofenac sodium or the
vehicle i.e. DMSO for the DCM extracts and normal saline for the aqueous extracts. The diclofenac, DCM extracts and the vehicle were administered intraperitoneally (i.p). The rats were injected with 50 micrograms of Escherichia coli’s lipopolysaccharides batch number 0111:B4 (Sigma Aldrich) in normal saline intraperitoneally (i.p.). This dose was chosen on basis of a previous study (Tavares et al., 2004).

The rectal was taken as the core temperature and it was measured using a digital thermometer model (DT-01(A)). The thermister probe of the thermometer was inserted 2 cm into the rectum. The temperature measurement was taken before injection with the herbal extracts, drugs and vehicle and then every 30 minutes after injection with the lipopolysaccharide for the next 2 hours. The end point during the temperature measurement was when the thermometer gave an automatic alarm.

4.2.4 Data analysis

The data for each set of experiment was expressed as means, standard errors of the mean. It was analysed using one way ANOVA with Scheffé post hoc test. A value of p < 0.05 was considered significant.
4.3 Results and discussion

In the study, the DCM extracts of *C. pumilum* failed to exhibit significant antipyretic effect to LPS induced fever (Fig. 4.1). Similar results were obtained for aqueous extract not shown here.

![Figure 4.1](image)

Figure 4.1: Effects of dichloromethane extract of *Craterostigma pumilum* on lipopolysaccharide induced fever. *p* < 0.05 relative to vehicle.
Similar observations were made with both DCM and water extracts of *E. divinorum* stem and root (Fig. 4.2 & 4.3; Appendix xxxiii).

![Figure 4.2](image)

**Figure 4. 2:** Effect of dichloromethane extracts of *Euclea divinorum* on lipopolysaccharide induced fever. *p* value less than 0.05 as compared to the vehicle.

![Figure 4.3](image)

**Figure 4. 3:** Effects of aqueous extract of *Euclea divinorum* on lipopolysaccharide induced fever. *p* value less than 0.05 relative to the vehicle.

Fever is produced via stimulation by both exogenous pyrogens such as breakdown products of bacterial cell wall by products e.g. lipopolysaccharide and also by endogenous pyrogen or cytokines. Cytokines such as interleukins-1, 6 and
tumor necrotic factor-α (TNF-α) signaling induce fever through activation of cyclooxygenase-2 pathway resulting in production of prostaglandin E₂ (PGE₂) that activates the hypothalamic PGE₂ receptors (Dinarello, 2004). Lipopolysaccharide (LPS) binds to its specific receptors toll-like receptor 4 (TLR4) that are expressed by cells participating in innate immunity such as macrophages, neutrophils and dendritic cells and stimulates synthesis of PGE₂ which crosses the blood brain barrier to initiate fever (Evans et al., 2015).

Most antipyretics inhibit febrile illness by down regulating the cyclooxygenase activity however; other modes of action for antipyretic drugs have been suggested that include their reduction proinflammatory mediators, enhancing anti-inflammatory signals at sites of injury, antipyretic signals within the hypothalamus. In this study, the extracts of C. pumilum and E. divinorum showed no antipyretic effects to LPS induced fever. This observation may mean that the extracts did not interfere with cyclooxygenase activity and release of proinflamatory cytokines suchs TNF-α and interleukins-1 and 6, which mediate the genesis of fever.
CHAPTER FIVE: EFFECT OF THE CRUDE EXTRACTS AND ALKALOIDS RICH FRACTION OF Solanum incanum AND Euclea divinorum ON LEUKOCYTE MIGRATION

5.1 Introduction

Leukocyte migration or accumulation of white blood cells at the site of injury or infection plays a pivotal role in development of an inflammatory process. It involves neutrophil recruitment, lymphocyte recirculation and monocytes trafficking through blood vessel walls via the process of diapedesis, where these cells crawl in an amoeboid fashion through endothelial pores and, sometimes, through the endothelium itself (Ley et al., 2007; Muller, 2011). During the process, neutrophils are the first group that arrives at injury site followed by monocytes T and B lymphocytes in that order (Thiemann & Baum, 2011).

Inflamed tissues produce several pro-inflammatory cytokines which cause up-regulation of the expression of adhesion molecules by the endotheliocytes such as selectins and integrins. These adhesion molecules aid in the entry and recruitment of the leukocyte into the inflammation tissues (Norman et al., 2014). Migrating cells have minimal effect by their presence alone. Nevertheless, they initiate a complex inflammatory process that includes release of chemotaxic substances, which attract more inflammatory cells, inhibitors that reduce the severity of the process, histotoxic agents that include proteases, reactive oxygen metabolites and
cations. They also signal to the adjacent cells and tissue to trigger complex inflammatory reaction (Barbosa-Filho et al., 2006). Most of the drugs used in management of inflammatory conditions acts via inhibition of cyclooxygenase activity (Vane, 1976; Giovanni & Luca, 2015) while the steroids inhibit white blood cells diapedesis and concomitant degranulation which is associated with release of vasoactive compounds such as serotonin, histamine and leukotrienes or slow reacting substance of anaphylaxis (Guyton and Hall, 2006).

The differential and whole white blood cells count can either be done manually using hemocytometers or counting chambers e.g. improved Neubauer chambers or by use of automated hematological analyzer procedures that are carried out after lyses of red blood cells using a diluents fluid. Nevertheless both methods have some shortcomings for example the manual methods has lower degree of accuracy as compared to the automated method (Perkins, 2009). However, leukocyte count is falsely elevated with automated method especially in situations such as presence of cryofiblinogen and cryoglobulins (circulating immunoglobulins or their complexes that exhibit cold induced reversible precipitation between 4 and 37 °C (Fohlen–Walter et al., 2002) and in cases of aggregated platelets (Lombarts & de Kieviet, 1988).
False positive results may also be obtained with automated methods in cases of incomplete lysis of red blood cells as well as due to granulocyte agglutination (Zelster et al., 2000; Berliner et al., 2001).

Inflammatory conditions are associated with pathological conditions some with poor prognosis such as coronary artery disease and atherosclerosis (Göran, 2005), depressive illness in humans (Maes et al., 2012, Berk et al., 2013) and chronic pain states, e.g. osteoarthritis, rheumatoid arthritis (Laar et al., 2012) and gout (Cronstein & Terkeltaub, 2006). It has also been suggested that chronic subclinical inflammation is an etiology of insulin resistance syndrome in non-diabetic individuals (Festa, 2000). Inflammatory ailments are managed through several conventional ways which include administration of non-steroid anti-inflammatory agents such as ibuprofen, naproxen, diclofenac, and celecoxib which inhibit the cyclooxygenase activity. These drugs are associated with gastrointestinal bleeding, increased cardiovascular disorders and rise in blood pressure especially in hypertensive patients (Laar et al., 2012) and steroids such as hydrocortisone, dexamethasone etc. which posses myriad of undesirable side effects (Cronstein & Terkeltaub, 2006).

Alternative management of the condition involves use of plant parts of extracts. It is believed that remedies from plant sources are less toxic than their conventional counterparts. Among the plants used as folklore remedy for inflammatory process
include *S. incanum* and *E. divinorum* (Kokwaro, 1993; Matu, 2008) though there is little or no reports on scientific studies done on their activity, their effects on white blood cells chemotaxis and cellular behavior during the process of inflammation. This study aimed at evaluating the effects of *S. incanum* and *E. divinorum* extracts and their alkaloids rich fractions on leukocyte migration in vivo.

### 5.2 Materials and Methods

#### 5.2.1 Leucocytes migration of assay

Investigation of the effect of the DCM, aqueous extracts and alkaloid rich fractions of *S. incanum* and *E. divinorum* on leucocytes migration was carried out using the method described by Ferrandiz & Alcaraz (1991) with some modifications. Various doses of the DCM and alkaloid rich fractions and their controls were administered subcutaneously (s.c.) while the aqueous extracts was administered orally in mice 30 minutes prior to intraperitoneal injection of 0.25 ml of 1% carrageenan. Dimethyl sulphoxide (DMSO) was used as negative control (vehicle) in the DCM extracts assays while normal saline was used in the study using the other extracts. Dexamethasone which was used as positive control was injected subcutaneously (s.c.). Four hours after administration of various treatments, the mice were euthanized by dropping them in to a jar containing cotton soaked with chloroform. Then 2 ml of modified normal saline containing
EDTA was injected into the peritoneal cavity followed by peritoneal lavage.

The total number of leukocytes in the lavaged peritoneal fluid was determined using a method described by Ferrandiz & Alcaraz (1991) where they were counted using an improved Neubauer chamber. The total cell count for the herbal doses and dexamethasone were compared with that of their respective negative control treated animals. The differences in the WBC count in the lavaged fluid in the vehicle and the test treated animals indicated the effect the various treatments on leukocyte migration.

5.3 Data analysis

The experimental data was expressed as means and standard errors of the mean. It was analysed using one way ANOVA with Scheffé as the post hoc test. A value of p < 0.05 was considered significant.
5.4 Results and discussion

5.4.1 Effects of S. incanum on carrageenan induced leukocyte migration

In the assay, 10, 25, and 50 mg of DCM extract of S. incanum exhibited significant (p < 0.05) inhibition of carrageenan induced chemotaxis in the peritoneal exudates (Fig. 5.1). Similar results were observed with aqueous extract of the herb where the 25 and 50 mg doses showed significantly delayed migration of the leukocytes (Fig.5.2; Appendix xxix).

![Graph showing leukocyte migration](image)

Figure 5.1: Effect of dichloromethane extract of *Solanum incanum* on leukocyte migration. * (p < 0.05) relative to the vehicle.
Figure 5.2: Effect of aqueous extract of *Solanum incanum* on leukocyte. * (p < 0.05) relative to the vehicle

In the study, the activity of the various doses of the *S. incanum* did not show a dose related response though higher doses had lower potency as compared to the lower ones which may be attributed to competing metabolite(s) for same carrier protein or enzyme and or higher substrate product inhibition besides others.

Steroids such as dexamethasone, with glucocorticoid activity and other steroids exert their anti-inflammatory effects by inhibiting migration and degranulation of leukocytes (Hofbauer *et al.*, 1999). They also block the expression of COX-2, which contributes to their anti-inflammatory activity (Vane, 2000). In this study dexamethasone exhibited a significant (p < 0.001) anti-leukocyte migration effects as compared to the vehicle.

*Solanum incanum* contains several secondary metabolites that include flavonoids,
saponins, phenolics, terpenes, and steroidal alkaloids (Eltayeb, 1997). Flavonoids have been shown to possess anti-inflammatory effects (Ferrandiz & Alcaraz, 1991) just like the alkaloids (Barbosa-Filho, 2006). Hence it is likely that the edema reduction effect and leukocyte migration inhibition seen in the study may be as a result of these secondary metabolites acting singly or in synergy with others.

The red blood cells float freely and flow with the blood fluid. However, the leukocytes tend to adhere and roll on the endothelial surface which aids in their extravasations through the endothelium. In acute inflammation, chemokines and lipid chemo-attractants are released and presented on the luminal surface of activated endothelial cells and together with other attractants are released by mast cells and activated platelets, which initiate the arrest of white blood cells rolling.

(Leys et al., 2007). Reduction of vascular resistance, increased blood flow and intravascular fluid exudates are normally associated with release of pro-inflammatory cytokines like interleukin-I and tumor necrotic factor–α, as well as vasoactive amines and peptides (Leys et al., 2007). Therefore, it is probable that the extracts alkaloid rich fraction of S. incanum inhibited WBC extravagation by blocking one of these mechanisms.
5.4.2 Effects of *E. divinorum* on leukocyte migration

In this study, the effect of both DCM and water extract as well as the alkaloid rich fraction of *E. divinorum* was investigated where the 25 and 50 mg doses of DCM stem extract exhibited significant (p < 0.05) inhibition of leukocyte chemotaxis following carrageenan administration (Fig. 5.3; Appendix xxxii). The activity of the 25 and 50mg doses was significantly different from the vehicle but comparable to the dexamethasone treatment. However, the doses of DCM extract of the *E. divinorum* root and aqueous extract of both stem and root did not have significantly different effect from the vehicle (Fig. 5.4).

Figure 5. 3: Effect of dichloromethane extracts of *Euclea divinorum* stem on leukocyte. * (p < 0.05) relative to the vehicle
In the study involving the aqueous extract of *E. divinorum*, both the stem and root extracts did not exhibit significant delay in leukocyte migration (Fig. 5.5).

Figure 5.5: Effects of aqueous extracts of *Eulea divinorum* on leukocyte migration. * (p < 0.05) relative to the vehicle
The 100 mg dose of both alkaloid rich fractions of *S. incanum* and *E. divinorum* root exhibited significant (p < 0.05) inhibition of leukocyte migration (Fig. 5.6).

![Figure 5.6: Effect of alkaloids rich fraction of *Solanum incanum* and *Euclea divinorum* on leukocyte migration. * and ** means (p < 0.05) and (p < 0.001) respectively](image)

A finding that indicates that the effect seen earlier with bioth DCM and aqueous extracts of *S. incanum* may solely be due alkaloid activity and orin synergy with other secondary metabolites. However it is surprising to note that the crude extracts of the *E. divinorum* root had no effect on leukocyte movement, an observation that is in contrast to that of its alkaloid rich fraction. This observation may mean that the crude extract may have contained an antagonistic metabolite that inhibited the activity of the alkaloids or its biotransformation.

Qualitative analysis of the plant extracts had shown that they contain several of
these metabolites that include tannins, terpenes, flavonoids, glycosides, alkaloids among others (Amusan et al., 2007; Omwenga et al., 2012; Ngari et al., 2013). Some of the phytochemicals isolated from *Euclea divinorum* include lupeol, lupene, betulin, 7-methyljuglone, isodiospyrin, shinalone, catechin and 3β-(5-hydroxyferuloyl) lup-20(30)-ene (Mebe et al., 1998), myricitrin (Hattas et al., 2011), a naphthalene derivative eucanal A & B (Ng’ang’a et al., 2012) and glycosides of aromadendrin, quercetin and myricetin (Njuguna, 2005). Many of the metabolites named belong to the flavonoids fraction.

There is no report on characterization of alkaloids in this plant genus where much of the work has focused on less polar fractions (Mebe et al., 1998; Hattas et al., 2011) and on the qualitative assays (Ngari et al., 2013). The flavonoids have been shown to exert anti-inflammatory effects (Ferrandiz & Alcaraz, 1991). Therefore, it can be inferred that these secondary metabolites that are abundant in this plant may have contributed to the anti-inflammatory effects observed in these extract alongside other potential but unidentified phytochemicals.

In this study, 100 mg *S. incanum*, 100mg *E. divinorum* root and 500 mg of *E. divinorum* stem showed no significant effect on leukocyte migration pattern as compared to the vehicle. Earlier on doses of *S. incanum* had shown significant reduction in white blood cell counts in the peritoneal fluid following treatment with carrageenan. Similar results were observed with DCM extract of *E.
*divinorum* stem, which may indicate that the inhibition of leukocyte chemotaxis may be due to other metabolites or their combination but not to purified alkaloid fraction alone.
CHAPTER SIX: ANTINOCICEPTIVE, ANTI-INFLAMMATORY, ANTIPYRETIC AND MODES OF ACTION EFFECTS OF ALKALOID RICH FRACTIONS OF Solanum incanum AND Euclea divinorum

6.1 Introduction

Alkaloids form the largest group of secondary metabolites in plants. They are compounds comprising of nitrogen bases (aglycone). They react with acids to form crystalline salts without the production of water (Firn, 2010). They mainly exist as solids like atropine, though some exist as liquids. Many of them are very soluble in alcohol and sparingly soluble in water. They normally play an important role in the protection of plants against predators.

This group of metabolites has wide application in medical practice for example opioids such as morphine and codeine are analgesics, vinblastine, vincristine and camptothecin are used as anticancer treatments, tubocurarine as a muscle relaxant, while ajmaline is used in the management of arrhythmias. Other alkaloids include caffeine, nicotine, cocaine and heroin (Keasling, 2008).

Many alkaloids are known to posses anti-inflammatory effects (Barbosa-Filho et al., 2005; Souto et al., 2010) while others such as opioids have very potent analgesic activity (Feng et al., 2012).
*Solanum incanum* contains steroidal glycoalkaloids solasonine and solamargine (Fukuhara & Kubo, 1991) while phytochemical analysis has shown that alkaloids are present in *E. divinorum* (Amusan *et al.*, 2007; Ngari *et al.*, 2014; Onyango *et al.*, 2015), though it appears that no attempt has ever been made to determine their types. Though analgesia and anti-inflammatory effect of the two plants may have been caused by one or more of the metabolites in them, it would not be appropriate to ignore the probable contribution of the alkaloid based on the available information.

Mode of action and target identification studies play important roles in drug discovery. The latter usually utilizes combinations of approaches such as biochemical methods, genetic interactions or computational inference, which help us understand mechanisms of action (Drews, 2000). There are various mechanism that are utilized by most analgesic and anti-inflammatory drugs such as inhibition of cyclo-oxygenase (COX) activity (Vane, 2000), stimulation of cholinergic system (Kruse *et al.*, 2014), cannabinoid (Guindon & Hohmann, 2008) and opiate (Al-Hassani, 2011) receptors. Anti-inflammatory effect may be induced via inhibition of COX activity, leukocyte migration, histamine and other vaso-active amine receptors. The antinociceptive effect of a drug on a receptor can be investigated by co-administration of agonists or antagonists and observation of their effect on pain behavior in an animal. Acetylcholine plays an important part by blocking pain transmission (Kruse *et al.*, 2014). It follows therefore that pre-
administration of an antagonists such as atropine an M2 cholinergic receptor blocker inhibits action of cholinergic analgesic hence exacerbating the perception of pain in an animal (Bernardini et al., 2002).

Glutamic acid is an excitatory amino acid neural transmitter that binds to the fast, ligand gated cation channels generating excitatory post synaptic potential on the post synaptic membrane (Connors, 2005, Guyton & Hall, 2006). When acting via N-methyl D-aspartate receptors (NMDARs) subtype glutamate plays a crucial role in excitatory synaptic transmission of pain in the central nervous system (Millan, 1999). The current study aimed at determining whether the alkaloid rich fractions, of S. incanum root and E. divinorum stem and root posse antinociceptive and anti-inflammatory effects and their respective modes of action in mice.

6.2 Materials and methods

6.2.1 Preparation of alkaloid rich fractions

The alkaloid rich fractions were prepared as per method described by Houghton & Roman, (1998). Two hundred grams of plant powder was defatted with petroleum ether then extracted with methanol. The extract was then concentrated using a rotor evaporator under reduced pressure to obtain the crude methanol extract. The extract was treated with 1M hydrochloric acid then partitioned using diethyl ether to remove the flavonoids. Excess ammonium hydroxide was added to the water
acid fraction until pH 8.0. The mixture was then partitioned using chloroform to obtain the alkaloid rich fraction containing primary, secondary and tertiary alkaloids in the chloroform layer and quaternary alkaloids in the aqueous layer as described by Houghton & Roman, (1998).

6.2.2 Antinociceptive assay of the alkaloid rich fraction

The antinociceptive assays were carried out using formalin test described earlier in chapter three in white albino mice. The various doses of the extract and controls were administered intraperitoneally.

6.2.3 Determination of anti-inflammatory effect

The anti-inflammatory assays were carried out using formalin as a phlogistic substance as described earlier in chapter three in the white albino mice. The various doses of the extract and controls were administered parenterally through the peritoneum.

6.2.4 Antipyretic effect assay

Antipyretic effect of *S. incanum* alkaloid rich fraction was investigated using LPS induced fever in white Wister male rat using method described earlier chapter four while the 50 and 100 mg doses of extract and controls were administered through the peritoneal route.
6.2.5 Determination of the mode of action assay

6.2.5.1 Antinociception mode of action assay

Antinociceptive mechanism of action was carried out using the method used by Mwangi et al. (2011) with some modifications. In the study either atropine or ketamine (receptor blockers) were injected intraperitoneally, 15 minutes prior to administration of the alkaloid rich fractions, and controls (normal saline and diclofenac sodium) in white albino mice. Thirty minutes after administration of the various treatments, the mice underwent the nociceptive formalin test where pain was induced by injection of 5% formalin solution (Hunskaar et al., 1985). All the treatments were administered intraperitoneally. For each experiment, the mice were divided in groups of five. The dose of atropine was 4 mg while that of ketamine was 2.5 mg/kg. All the animals underwent a sensory motor test before injection with formalin to rule out any sensory motor impairment induced by the treatments see chapter three.

6.2.5.2 Histamine induced pedal edema assay

In this study, inflammation was induced by injection of histamine instead of formalin in the paw of white albino mice thirty minutes after intraperitonal administration of various doses of the alkaloid rich fractions and controls.
Chlorpheniramine was used as the positive control and the paw diameter was measured using digital callipers as described in chapter three.

6.2.6 Data analysis

The data obtained for each set of experiment was pooled as a mean and standard errors of the mean and analyzed using unpaired Student’s t test with a value of p < 0.05 considered significant.
6.3 Results and discussion

6.3.1 Anti-inflammatory effects

On edema reducing effect, the 100 mg doses of *S. incanum* and *E. divinorum* root as well as the 500 mg of the stem exhibited significant (*p* < 0.05) effect at 120 minutes only (Fig.6.1; Appendix xvi). Apparently these results represent a decrease in activity when compared with the DCM and aqueous extract of the plants seen in Chapter three.

Figure 6.1: Effect of alkaloids rich fraction of *Solanum incanum* and *Euclea divinorum* paw edema. * value of *p* < 0.05 in respect to the vehicle
6.3.2 Antinociceptive effects

The alkaloid rich fractions of the *S. incanum* and *E. divinorum* exhibited significant antinociceptive effects in both the first and second phase of nociception (Fig.6.2; Appendix xvii). This observation seems to indicate that alkaloids fractions may be more potent than both the crude DCM and aqueous extracts.

![Figure 6.2: Anociceptive effect of alkaloid rich fractions of *Solanum incanum* and *Euclea divinorum*. * and ** implies (p < 0.05) and (p < 0.001) as compared to the vehicle respectively](image)

6.3.3 Antipyretic effects assay

In the study, the 50 mg of *S. incanum* failed to show significant antipyretic effect while the 100 mg dose exhibited significant (p < 0.05) effect at 180 minutes following injection of the LPS pyrogen an activity that was comparable to that of diclofenac (Figure 6.3; Appendix xvii). In a previous study, crude DCM
methanolic extract of *S. incanum* was reported to exhibit antipyretic effect to LPS pyrogen (Mwonjoria *et al*., 2011).

![](image)

**Figure 6. 3:** Effect of alkaloid rich fraction of *Solium incanum* on lipopolysacharide induced.*p* < 0.05 as compared to the vehicle

Some of the alkaloids isolated from *S. incanum* include the glycoalkaloid solasonine and solamargine (Fukuhara & Kubo, 1991 and Eltayeb *et al*., 1997) therefore it is highly likely that these alkaloids perhaps singly, together or in combination with others yet to be described may be responsible for the antipyretic activity observed in the study. LPS (exogenous pyrogen) causes release of pro-inflammatory cytokines (endogenous pyrogen) such as Interleukin-1 beta, Interleukin-6 by white blood cells a process that acts as the initial step in the development of fever (Scapini *et al*., 2000).

In the current study, LPS was used to induce the febrile state in the experimental animals. Therefore the alkaloid(s) exhibiting this effect may have interfered with
synthesis or release of these proinflammatory cytokines. However, the pyrogens also cause synthesis of prostaglandin E-2 in the pre-optic area of the anterior hypothalamus which together with prostaglandin dependent pathway which is mediated by macrophage chemotactic factor acts as the final step in the development of fever (Tavares, 2004) It can be speculated that *S. inanum* alkaloids may also have utilized these pathways in alleviation of the induced pyrexia.

6.3.4 Antinociceptive mode of action of *S. inanum* alkaloid rich fraction

Administration of 50 and 100 mg of the alkaloid rich fraction of *S. inanum* resulted in a highly significant (p < 0.001) reduction of time spent in pain behavior in the first phase as compare to the vehicle (Figure 6.4; Appendix xix). Conversely, pretreatment with atropine prior to administration of 50 and 100 mg doses of the alkaloids resulted in a significant (p < 0.05) extension of the time spent in nociception as compare to the respective doses of the herb (Figure 6.4). Atropine antagonizes the effect of acetylcholine by binding and inhibiting its activity on muscarinic M2 acetylcholine receptors subtype which are expressed on nerve endings and are associated with peripheral antinociception, (Bernardini *et al.*, 2002). Stimulation of these receptors either in the central nervous system or the periphery causes antinociception.
These receptors are also stimulated by various agonists leading to analgesic effect. However atropine selectively blocks various types of M acetylcholine receptors in the body including the M2 type and hence exacerbates the feeling of pain by a subject and extends the time spent in nociceptive behavior in animals. In this study, administration of atropine significantly (p < 0.001) extended the nociceptive period after co-administration of the herbal doses (Figure 6.4).

Figure 6.4: Effect of atropine on antinociceptive effects of Solanum incanum alkaloid rich fraction. ‘a’ and ‘b’ implies a value of p < 0.05 while ‘z’ and ‘f’ show p > 0.05

This meant that atropine could have inhibited the effects of the herbal extracts by blocking the M2 cholinergic receptors and thereby obliterating the cholinergic antinociception. Therefore, it is highly probable that the alkaloid rich fraction of S. incanum contains metabolites whose antinociceptive mode of action involves stimulation of the M2 receptors of acetylcholine in the peripheral nervous system.
Muscarinic acetylcholine receptors belong to the G protein-coupled receptors subfamily that regulates many important functions in the peripheral and central nervous system. As a result, there is a great interest in development of a novel selective agonist ligands of muscarinic receptor subtype that can be useful in management of severe pathophysiological pain states (Kruse et al., 2014). Hence the findings study may present a good candidate for that purpose.

**6.3.5 Antinociceptive mode of action of *E. divinorum* alkaloid rich fractions**

In the current study, the the 250 mg dose of the alkaloid rich fraction from the stem antinociception was obliterated by pretreatment with ketamine (Fig.6.5; Appendix xx).

![Figure 6.5: Effect of ketamine on the antinociceptive effect of *Euclea divinorum* stem alkaloids. ‘a’ p < 0.05 against the vehicle, ‘b’ significant difference between the two values, ‘c’ no significant difference between the values, z significant difference between the values](image-url)
In this study both ketamine and 100 mg dose of the alkaloid rich fraction of *E. divinorum* root significantly (p < 0.001) attenuated the formalin induced pain behavior in both the first and the second phases of formalin induced nociception. Pretreatment of animals with ketamine before administration of 100 mg dose (ketamine +100) had a significant (p < 0.05) inhibitory effect but did not abolish the analgesic effect of the alkaloid rich fraction in the early phase of the test. Nevertheless it had no significant (p > 0.05) effect on the analgesic effect of the alkaloid fraction in late phase of the formalin test. However when administered together with the 100 mg dose, ketamine significantly (p < 0.001) lengthened the time spent in pain behavior in the second phase relative to the alkaloids rich fraction (Fig. 6.6; Appendix xxi).

![Figure 6.6: Effect of ketamine on the antinociceptive effect of Euclea divinorum root alkaloids. * and ** implies (p < 0.05) and (p < 0.001) respectively as compared to the vehicle, ‘a’ significant differenc between the values at p > 0.05](image-url)
Ketamine and N-methyl-D-aspartate (NMDA) receptor inhibitor was shown to produce potent analgesia during administration (Niesters et al., 2014) while low dose of the drug induce antinociceptive in the rat formalin test (Ahmad et al., 2008).

In this, study, the antinociceptive effect of ketamine alone and ketamine +100 mg of the extract was not significantly different in the early phase of nociception, however it differed significantly from the 100 gm dose of the alkaloid fraction. Since ketamine is a non specific N-methyl-D-aspartate receptor antagonist, it is likely that the alkaloid rich fraction of the *E. divinorum* root bark and ketamine competitively inhibited the same receptor. Hence the antinociceptive activity of alkaloids from the bark extract probably is mediated via the glutamatergic (N-methyl-D-aspartate) receptor blockade. When the treatments were co-administered, both the early and late phases of formalin nociception were not inhibited (Fig. 6.6). A possible explanation could be that the two treatments competed for the same transport mechanisms and or same enzymatic pathway during biotransformation in the CNS. This assertion may be supported by the fact that NMDA receptors are believed to play a pivotal role in the wind-up and central sensitization which is associated with pathogenesis of chronic pain states in the dorsal column of the spinal cord (Woolf & Thompson, 1991).
6.3.7 Effect of alkaloids rich fraction of *E. divinorum* stem on histamine induced pedal edema

In the study, it was observed that the significant (p < 0.05) anti-edema response occurred after one hour following administration of histamine after pretreatment with chlorpheniramine and persisted for the next 30 minutes. However the rich alkaloid fraction of *E. divinorum* exhibited significant (p < 0.05) anti-edema effect after one and half hours which lasted for 30 minutes (Figure 6.7; Appendix xxii).

Figure 6. 7: Effect of alkaloid rich fraction of *Euclea divinorum* stem on histamine induced paw edema in mice. (*p < 0.05)
Histamine is a vasoactive amine formed from decarboxylation of histidine (Connors, 2005). It binds competitively and non-selectively on histamine H1 receptors and perhaps in synergy with H4 receptors causes inflammation (Thurmond et al., 2008, Huang & Thurmond, 2008; Jutel et al., 2009). Binding of the amine on the capillaries causes opening of pores and massive exudates of fluid from the vasculature to the interstitium. It is this escape of fluid and resultant swelling that is referred to as tissue edema a component of acute inflammation (Owen et al., 1980).

Drugs such as chlorpheniramine exert their anti-edema effect by blocking H1 receptors (Owen et al., 1980). In this study the alkaloid rich fraction of E. divinorum stem antagonized the acute edema inducing effect of histamine after 90 minutes. This phenomenon indicates that probably the extract had a histamine blocking effect which probably involved blockade of the H1 receptors or both H1 and H4 receptors.
CHAPTER SEVEN: EVALUATION OF TOXICITY OF ORALLY ADMINISTERED ALKALOID RICH FRACTIONS OF S. incanum AND E. divinorum STEM AND ROOT BARK IN MICE

7.1 Introduction

Consumption of plants extracts for health benefits has been practiced for several millennia. It is therefore not surprising that they are generally believed to be safe for consumption since they are produced from natural source. However, this is not always the case with many phytochemicals possessing potentially harmful side effect that include carcinogenic activity (Bode & Dong, 2014) and perhaps the extracts of S. incanum and E. divinorum may not be an exception. This study aimed at establishing the toxic effect of orally administered dose of alkaloid rich fractions of S. incanum and E. divinorum stem and root on biochemical parameters, hemopoetic effects, and tissue histological appearance in mice.

7.2 Materials and methods

The toxicity test was carried out using the method previously used by Ngari et al. (2013) with some modifications. Briefly one month old male Swiss albino mice divided into groups of five were given either 1000 mg/kg doses of alkaloid rich fractions of the S. incanum root, E. divinorum stem and root or normal saline orally daily for 28 days. The body weight of the animals was taken before administration then every 7 days during the experimental period. On the
28 days, the animals were bled from the tail and blood placed in vials without coagulant for biochemical analysis, and from cardiac puncture and placed in heparinized vials for assay of hematological parameters after which they were euthanized by dropping them in a jar containing cotton wool soaked in chloroform. From the euthanized animals brain, spleen, liver, kidneys, testes, heart and, lungs were harvested, weighed and placed in 10% formalin. They were later processed for histological purposes, sectioned, mounted on a glass slide and stained using hematoxylin and eosin.

7.2.1 Hematological analysis

The assay for the hematological parameters was carried out using an automated hemoanalyzer and the parameters checked for included both white blood cells (WBC) and red blood cells (RBC) count, packed cell volume (PCV), hemoglobin concentration (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), number of platelets, differential white blood cell count, red cell distribution width (RDW) and plateletcrit (PCT).

7.2.2 Biochemical analysis

The blood samples for biochemical analysis were centrifuged for 10 minutes at 3000 revolutions per minute to obtain plasma which was stored in the refrigerator at negative 20°C until analyzed for the following parameters; alanine
aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (Alb) both total (Tbl) and direct bilirubin (DbI), and gamma glutamate aminotransferase (λGT) using a clinical chemistry auto analyzer.

7.2.3 Data analysis

The data was expressed as mean and standard errors. Unpaired Student’s t-test was used to compare both the test and vehicle treated values with a value of p < 0.05 being considered significant.
7.3 Results and discussion

7.3.1 Effect of the alkaloid rich fractions on some biochemical parameters

The three treatments namely alkaloid rich fractions of *S. incanum* and *E. divinorum* caused no significant (*p* ≥ 0.05) variation in levels of the biochemical parameters tested namely alanine aminotransferase, aspartate aminotransferase, total protein, albumin, both total and direct bilirubin, gamma glutamyltransferase and alkaline phosphatase (Fig. 7.1, 7.2 & 7.3; Appendix xxiii).
Figure 7. 1: Effect of alkaloid rich fraction of *Solanum incanum* on some biochemical parameters


Figure 7. 2: Effect of alkaloid rich fraction of *Euclea divinorum* stem on some biochemical parameters.

Figure 7.3: Effect of alkaloid rich fraction of *Euclea divinorum* root on some biochemical parameters.


Alanine aminotransferase (ALT) is a transaminase that catalyses the transfer of amino groups from alanine to α-ketoglutarate with formation of pyruvate and glutamate. High levels of the enzyme occur in the cytosolic fluid of the hepatocytes though small levels exist in other tissues such as muscles, adipose and brain. It follows therefore that when liver injury occurs, it is released from the hepatocytes resulting in significant elevation of its serum activity level (Xing-Jiu *et al.*, 2006; Liu *et al.*, 2014).

In the study the three treatments i.e. alkaloid rich fractions of *S. incanum* and *E. divinorum* did not alter the levels of activity of the enzyme this probably means that the alkaloid fractions had minimal adverse effect on hepatocytes. Aspartate aminotransferase (AST) is a transaminase which transfers amino group from aspartate to α-ketoglutarate. It mainly occurs in the liver, though small level
is also found in red blood cells, heart cells, muscle tissue, pancreas and kidneys. When the liver is damaged, AST is released into the bloodstream, causing corresponding rise in serum levels of the enzyme activity. It should be noted that its activity though not specific for liver disease, is valuable when used in combination with other enzymes in monitoring the course of various hepatic disorders (Xing-Jiu et al., 2006). The three alkaloid rich fractions hardly increased the AST activity in serum, therefore it is likely that the cells forming the liver parenchyma were not adversely affected.

Gamma-glutamyl transferase (γGT) is mainly involved in the metabolism of glutathione and glutathionylated xenobiotics. Traditionally it has been regarded as a major marker of liver dysfunction, biliary disorders, and alcohol consumption (Emdin et al., 2005; Koenig & Seneff, 2015). It is also an early predictive marker for atherosclerosis, heart failure, arteriosclerosis and plaque, gestational diabetes, and various hepatic disorders such as viral hepatitis, other infectious diseases as well as several cancers with poor prognosis (Koenig & Seneff, 2015). Its elevation is also associated with increased risk of coronary heart disease and hemorrhagic stroke (Emdin et al., 2005).

The results from this study show no significant variation in the activity level of the enzyme (Fig. 7.1, 7.2 & 7.3) which indicates that injury to the hepatocytes and or other associated disorder did not take place. Alkaline phosphatases (ALP) are
metallo-enzymes that hydrolyse organic phosphate esters in an alkaline medium (Ren et al., 2015). This enzyme exhibit low substrate specificity and are commonly elevated in metastatic osteosarcoma hence it is an effective and convenient biomarker of prognosis for the same (Ren et al., 2015), it is also a biomarker for vascular calcification (Lee et al., 2015).

Bilirubin, a yellowish green pigment which is the main degradation product of heme in the hepatocytes after red blood cells are hemolyzed and may be conjugated or unconjugated. Un-conjugated bilirubin is referred to as indirect bilirubin to distinguish it from direct or conjugated type (Dbil). Total bilirubin refers to the sum total of the two types of bilirubins. Conjugation which makes bilirubin more soluble and easily excreted in urine or bile occurs in the liver. Accumulation of the pigment causes jaundice and may be deposited in the basal ganglia of neonate causing a fatal condition known as kernicterus (Hardiker & Suchy, 2006; Guyton & Hall, 2006b; Walter et al., 2013). Normal values of bilirubin even after administration of the extracts meant that there was no hemolytic activity beyond what is normal an indication that the extracts were to be safe (Fig. 7.1, 7.2, & 7.3).

The total plasma protein consists of mainly albumin and globulin fractions. The albumin fraction is mainly synthesized in the liver while the globulin fraction is produced by the B-cells. One of their main functions is maintenance of plasma
oncotic pressure which retains blood within the vascular space. Any damage to
the liver for example, liver cirrhosis and hepatitis result in decreased plasma
proteins levels. The main plasma protein, albumin play a vital role in
maintenance of the plasma colloidal oncotic pressure thus, decrease in this protein
fraction ultimately leads to development of ascites which may be fatal (Walker et
al., 2013). In the current study there was no change in the levels of total proteins
as well as albumin fraction meaning that the hepatocytes were not affected
adversely (Fig. 7.1). Looking back to all these parameters (Fig. 7.1, 7.2 & 7.3
above) it is evident that the hepatocytes damage did not take place after
administration of the various treatments. Therefore it may suffice to say that the
alkaloid rich fractions of these plants are not hepatotoxic.

7.3.2 Effect of the alkaloid rich fractions on hematological parameters

All the herbal treatment with exception of E. divinorum root had no significant
effects on the total red blood cell count with the alkaloids from S. inca
num showing no effects on the quantity of all the formed elements of blood
(Fig. 7.4 & 7.5; Appendix xxiv & xxv).
Figure 7.4: Effect of alkaloids from *Solanum incanum* and *Euclea divinorum* on quantity of formed elements of blood.

Figure 7.5: Effect of alkaloids from *Solanum incanum* and *Euclea divinorum* on differential white blood cell count.

The *E. divinorum* stem highly elevated the number of platelets (thrombocythemia) (Fig. 7.4) while the root exhibited significant reduction in the number of WBC (Fig. 7.4) without alteration in their respective proportions (Fig. 7.5). Platelets play an important role in hemostasis, hence thrombocythemia may result in
hypercoagulable state (Nakashima & Rogers, 2014) that can cause thrombosis and thrombo-embolism in the brain, coronaries, lungs muscles. Large arterial thrombosis may cause mortality, severe neurological, cardiac or peripheral arterial disorders. Thrombosis may also occur in the veins such as deep venous thrombosis, a painful and potentially life threatening condition (Brière, 2007). Hence the *E. divinorum* stem alkaloid rich fractions is likely to aggravated the condition of patients with essential thrombocythemia an acquired myeloproliferative disorder characterized by chronic elevated quantity of platelet with tendency for thrombosis and hemorrhage (Brière, 2007).

Further the extracts from the three herbs did not significantly alter the blood parameters namely; Hb, PCV, MCV, MCHC, RCDW and PCT (Fig. 7.6; Appendix xxvi).
Figure 7. 6: Effect of alkaloids from *Solanum incanum* and *Euclea divinorum* on some blood parameters.


Therefore, it suffices to say that the alkaloid rich fractions of these plants have no deleterious effect on formed elements of blood which are good indicators of the anemic state of the body. Since the treatments showed that no adverse effect on the quantities, it can be argued that they can neither cause anemia nor polycythemia. The treatments also had no effects on the differential cell count (Fig. 7.5), which implies that the leukopenia observed with *E. divinorum* root involved all the fractions of leukocyte.

### 7.3.3 Effect of alkaloid rich fractions of *S. incanum* and *E. divinorum* on histology of selected organs in mice

Table 7.1 shows the effect of administration of the vehicle normal saline on the histological appearance of selected organs. All the tissues showed normal
structure and no sign of inflammatory activity.

Table 7.1: Effect of the normal saline on histology of selected body organs

<table>
<thead>
<tr>
<th>Mice number</th>
<th>Testes</th>
<th>Brain</th>
<th>Liver</th>
<th>Kidney</th>
<th>Intestine</th>
<th>Lungs</th>
<th>Heart</th>
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</table>

-No inflammation, + mild inflammation, ++ moderately severe inflammation, +++ severe inflammation

Table 7.2 shows that orally administered alkaloid rich fraction of *S. incanum* had minimal or no effects on the tissue neutrophil infiltration in the testes, brain, liver, kidney, intestine, lungs, heart and spleen. These results are comparable to those from the control treated animals.

Table 7.2: Effect of *Solanum incanum* alkaloids on selected organs’ histology

<table>
<thead>
<tr>
<th>Mice number</th>
<th>Testes</th>
<th>Brain</th>
<th>Liver</th>
<th>Kidney</th>
<th>Intestine</th>
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-No inflammation, + mild inflammation, ++ moderately severe inflammation, +++ severe inflammation
Table 7.3 shows the effects of orally administered alkaloid rich fraction of *E. divinorum* stem on selected organs in mice. The liver and testes of mice treated with *E. divinorum* stem alkaloids were characterized by severe inflammatory process which involved increased neutrophil infiltration. The treatment had moderately severe inflammatory process on the kidney and the brain and minimal or no effects on intestine, lungs, heart and spleen.

**Table 7.3: Effect of Euclea divinorum stem alkaloids on selected organs histology**

<table>
<thead>
<tr>
<th>Mice number</th>
<th>Testes</th>
<th>Brain</th>
<th>Liver</th>
<th>Kidney</th>
<th>Intestine</th>
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- No inflammation, + mild inflammation, ++ moderately severe inflammation, +++ severe inflammation

Table 7.4 shows the effects of orally administered alkaloid rich fraction of *E. divinorum* root on selected organs in mice. The liver and kidney from animals treated with alkaloids from *E. divinorum* root were characterized by a moderately severe inflammatory process while the testes and intestines had mild effect. The treatment had no apparent effect on the lungs, heart, brain and spleen.
Table 7.4: Effects of *Euclea divinorum* root alkaloids on selected organs histology

<table>
<thead>
<tr>
<th>Mice number</th>
<th>Testes</th>
<th>Brain</th>
<th>Liver</th>
<th>Kidney</th>
<th>Intestine</th>
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</table>

- No inflammation, + mild inflammation, ++ moderately severe inflammation, +++ severe inflammation

Plate 7.1 is a photomicrograph of normal live tissue of mice showing sinusoids intact central lobule, hepatocytes and sinusoids (Eroschenko & Di Fiore, 2013).

Plate 7.1: Effect of normal saline on liver histology x200
Plates 7.2 & 7.3 are a photomicrographs of mice liver treated with alkaloid rich fraction of *E. divinorum* stem. There is no destruction of the general structure of the liver for example the sinusoids are preserved and appears normal just like in the control. Therefore there was minimal damage to the hepatocytes.

**Plate 7. 2: Effect of Euclea divinorum stem alkaloids on liver histology x200**
Plate 7.3: Effect of *Euclea divinorum* root alkaloids on liver histology x400

Plate 7.4 is a photomicrograph of mice liver treated with oral doses of alkaloid rich fraction of *E. divinorum* root. The alkaloid from *E. divinorum* root did not alter the structure of the liver parenchyma or cause significant injury to the hepatocytes. However they increased the tissue neutrophil infiltration which is a sign of acute inflammatory process.
Plate 7.4: Effect of *Euclea divinorum* root’s alkaloids on liver histology

(a) Control x200  (b) *E. divinorum* root x200  (c) *E. divinorum* root x400

*The arrow shows region with acute inflammatory process in the liver*

7.3.4 Effects of alkaloid rich fractions on live and selected organ weights

The organ weight from mice treated with alkaloids rich fractions from *S. incanum* were comparable to those of the saline treated group (Fig. 7.7; Appendix xxviii), similar results were obtained with *E. divinorum* stem and root (Fig. 7.8 & 7.9; Appendix xxviii).

![Graph showing organ weight gain](image-url)

**Figure 7.7:** Effect of *Solanum incanum* alkaloids on selected organ weights
Figure 7.8: Effect of *Euclea divinorum* root’s alkaloid on selected organ weights

Figure 7.9: Effect of *Euclea divinorum* root’s alkaloid on selected organ weights

These finding indicates that these metabolites caused neither atrophy nor hypertrophy of these tissues. The same trend was observed when it came to the total live body weight in *S. incanum* and *E. divinorum* stem treated animals. In these cases, all the alkaloid rich fractions failed to exhibit significant (*p < 0.05*) increase in weight (Fig.7.10; Appendix xxvii).
Nevertheless, *E. divinorum* root treated animals had significantly (p < 0.05) higher weight than in the control (Fig. 7.10; Appendix xxvii).

![Bar chart showing body weight in grams for different treatments](image)

**Figure 7.10: Effect of Solanum inanum and Euclea divinorum alkaloids on live weight.** * implies p < 0.05 as compared to the vehicle

This scenario may have resulted from increased growth rate, decreased metabolic rate or perhaps even edema. Therefore it appears that *E. divinorum* root the extract contains metabolite(s) with weight enhancing effect as one of their toxic effect in mice but with no effect on the selected individual organs. Therefore it is likely that the observed weight gain may have resulted from systemic edema or weight gain in the other organs not tested in this study. The other two plant parts metabolites probably had no weight enhancing effect.

The *E. divinorum* root significantly increased the mean live weight in mice. However *E. divinorum* stem and *S. inanum* had no effect the body weight. Increase in total body weight may be due to in elevated growth rate and food
consumption or perhaps generalized edema. In the current scenario, the root extract of \textit{E. divinorum} have caused either of the two effects. However, increase in growth is usually associated with change in organs weight which was not observed in this case. Therefore the most likely explanation for the weight gain may be edema development.
CHAPTER EIGHT: PHYTOCHEMICAL ANALYSIS OF S. incanum, C. pumilum AND E. divinorum EXTRACTS

8.1 Introduction

Plants contain a variety of secondary metabolites that are responsible for their pharmacological and physiological activities. These metabolites or phytochemicals are grouped into several classes namely the alkaloids, saponins, flavonoids, phenolics, terpenes and tannins. The alkaloids are cyclic compounds that contain one or more basic nitrogen group in their rings (Barbosa-Filho et al., 2006). Flavonoids (originally “vitamin P”) are phenol derivatives occurring in a wide range of plants.

Plant extracts including alkaloids exert a variety of pharmacological activities that include antioxidants (Dilipkuma & Preeti, 2013), analgesia (Feng et al., 2012) and anti-inflammatory effects (Ferrandiz & Alcaraz, 1991). The tannins are also powerful antioxidants (Vinson et al., 1995). The terpenes are associated with the term essential oils. They consist of hydrocarbons chain with one or more C=C double bonds, while their derivatives are referred to as terpenoids. These secondary metabolites differ in composition and quantities in different plants as well as plants from different climatic condition and ecological zones which may also impart on the pharmacological effects of the plants. These secondary metabolite help the plants mitigate the effects of ecological stresses. These
metabolites are responsible for the pharmacological activity observed in plant extracts and are less likely to be as toxic as their synthetic counterparts. They are also important as raw materials in synthesis or semi synthesis of various drugs. The alkaloids, which comprise one of the largest single class of secondary plant metabolite, posses a significant range of pharmacological activity which includes anti-inflammatory activity (Barbosa-Filho et al., 2006; Souto et al., 2010) and antinociceptive effects (Radulovic et al., 2013). Therefore, it is highly probable that the analgesia and anti-inflammatory effects observed in the current study may be due to these phytochemicals individually or combined with others.

The aim of this study was to determine the phytochemicals composition as well as the major types of alkaloids present in S. incanum and E. divinorum.

8.2 Materials and methods

8.2.1 Qualitative phytochemical screening of S. incanum, C. pumilum and E. divinorum extracts

The qualitative phytochemical screening was carried out using the methods used by Rasool et al. (2010). It involved detection of various groups of secondary metabolites present in the plant materials with minimal emphasis on their quantities as follows:
8.2.1.1 Tannins

About 2 ml of 5% FeCl₃ was added to 2 ml of each of the aqueous extracts of the plant. Formation of yellow brown precipitate indicated that tannins are present (Rasool et al., 2010).

8.2.1.2 Glycosides

About 1 ml glacial acetic acid and 1-2 drops of FeCl₃ was added to about 2 ml alcoholic filtrate of each of the plant extracts, followed by 1 ml of concentrated sulphuric acid. Appearance of brown ring at the interface indicated presence of cardiac glycosides (Rasool et al., 2010).

8.2.1.3 Terpenes

About 2 ml of each of the three aqueous extracts was measured and placed in a test tube, then 5 ml chloroform, 2 ml acetic anhydride and concentrated sulphuric acid was added carefully to form layer. Reddish brown coloration of the interface indicated the presence of terpenes (Rasool et al., 2010).
8.2.1.4 Flavonoids

Qualitative assay for flavonoids was carried out using Shimoda’s test where a few drops of concentrated hydrochloric acid followed by 0.5 g of Zinc turnings were added to a tube containing 2 ml of each of the three aqueous extracts. The tubes were placed in a boiling water bath for few minutes. Formation of magenta, red or pink color indicated presence of flavonoids (Rasool et al., 2010).

8.2.1.5 Phenolics

About 1ml of 1% iron (III) chloride solution was added to 2ml of each of the three aqueous extracts, development of blue or green color indicated presence of phenols.

8.2.1.6 Alkaloids

About 1.5 ml of 1% hydrochloric acid was added to 2 ml of methanolic filtrate of each of the three samples. Then, the solution was heated and this was followed by addition of 6 drops of Dragendorff’s reagent. An orange precipitate confirmed the presence of alkaloids (Rasool et al., 2010).

8.2.1.7 Saponins

A few drops of sodium hydrogen carbonate solution was added to 2 ml of each of the aqueous extract of all samples and shaken to mix. Appearance and persistence of froth indicated presence of saponins.
8.2.2 Quantitative phytochemical screening

Quantitative phytochemical involved detection and quantification of various selected groups of secondary metabolites in the plant extracts was carried out using the methods described by Rasool et al. (2013) as follows;

8.2.2.1 Tannins

The determination of tannins was carried out using the method described by Graham, (1992). Briefly 2g of plant powder was extracted with 70% acetone, decanted then centrifuged. The supernatant was mixed with 1ml of 0.016 molar potassium ferricyanide (K₃F (CN)₆) followed by 1ml of 0.02 molar of ferric chloride (FeCl₃) in 0.1 molar hydrochloric acid. The tubes were placed on a vortex mixer for 15 minutes. This was followed by addition of 5ml of a stabilizer i.e. water, phosphoric acid and 1% gum Arabica in the ratio of 3:1:1 and mixing repeated. The absorbance of the mixture was determined at 700 nanometres (nm) against a blank. Gallic acid was used as a control and it’s absorbance at various concentrations was used to plot the standard curve.

8.2.2.2 Alkaloids

About 2.5 grams of crude herbal powder was extracted using a deciliter of 20% acetic acid in acetyl alcohol. The solution was covered and allowed to stand for 4 hours before filtration. The filtrate was then concentrated to a volume of 25 ml and concentrated ammonium solution added drop wise. Then the solution was left
to stand for a while to allow the precipitate to sediment. This was followed by washing of the precipitate with dilute ammonium solution and filtration. The pellets that formed were dried to a constant weight.

8.2.2.3 Saponins

Approximately 100 ml of 20% aqueous ethanol was added to 10 g the sample powder place on a waterbath shaker for 4 hours at 55° C. It was then filtered and concentrated to 40 ml in a water bath at 90° C. The solution obtained was put into a separating funnel. Then 10 ml of diethyl ether was added and shaken vigorously after which the aqueous layer was obtained while the ether layer was discarded. The process was repeated again after which n-butanol was added to the aqueous layer. The mixture was washed twice with 10 ml of 5% aqueous sodium chloride solution in a separating funnel. The upper layer was separated and placed in a hot water bath for evaporation then placed in an oven and allowed to dry to constant weight.

8.2.2.4 Phenolics

About 1 g of the herb powder was extracted with 10 ml of 80% ethanol. The supernatant was evaporated to dryness. The extract was dissolved in 5 ml of distilled water then different aliquots 0.1-1 ml was transferred to test tubes using a pipette. Distilled water was added to the aliquots to a final volume of 3 ml followed by 0.5 ml Folin’s reagent and 2 ml of 20% sodium carbonate the tubes
were vortexed and placed in boiling water bath for 1 minute. On cooling, the absorbance was read at 650 nm against the blank and a standard curve plotted using different concentrations of 1001 % catechol (standard).

8.2.3 Determination of types of alkaloids

Determination of the major alkaloid composition in extracts was carried out using Liquid chromatography-Quadrupole Time of flight MS (LC Q-ToF MS) in full scan MS⁸ in positive mode based on accurate mass measurements, fragmentation pattern and reference spectra database as described in method used by Wamalwa et al. (2014).
8.3 Results and Discussion

8.3.1 Qualitative phytochemical analysis

The plant materials examined contained alkaloids, tannins, saponins, glycosides, terpenes, flavonoids and phenolics. *Solanum incanum* contained all the tested type of secondary metabolites. Similar results were obtained in other studies (Muriithi *et al.* 2015). However; *E. divinorum* had all the secondary metabolites except terpenes a finding which concurs with observation made by (Ngari *et al.*, 2014), while *C. pumilum* contained all the tested phytochemicals with exception of alkaloids. (Table 6.0).

Table 8.1: Results of phytochemical analysis of the three plant extracts

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th><em>S. incanum</em></th>
<th><em>E. divinorum</em> root</th>
<th><em>E. divinorum</em> stem</th>
<th><em>C. pumilum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>Tannins</td>
<td>+</td>
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<td>Saponins</td>
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<td>Flavanoids</td>
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<tr>
<td>Phenolics</td>
<td>+</td>
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* + Present, - Absent
8.3.2 Quantitative phytochemical analysis

Figure 8.1: Quantity of secondary metabolites in *Solanum incanum* root

Figure 8.2: Quantity of secondary metabolites in *Euclea divinorum* stem bark
Figure 8.3: Quantity of secondary metabolites in *Euclea divinorum* root bark

All the tested secondary metabolites were present in variable quantities in the three plant extracts. A large proportion of the secondary metabolites in *S. incanum* consisted of flavonoids at 340.45 mg/g, followed by saponins at 10.49 mg/g, tannins at 7.36 mg/g and phenols at 2.171 mg/g. The alkaloids fraction at 0.05 contributed the least amount proportionally (Fig. 8.1).

In the *E. divivnorum* stem bark, the alkaloids showed a similar trend again representing the least amount at 0.18 mg/g. The tannins followed with 3.07 mg/g, then saponins at 7.41 mg/g. Total phenols contributed 17.955 mg/g while the again the Flavonoids had the highest amount at 143.9 mg/g (Fig. 8.2).
The Flavonoids and the phenols at 483.71 and 18.701 mg/g respectively formed the bulk of secondary metabolites in *E. divinorum* root bark extract. The alkaloids at 0.19 mg/g represented the least fraction. The plant extract had substantial amount of tannins and saponins at 6.328 and 7.09 mg/g respectively (Fig. 8.3).

### 8.3.3 Liquid chromatography quadrapple time of flight Mass spectrometry results

#### 8.3.3.1 *Solanum incanum* alkaloids

Q -ToF-MS major peaks for *S. incanum* (Fig. 8.4) at m/z 868 ([M+H]⁺ equivalent to m/z 867 or beta solamargine then at m/z 1002 [M+H]⁺ equivalent mass m/z 1001 ([M+H]+ 133 units) which corresponded to solamargine plus a xylose. The peak at m/z 722 ([M+H] -146 units) corresponding to beta solamargine that had lost a rhamnosyl group. Another peak at m/z 576 ([M+H]-146-146 units) due to loss of 2 rhamnose and finally at m/z 414 ([M+H]-146-146-162 units) this corresponds to loss of (2-rhamnose & 1-hexose residues) this corresponds to beta solamargine with 3 sugars. The m/z 414 corresponded to an aglycone solasodine. A peak m/z 1016 [M+H]⁺ corresponding to mass m/z 1015 (Fig. 8.5) corresponds to solamargine with a terminal rhamnose residue. This fragmentation pattern is characteristic of (solamargine) steroidal alkaloids with 4 sugars found in *Solanum* spp. (Wanyonyi *et al.*, 2002).
The Q-ToF m/s (Fig. 8.5) shows intense peak at m/z 1016 [M+H]⁺ equivalent to m/z 1015, at m/z 882 corresponding to ([M+H] -133 units). This is equivalent to loss of xylose, then m/z 720 here 147 and 162 units probably due loss of a rhamnose and hexose residues, at m/z 574 where 147, 162, and 146 units are lost indicating loss of a rhamnose, a hexose and another rhamnose residue. Finally a peak at m/z 413 (solasodine) representing a loss of 162 units which corresponds to loss of a hexose (either galactose or glucose) residues. Over all the metabolite lost a total of 2 rhamnose and 2 hexose molecules as per the fragmentation pattern.
This may indicate that the compound is a glycoside of solasodine containing four sugars namely 2 rhamnosyl and 2 hexose residues (Wanyonyi et al., 2002).

Figure 8. 5: Q-ToF fragmentation pattern of Solanum incanum alkaloids
8.3.3.2 *Euclea divinorum* alkaloids

The alkaloid fraction from the stem bark of *E. divinorum* had relatively fewer peaks in Q ToF-MS (Figure 8.6). An intense peaks occurred at m/z 473 corresponding to [M+H] attributed molecular mass of 472, and m/z 457(M+H –15 units) probably due to loss of either a methyl group. Another peak is seen at m/z 317(M+H – 15 -140) attributed to loss of yet to be identified compound with a relative molecular mass of 140 units. The aglycone hand a molecular mass of 317.

![Figure 8.6: Q-ToF MS fragmentation pattern of *Euclea divinorum* stem alkaloids](image-url)
The Q-ToF MS for the *E. divinorum* root bark alkaloids had a intense peak at $m/z$ 863 $[\text{M}+\text{H}]^+$ equivalent to mass $m/z$ 862, then $m/z$ 731 $([\text{M}+\text{H}]-133$ units) corresponding to loss of 1 xylose, $m/z$ 599 $([\text{M}+\text{H}-133]-133$ units) corresponding to loss of 2 xylose, 467 $([\text{M}+\text{H}-133-133]-133$ units) corresponding to loss of 3 xylose and an aglycone $m/z$ 335 $([\text{M}+\text{H}-133-133-133]-133$ units) corresponding to loss of xylose due to loss of 4 xylose residues.

Figure 8.7: Q-ToF fragmentation pattern of *Euclea divinorum* root alkaloids
Several studies have reported the presence of alkaloids in the *E. divinorum* extracts (Amusan *et al.*, 2007; Ngari *et al.*, 2014) though there is no report on their quantities or the types.

From the results of the quantitative assay, the three plants contained a variety of secondary metabolites that differed in their proportions with the alkaloids contributing the least amount. From the fragmentation patterns the *S. incanum* mainly contained the steroidal alkaloid solamargine. The *E. divinorum* stem yielded an alkaloid that probably contains a methyl group and another group with 140 as its molecular mass while the root contained a glycoside alkaloid with four xylose residues. The aglycone from this plant could not be identified from the MS database and therefore more chemical studies especially x-ray crystallography and nuclear magnetic resonance need to be conducted to shed light on its structure. The rich fractions of these alkaloids showed significant antinociceptive as well as anti-inflammatory effects, hence it can be assumed that these effects were as a result of the activity of the alkaloids.
CHAPTER NINE: RESULTS SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

9.1 Summary of the results

From the results, both DCM and aqueous extracts of *S. incanum* inhibited development of paw edema and nociception in rats as well as migration of leukocytes in mice. Its alkaloid rich fraction exhibited similar activity besides attenuating the LPS induced fever. Its antinociception was antagonized by pre-administration of atropine. The drug (atropine) blocks M2 cholinergic receptors hence cause elevated feeling of pain. The alkaloid rich fraction did not cause significant abnormalities in the mice. Phytochemical analysis showed that the plant contains several groups of metabolites of varying quantities with the alkaloid rich fraction mainly containing solamargine linked to several sugars. This is evidence that the plant part contains alkaloids and perhaps other secondary metabolites with anti-inflammatory activity whose mechanism includes inhibition of white blood cell migration and analgesic/antinociceptive activity which may be partly due to stimulation of this M2 receptor subtype.

In this study, *C. Pumilum obliterated* edema development but aggravated the nociception. Hence it can be inferred that all these extracts probably contains metabolites with anti-inflammatory effects probably similar in activity to capsaicin.
Both DCM and aqueous extracts of *E. divinorum* stem and root barks inhibited development of paw edema and nociception in rats but had no effect on development of fever following administration of the pyrogen. The DCM extract of the stem and the alkaloid rich fraction of the root significantly delayed the leukocyte migration. The alkaloid rich fraction of the stem delayed paw edema development in mice following injection with histamine while ketamine an N-methyl D aspartate receptor antagonist competitively blocked nociceptive effect of the alkaloid rich fractions of both stem and root. The alkaloid rich fraction caused appreciable acute inflammatory effect in several organs.

Phytochemical analysis showed that the plant contains several groups of metabolites of varying quantities with the alkaloid rich fraction mainly containing glycoside with xylose residues. Therefore it can be inferred that plant stem and root bark contains alkaloids and probably other secondary metabolites with both anti-inflammatory and antinociceptive but no antipyretic effects. The anti-inflammatory mode of action of the crude stem extract and alkaloids rich fraction of the root involved delayed leukocyte diapedesis while those (alkaloids) of the stem involved antagonizing the H1 and H4 histamine receptors. The alkaloids rich fraction of the stem and root bark exhibited antinociception that was blocked by pretreatment with ketamine hence it is likely that the active principles in these plant parts caused analgesia via stimulation of the N-methyl D aspartate
receptor (glutamic acid receptors). The crude extract of E. divinorum root had no effect on the white blood cell migration, an observation that was contrary to the effect of alkaloid rich fraction. Therefore it is likely that the crude extract contained metabolites which antagonized the effect of the alkaloids on leukocyte behavior.

9.2 Conclusions

Solanum incanum contained secondary metabolites that included alkaloids which inhibited nociception, paw edema, leukocyte migration and lipopolysaccharide induced fever. The main alkaloids include solamargine and its derivatives with varied glycosidic linkages which probably caused analgesia through inhibition of muscarinic (M2) receptors of acetylcholine.

Craterostigma pumilum contained several groups of metabolites but no significant levels of alkaloids. One or several of these phytochemicals may have been responsible for the hyperalgesic and anti-inflammatory effects seen in animals treated with the herbs extracts.

Euclea divinorum stem and root barks contained phytochemicals with both antinociceptive and anti-inflammatory effect but no antipyretic effects. One of the antinociceptive mode of action involved inhibition of N-methyl D aspartate receptor while the anti-inflammatory activity of stem alkaloid rich fraction involved antagonizing the H1 and H4 histamine receptors but those for the root it
involved delaying the process of the leukocyte migration. The plant contains several groups of metabolites of varying quantities with the alkaloid rich fraction mainly containing unidentified glycosidic alkaloids with xylose residues.

Therefore it can be inferred that the; extracts of *S. incanum* and *E. divinorum* has antinociceptive effects in rats while that of the extracts of *S. incanum*, *C. pumilum* and *E. divinorum* has metabolites that exhibit anti-inflammatory effects in mice an observation that supports the folklore use of the these plant extracts for pain and inflammation.

**9.3. Recommendations**

**9.3.1 Recommendations from the study**

**On Solanum incanum**

(i) Since *S. incanum* alkaloids rich fraction exhibited both analgesia, anti-inflammatory and antipyretic effects. These are desirable effects which require more studies with the aim of developing a novel drug for these ailments.

(ii) Most analgesic drugs used today target arachidonic acid metabolites however cholinergic system which is an important pain integrating mechanism may offer an alternative line of therapy. This places the alkaloid rich fraction of *S. incanum* in a unique position as a potential candidate for development of a drug targeting that system. Therefore more research on specificity and clinical application of these alkaloids is indicated.
iii) The scope of the current study did not allow all aspects of toxicity studies, therefore more requires to be done before the plant extract can be candidly declared save to consume.

**On Craterostigma pumilum**

*C. pumilum* showed elevated anti-inflammatory activity therefore it is likely to be a good source of an anti-inflammatory remedy hence more investigation is needed to isolate elucidate and determine the activity as well as the toxicity of the metabolite(s) responsible for this activity. However it highly elevated the agonizing or chronic phase of nociception which may indicate that the crude extract or a metabolite may have been responsible for this effect. Therefore more studies require to be done targeting both the mechanism and toxicity and applicability of these plant metabolites in research and use as a substitute for capsaicin as an agonist instudy of vanellloid receptor studies and use as a component of tear gas during riot control.

**On Euclea divinorum**

(i) The studies on *E. divinorum* stem and root extract showed a high analgesic and anti-inflammatory activity. Though the alkaloids may have inhibited pain via N-methyl D aspartate receptors more studies need to be initiated to determine whether other mechanisms or receptors exist for the same function.

(ii) The alkaloids had analgesic and anti-inflammatory activity, however they
caused acute inflammatory reaction in several organs a phenomenon that indicates that some metabolites present are toxic, therefore more studies needs to be done in order to identify and isolate them.

(iii) The Q-TOF MS study showed that *E. divinorum* alkaloids rich fractions probably contained xylopyanosyl glycosides whose identity is unknown. There is need for more chemical studies on these plants to try and elucidate the structure of these alkaloids.

**9.3.2 Recommendations for further work**

(i) Cholinergic system is an important player in pain processing in the nervous system. However it also plays a vital role in many cellular functions, therefore most cholinergic receptor agonists with nociceptive activity end up possessing numerous side effects. There is a great interest in development of novel M2 specific cholinergic receptor agonist for use as an analgesic. Alkaloid rich fraction of *S. incanum* caused analgesia by stimulating the M2 receptors therefore more research require to be done to determine the extent of its specificity and also on chemical modification that can be done to improve its activity.

(ii) Cancer causes a very severe intractable type of chronic pain which is believed to be mediated through NMDA receptor (Mehrotra & Koiri, 2015; Gu *et al.*, 2009) containing NR2B subunit in the spinal cord. *E. divinorum* alkaloid rich fractions caused analgesia via inhibition of NMDA receptors however it is
not known whether it has any effect on NR2B subunit. Therefore more work need to be done on it receptor selectivity and effect on the intractable cancer pain.

(iii) Both qualitative and quantitative phytochemical analysis showed that several other secondary metabolites beside the alkaloids were present in the plant materials. The analgesic and phlogistic effects were no screened for. Hence future study should endeavor to determine whether some or all metabolites have the similar activity.
REFERENCES


APPENDICES

Appendix i

Table 3.4.1a. Show the effect of DCM extract of *S. incanum* root on formalin induced paw edema in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>10mg</td>
<td>2.02 ± 0.22</td>
<td>2.04 ± 0.24</td>
<td>2.03 ± 0.15</td>
<td>2.16 ± 0.28*</td>
<td>2.13 ± 0.24*</td>
<td>2.3 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>25mg</td>
<td>1.12 ± 0.16*</td>
<td>1.31 ± 0.16*</td>
<td>1.84 ± 0.10*</td>
<td>1.76 ± 0.17**</td>
<td>1.64 ± 0.12**</td>
<td>1.79 ± 0.12*</td>
<td></td>
</tr>
<tr>
<td>50mg</td>
<td>1.63 ± 0.18*</td>
<td>1.92 ± 0.20</td>
<td>2.23 ± 0.31</td>
<td>2.21 ± 0.31*</td>
<td>2.07 ± 0.35*</td>
<td>2.18 ± 0.33</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>2.09 ± 0.09</td>
<td>2.43 ± 0.1</td>
<td>2.13 ± 0.16</td>
<td>1.94 ± 0.12*</td>
<td>1.76 ± 0.08*</td>
<td>2.31 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Vehide</td>
<td>2.15 ± 0.12</td>
<td>2.75 ± 0.18</td>
<td>2.86 ± 0.18</td>
<td>3.37 ± 0.19</td>
<td>3.35 ± 0.24</td>
<td>3.09 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.83 ± 0.34</td>
<td>3.26 ± 0.41</td>
<td>3.25 ± 0.43</td>
<td>3.38 ± 0.38</td>
<td>3.58 ± 0.38</td>
<td>3.22 ± 0.09</td>
<td></td>
</tr>
</tbody>
</table>

Values in the table represent change in edema diameter in millimeters.

Appendix ii

Table 3.4.1b. Show effect of *S. incanum* aqueous extract on formalin induced paw edema in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>25mg</td>
<td>0.79 ± 0.05**</td>
<td>0.99 ± 0.11**</td>
<td>1.04 ± 0.13**</td>
<td>0.99 ± 0.15**</td>
<td>0.81 ± 0.15**</td>
<td>0.77 ± 0.23**</td>
<td></td>
</tr>
<tr>
<td>50mg</td>
<td>1.84 ± 0.11</td>
<td>1.85 ± 0.17</td>
<td>1.88 ± 0.19</td>
<td>1.94 ± 0.10</td>
<td>1.62 ± 0.19</td>
<td>1.44 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>100mg</td>
<td>1.65 ± 0.12</td>
<td>1.75 ± 0.11*</td>
<td>1.86 ± 0.06</td>
<td>1.94 ± 0.07</td>
<td>2.17 ± 0.12</td>
<td>1.49 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1.45 ± 0.18*</td>
<td>1.14 ± 0.19*</td>
<td>0.84 ± 0.26**</td>
<td>0.65 ± 0.19**</td>
<td>0.55 ± 0.26**</td>
<td>0.43 ± 0.18**</td>
<td></td>
</tr>
<tr>
<td>Vehide (NS)</td>
<td>2.26 ± 0.19</td>
<td>2.52 ± 0.17</td>
<td>2.52 ± 0.11</td>
<td>2.4 ± 0.16</td>
<td>2.33 ± 0.07</td>
<td>2.2 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>Formalin</td>
<td>2.44 ± 0.18</td>
<td>2.82 ± 0.27</td>
<td>2.84 ± 0.25</td>
<td>2.8 ± 0.29</td>
<td>2.75 ± 0.23</td>
<td>2.69 ± 0.22</td>
<td></td>
</tr>
</tbody>
</table>

Values in the table represent change in edema diameter in millimeters.
Appendix iii

Table 3.4.2a. Show effect of DCM extract of *C. pumilum* on formalin induced paw edema in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg</td>
<td>0.17 ± 0.13</td>
<td>1.76 ± 0.13</td>
<td>2.25 ± 0.9</td>
<td>2.16 ± 0.16</td>
<td>2.15 ± 0.21</td>
<td>2.15 ± 0.21**</td>
<td>2.27 ± 0.36</td>
</tr>
<tr>
<td>50 mg</td>
<td>1.28 ± 0.11**</td>
<td>1.17 ± 0.11**</td>
<td>1.11 ± 0.11**</td>
<td>1.17 ± 0.23**</td>
<td>1.17 ± 0.16*</td>
<td>1.18 ± 0.11**</td>
<td>1.29 ± 0.11**</td>
</tr>
<tr>
<td>100 mg</td>
<td>1.48 ± 0.11</td>
<td>1.52 ± 0.19*</td>
<td>1.55 ± 0.13*</td>
<td>1.05 ± 0.17**</td>
<td>0.96 ± 0.22**</td>
<td>0.84 ± 0.15**</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>2.09 ± 0.09</td>
<td>2.43 ± 0.1</td>
<td>2.38 ± 0.16</td>
<td>2.38 ± 0.12</td>
<td>1.74 ± 0.08**</td>
<td>2.51 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Vehicle(DMSO)</td>
<td>2.15 ± 0.19</td>
<td>2.75 ± 0.17</td>
<td>2.86 ± 0.11</td>
<td>3.37 ± 0.19</td>
<td>3.35 ± 0.16</td>
<td>3.09 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Formalin</td>
<td>2.83 ± 0.34</td>
<td>3.29 ± 0.41</td>
<td>3.52 ± 0.43</td>
<td>3.58 ± 0.38</td>
<td>3.56 ± 0.38</td>
<td>3.23 ± 0.09</td>
<td></td>
</tr>
</tbody>
</table>

Values in the table represent change in edema diameter in millimeters.

Appendix iv

Table 3.4.2b. Show effect of aqueous extract of *C. pumilum* on formalin induced change in paw edema in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg</td>
<td>1.76 ± 0.18</td>
<td>2.17 ± 0.28</td>
<td>2.37 ± 0.53</td>
<td>1.94 ± 0.21</td>
<td>2.04 ± 0.39</td>
<td>2.10 ± 0.33</td>
<td></td>
</tr>
<tr>
<td>50 mg</td>
<td>1.29 ± 0.06*</td>
<td>1.22 ± 0.06*</td>
<td>1.29 ± 0.1</td>
<td>1.19 ± 0.13*</td>
<td>1.05 ± 0.15</td>
<td>1.13 ± 0.17*</td>
<td></td>
</tr>
<tr>
<td>100 mg</td>
<td>1.16 ± 0.11**</td>
<td>1.184 ± 0.21**</td>
<td>1.32 ± 0.12</td>
<td>1.17 ± 0.17**</td>
<td>1.08 ± 0.19*</td>
<td>0.78 ± 0.22*</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1.45 ± 0.18*</td>
<td>1.14 ± 0.19**</td>
<td>0.83 ± 0.26*</td>
<td>0.65 ± 0.19**</td>
<td>0.55 ± 0.26**</td>
<td>0.43 ± 0.18**</td>
<td></td>
</tr>
<tr>
<td>Vehicle(NS)</td>
<td>2.26 ± 0.19</td>
<td>2.52 ± 0.17</td>
<td>2.52 ± 0.11</td>
<td>2.4 ± 0.16</td>
<td>2.32 ± 0.07</td>
<td>2.2 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>Formalin</td>
<td>2.44 ± 0.18</td>
<td>2.82 ± 0.27</td>
<td>2.84 ± 0.25</td>
<td>2.8 ± 0.29</td>
<td>2.75 ± 0.23</td>
<td>2.69 ± 0.22</td>
<td></td>
</tr>
</tbody>
</table>

Values in the table represent change in edema diameter in millimeters.

Appendix v

Table 3.4.3a. Shows effect of DCM extract of *E. divinorum* stem on formalin induced paw edema in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mg</td>
<td>1.9 ± 0.19</td>
<td>2.19 ± 0.11*</td>
<td>2.08 ± 0.14**</td>
<td>1.85 ± 0.26**</td>
<td>1.9 ± 0.18**</td>
<td>1.41 ± 0.24**</td>
<td></td>
</tr>
<tr>
<td>100 mg</td>
<td>1.18 ± 0.17**</td>
<td>1.55 ± 0.09**</td>
<td>1.29 ± 0.22**</td>
<td>1.25 ± 0.25**</td>
<td>1.24 ± 0.15**</td>
<td>1.65 ± 0.13**</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>2.09 ± 0.09</td>
<td>2.43 ± 0.1</td>
<td>2.13 ± 0.16</td>
<td>1.94 ± 0.12*</td>
<td>1.7 ± 0.08**</td>
<td>2.51 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>2.15 ± 0.12</td>
<td>2.75 ± 0.18</td>
<td>2.86 ± 0.17</td>
<td>3.37 ± 0.19</td>
<td>3.35 ± 0.24</td>
<td>3.09 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Formalin</td>
<td>2.83 ± 0.34</td>
<td>3.29 ± 0.41</td>
<td>5.25 ± 0.43</td>
<td>3.58 ± 0.38</td>
<td>3.56 ± 0.38</td>
<td>3.23 ± 0.9</td>
<td></td>
</tr>
</tbody>
</table>

Values in the table represent change in edema diameter in millimeters.
Appendix vii

Table 3.4.3b. Shows effect of aqueous extract of *E. divinorum* stem on formalin induced paw edema in rats.

<table>
<thead>
<tr>
<th>Doses</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg</td>
<td>1.2±0.12**</td>
<td>1.2±0.11**</td>
<td>1.22±0.14**</td>
<td>1.25±0.2**</td>
<td>0.99±0.15**</td>
<td>0.91±0.22**</td>
<td></td>
</tr>
<tr>
<td>50 mg</td>
<td>1.4±0.1*</td>
<td>1.47±0.07**</td>
<td>1.22±0.05**</td>
<td>1.29±0.07**</td>
<td>1.2±0.1*</td>
<td>1.05±0.1**</td>
<td></td>
</tr>
<tr>
<td>100 mg</td>
<td>1.37±0.11*</td>
<td>1.6±0.25**</td>
<td>1.18±0.09**</td>
<td>1.04±0.05**</td>
<td>0.81±0.13**</td>
<td>0.75±0.09**</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1.45±0.18*</td>
<td>1.4±0.19**</td>
<td>0.84±0.26**</td>
<td>0.65±0.19**</td>
<td>0.55±0.26**</td>
<td>0.43±0.18**</td>
<td></td>
</tr>
<tr>
<td>Vehicle(N/S)</td>
<td>2.26±0.19</td>
<td>2.52±0.17</td>
<td>2.52±0.11</td>
<td>2.4±0.16</td>
<td>2.33±0.07</td>
<td>2.2±0.22</td>
<td></td>
</tr>
<tr>
<td>Formalin</td>
<td>2.44±0.18</td>
<td>2.82±0.27</td>
<td>2.84±0.25</td>
<td>2.8±0.29</td>
<td>2.75±0.23</td>
<td>2.69±0.22</td>
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</tr>
</tbody>
</table>

Values in the table represent change in paw diameter in millimeters

Appendix viii

Table 3.4.4a. Shows effect of DCM extract of *E. divinorum* root on formalin induced paw edema in rats.

<table>
<thead>
<tr>
<th>Doses</th>
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<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mg</td>
<td>2.42±0.06</td>
<td>2.95±0.21</td>
<td>2.6±0.13</td>
<td>2.87±0.18</td>
<td>3.18±0.17</td>
<td>2.82±0.12</td>
<td></td>
</tr>
<tr>
<td>100 mg</td>
<td>1.68±0.14**</td>
<td>1.78±0.17**</td>
<td>2.09±0.19*</td>
<td>2.18±0.17**</td>
<td>2.18±0.17***</td>
<td>1.89±0.18***</td>
<td>1.83±0.27***</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>2.09±0.09</td>
<td>2.43±0.1</td>
<td>2.13±0.16</td>
<td>1.94±0.12**</td>
<td>1.7±0.08**</td>
<td>2.51±0.06</td>
<td></td>
</tr>
<tr>
<td>Vehicle(N/S)</td>
<td>2.15±0.12</td>
<td>2.75±0.18</td>
<td>2.86±0.17</td>
<td>3.7±0.19</td>
<td>3.55±0.24</td>
<td>3.09±0.09</td>
<td></td>
</tr>
<tr>
<td>Formalin</td>
<td>2.83±0.34</td>
<td>3.29±0.41</td>
<td>3.25±0.43</td>
<td>3.58±0.38</td>
<td>3.56±0.38</td>
<td>3.23±0.09</td>
<td></td>
</tr>
</tbody>
</table>

Values in the table represent change in paw diameter in millimeters

Appendix viii

Table 3.4.4b. Shows effect of aqueous extract of *E. divinorum* root on formalin induced paw edema in rats.

<table>
<thead>
<tr>
<th>Doses</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg</td>
<td>0.77±0.12**</td>
<td>0.84±0.11**</td>
<td>0.94±0.05**</td>
<td>0.81±0.11**</td>
<td>0.49±0.06**</td>
<td>0.89±0.14**</td>
<td></td>
</tr>
<tr>
<td>50 mg</td>
<td>1.45±0.26*</td>
<td>1.66±0.17**</td>
<td>1.66±0.17**</td>
<td>1.66±0.23*</td>
<td>1.42±0.15*</td>
<td>1.29±0.08**</td>
<td></td>
</tr>
<tr>
<td>100 mg</td>
<td>0.65±0.07**</td>
<td>1.2±0.14**</td>
<td>1.15±0.17**</td>
<td>0.98±0.25**</td>
<td>1.05±0.2**</td>
<td>1.25±0.24**</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1.45±0.18**</td>
<td>1.14±0.19**</td>
<td>0.84±0.26**</td>
<td>0.65±0.19**</td>
<td>0.55±0.26**</td>
<td>0.43±0.18**</td>
<td></td>
</tr>
<tr>
<td>Vehicle(N/S)</td>
<td>2.26±0.19</td>
<td>2.52±0.17</td>
<td>2.52±0.11</td>
<td>2.4±0.16</td>
<td>2.33±0.07</td>
<td>2.2±0.22</td>
<td></td>
</tr>
<tr>
<td>Formalin</td>
<td>2.44±0.18</td>
<td>2.82±0.27</td>
<td>2.84±0.25</td>
<td>2.8±0.29</td>
<td>2.75±0.23</td>
<td>2.69±0.22</td>
<td></td>
</tr>
</tbody>
</table>

Values in the table represent change in paw diameter in millimeters
Appendix ix

Table 3.4.5a. Show the effect of DCM extract of *S. ineanum* root on formalin induced nociception

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg</td>
<td>228 ± 12</td>
<td>228 ± 12</td>
</tr>
<tr>
<td>25 mg</td>
<td>216 ± 14.7</td>
<td>144 ± 36*</td>
</tr>
<tr>
<td>50 mg</td>
<td>84 ± 14.7**</td>
<td>144 ± 36**</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>132 ± 12**</td>
<td>84 ± 24**</td>
</tr>
<tr>
<td>Vehicle (DCM)</td>
<td>204 ± 30.6</td>
<td>312 ± 29.4</td>
</tr>
<tr>
<td>Formalin</td>
<td>228 ± 12</td>
<td>336 ± 40.7</td>
</tr>
<tr>
<td>Morphine</td>
<td>42.4 ± 7.49**</td>
<td>23.6 ± 3.08**</td>
</tr>
</tbody>
</table>

Appendix x

Table 3.4.5b. Show the effect of aqueous extract of *S. ineanum* on formalin induced nociception in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg</td>
<td>246 ± 8.06*</td>
<td>175.8 ± 8.84*</td>
</tr>
<tr>
<td>50 mg</td>
<td>241 ± 6.4*</td>
<td>209.2 ± 36.51*</td>
</tr>
<tr>
<td>100 mg</td>
<td>243.4 ± 7.48*</td>
<td>215.6 ± 23.65 *</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>228.6 ± 9.43**</td>
<td>184.8 ± 17.69*</td>
</tr>
<tr>
<td>Vehicle (N/S)</td>
<td>282.8 ± 3.61</td>
<td>452 ± 82.06</td>
</tr>
<tr>
<td>Formalin</td>
<td>252 ± 12</td>
<td>336 ± 40.7</td>
</tr>
<tr>
<td>Morphine</td>
<td>42.4 ± 7.49**</td>
<td>23.6 ± 3.08**</td>
</tr>
</tbody>
</table>
Appendix xi

Table 3.4.6a show the effect of DCM extract of C. pumilum on formalin induced nociception in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg</td>
<td>269.8 ± 1.85</td>
<td>725 ± 29.92</td>
</tr>
<tr>
<td>50 mg</td>
<td>269.6 ± 3.26</td>
<td>556.8 ± 2.64</td>
</tr>
<tr>
<td>100 mg</td>
<td>268. ± 63.31</td>
<td>736.6 ± 19.74</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>132 ± 12*</td>
<td>84 ± 19.8**</td>
</tr>
<tr>
<td>Vehicle (DMSO)</td>
<td>204±30.6</td>
<td>312 ± 24.6</td>
</tr>
<tr>
<td>Formalin</td>
<td>260 ± 12.65</td>
<td>336 ± 40.69</td>
</tr>
<tr>
<td>Morphine</td>
<td>42.4 ± 7.49 **</td>
<td>23.6 ± 3.08 **</td>
</tr>
</tbody>
</table>

Appendix xii

Table 3.4.6b show the effect of aqueous extract of C. pumilum on formalin induced nociception in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg</td>
<td>245.0 ± 10.69</td>
<td>512.4±68.21</td>
</tr>
<tr>
<td>50 mg</td>
<td>241.2 ± 11.32</td>
<td>311.8 ± 11.67</td>
</tr>
<tr>
<td>100 mg</td>
<td>243.4 ± 9.36</td>
<td>339.4 ± 36.94</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>228.6 ± 9.43 *</td>
<td>184.8 ± 17.69**</td>
</tr>
<tr>
<td>Vehicle (N/S)</td>
<td>282.8 ± 3.61</td>
<td>452.0 ± 36.14</td>
</tr>
<tr>
<td>Formalin</td>
<td>252.0±12</td>
<td>336.0 ± 40.60</td>
</tr>
<tr>
<td>Morphine</td>
<td>42.4 ± 7.49 **</td>
<td>23.6 ± 3.08 **</td>
</tr>
</tbody>
</table>
Appendix xiii

Table 3.4.7a show the effect of DCM extract of *E. divinorum* stem bark on formalin induced nociception in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mg</td>
<td>240 ± 19.2</td>
<td>96 ± 24.0**</td>
</tr>
<tr>
<td>100 mg</td>
<td>240 ± 21.0</td>
<td>72 ± 4.2**</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>132 ± 12.0</td>
<td>84 ± 19.8**</td>
</tr>
<tr>
<td>Vehicle (DMSO)</td>
<td>204 ± 30.6</td>
<td>312 ± 24.6</td>
</tr>
<tr>
<td>Formalin</td>
<td>260 ± 122.7</td>
<td>336 ± 40.7</td>
</tr>
<tr>
<td>Morphine</td>
<td>42.4 ± 7.5**</td>
<td>23.6 ± 3.1**</td>
</tr>
</tbody>
</table>
Appendix xiv

Table 3.4.7b. Show the effect of aqueous extract of *E. divinorum* stem bark on formalin induced nociception in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg</td>
<td>255 ± 11</td>
<td>226.4 ± 58.6*</td>
</tr>
<tr>
<td>50 mg</td>
<td>228 ± 7.4**</td>
<td>213 ± 18.7*</td>
</tr>
<tr>
<td>100 mg</td>
<td>235.4 ± 12.8</td>
<td>260.4 ± 38.7**</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>222.6 ± 5.7**</td>
<td>184.8 ± 17.7*</td>
</tr>
<tr>
<td>Vehicle (N/S)</td>
<td>282.8 ± 3.6</td>
<td>388 ± 21.3</td>
</tr>
<tr>
<td>Formalin</td>
<td>278 ± 3.7</td>
<td>360 ± 35.2</td>
</tr>
<tr>
<td>Morphine</td>
<td>42.4 ± 7.49**</td>
<td>23.6 ± 3.08**</td>
</tr>
</tbody>
</table>

Appendix xv

Table 3.4.8a. Show the effect of DCM extract of *E. divinorum* root bark on formalin induced nociception in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mg</td>
<td>108 ± 12*</td>
<td>132 ± 22.5**</td>
</tr>
<tr>
<td>100 mg</td>
<td>120 ± 19*</td>
<td>128 ± 14.6**</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>132 ± 12.0*</td>
<td>84 ± 19.8**</td>
</tr>
<tr>
<td>Vehicle (DMSO)</td>
<td>204 ± 30.6</td>
<td>312 ± 24.6</td>
</tr>
<tr>
<td>Formalin</td>
<td>278 ± 3.7</td>
<td>336 ± 40.7</td>
</tr>
<tr>
<td>Morphine</td>
<td>42.4 ± 7.5**</td>
<td>23.6 ± 3.1**</td>
</tr>
</tbody>
</table>
Table 3.4.8b show the effect of aqueous extract of *E. divinorum* root bark on formalin induced nociception in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg</td>
<td>206 ± 5.1</td>
<td>232.2 ± 33.2*</td>
</tr>
<tr>
<td>50 mg</td>
<td>256 ± 13.3**</td>
<td>166.4 ± 13.6*</td>
</tr>
<tr>
<td>100 mg</td>
<td>248.6 ± 6.1</td>
<td>201.4 ± 20.7*</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>222.6 ± 5.7**</td>
<td>184.8 ± 17.7*</td>
</tr>
<tr>
<td>Vehicle (N/S)</td>
<td>282.8 ± 3.6</td>
<td>388 ± 21.3</td>
</tr>
<tr>
<td>Formalin</td>
<td>278 ± 3.7</td>
<td>360 ± 35.2</td>
</tr>
<tr>
<td>Morphine</td>
<td>42.4 ± 7.49**</td>
<td>23.6 ± 3.08**</td>
</tr>
</tbody>
</table>
Appendix xvi

Figure 6.1. Anti-inflammatory effects of alkaloid rich fractions of *S. incanum* and *E. divinorum*

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle(NS)</td>
<td>0.00 ± 0.03</td>
<td>1.35 ± 0.04</td>
<td>1.49 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>100 mg <em>S. incanum</em></td>
<td>0.31 ± 0.11</td>
<td>1.34 ± 0.09</td>
<td>1.12 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>500 mg <em>E. divinorum</em> stem</td>
<td>1.29 ± 0.11</td>
<td>1.26 ± 0.04</td>
<td>1.2 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>100 mg <em>E. divinorum</em> root</td>
<td>1.25 ± 0.07</td>
<td>1.16 ± 0.12</td>
<td>1.12 ± 0.09</td>
<td></td>
</tr>
</tbody>
</table>
Appendix xvii

Figure 6.2. Antinociceptive effect of alkaloid rich fractions of *S. incanum* and *E. divinorum*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg <em>S. incanum</em></td>
<td>163 ± 14.62</td>
<td>95.6 ± 9.41</td>
</tr>
<tr>
<td>500 mg <em>E. divinorum</em> stem</td>
<td>167.2 ± 10.07</td>
<td>46 ± 6.20</td>
</tr>
<tr>
<td>100 mg <em>E. divinorum</em> root</td>
<td>159 ± 7.94</td>
<td>52 ± 3.81</td>
</tr>
<tr>
<td>Vehicle</td>
<td>251.8 ± 13.16</td>
<td>330.8 ± 23.50</td>
</tr>
</tbody>
</table>

Appendix xviii

Table 6.3 Show the effect of alkaloid rich fractions of *S. incanum* lipopolysaccharide induced fever in rats

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mg dose</td>
<td>37.3 ± 0.00</td>
<td>37.18 ± 0.04</td>
<td>37.48 ± 0.14</td>
<td>37.82 ± 0.3</td>
<td>37.66 ± 0.11</td>
</tr>
<tr>
<td>100 mg dose</td>
<td>37.09 ± 0.00</td>
<td>37.12 ± 0.06</td>
<td>37.18 ± 0.16</td>
<td>37.26 ± 0.1</td>
<td>37.32 ± 0.09</td>
</tr>
<tr>
<td>Vehicle</td>
<td>37.24 ± 0.00</td>
<td>37.62 ± 0.22</td>
<td>37.8 ± 0.25</td>
<td>37.85 ± 0.21</td>
<td>37.94 ± 0.12</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>37.1 ± 0.00</td>
<td>37.64 ± 0.05</td>
<td>37.72 ± 0.06</td>
<td>37.54 ± 0.07</td>
<td>37.46 ± 0.05</td>
</tr>
</tbody>
</table>

(a) Values in the table represent the mean rectal temperature in °C ± SEM
Appendix xix

Effect of atropine on antinociceptive effects of alkaloid rich fraction of S. incanum

<table>
<thead>
<tr>
<th></th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>251.8</td>
<td>330.8</td>
<td>13.16</td>
<td>23.5</td>
</tr>
<tr>
<td>Diclofenac 132</td>
<td>84</td>
<td>12</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>50mg</td>
<td>154</td>
<td>76</td>
<td>10.77</td>
<td>12.08</td>
</tr>
<tr>
<td>50mg+atropine</td>
<td>237</td>
<td>139.4</td>
<td>17.66</td>
<td>9.52</td>
</tr>
<tr>
<td>100mg</td>
<td>192.6</td>
<td>187.6</td>
<td>11.73</td>
<td>60.06</td>
</tr>
<tr>
<td>100mg+atropine</td>
<td>265.8</td>
<td>166</td>
<td>4.96</td>
<td>69.47</td>
</tr>
</tbody>
</table>

Appendix xx

Effect of ketamine on alkaloids rich fraction of E. divinorum root antinociception

<table>
<thead>
<tr>
<th></th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (NS)</td>
<td>251.8</td>
<td>330.8</td>
<td>13.16</td>
<td>23.5</td>
</tr>
<tr>
<td>100 mg</td>
<td>167.6</td>
<td>27.4</td>
<td>9.58</td>
<td>3.5</td>
</tr>
<tr>
<td>Ketamine only</td>
<td>191.8</td>
<td>152.6</td>
<td>5.37</td>
<td>11.9</td>
</tr>
<tr>
<td>Ketamine+100</td>
<td>205.8</td>
<td>41.6</td>
<td>2.46</td>
<td>20.84</td>
</tr>
<tr>
<td>100+Ketamine</td>
<td>239.6</td>
<td>336.6</td>
<td>10.78</td>
<td>65.04</td>
</tr>
</tbody>
</table>

Appendix xxi

Effect of ketamine on alkaloids rich fraction of E. divinorum stem antinociception
Appendix xxii

Effect of alkaloids rich fractions of *E.divinorum* on histamine induced edema

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>0.88</td>
<td>0.85</td>
<td>0.97</td>
<td>0.85</td>
<td>0</td>
<td>0.12</td>
<td>0.08</td>
<td>0.04</td>
<td>0.1</td>
</tr>
<tr>
<td>250 mg</td>
<td>0</td>
<td>0.61</td>
<td>0.78</td>
<td>0.51</td>
<td>0.58</td>
<td>0</td>
<td>0.11</td>
<td>0.13</td>
<td>0.1</td>
<td>0.07</td>
</tr>
<tr>
<td>500 mg</td>
<td>0</td>
<td>0.86</td>
<td>0.69</td>
<td>0.79</td>
<td>0.7</td>
<td>0</td>
<td>0.14</td>
<td>0.15</td>
<td>0.14</td>
<td>0.11</td>
</tr>
<tr>
<td>Histamine</td>
<td>0</td>
<td>0.91</td>
<td>1.02</td>
<td>1.03</td>
<td>1.04</td>
<td>0</td>
<td>0.11</td>
<td>0.11</td>
<td>0.08</td>
<td>0.15</td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>0</td>
<td>0.84</td>
<td>0.51</td>
<td>0.54</td>
<td>0.53</td>
<td>0</td>
<td>0.04</td>
<td>0.08</td>
<td>0.15</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Appendix xxiii

Table 7.1. Show the effect of alkaloid rich fractions of *S. incanum* and *E. divinorum* on some biochemical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>S. incanum</th>
<th>E. div stem</th>
<th>E. div root</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>62.4</td>
<td>108.8</td>
<td>48</td>
<td>53</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>242.4</td>
<td>263.2</td>
<td>314.4</td>
<td>241.4</td>
</tr>
<tr>
<td>TP (g/L)</td>
<td>28.42</td>
<td>30.4</td>
<td>32.6</td>
<td>25.04</td>
</tr>
<tr>
<td>ALB (g/L)</td>
<td>20.2</td>
<td>21.16</td>
<td>21.26</td>
<td>17.74</td>
</tr>
<tr>
<td>TBIL (µM/L)</td>
<td>261.4</td>
<td>168.75</td>
<td>287.96</td>
<td>221.48</td>
</tr>
<tr>
<td>DBIL (µM/L)</td>
<td>99.18</td>
<td>110.92</td>
<td>112.84</td>
<td>83.4</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>-1.2</td>
<td>-1.6</td>
<td>-0.8</td>
<td>-1</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>-2.6</td>
<td>0</td>
<td>-1.4</td>
<td>-1.2</td>
</tr>
</tbody>
</table>

Appendix xxiv

Figure 7.2. Effect of alkaloid rich fractions of *S. incanum* and *E. divinorum* on formed elements of blood

<table>
<thead>
<tr>
<th>Treatment</th>
<th>White blood cells</th>
<th>Erythrocytes</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>16.3</td>
<td>7.92</td>
<td>1017.8</td>
</tr>
<tr>
<td><em>S. incanum</em></td>
<td>15.62</td>
<td>8.41</td>
<td>1079</td>
</tr>
<tr>
<td><em>E. divinorum</em></td>
<td>11.56</td>
<td>8.42</td>
<td>1148.8</td>
</tr>
<tr>
<td><em>E. divinorum</em></td>
<td>14.02</td>
<td>8.08</td>
<td>8804</td>
</tr>
</tbody>
</table>
Appendix xxv

Table 7.3. Show the effect of alkaloid rich fractions of *S. incanum* and *E. divinorum* on differential cell count in mice

<table>
<thead>
<tr>
<th></th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Neutrophils</th>
<th>Basophils</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>70.82</td>
<td>15.56</td>
<td>13.62</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>S. incanum</em></td>
<td>68.64</td>
<td>16.84</td>
<td>14.52</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>E. divinorum</em> root</td>
<td>60.58</td>
<td>19.55</td>
<td>19.95</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>E. divinorum</em> stem</td>
<td>67.46</td>
<td>17.62</td>
<td>15.76</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Appendix xxvi

Table 7.4. Show the effect of alkaloid rich fractions of *S. incanum* and *E. divinorum* on some blood parameters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hb (g/dl)</th>
<th>PCV (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
<th>RDW</th>
<th>PCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>12.98</td>
<td>59</td>
<td>74.48</td>
<td>17.54</td>
<td>22</td>
<td>17.56</td>
<td>0.57333</td>
</tr>
<tr>
<td><em>S. incanum</em></td>
<td>13.12</td>
<td>59.94</td>
<td>71.46</td>
<td>15.64</td>
<td>21.88</td>
<td>18.08</td>
<td>0.57</td>
</tr>
<tr>
<td><em>E. div. root</em></td>
<td>13.72</td>
<td>56.98</td>
<td>68.7</td>
<td>16.44</td>
<td>16.02</td>
<td>16.82</td>
<td>0.73</td>
</tr>
<tr>
<td><em>E. div. stem</em></td>
<td>13.58</td>
<td>59.04</td>
<td>68.88</td>
<td>15.3</td>
<td>16.82</td>
<td>16.82</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Appendix xxvii

Table 7.5. Show the effect of alkaloid rich fractions of *S. incanum* and *E. divinorum* on body weight in mice

<table>
<thead>
<tr>
<th>Time in days</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>4.28</td>
<td>6.92</td>
<td>8.9</td>
<td>9.65</td>
</tr>
<tr>
<td><em>S. incanum</em></td>
<td>0</td>
<td>2.96</td>
<td>4.48</td>
<td>6.42</td>
<td>7.62</td>
</tr>
<tr>
<td><em>E. divinorum</em> stem</td>
<td>0</td>
<td>3.4</td>
<td>4.06</td>
<td>5</td>
<td>5.78</td>
</tr>
<tr>
<td><em>E. divinorum</em> root</td>
<td>0</td>
<td>2.6</td>
<td>3.96</td>
<td>5.78</td>
<td>7.12</td>
</tr>
</tbody>
</table>

Values represent changes in live weight in grams
Appendix xxviii

Table 7.6. Show the effect of alkaloid rich fractions of *S. incanum* and *E. divinorum* on some organ weight

<table>
<thead>
<tr>
<th></th>
<th>Brain</th>
<th>Liver</th>
<th>Kidney</th>
<th>Lungs</th>
<th>Spleen</th>
<th>Testes</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.432</td>
<td>1.818</td>
<td>0.392</td>
<td>0.31</td>
<td>0.222</td>
<td>0.16</td>
<td>0.108</td>
</tr>
<tr>
<td><em>S. incanum</em></td>
<td>0.458</td>
<td>1.738</td>
<td>0.426</td>
<td>0.284</td>
<td>0.208</td>
<td>0.2</td>
<td>0.15</td>
</tr>
<tr>
<td><em>E. divinorum</em> stem</td>
<td>0.52</td>
<td>1.84</td>
<td>0.452</td>
<td>0.404</td>
<td>0.268</td>
<td>0.224</td>
<td>0.132</td>
</tr>
<tr>
<td><em>E. divinorum</em> root</td>
<td>0.522</td>
<td>1.882</td>
<td>0.48</td>
<td>0.354</td>
<td>0.186</td>
<td>0.528</td>
<td>0.142</td>
</tr>
</tbody>
</table>

Values represent weight change in grams

Appendix xxix

Effects of water extract *S. incanum* on carrageenan induced leukocyte migration

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>sem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone</td>
<td>312</td>
<td>12.22</td>
</tr>
<tr>
<td>25mg</td>
<td>492</td>
<td>52.76</td>
</tr>
<tr>
<td>50mg</td>
<td>352</td>
<td>63.75</td>
</tr>
<tr>
<td>100mg</td>
<td>736</td>
<td>51.54</td>
</tr>
<tr>
<td>Vehicle</td>
<td>976</td>
<td>217.84</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>1208</td>
<td>101</td>
</tr>
</tbody>
</table>
Appendix xxx

Effects of water extract *E. divinorum* on carrageenan induced leukocyte migration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean</th>
<th>sem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>296</td>
<td>24</td>
</tr>
<tr>
<td>25 mg. <em>E. div</em> stem</td>
<td>224</td>
<td>24</td>
</tr>
<tr>
<td>50 mg <em>E. div</em> stem</td>
<td>248</td>
<td>20.4</td>
</tr>
<tr>
<td>25 mg <em>E. div</em> root</td>
<td>224</td>
<td>24</td>
</tr>
<tr>
<td>50 mg <em>E. div</em> root</td>
<td>256</td>
<td>89.98</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>128</td>
<td>15</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>336</td>
<td>45.61</td>
</tr>
</tbody>
</table>

Appendix xxxi

Effects of DCM extract *S. incanum* on carrageenan induced leukocyte migration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>10 mg</th>
<th>25 mg</th>
<th>50 mg</th>
<th>Dexa</th>
<th>Vehicle</th>
<th>carrageenan</th>
<th>Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean no. of wbc</td>
<td>256</td>
<td>280</td>
<td>248</td>
<td>296</td>
<td>424</td>
<td>1208</td>
<td>120</td>
</tr>
<tr>
<td>Sem</td>
<td>37</td>
<td>25</td>
<td>23</td>
<td>10</td>
<td>27</td>
<td>101</td>
<td>18</td>
</tr>
</tbody>
</table>
Appendix xxxii

Effects of DCM extract *E. divinorum* stem on carrageenan induced leukocyte migration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean no. of leukocytes</th>
<th>Sem</th>
</tr>
</thead>
<tbody>
<tr>
<td>25mg</td>
<td>208</td>
<td>34</td>
</tr>
<tr>
<td>50mg</td>
<td>152</td>
<td>20</td>
</tr>
<tr>
<td>100mg</td>
<td>408</td>
<td>47</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>296</td>
<td>10</td>
</tr>
<tr>
<td>Vehicle</td>
<td>424</td>
<td>27</td>
</tr>
<tr>
<td>Baseline</td>
<td>120</td>
<td>18</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>1208</td>
<td>101</td>
</tr>
</tbody>
</table>

Appendix xxxii

Effects of DCM extract *E. divinorum* root on carrageenan induced leukocyte migration

<table>
<thead>
<tr>
<th></th>
<th>Mean no. of leukocytes</th>
<th>sem</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mg</td>
<td>480</td>
<td>58</td>
</tr>
<tr>
<td>100 mg</td>
<td>400</td>
<td>28.28</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>296</td>
<td>10</td>
</tr>
<tr>
<td>Vehicle</td>
<td>424</td>
<td>27</td>
</tr>
<tr>
<td>Baseline</td>
<td>120</td>
<td>18</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>1208</td>
<td>101</td>
</tr>
</tbody>
</table>

Appendix xxxiii

Antipyretic effect of DCM extract of *E. divinorum*

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Diclofenac</th>
<th><em>E. div root</em></th>
<th><em>E. div stem</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>37.18</td>
<td>37.32</td>
<td>37.05</td>
</tr>
<tr>
<td>30</td>
<td>37.48</td>
<td>37.89</td>
<td>37.64</td>
</tr>
<tr>
<td>60</td>
<td>37.68</td>
<td>37.98</td>
<td>37.82</td>
</tr>
<tr>
<td>120</td>
<td>37.86</td>
<td>38.32</td>
<td>38.15</td>
</tr>
<tr>
<td>180</td>
<td>37.82</td>
<td>38.30</td>
<td>38.16</td>
</tr>
</tbody>
</table>