Evaluation of the analgesic and anti-inflammatory activities of 80% methanol leaf extract of *Stephania abyssinica* (Quart.-Dill. & A. Rich.) Walp. (Menispermaceae) in mice

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This is to certify that the thesis prepared by Tigist Leyikun, entitled 'Evaluation of the analgesic and anti-inflammatory activities of 80% methanol leaf extract of *Stephania abyssinica* (Quart.-Dill. & A. Rich.) Walp. (Menispermaceae) in mice' and submitted in partial fulfillment of the requirements for the degree of Masters of Science in Pharmacology complies with regulations of the university and meets the accepted standards with respect to originality and quality.

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Abstract

Stephania abyssinica is used in Ethiopian traditional medicine for the treatment of stomachache, headache and inflammation. The present study evaluated the 80% methanol leaf extract of *S. abyssinica* for its analgesic and anti-inflammatory activities. Dried leaves of the plant were extracted using maceration technique. Antinociceptive and antinflammatory activity were evaluated using hot plate test, acetic acid induced writhing test, formalin test and carrageenan induced paw edema test. Swiss albino mice of either sex were randomly assigned into five groups of six mice each. The extract reduced the number of writhing at all doses though to a lesser extent than aspirin. Increase in latency time was observed throughout the observation time. Greater increase in latency was observed at the 30 minute and lesser on the other time intervals. In the formalin test, 200 mg/kg and 400 mg/kg of the extract reduced paw-licking time more significantly in the second phase. During carrageenan-induced paw edema test, paw volume was considerably decreased in the second phase. The plant extract contained alkaloids, flavonoids, tannins and saponins. This study showed that the extract had potential analgesic and anti-inflammatory activity.

Key words: Analgesic activity, Anti- inflammatory activity, *Stephania abyssinica*

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List of Abbreviations /Acronyms

ASA	Acetyl Salicylic Acid
CNS	Central Nervous System
COX	Cyclooxyginase
DRG	Dorsal Root Ganglion
5-HT	5-Hydroxytryptamine
IASP	International Association for Study of Pain
IBD	Inflammatory Bowel Disease
IL	Interleukin
NO	Nitric Oxide
NSAIDs	Non-Steroidal Anti -Inflammatory Drugs
NRM	Nucleus Raphe Magnus
PDGF	Platelet Derived Growth Factor
PG	Prostaglandin
PMN	Polymorphonuclear cell
(PAG)	periaqueductal grey matter
TGF-β	Transforming Growth Factor-β
TNF-α	Tumor Necrosis Factor-α
WHO	World Health Organization

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1 Introduction

1.1 Definition of pain and inflammation

The International Association for Study of Pain (IASP) defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (Bridges, 2011; Rajagopal, 2006). Pain is a specific enteroceptive sensation with the following features; i) it can be perceived as arising from a particular portion of the body, ii) its temporal properties can be detailed, iii) it can be differentiated qualitatively, and iv) it involves dedicated subsets of peripheral and central neurons. The experience of pain has a distinctly unpleasant character, that is, an affective or motivational aspect that can be distinguished from its discriminative sensory aspects (Craig and Sorkin, 2001). Pain is the result of a complex interplay between signaling systems, modulation from higher centers and the unique perception of the individual. It is a subjective experience, which cannot be easily measured. It requires consciousness. Pain comes in many flavors such as burning, shooting, dull, sharp, prickly and many others (Steeds, 2009; Mills, 2007).

Inflammation is a pervasive phenomenon that operates during severe perturbations of homeostasis and is triggered by innate immune receptors that recognize pathogens and damaged cells (Ashley, 2012). It is defined by the presence of five macroscopic pathological phenomena; these are swelling of the tissue, elevated tissue temperature, blood color-like redness of vascularized tissue at the inflammation site, intensive sensation of a noxious stimulus and impaired function of the organ affected (Stankov, 2012). The sensation of heat is caused by the increased movement of blood through

dilated vessels into the environmentally cooled extremities, also resulting in the increased redness. The swelling is the result of increased passage of fluid from dilated and permeable blood vessels into the surrounding tissues, infiltration of cells into the damaged area and in prolonged inflammatory responses due to deposition of connective tissue (Punchard *et al.*, 2004). Inflammation is caused by microbes, physical agents (burns, radiation and trauma), chemicals (toxins, caustic substances), necrotic tissue and/or immunological reactions (Villarreal *et al.*, 2001).

1.2 Classification of pain and inflammation

The experience of pain and inflammation can be divided into acute and chronic types (Helms and Barone, 2008; Wakefield and Kumar, 2001).

Acute pain

Acute pain has sudden onset, felt immediately following injury, is severe in intensity, is usually short-lasting (less than 30 days). It arises as a result of tissue injury stimulating nociceptors and generally disappears when the injury heals (WHO, 2012). It typically has an identifiable cause. The pain is usually confined to the affected area and is limited over time (Renn and Dorse, 2005; Helms and Barone, 2008). Acute pain is useful and a vital physiological sensation, which alerts a person to a harmful environment that should be avoided. If tissue injury occurs following a noxious stimulus, it induces a reversible state of localized hypersensitivity in and around the injured area, resulting in an avoidance of the damaged part and promotes healing (Bader, 2010).

Chronic pain

Chronic pain is recognized as pain, which persists past the normal time of healing. Three months is the most convenient point of division between acute and chronic pain (IASP, 2002; Lamont *et al.*, 2000). Chronic pain is classified by its pathophysiology as nociceptive or neuropathic, with mixed or undetermined causes as well. The pain may begin as acute pain and persist for long periods or may recur due to persistence of noxious stimuli or repeated exacerbation of an injury (WHO, 2012; American Chronic Pain Association, 2013). Chronic pain does not exist to protect from an illness or injury. Due to the apparent changes in neurophysiology, it can be considered a pathological condition itself (Lamont *et al.*, 2000; Australian Physiotherapy Association, 2012).

Acute inflammation

Acute inflammation usually has a sudden onset and characterized by the classical symptoms of redness, heat, edema and pain (Wakefield and Kumar, 2001). Acute inflammation tends to reverse tissue homeostasis towards normality and regarded as a true defensive reaction. It is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process (Stankov, 2012; Phanse, 2012). As soon as the risk factor is removed, the acute inflammatory response will stop. Acute inflammation does not lead to oxidative and nitrosative stress or any serious adverse effect. However, when the risk factor continues to exist, it will progress to chronic inflammation (James and Lily, 2007; Percival, 1999).

Chronic inflammation

Chronic inflammation defined in terms of the process, in which continuing inflammation and attempted tissue healing by repair occur simultaneously. Because the irritant fails to be eliminated in chronic inflammation, it may cause continuing tissue damage in its own right. In addition, most persistent irritants are recognized as foreign antigens by the host immune response, which contributes to the chronic inflammatory process and may add to the tissue destruction. In chronic inflammation, there is typically a less pronounced exudative response and increased inflammatory cellular recruitment, which may be accompanied by local cellular proliferation (Wakefield and Kumar, 2001).

1.3 Prevalence of pain and inflammatory diseases

Pain is an enormous problem globally. Estimates suggest that 20% of adults suffer from pain globally and 10% are newly diagnosed with chronic pain each year (Goldberg and McGee, 2011). The prevalence is rising, with more cases compared with 40 years ago. Studies suggest that it is more commonly reported by women and those from socially or financially disadvantaged groups. The prevalence of chronic pain increased with age, from 14% of men and 18% of women aged 16-34 to 53% of men and 59% of women aged 75 and over (Bridges, 2011). In US, one in three Americans experiences chronic pain. In Europe, the prevalence of chronic pain reached 25-30% (Cohen and Mao, 2014). Chronic pain after surgery is estimated from 10% to 50% (Bridges, 2011). Globally, the prevalence of the adult population with active headache disorders is found to be 46% (Stovner *et al.,* 2007). In Africa, the average lifetime prevalence of low back pain among the adolescents was 36% and among adults was 62% (Louw *et al.,* 2007). In population

based urban study in Ethiopia, prevalence of primary headache disorders was found to be 21.6% and that for migraine was 10% (Mengistu and Alemayehu, 2013). A study in rural southwest Ethiopia reported a prevalence of 16.7% for back pain and 5.0% for neck pain (El-Sayed *et al.*, 2010).

Non-healing wounds are a significant problem for healthcare systems all over the world; it is estimated that in Australia alone 270,000 people suffer from chronic wounds (Rajan and Murray, 2008). Higher rates of inflammatory bowel disease (IBD) are seen in industrialized countries. In the US, specifically about 30,000 new IBD cases are reported each year (Hanauer, 2006). Rheumatoid arthiritis is a chronic debilitating autoimmune disorder that affects about 1% of the population in developed countries (Cardinali and Esquifino, 2003). The global prevalence of allergic diseases seems to have increased with time particularly in the last 3 decades (Potter *et al.*, 2009). In Africa, prevalence of allergic diseases is reported to be in the range of 20–30%. It is higher than commonly reported diseases like tuberculosis and HIV/AIDS (Mbugi and Chilongola, 2010). The incidence of atopic dermatitis appears to be increasing in urban areas and developed countries. Its prevalence in US is 11%. It is estimated that 10-20% of children and 1-3% of adults in developed countries are affected by atopic dermatitis (Watson and Kapur, 2011).

1.4 Pain processing and the inflammatory phase

Pain processing

Interposed between the initial detection of a noxious stimulus by nociceptors and the conscious appreciation of pain is a complex series of mechanisms (Fein, 2012). Pain pathway can be considered as a three-neuron chain, with the first-order neuron originating in the periphery and projecting to the spinal cord, the second-order neuron ascending the spinal cord and the third-order neuron projecting to the cerebral cortex (Lamont *et a1*., 2000). The normal processing of acute, nociceptive pain begins with primary afferent neurons found in the peripheral nervous system. These neurons, known as nociceptors, are specialized to respond to mechanical, thermal and chemical noxious stimuli (Hainline, 2005). Whenever a noxious stimulus hits a nociceptor, a generator potential is elicited after a certain threshold. This evokes an action that travels along the peripheral nerve to the spinal cord dorsal horn (Zeilhofer, 2005). Nociceptors form synapses with second order neurons in the grey matter of the dorsal horn. A proportion of second-order neurons have ascending axons and project to the brain stem or to thalamocortical system that produces conscious pain response. Second-order neurons ascend to higher centers via the contralateral spinothalamic and spinoreticular tracts. Ascending spinothalamic and spinoreticular tracts convey pain to the brain, where pain signals are processed by the thalamus and sent to the cortex (Steeds, 2009). From brain stem nuclei, impulses "descend" onto the spinal cord and influence the transmission of pain signals. Concerning descending inhibition, the periaqueductal grey matter (PAG) is a key region. PAG neurons excite cells in the nucleus raphe magnus (NRM) that in turn

project down to the spinal cord to block pain transmission by dorsal horn cells (Steeds, 2009; Schaible, 2006).

The inflammatory phase

Damage to the blood vessels is an initial consequence of tissue trauma and possibly during microbial invasion, necessitating blood containment and vessel repair. Local macrophages and other cells then sense the insult and produce a panel of inflammatory mediators such as cytokines and chemokines that stimulate the nearby microvasculature and attract large numbers of Polymorphonuclear cell (PMN) to migrate across the vascular wall and infiltrate into tissues (Bian *et al*. 2012). Neutrophils and macrophages that migrate into the wound are responsible for bacterial destruction and removal of foreign material and cell debris (Villarreal *et al.*, 2001). During the repair process, Martin and Leibovich, cited by (Rajan and Murray, 2008), explained that macrophages are thought to play a pivotal role in fibrosis and scarring. They clear the matrix and cell debris. They also secrete a variety of cytokines, growth factors and mediators of inflammation that can coordinate fibroblasts proliferation and angiogenesis during wound closure. Once the wound site is cleaned out, fibroblasts migrate to begin the proliferative phase. By the third day after wounding, they are the predominant cell type in the healing wound. These cells are responsible for producing the new matrix needed to restore structure and function of the injured tissue. The new collagen matrix cross-linked and organized during the final remodeling (Diegelmann and Evans, 2004). Fibroblasts produce the extracellular matrix that fills the healing scar and provides a platform for keratinocyte migration (Gurtner, 2007).

1.5 Pathophysiology of pain and inflammation

Pathophysiology of pain

Pathophysiologic pain occurs in response to damage to the nervous system or result from abnormal operation of the nervous system (Woolf, 2004; Lamont *et al.*, 2000). After severe tissue injury and in inflammatory diseases, a sensory system become sensitized and pain then turn into a disease (Zeilhofer, 2005). An inflammatory response triggers both peripheral sensitization of nociceptors and central sensitization of dorsal horn neurons (American Medical Association, 2013). Inflammation results in profound changes to the chemical environment of the peripheral terminal of nociceptors (Woolf, 2004). The excitation threshold of polymodal nociceptors drops such that normally innocuous stimuli activate the fibers and noxious stimuli evoke stronger responses than in the non-sensitized state (Schaible *et al.*, 2011). During central sensitization, there is an increased excitability of spinal and supraspinal neural circuits (Bennett, 1999). It is triggered in central neurons by inflammation-induced repetitive activation of primary afferent fibers input into the spinal cord (Woolf, 2004; Kidd and Urban, 2001).

Peripheral nerve damage due to trauma or to pathogenic processes can also initiate cellular changes in primary afferent fibres that result in ectopic neural activity, molecular phenotype changes and anatomical sprouting in the spinal dorsal horn (Craig and Sorkin, 2001). Peripheral nociceptors become sensitized by injury so that they: have a lower threshold for firing, increase their response to noxious stimuli, and can fire in response to non-noxious stimuli. Thus, damage at the site of nerve injury or in the cell body in DRG

becomes the foci of hyperexcitability and ectopic discharge (Steeds, 2009; Schaible, 2006).

Pathophysiology of inflammation

The inflammatory pathway is a complex biochemical pathway, which, once stimulated by injury, leads to the production of inflammatory mediators whose initial effect is pain and tissue destruction (Maroon *et al*., 2010). Disruption of one or more of the healing stages can result in prolonged and incomplete repair, with lack of restoration of integrity (Rajan and Murray, 2008). In pathologic conditions, efficient and orderly inflammatory process is lost and the ulcers are locked into a state of chronic inflammation characterized by abundant neutrophil infiltration with associated reactive oxygen species and destructive enzymes (Diegelmann and Evans, 2004). A coordinated series of common effector mechanisms of inflammation contribute to tissue injury, oxidative stress, remodeling of the extracellular matrix, angiogenesis and fibrosis in target tissues (Libby, 2007). During prolonged exposure to risk factor (s), the onset of chronic systemic inflammation generates proinflammatory mediators from the primary site of inflammation, travels via blood vessel to various distant sites of the body, attacks cells at distant organs and tissues, creating new inflammation sites and new inflammatory diseases (James and Lily, 2007; Percival, 1999). The acute inflammatory response is self-limiting and normally results in tissue restoration and the return of tissue homeostasis. However, persistent inflammatory stimuli or dysregulation of mechanisms of the resolution phase results in chronic inflammation recognized to be a key underlying factor in the progression of a range of diseases (Maskrey *et al*., 2011).

1.6 Management of pain and inflammation

1.6.1 Non-pharmacological management

Pain and inflammation management can be divided into pharmacological and non-pharmacological interventions. Studies suggested that nonpharmacological interventions were used in 22% of the pain episodes evaluated (Nadler, 2004). A variety of comfort-producing measures were implemented, including endotracheal tube suctioning, repositioning in bed, bed rest, massage and oral care. Other non-pharmacological measures for critically ill patients include application of heat or cold, therapeutic touch, guided imagery and relaxation techniques, use of manipulation and mobilization, exercise, psychological intervention, bracing and therapeutic modalities including; transcutaneous electrical nerve stimulation, electrical stimulation, ultrasound, superficial heat, and cryotherapy (Helms and Barone, 2008; Nadler, 2004).

1.6.2 Pharmacological management

Based on the understanding of pain neurophysiology, treatment could be aimed toward either reducing excitatory mechanisms or enhancing inhibitory activity (Marchand, 2008). Analgesics are substances which decrease pain sensation by increasing pain threshold to external stimuli (Tripathi, 2003). These drugs include many categories like NSAIDs and opioid analgesics (Gautam *et al.*, 2013).

i) Opioid analgesics

The opioid analgesics are potent analgesics with both peripheral and central actions. In the spinal cord, they produce presynaptic inhibition of nociceptive afferents and

postsynaptic inhibition of nociceptive dorsal horn cells (Baron *et al.*, 2010). Opioid analgesics take advantage of the innate opioid receptor system in the central nervous system (Hainline, 2005). There is evidence for the action of opioids on peripheral nociceptor terminals after tissue damage. Peripheral receptors become active within hours of local tissue damage. This occurs with unmasking of opioid receptors and the arrival of immunocompetent cells that possess opioid receptors and have the ability to synthesize opioid peptides. This finding led to the peripheral administration of opioids for postoperative analgesia (Hudspith *et al.*, 2006). In contrast to NSAIDs, both sensitized release and normal pain transmission are reduced by opiates (Craig and Sorkin, 2001).

ii) Non-steroidal anti -inflammatory drugs (NSAIDs) and Paracetamol

NSAIDs are useful for acute and chronic pain resulting from a variety of disease processes including trauma, arthritis, surgery and cancer. They are both analgesic and anti-inflammatory. They suppress production of inflammatory prostaglandins (Munir *et al.*, 2007). Although they are classified as mild analgesics, NSAIDs have a more significant effect on pain resulting from increased peripheral sensitization during inflammation, which leads to a lowering of the response threshold of polymodal nociceptors (Dugowson and Gnanashanmugam, 2006). Paracetamol acts at all levels of pain stimulus conduction from the tissue receptors through the spinal cord to the thalamus and the cerebral cortex in which pain sensations are evoked. It is a recommended analgesic of first choice to be used for a longtime in symptomatic treatment of slight and moderate pain sensation (Bebenista and Nowak, 2014).

iii) Glucocorticoids

Glucocorticoids act principally through their binding to the specific glucocorticoid receptor and consequent inhibition of cellular signalling pathways that regulate the production of inflammation (Rainsford, 2007). Glucocorticoid receptor mediated pathways directly regulate gene expression. The glucocorticoid-receptor complex can either induce key anti-inflammatory genes that encode anti-inflammatory molecules or selectively repress specific inflammatory genes that encode cytokines, chemokines, adhesion molecules, inflammation associated enzymes, lipid mediators of inflammation and receptors (Derendorf and Meltzer, 2008).

iv) Newer anti-inflammatory and analgesic agents

Cannabinoids

Cannabinoids are a group of compounds that mediate their effects through cannabinoid receptors. Cannabinoid CB (2) receptors are considered to be targets for novel analgesic drugs. In the gut, CB2 receptors are also involved in visceral pain, inflammation, and motility disturbances (Wright *et al.*, 2008). Several studies showed that cannabinoids down regulate cytokine and chemokine production and upregulate T-regulatory cells (Tregs) as a mechanism to suppress inflammatory responses (Nagarkatti *et al.*, 2009). Sativex (THC (delta-9-tetrahydrocannabinol) and CBD (cannabidiol)) and Dronabinol / Marinol (Synthetic Delta-9 THC) are licensed to be used in the market (Reilly, 2013).

Anti - inflammatory cytokines

The net effect of the inflammatory response by cytokine inhibitors is determined by a delicate balance between pro- and anti-inflammatory cytokines. Perturbations in this equilibrium drive the host defence immune response either towards chronic inflammation or towards healing. To date, various anti-inflammatory cytokines have been acknowledged and these include IL-4, IL-6, IL-10, IL-11, IL-13, TGF-β, and various soluble cytokine receptors (Sultani *et al.,* 2012). Five anti-TNF agents are also approved by regulatory agencies for treating rheumatoid arthritis (Moreland, 2009). These are Infliximab (Remicade ®), Etanercept (Enbrel ®), Adalimumab (Humira ®), Certolizumab *(Cimzia ®)* and Golimumab (Simponi ®) (González-Rivera, 2012).

1.7 The experimental plant

The genus S*tephania* belongs to a family Menispermaceae, a large family of about 65 genera and 350 species (Semwal *et al.,* 2010). Two varieties are recognised in *Stephania abyssinica*: var. *abyssinica* and var. *tomentella* (Oliv.) Diels, both with a wide distribution. Var. *abyssinica* is nearly glabrous, whereas various plant parts are hairy in var. *tomentella*. *S. abyssinica* (Figure 1) is a liana wood at the base and 2-3 m high (Anyango, 2011). It occurs in grassland, usually in shady, damp localities, but not in rainforest, up to 3500 m altitude (Grace and Fowler, 2008). Phytochemical investigations on *S. abyssinica* root extract revealed the presence of hasubanan alkaloids: stephaboline, stephavamine, methaphamine and stephabyssine (Anyango, 2011).

This plant traditionally has been used for the treatment of asthma, tuberculosis, hyperglycemia, cancer, fever, sleep disturbances and inflammation (Semwal *et al.*, 2010). In Africa, the juice stem or leaf of *Stephania abyssinica* is taken to treat contusion, dysentery, fracture, cystitis, anemia, rachitis, stomach pains, collitis, diabetes and sterility (Masi *et al.*, 2012). Throughout eastern Africa a root extract is used in malaria therapy and against internal parasites. It is also used as an antidote to snakebites and applied to tortoise bites (Masi *et al.*, 2012). Fruits and roots of *S. abyssinica* used as aphrodisiac and for roundworm (Cousins and Huffman, 2002). In Ethiopia, *S. abyssinica* has a vernacular name Kib kitel or Etse eyesus (in Amharic) and Hidda kalaalaa (in Oromiffa) (Amenu, 2007; Teklehaymanot and Giday, 2007). Leaves and stem of the plant used for treatment of headache and stomach pains (Masi *et al.*, 2012). Fresh stem chewed and extracts swallowed to suppress cough (Beyene, 2011). The extract of the whole plant is used to treat mastatis in cattle by natives (Amare, 1976). Dried leaves of *S. abyssinica* used for stabbing pain by Oromo ethnic group (Yineger, 2008). *S. abyssinica* is used by Zegie people in northern Ethiopia for headache and for stomachache by drinking juice of leaves and stems (Teklehaymanot and Giday, 2007).

Figure 1: Photograph of *Stephania abyssinica* (Grace and Fowler, 2008)

1.8 Rationale for the study

Worldwide about 20% of adults suffer from chronic pain. The current pain therapy is not sufficient for different reasons (Craig and Sorkin, 2001; Schaible *et al.,* 2011). Although several agents are known to treat inflammatory disorders, their prolonged use often lead to a number of side effects (Matthew *et al.*, 2013). Use of NSAIDs is associated with a number of adverse effects such as gastric irritation and gastric ulcer, alterations in renal function, effects on blood pressure, hepatic injury, and platelet inhibition, which may result in increased bleeding. In addition, steroids can disrupt cytokine networks involved in lymphocyte functions, resulting in immuno-suppression (Barrison and Wolfe, 1999).

Despite the progress that has made in past in the development of pain therapy, there is a need for effective and potent analgesic. Gupta *et al.*, cited by (Kaushik *et al.*, 2012), noted that medicinal plants are important sources of new chemical substances with potential therapeutic effects. Many herbal medicines for inflammation and rheumatism have not undergone scientific investigations. The research into plants with alleged folkloric use as pain relievers and anti-inflammatory agents should therefore be viewed as logical research strategy in the search for new and safe analgesic and anti-inflammatory drugs. *S. abyssinica* has many medicinal uses, but little pharmacological research has been done so far (Grace and Fowler, 2008). It has been used to alleviate pain and inflammatory condition for so long but scientific studies were not done so far to support its traditional claim. The present study was done to provide baseline information on the traditional claim of *S. abyssinica* for pain and/or inflammation.

2 Objective

2.1 General objective

- To evaluate analgesic and anti-inflammatory activities of 80% methanol leaf extract of S*tephania abyssinica* in mice

2.2 Specific objectives

- To assess acute toxicity of the leaf extract of S*tephania abyssinica* in mice
- To evaluate the peripheral analgesic activity of the leaf extract of S*tephania abyssinica* using acetic acid induced writhing test in mice
- To evaluate the central analgesic activity of the leaf extract of S*tephania abyssinica* using hot plate test in mice
- To evaluate the analgesic activity against inflammation induced pain of the leaf extract of S*tephania abyssinica* using formalin test in mice
- To evaluate the anti-inflammatory activity of the leaf extract of S*tephania abyssinica* using carrageenan induced mouse paw edema test in mice
- To identify secondary metabolites of the leaf extract of S*tephania abyssinica* through preliminary phytochemical analysis

3 Materials and methods

3.1 Materials

3.1.1 Drugs and chemicals

Carrageenan (Sigma Chemicals Co., St Louis, USA), Tween 80 (Atlas Chemical Industries Inc, USA), formalin (Taflen Industry, Ethiopia), normal saline (H. R. Leuven, Belgium), distilled water (Ethiopian Pharmaceutical Manufacturing Factory, Ethiopia), absolute methanol (Indenta chemicals, India) and Glacial acetic acid (Sigma – Aldrich laborchemikalien, Germany), indomethacin (Cadilla pharmaceuticals Ethiopia), aspirin and morphine (Ethiopian Pharmaceutical Manufacturing Factory, Ethiopia) obtained from the respective vendors were used in the experiment.

3.1.2 Plant collection

The leaf of S*tephania abyssinica* was collected from Wondo Genet area of Oromia region in April 2014. The collected leaves were packed with a plastic bag and transported in the same day after collection. Identification and authentication of the plant specimen was done by a taxonomist (Melaku Wondafrash) at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University and a voucher specimen (TL001) was deposited for future reference. The leaves of *S. abyssinica* were carefully cleaned to remove dirt and soil. The leaves were then dried at room temperature under shade and reduced to appropriate size and powdered finely using mortar and pestle.

3.1.3 Experimental animals

The experiment was performed using Swiss albino mice of either sex weighing (20-25g) obtained from animal unit of College of Veterinary Medicine and School of Pharmacy, Addis Ababa University, Ethiopia. The animals were maintained under standard laboratory condition (room temperature with 12:12 hour light–dark cycle) and provided with the standard animal feed and water ad libitum. Animals were acclimatized to the laboratory conditions for 7 days before the experiment. All animals used in this study were handled in accordance with the internationally accepted standard guidelines for use of animals (OECD, 2001).

3.2 Methods

3.2.1 Extraction

The 80% methanol extract was prepared using maceration technique by soaking 150 g dry powder of *Stephania abyssinica* leaves with 80% methanol solution (1.8 L) in Erlenmeyer flask for 72 h at room temperature. The mixture was filtered through (Whatman No. 1) filter paper. The mark was re-extracted successively two times for a total of 6 days (ICS-UNIDO, 2008). The filtrates were combined and the solvent was removed by evaporation using a rotary evaporator (Buchi labortechnik, Switzerland) at 40 °C under reduced pressure. The remaining solvent was removed in a water bath to obtain a complete dry powdered residue and the yield was calculated to be 27.5 gm.

3.2.2 Acute toxicity study

Acute toxicity test was performed based on the limit test recommendations of OECD Guideline 425 (OECD, 2001). On day one, a single female mouse was fasted for 3-4 h and given 2000 mg/kg of the extract orally. The mouse was then kept under strict observation for any physical or behavioural changes within 24 h, with special attention during the first 4 h. Following the results from the first mouse, other four female mice were fasted for 3-4 h and administered a single dose of 2000 mg/kg. They were then observed in the same manner. The observation continued for further 14 days for any signs of toxicity. Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern.

3.2.3 Animal grouping and dosing

Mice were randomly divided into five groups of six mice per group. Group I served as negative control and received vehicle (2% of Tween 80, p.o, 0.1 ml/10 g). Group II served as positive control and treated with standard drugs; morphine (5mg/kg, s.c) for hot plate test and (4 mg/kg, s.c) for formalin test, indomethacin (10 mg/kg, p.o) for carrageenan test, aspirin (30 mg/kg, p.o) for writhing test. Group III-V were treated with the extract at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg, respectively. Doses were selected based on acute toxicity study.

3.2.4 Acetic acid induced writhing test

This test was performed as described by Koster *et al.*, cited by (Chattopadhyay *et al.*, 2012). Albino mice of either sex were divided into their respective groups and treated as

described under grouping and dosing. One hour following treatment, mice received i.p. injection of 0.6% v/v acetic acid solution at a dose of 10 ml/kg. Five minutes after administration of acetic acid, the number of writhes or stretches (a syndrome, characterized by a wave of contraction of the abdominal musculature followed by extension of hind limbs) was counted for 15 min. A reduction in the number of writhes as compared to the control group was considered as evidence for the presence of analgesia, expressed as percent inhibition of writhing, which was calculated according to the following formula:

$$\textbf{\% Inhibition} = \left(\frac{\textbf{Mean no. of writhes in control} - \textbf{Mean no.of writhes in test}}{\textbf{Mean number of writhes in control}}\right) * 100$$

no.- number

3.2.5 Hot plate test

Franzotti *et al.*, cited by (Dutra *et al*, 2008), stated that for the hot plate test, mice were placed on a hot-plate set at 55 ± 0.5 °C. When the mice licked their fore- and hind paws or jumped, reaction time was recorded at different times after oral administration of the extract or morphine (5 mg/kg, s.c.) or vehicle (2% Tween 80). Latency time or reaction time (in seconds) on the hot plate for each mouse was determined at intervals of 30, 60, 90 and 120 minutes after treatment with the respective agent (De Sá *et al*., 2012). A cut of period of 15 seconds was observed to avoid damage to the paw (Mamta *et al*, 2011).

Percentage latency was obtained by using this formula:

$$\% \textbf{ latency} = \left(\frac{\textbf{To} - \textbf{Tt}}{\textbf{To}}\right) * \textbf{100}$$

T_o= mean latency time for the control group

T_t = mean latency time for the test group

3.2.6 Formalin Test

This was done as described by Hunskaar and Hole (1987), cited by (Silva *et al.*, 2012). A volume of 20 µl of a 1% formalin solution was injected in the plantar surface of the right hind paw to mice pre-treated orally 60 min earlier with the vehicle or extract or pre-treated 30 min earlier with morphine (4 mg/kg, s.c.). After formalin injection, the mice were individually placed in a glass cylinder and were observed from 0-5 min (neurogenic phase) and 15-30 min (inflammatory phase). The time spent licking the injected paw was recorded for both phases and considered as indicative of nociception.

The percentage inhibition was calculated using the formula given below:

$$\% \textbf{ inhibition } = \left(\frac{A - B}{A}\right) * 100$$

Where A is mean paw licking time (sec) for the control group and B is mean paw licking time (sec) for the treated group.

3.2.7 Carrageenan induced mouse paw edema test

The test was conducted according to the method of Winter *et al*., cited by (Kaushik *et al.*, 2012), with slight modification. Fifty microliters of 1% carrageenan suspended in saline was injected into the plantar side of the right hind paw, different dose of the extract, the vehicle and the standard drug (Indomethacin 10 mg/kg, p.o) administered one hour prior to carrageenan injection and the paw volume was measured at 0, 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} hour after the injection using a plethysmometer (Ugo Basile, 7140) (Liao *et al*, 2012). Percentage inhibition of inflammation was calculated as (Mamta *et al*, 2011):

$$\% \textbf{ inhibition } = \left(\frac{\textbf{Vc} - \textbf{Vt}}{\textbf{Vc}}\right) * 100$$

Where V_t is the paw edema volume (ml) in test or standard compound at the corresponding time and V_c is the paw edema volume (ml) in control

3.2.8 Preliminary phytochemical screening

The methanol extract of *S. abyssinica* leaves was screened according to Trease and Evans (1996), cited by (Musa *et al*., 2009), for its phytochemical constituents according to standard procedures

Test for sterols/terpenes

Salkowski test: A little quantity of the extract was dissolved in 1 ml chloroform and to it 1 ml of concentrated sulfuric acid was added down the test tube to form two phases. Formation of red or yellow coloration was taken as an indication for the presence of sterols.

Test for flavonoids

Sodium hydroxide test: Two ml of the extract was dissolved in 10% aqueous sodium hydroxide solution and filtered to give yellow color, a change in color from yellow to colorless on addition of dilute HCl indicated the presence of flavonoids.

Test for alkaloids

The extract (0.5 g) was stirred with 5 ml of 1% aqueous hydrochloric acid on a water bath and filtered. Three ml of the filtrate was divided into three. To the first 1 ml few drops of Dragendoff reagent was added and observed for formation of orange to brownish precipitate. To the second, 1 drop of Mayer reagent was added and observed for formation of white to yellowish or cream color precipitate.

Test for tannins

A small quantity of the extract was boiled with water and filtered. Two drops of ferric chloride was added to the filtrate, formation of a blue-black, or green precipitate was taken as evidence for the presence of tannins.

Test for anthraquinones

Free anthraquinones: The extract was shaken with 10 ml of benzene, the content was filtered, and 5 ml of 10% ammonia solution was added to the filtrate, the mixture was shaken. Presence of a pink, red or violet color in the ammoniacal layer (lower phase) indicated the presence of free anthraquinone.

Test for saponins

About 0.5 g of the extract was shaken with water in a test tube. Frothing which persisted for 15 min indicated the presence of saponins.

3.3 Statistical analysis

Results were expressed as mean ± standard error of mean (SEM) of responses. The results were analyzed statistically using SPSS Software Ver. 16.0. The statistical significance was determined using One-way Analysis of Variance (ANOVA) followed by Tukey post Hoc test. Two way ANOVA was also used to analyze the progression of latency and formation of edema over time. The value, $p<0.05$ was considered as statistically significant.

4 Results

4.1 Acute toxicity study

The acute toxicity study indicated that the leaf extract of *Stephania abyssinica* at a dose of 2000 mg/kg caused no mortality within the first 24 h and for the next 14 days. Physical and behavioural observations of the experimental mice also revealed no visible overt signs of acute toxicity like lethargy, tremor, fatigue, paralysis, autonomic and behavioral changes. The data suggest that LD50 of the leaf extract is more than 2000 mg/kg.

4.2 Acetic acid induced writhing test

Peripheral antinociceptive activity was measured as a reduction in the number of writhes. In this test, treatment with the extract (100, 200 and 400 mg/kg) showed a significant reduction in the number of writhing compared to the negative control (Table 1). The different doses of the extract produced increased inhibition with dose, with maximum inhibition observed with the higher dose (400 mg/kg). It is worth noting that the inhibition produced by the higher dose was significantly greater than the middle ($p< 0.001$) and lower dose ($p<0.001$) used in the study. Although inhibition of writhing produced by the extract, the extent of reduction was significantly lower ($p<0.001$) for (100 and 200 mg/kg) and ($p<0.05$) for (400 mg/kg) when compared to the standard drug.

Table 1: Effect of 80% methanol leaf extract of *Stephania abyssinica* in acetic acid induced writhing test

Group	Mean No. of writhing ± S.E.M	% Inhibition
Tween 80 2%	34.67±1.63	-
SM 100 mg/kg	19.83±1.05 a^3 b^3	43
SM200 mg/kg	13.83±1.17 a^3 b^3	60.2
SM400 mg/kg	8.33±0.81 a^3b^1	76
ASA (30 mg/kg)	3.67±0.33 a^3	89.4

Data represent mean± S.E.M (n = 6); [1]p<0.05, [2]p<0.01, [3]p<0.001; [a]: relative to control; [b]: relative to standard; [c]: relative to 100 mg/kg; [d]: relative to 200 mg/kg; [e]: relative to 400 mg/kg. ASA, acetyl salycilic acid; SM, 80% methanol extract of *Stephania abyssinica*

4.3 Hot plate test

Hot plate method was used to evaluate central antinociceptive activity. All doses of the extract produced central analgesic effect by increasing the latency time as compared with negative control at all time intervals (Table 2). The increase in latency time observed by the lower dose was significantly less than the middle ($p< 0.001$) and higher dose ($p<0.001$) at all time intervals. The increase in latency time observed by the higher dose was significantly greater than the middle ($p< 0.001$). But the latency shown by all doses of the extract was significantly lesser ($p< 0.001$) than that of the standard drug. Maximum percent inhibition was observed at 30 min and there appeared to be a decrease in effect with time in all doses of the extract, Two way repeated measures of ANOVA analysis of mean latency showed significant ($p<0.001$) difference in mean latency of the extract and

the standard drug across time (Figure 2). There was a difference in the latency of the different treatment groups in time. At 30 min, the standard drug resulted in a higher effect than all doses of the extract, however for the rest of the time intervals although the standard drug resulted in higher effect than the extract that difference was much less.

Table 2: Effect of 80% methanol leaf extract of *Stephania abyssinica* in hot plate test

Group	Mean latency time (sec) ± S.E.M				
	0 min	**30 min**	**60 min**	**90 min**	**120min**
Tween 80 2 %	3.64±0.30	3.96±.03	3.73±.08	3.69±0.06	3.07±0.14
SM100 mg/kg	2.85±0.22	5.18±0.30 a^1 b^3 d^3 e^3 (81.8)	4.86±0.08 a^2 b^3 d^3 e^3 (70.5)	4.68±0.06 a^3 b^3 d^3 e^3 (64.2)	4.71±0.41 a^3 b^3 d^3 e^3 (53.3)
SM200 mg/kg	3.65±0.14	6.75±0.25 a^3 b^3 c^3 e^3 (84.9)	6.27±0.07 a^3 b^3 c^3 e^3 (71.8)	5.37±0.16 a^3 b^3 c^3 e^3 (47.1)	5.19±0.06 a^3 b^3 c^3 e^3 (42.2)
SM400 mg/kg	3.93±0.11	8.36±0.34 a^3 b^1 c^3 d^3 (112.5)	7.30±0.32 a^3 b^3 c^3 d^3 (85.8)	6.61±0.10 a^3 b^1 c^3 d^3 (68.2)	5.98±0.16 a^3 b^3 c^3 d^3 (54.5)
MOR 5mg/kg	3.86±0.17	14.32±0.1a^3 (271)	13.76±0.22a^3 (256.5)	12.03±0.26a^3 (211.7)	9.89±0.32a^3 (156.2)

Data represent mean± S.E.M (n = 6) ; [1]p<0.05, [2]p<0.01, [3]p<0.001; [a:]relative to control [b]: relative to standard [c]: relative to 100 mg/kg; [d]: relative to 200 mg/kg; [e]: relative to 400 mg/kg MOR - morphine 10 mg/kg, data in parenthesis show percentage increase in latency of licking

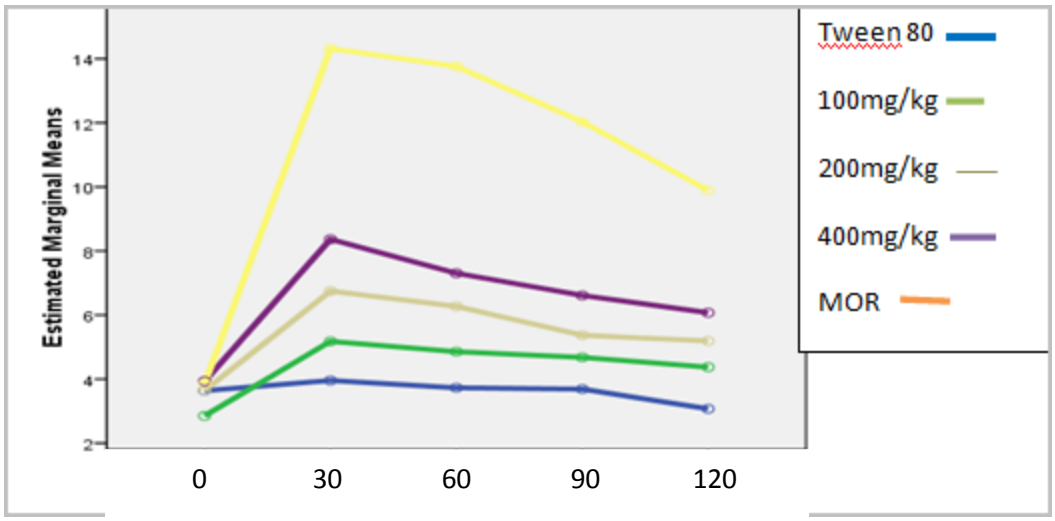

Figure 2: Estimation of mean latency of 80% methanol leaf extract of *Stephania abyssinica* versus time

4.4 Formalin test

Formalin test was performed to distinguish between the central and peripheral antinociceptive action of the extract. Detection of the time of paw-licking showed that extract exhibited significant reduction ($p<0.001$) at (100 and 200 mg/kg) and ($p<0.05$) at (400 mg/kg) doses in both early and late phase of pain responses compared to the negative control (Table 3). The middle dose of the extract reduced licking time significantly which was greater than the lower ($p< 0.001$) dose and lower than the higher dose ($p<0.001$) in both phases. The maximum reduction was produced at the highest dose for the extract but the reduction of licking by all doses was significantly lower ($p<0.001$) when compared to the standard drug. The percentage inhibition of licking time revealed that, the extract doses reduced paw-licking time more significantly in the second phase than the first phase.

Table 3: Effect of 80% methanol leaf extract of *Stephania abyssinica* in formalin test

Group	Licking time (sec)	
	First phase	**Second phase**
Tween 80 2%	114.77±1.01	120.24 ± 1.18
SM100 mg/kg	109.64 ±.75 a^1 b^3 d^3 e^3 (4.5)	114.47±1.51 a^1 b^3 d^3 e^3 **(**4.8**)**
SM200 mg/kg	105.26±0.64a^3 b^3 c^3 e^3 (8.3)	104.60 ± 0.85 a^3 b^3 c^3 e^3 **(**13.0**)**
SM400 mg/kg	97.25 ± 0.79a^3 b^3 c^3 d^3 (15.3)	87.92 ± 1.27 a^3 b^3 c^3 d^3 **(**26.9**)**
Morphine 4mg/kg	50.48 ± 1.65 a^3 (56.0)	17.55 ± 1.05 a^3 **(**85.4**)**

Data represent mean± S.E.M (n = 6) ;[1]p<0.05, [2]p<0.01, [3]p<0.001; [a:]relative to control [b]: relative to standard [c]: relative to 100 mg/kg; [d]: relative to 200 mg/kg; [e]: relative to 400 mg/kg Data in parenthesis show the percentage decrease of licking time

4.5 Carrageenan induced paw edema test

To determine acute-phase inflammation in vivo, a carrageenan-induced paw edema experiment was conducted. The effect of all doses of the extract on carrageenan induced paw edema test produced significant (p<0.001) reduction of paw edema compared with the negative control throughout the observation time (Table 4). Reduction produced by the higher dose was significantly greater than the middle (p< 0.001) and lower dose (p<0.001) used in the study at all time intervals. The extent of reduction at 100 mg/kg and 200mg/kg doses was significantly lower (p<0.001) at all time intervals when compared to the standard drug. And 400 mg/kg produced significantly lower reduction in paw volume

($p<0.05$) at 1st, 2nd and 3rd h and ($p<0.001$) at 4th and 5th h when compared to the standard drug. The percentage reduction of edema was higher in the second phase than the first phase. Two way repeated measures ANOVA analysis of edema showed significant ($p<0.05$) value and doses of the plant extract showed a difference in paw volume (ml) across the observation time (Figure 3). At the different time intervals, the higher dose of the extract produced higher effect than the middle and the lower doses, on the other hand at all time intervals the effect for the standard drug was higher than the extract.

Table 4: Effect of 80% methanol leaf extract of *Stephania abyssinica* in carrageenan induced mouse paw edema test

Group	Change in paw volume (ml)					
	0 hour	1 hour	2 hour	3 hour	4 hour	5 hour
Tween 80 2 %	0.78±0.02	0.59±0.02	0.74±0.01	0.77±0.01	0.70±0.01	0.65±0.01
SM100 mg/kg	0.67±0.01	0.50±0.01 a^3 b^3 d^3 e^3 (15.3)	0.60±0.01 a^3 b^3 d^3 e^3 (18.9)	0.64±0.02 a^3 b^3 d^3 e^1 (16.9)	0.58±0.01 a^3 b^3 d^3 e^3 (17.14)	0.50±0.01 a^3 b^3 d^3 e^3 (23.03)
SM200 mg/kg	0.63±0.01	0.44±0.01 a^3 b^3 c^3 e^3 (25.4)	0.52±0.01 a^3 b^3 c^3 e^3 (29.7)	0.57±0.02 a^3 b^3 c^3 e^3 (25.97)	0.52±0.01 a^3 b^3 c^3 e^3 (25.71)	0.46±0.01 a^3 b^3 c^3 e^3 (29.23)
SM400 mg/kg	0.57±0.02	0.39±0.01 a^3 b^1 c^3 d^3 (33.9)	0.49±0.01 a^3 b^1 c^3 d^3 (33.8)	0.52±0.02 a^3 b^1 c^1 d^3 (32.47)	0.45±0.01 a^3 b^3 c^3 d^3 (35.71)	0.41±0.01 a^3 b^3 c^3 d^3 (36.92)
IND.10 mg/kg	0.50±0.01	0.34±0.01 a^3 (42.4)	0.42±0.01 a^3 (43.2)	0.43±0.02 a^3 (44.16)	0.40±0.01 a^3 (42.86)	0.36±0.01 a^3 (44.62)

Data represent mean± S.E.M (n = 6) ; [1]$p<0.05$, [2]$p<0.01$, [3]$p<0.001$; [x:] relative to control [y]: relative to standard; [c]: relative to 100 mg/kg; [d]: relative to 200 mg/kg; [e]: relative to 400 mg/kg IND- indomethacin. The data in parenthesis show the percentage inhibition of paw volumey

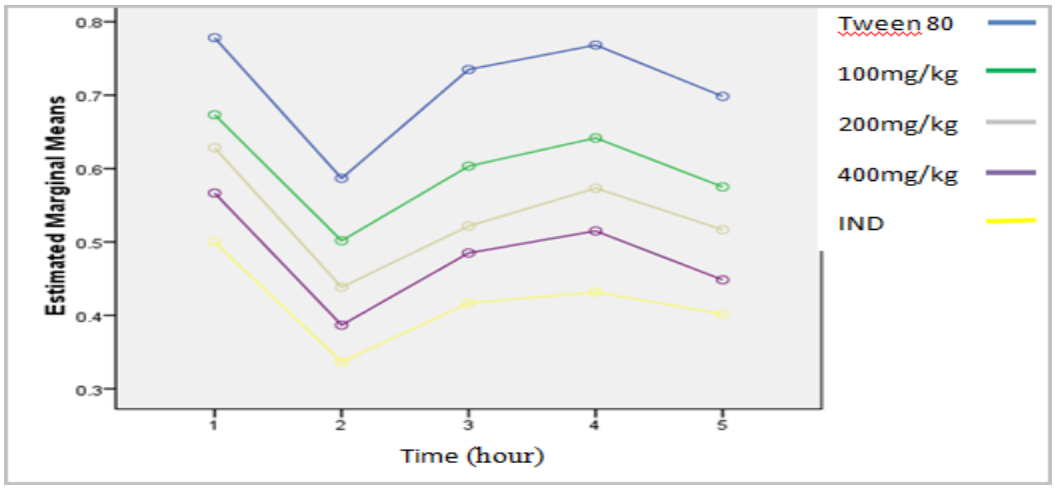

Figure 3: Estimation of mean paw volume of 80% methanol leaf extract of *Stephania abyssinica* versus time in carrageenan induced mouse paw edema

4.6 Preliminary phytochemical screening

From the preliminary phytochemical study, it was observed that the 80% methanol leaf extract of *S. abyssinica* contained the following secondary metabolites (Table 5).

Table 5: Preliminary phytochemical screening of 80% methanol leaf extract of *Stephania abyssinica*

Secondary metabolites	Methanol extract
Alkaloids	+
Flavonoids	+
Tannins	+
Cardiac Glycosides	-
Saponins	+
Terpenoids	-
Steroids	-
Anthraquinones	-
coumarins	-

5 Discussion

Collier *et al.* and Bentley *et al*., cited by (Mbiantcha *et al.,* 2012), stated that the acetic acid-induced abdominal constriction method was employed to evaluate peripheral antinociceptive activity because it is very sensitive and able to detect antinociceptive effects of compounds at dose levels that may appear inactive in other methods. In this test, the 80% methanol leaf extract of *S. abyssinica* significantly inhibited chemical induced pain. The higher dose of the extract produced lesser effect compared with the standard drug aspirin. Acetic acid irritates the peritoneal cavity leading to stimulation of local nociceptors (Konaté *et al.*, 2012). Bentley *et al*., cited by (Mishra *et al.*, 2011) explained that acetic acid induces liberation of endogenous substances such as 5-HT, histamine, PGs, bradykinins and substance P, from peripheral sensory nerve endings. This suggests that the extract of S. *abyssinica* leaves might work by suppressing the release of these endogenous substances and inflammatory mediators. The writhing test shows good sensitivity, as it allows for the effects of weak analgesics, but this test alone cannot specify the involvement of central or peripheral activity (Oliveira *et al.*, 2011; Okokon *et al.,* 2012).

In the second model, hot plate test, the extract of *S. abyssinica* leaves showed central analgesic effect. This is evidenced by increase in the latency time or increase in threshold of pain by thermal stimuli in a dose-dependent manner. The hot plate test was used to test supra-spinal nociception and for determining the involvement of central antinociceptive mechanism. The exposure of animal paws to thermal stimuli in the hot plate test leads to the development of non-inflammatory, acute nociceptive response (Dutra *et al*, 2008). Greater increase in the latency time was observed at 30 min of interval. Besides there was

marked reduction in the percentage latency at 120 minute interval compared with the percentage latency at 30 minute interval, which implies that there might be a time dependent decrease in activity of the extract. As the hot plate test is a specific central antinociceptive test, it was possible that the extract exerted an antinociceptive effect at least in part through central mechanisms. The plant extract might do so by acting at the periaqueductal gray matter (PAG) of the central nervous system (Imam and Sumi, 2014).

The formalin test was employed to evaluate the plant extract, since it encompasses inflammatory, neurogenic and central mechanisms of nociception (Konaté *et al*., 2012; Meunier *et al*, 1998). In the test, the plant extract inhibited licking time in both phases of nociception. It might be because the plant extract has a characteristic of drugs that act centrally and shows a possible interaction with opioid receptors. When comparing the percentage reduction of paw licking time of the plant extract between the first and the second phases, significant reduction was observed in the late phase of the response for all doses. The extract was more effective in acetic acid induced test and on the late phase of formalin test. Thus, the leave extract might possess profound peripheral analgesic and anti-inflammatory activities than central analgesic effect which involves inhibition of inflammatory mediators such as histamine, serotonin, prostaglandins and bradykinin, which facilitate sensitization of nociceptive neurons (Imam and Sumi, 2014; Oliveira *et al.*, 2011; Konaté *et al.*, 2012).

Carrageenan- induced paw edema is a highly sensitive tool to evaluate the efficacy of acute inflammation (Xu *et al*, 2012). Brooks *et al*., cited by (Chattopadhyay *et al.*, 2012)

explained that in the early hyperemia, 0–2 hour after carrageenan injection, there is a release of histamine, serotonin and bradykinin to increase vascular permeability. The second accelerating phase of swelling appears in 2-3 h and sustained by release of bradykinin, leukotrienes, poly-morphonuclear cells, protease, lysosome like substances and PGs produced by tissue macrophages. The extract produced significant reduction in paw volume at all doses. Huang *et al*., cited by (Paviaya *et al*, 2013), believed that herbal preparations may involve multiple sites in their actions. Thus, the extract's effect might involve inhibition of one or many of the inflammatory mediators. The extract doses inhibited paw edema induced by carrageenan more significantly in the second phase comparing the results of percentage inhibition of edema in the first phase. There is an increase in paw volume on the 3rd hour compared with the other time intervals this might be due to the inflammatory edema produced by carrageenan reached its maximum level at the third hour (Chiu *et al.,* 2012). The percentage inhibition was increased and reached maximally at 5 hour of carrageenan injection. one hour after carrageenan injection, reduction in paw volume was measured for all doses of the plant extract and the reference drug.

Seibert *et al*., cited by (Paviaya *et al*, 2013), noted that several plant extracts showed analgesic and anti-inflammatory effect in animal models and their effects have been attributed to the presence of triterpenoids, alkaloids, glycosides, tannins and sterols. Based on the result, S. *abyssinica* leave extract possess phytochemical constituents which includes flavonoids, tannins, saponins and alkaloids. It seems likely that the *S. abyssinica* analgesic effect observed in the present study could be attributed to one of these active

constituents. Alkaloids have anti-inflammatory activity (Chiu *et al.*, 2012). Flavonoids and saponins are well known for their ability to inhibit pain perception as well as anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation (Kaushik *et al.*, 2012). Flavonoids may also increase the amount of endogenous serotonin or may interact with 5-HT_2A and 5-HT_3 receptors, which may be involved in the mechanism of central analgesic activity (Lee *et al.*, 2005). Flavonoids are also found to target the prostaglandins, which are involved in the late phase of acute inflammation and pain. Thus, in the 80% methanol leaf extract of *S. abyssinica*, flavonoids could contribute to suppression of activation and sensitization of peripheral chemo-sensitive nociceptors by acetic acid and inhibition of inflammatory pain in the late phase of formalin test.

6 Conclusion

In conclusion, the plant extract produced pronounced peripheral analgesic activity than central pain inhibition. The plant extract was also exhibited anti inflammatory effects in the present study. This might imply that the plant extract was involved in inhibition of inflammatory mediators and pain mediators. The evaluation of analgesic and anti-inlammatory activity support the traditional claim of the extract of *S. abyssinica* leaves for relieving pain and inflammation in Ethiopian folklore medicine

7 Recommendation

Further studies should be done in the future on the following aspects;

- Investigation to know the subacute and chronic toxicity of 80% methanol extract of S. *abyssinica* leaves
- Identification of the particular pharmacological mechanisms that could contribute to anti-inflammatory and antinociceptive activity
- Isolation and fractionation of the crude extract to find the active constituents that contributed for reduction of pain and inflammation
- Evaluation of the analgesic and anti-inflammatory activity of the plant on chronic pain and inflammatory models

8 References

Amare G (1976). Some common medicinal and poisonous plants used in Ethiopian folk medicine. Unpublished Master Thesis, Addis Ababa University, Addis Ababa.

Amenu E (2007). Use and management of medicinal plants by indigenous people of Ejaji area (Chelya woreda) West Shoa, Ethiopia. Unpublished Master Thesis, Addis Ababa University, Addis Ababa.

American Chronic Pain Association (2013). *ACPA* resource guide to chronic pain medication and treatment. [Online] Available at: http://www.theacpa.org [Accessed 7 February 2015].

American Medical Association (2013). Pathophysiology of pain and pain management. [Online] Available at: https://cme.ama-assn.org/Activity/ [Accessed 2 February 2015].

Anyango OR (2011). Anti-malarial activity and phytochemical studies of C*issampelosmucronata* and S*tephania abyssinica.* Unpublished Master Thesis, Kenyatta University.

Ashley NT (2012). Inflammation: Mechanisms, costs, and natural variation. *Annu. Rev. Ecol. Evol. Syst* **43**:385–406.

Australian Physiotherapy Association (2012). *Pain Management.* [Online] Available at: http:// www.physiotherapy.asn.au [Accessed 12 February 2015].

Bader P, Echtle D, Fonteyne V, Livadas K, De Meerleer G, Borda AP *et al.* (2010). Guidelines on pain management. *EurAssUro*:1-92

Baron R, Binder A, Wasner G (2010). Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. *Lancet Neurol* **9**: 807–19.

Barrison AF, Wolfe MM (1999). Management of NSAID- related gastrointestinal mucosal injury. *Inflammopharm* **7**: 277-286.

Bebenista MJ, Nowak JZ (2014). Paracetamol: mechanism of action, applications and safety concern. *Acta Pol Pharm* (71)**1**:11-23.

Bennett (1999). Emerging concepts in the neurobiology of chronic pain: evidence of abnormal sensory processing in fibromyalgia. *Mayo ClinProc***74**:385-398.

Beyene ST (2011). An ethnobotanical study of medicinal plants in wondo genet natural Forest and adjacent kebeles, Sidama zone, SNNP region, Ethiopia. Unpublished Master Thesis, Addis Ababa University, Addis Ababa.

Bian Z, Guo YL, Ha B, Zen K, Liu Y (2012). Regulation of the inflammatory response: enhancing neutrophil infiltration under chronic inflammatory conditions. *J Immunol* **188**:844-853.

Bridges S (2011). Chronic pain. *HSE*. **8**:1-33.

Cardinali PD and Esquifino IA (2003). Circardian disorganization in experimental arthritis. *Neuro Signals* **12**:267-282.

Chattopadhyay C, Chakrabarti N, Chatterjee M, Chatterjee S, Bhattacharyay D, Ghosh D (2012). Evaluation of acute anti-inflammatory and analgesic activities of green tea decoction on experimental animal models. *Int J Nutr Pharma Neurol Dis* **2**: 20-5.

Chiu YJ, Huang TH, Chiu CS, Lu TC, Chen YW, Peng WH *et al*. (2012). Analgesic and antiinflammatory activities of the aqueous extract from *Plectranthusamboinicus (Lour.) Spreng*.both in vitro and in vivo. *Evid Based Comp Alt Med.* 11.

Cohen SP, Mao J (2014). Neuropathic pain: mechanisms and their clinical implications. *BMJ* **348**:1-12.

Cousins D, Huffman MA (2002). Medicinal properties in the diet of gorillas: an Ethno-pharmacological evaluation. *Afr St Monog* **23**(2): 65-89.

Craig AD, Sorkin LS (2001). Pain and analgesia. *Ency Life Sci*.1-8.

Derendorf H, Meltzer EO (2008). Review article Molecular and clinical pharmacology of intranasal corticosteroids: clinical and therapeutic implications. *Allergy* 63:1292-1300.

DeSá PGS, Nunes XP, De Lima JT, DeSiqueira JAS, Fontana AP, De Souza SJ (2012). Antinociceptive effect of ethanolic extract of *Selaginella Convoluta* in mice. *BMC Comp Alt Med* **12**:187.

Diegelmann RF, Evans MC (2004). Wound healing: an overview of acute, fibrotic and delayed healing. *Fron Biosci* **9**:283-289.

Dugowson CE, Gnanashanmugam P (2006). Nonsteroidal Anti-Inflammatory Drugs. *Phys Med Rehab Clin N Am* **17**:347–354.

Dutra RC, Trevizani R, Pittella F, Barbosa NR (2008). Antinociceptive activity of the essential oil and fractions of *Pterodonemarginatus* Vogel seeds. *Lat Am J Pharm* **27**(6): 865-70.

El-Sayed AM, Hadley C, Tessema F, Tegegn A, Cowan JA Jr, Galea S (2010). Back and neck pain and psychopathology in rural sub-Saharan Africa: evidence from the Gilgel Gibe Growth and Development Study, Ethiopia. *Spine.* 35(6):684-9.

Fein A (2012). Nociceptors and the perception of pain. [Online] Available at: cell.uchc.edu/pdf/fein/nociceptors_fein_2012.pdf [Accessed 12 September 2015].

Gautam GK, Vidhyasagar G, Das S, Dwivedi B (2013). Comparative analgesic activity of selected medicinal plants from Indian origin. *Int J Pharm Sci Res* **4**(7):2726-2729.

Gawade SP (2012). Acetic acid induced painful endogenous infliction in writhing test on mice. *J Pharmacol Pharmacother* **3**(4):348.

Goldberg DS, McGee SJ (2011). Pain as a global public health priority. *BMC Public Health.* 11:770.

González-Rivera TC (2012). Anti-TNF. [Online] Available at: http://www.rheumatology.org/Practice/Clinical/Patients/Medications/Anti-TNF/ [Accessed 10 March 2015].

Grace OM, Fowler DG (2008). *Stephania abyssinica (Quart. Dill. & A.Rich.)Walp*. *Prota* **11**(1).

Gurtner GC (2007). Wound healing: normal and abnormal. *Lipp Will Wilk***15**-22.

Hainline B (2005). Chronic pain: Physiological, diagnostic, and management considerations. *Psychiatr Clin N Am* **28**:713–735.

Hanauer SB (2006). Inflammatory bowel disease: epidemiology, pathogenesis and therapeutic opportunities. *Inflamm Bowel Dis* **12**(1):3-9.

Helms JE, Barone CP (2008). Physiology and Treatment of Pain. *Crit Care Nurse* **28**:38-49.

Hudspith MJ, Siddall PJ, Munglani R (2006). Physiology of Pain. *Found Anes* :1-22.

IASP (2002). *Classification of chronic pain.* Task force on taxonomy of the international association for the study of pain. [Online] Available at: http://www.iasp-pain.org [Accessed 10 March 2015].

ICS-UNIDO (2008). Extraction Technologies for Medicinal and Aromatic Plants, AREA Science Park Padriciano 99, 34012 Trieste, Italy.

Imam MZ, Sumi CD (2014). Evaluation of antinociceptive activity of hydromethanol extract of Cyperusrotundus in mice. *BMC Comp Alt Med* **14**:83.

James T Wu, Lily L Wu (2007). Acute and chronic inflammation: Effect of the risk factor (s) on the progression of the early inflammatory response to the oxidative and nitrosative stress. *J Biomed Lab Sci* **19** (3):71-75.

Kabir A, Samad MB, D'Costa NM, Hannan JM (2012). Investigation of the central and peripheral analgesic and anti-infl amatory activity of Draksharishta an Indian Ayurvedic formulation. *J Basic Clin Pharma* **3**:336-40.

Kaushik D, Kumar A, Kaushik P, Rana AC (2012). Analgesic and anti-Inflammatory Activity of *Pinusroxburghii Sarg. Adv Pharmaco Sci* **6**.

Kidd, Urban (2001). Mechanisms of inflammatory pain. *Br J Anaesth* **87**:3-11.

Konaté K, Bassolé IHN, Hilou A, Aworet-Samseny RRR, Souza A, Barro N (2012). Toxicity assessment and analgesic activity investigation of aqueous acetone extracts of *Sidaacuta Burn F.* and *Sidacordifolia L.* (Malvaceae), medicinal plants of Burkina Faso. *Comp Alt Med* **12**:120.

Kumar S, Bajwa BS, Kuldeep S, Kalia AN (2013). Anti-inflammatory activity of herbal plants. *IJAPBC*2(2):272-281.

Lamont LA, Tranquilli WJ, Grimm KA (2000). Physiology of pain.Management of pain.*Vet Clin N Amer* **30**:4.

Lee BH, Jeong SM, Lee JH, Kim JH, Yoon IS, Lee JH *et al.* (2005). Quercetin inhibits the 5-hydroxytryptamine type 3 receptor-mediated ion current by interacting with pre-transmembrane domain I. *Mol Cells* 20(1):69-73.

Liao CR, Kao CP, Peng WH, Chang YS, Lai SC, Ho YL (2012). Analgesic and anti-inflammatory activities of methanol extract of *Ficuspumila L.*inmice. *Evid-Based Comp Alt Med* 9.

Libby P (2007). Inflammatory mechanisms: the molecular basis of inflammation and disease. *Nutr Rev* **65**:140-6.

Louw QA, Morris LD, Grimmer-Somers K (2007). The prevalence of low back pain in Africa: systematic review. *BMC Musculosk Dis* **8**:105. [Online] Available at: http://www.biomedcentral.com/1471-2474/8/105 [Accessed 7 December 2015].

Mamta S, Parminder N, Manisha M (2011). Evaluation of anti-inflammatory and analgesic activity of *Punica Granatum Linn* leaves. *IJRAP* **2**(3):987-990.

Marchand S (2008). The Physiology of pain mechanisms: from the periphery to the brain. *Rheum Dis Clin N Am* **34**:285–309.

Maroon J C, Bost J W, Maroon A (2010). Natural anti-inflammatory agents for pain relief. *Surg Neurol Int*. 1: 80.

Masi S, Gustafsson E, Jalme MS, Narat V, Todd A, Bomsel MC *et al* (2012). Unusual feeding behavior in wild great apes, a window to understand origins of self-medication in humans: Role of sociality and physiology on learning process. *Physio Beh* **105**:337–349.

Maskrey BH, Megson IL, Whitfield PD, Rossi AG (2011). Mechanisms of resolution of inflammation. *ATVB* **31**:1001-1006.

Matthew S, Jain AK, James M, Matthew C, Bhowmik D (2013). Analgesic and anti-inflammatory activity of *Kalanchoe Pinnata (Lam.) Pers*. *J Med Pl St* **1**(2):24-28.

Mbiantcha M, Kamanyi A, Teponno RB, Tapondjou AL, Watcho P, Nguelefack TB (2012). Analgesic and anti-inflammatory properties of extracts from the bulbils of Dioscorea bulbifera L. var sativa (Dioscoreaceae) in mice and rats. *Evi-Based Comp Alt Med*, 9.

Mbugi C (2010). Allergic disorders in Africa and Africans: Is it primarily a priority? *WAO* **3**:175–181.

Mengistu G, Alemayehu S (2013). Prevalence and burden of primary headache disorders among a local community in Addis Ababa, Ethiopia. *J Head Pa* **14**:30.

Meunier CJ *et al* (1998). Evaluation of the formalin test to assess the analgesic activity of diflunisal in the rat. *Euro J Pharm Sci* 307–312.

Mills D (2007). Managing Chronic Pain: A Guide for Patients. [Online] Available at: https://www.acponline.org/patients_families/pdfs/misc/pain [Accessed 2 February 2015].

Mishra D, Ghosh G, Kumar PS, Kumar P (2011). An experimental study of analgesic activity of selective COX-2 inhibitor with conventional NSAIDs. *As J Pharm Cl Res* **4**:1.

Moreland LW (2009). Cytokines as targets for anti-inflammatory agents. *Ann N Y Acad Sci.***1182**:88-96.

Munir MA, Enany N, Zhang JM (2007). Nonopioid analgesics. *Anesth Clin* **25**:761–774.

Musa AM, Aliyu AB, Yaro AH, Magaji MG, Hassan HS, Abdullahi MI (2009). Preliminary phytochemical, analgesic and anti-inflammatory studies of the methanol extract of *Anisopusmannii* (N.E.Br) (Asclepiadaceae) in rodents. *Afr J Pharm Pharmacol* **3**(8):374-378.

Musa YM, Haruna AK, Ilyas M, Yaro AH, Ahmadu AA, UsmanH (2008). Phytochemical, analgesic and anti-Inflammatory effects of the ethylacetate extract of the leaves of *Pseudocedrella Kotschyii*. *Afr J Tradit* **5**(1): 92–96.

Nagarkatti (2009). Cannabinoids as novel anti-inflammatory drugs. *Fut Med Chem***1**(7): 1333–1349.

Nadler SF (2004). Nonpharmacologic Management of Pain. *J Am Osteopath Assoc104* (11):*6S-12S.*

OECD (2001). Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No 425.

Okokon JE, Udoh AE, Frank SG, Amazu LU (2012). Anti-inflammatory and analgesic activities of *Melantherascandens. Asian Pac J Trop Biomed* **2**(2):144-148.

Oliveira AM, Conserva LM, Souza Ferro JN, Almeida Brito F, LyraLemos RP, Barreto E (2011). Antinociceptive and anti-inflammatory effects of Octacosanol from the leaves of *Sabiceagrisea*var. *Grisea* in mice. *Int. J. Mol. Sci.**13***:1598-1611.

Paviaya US, Kumar P, Wanjari MM, Thenmozhi S, Balakrishnan BR. (2013). Analgesic and anti‑inflammatory activity of root bark of *Grewiaasiatica* Linn. in rodents. *Anc Scie Lif* **32**:I3.

Percival M (1999). Understanding the natural management of pain and inflammation. *NUT **30**:4.*

Phanse MA, Patil MJ, Chaudhari KA, Patel B (2012). *In-vivo* and *in-vitro* screening of medicinal plants for their anti-inflammatory activity: an overview. *JAPS* **2**(06):19-33.

Potter PC, Warner JO, Pawankar R (2009). Recommendations for competency in allergy training for undergraduates qualifying as medical practitioners: a position paper of the World Allergy Organization. *World Allergy Org J* 2:150 –154.

Punchard NA, Whelan CJ, Adcock I (2004). The journal of inflammation. *J Inf* **1**:1.

Rainsford.KD (2006). Influenza "Bird 'Flu"inflammation and anti inflammatory/analgesic drugs. *Inflammopharm***14**:2–9.

Rainsford KD (2007). Anti-inflammatory drugs in the 21st century. *Inf Path Chr Dis* **3**–27.

Rajagopal MR (2006). Pain - basic considerations. *Indian J. Anaesth*.**5**(50):331-334.

Rajan V, Murray RZ (2008). The duplicitous nature of inflammation in wound repair. *Wound Pr Res***16**(3):122-129.

Reilly R (2013). Marijuana pill is more effective at relieving pain and less harmful than smoking the drug. Available at: http://www.dailymail.co.uk/health/article-2313425/Marijuana-pill-MORE-effective-relieving-pain-harmful-smoking-drug.html [Accessed 7 February 2015].

Renn C, Dorsey SG (2005). The physiology and processing of pain: *A review*. *AACN* 3277–290.

Schaible HG (2006). Peripheral and central mechanisms of pain generation. *HEP* **177**:3–28.

Schaible HG, Ebersberger A, Natura G (2011). Update on peripheral mechanisms of pain: beyond prostaglandins and cytokines. *Arth Res Ther* **13**:210.

Semwala DK, Badonib R, Semwal R, Kothiyalb SK, Singha GJP, Rawatb U (2010). The genus *Stephania* (Menispermaceae): Chemical and pharmacological perspectives. *J Ethnopharm***132**:369–383.

Silva ASB, Pinheiro BG, Figueiredo JG, Souza GEP, Cunha FQ, Lahlou S *et al.* (2012). Antinociceptive and anti-inflammatory activities of the aqueous extract of *MikaniaLindleyana*in rodents. *IJPSR* **3**(6):1637-1646.

Stankov SV (2012). Definition of inflammation, causes of inflammation and possible anti-inflammatory strategies. *Op Inf J 5*:1-9.

Steeds CE (2009). The anatomy and physiology of pain.*Surg***27**:12.

Stovner Lj, Hagen K, Jensen R, Katsarava Z, Lipton R, Scher A *et al.* (2007). The global burden of headache: a documentation of headache prevalence and disability worldwide. *Cephalalg* 27(3):193-210.

Sultani M, Stringer AM, Bowen JM, Gibson RJ (2012). Anti-Inflammatory cytokines: Important immunoregulatory factors contributing to chemotherapy-induced gastrointestinal mucositis. *Chemother Res Pr.*

Teklehaymanot T, Giday M (2007). Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia. *J Ethnobio Ethnomed* **3**:12.

Tripathi KD (2003). Essential of Medical Pharmacology, 5th edn, Jayapee Brother Medical Publisher:219- 220.

Villarreal G, Zagorski J, Wahl SM (2001). Inflammation: Acute. *Ency lif sci.1-8*

Wakefield D, Kumar RK (2001). Inflammation: Chronic. *Ency lif sci.1-6*

;Watson W, Kapur S (2011). Atopic dermatitis *AACI* **7**(1):S4.

WHO (2012).*WHO guidelines on the pharmacologicaltreatment of persisting pain in children with medical illnesses.*WHO Library Cataloguing-in-Publication Data.

Woolf CJ (2004). Pain: moving from symptom control toward mechanism-specific pharmacologic management. *Ann Intern Med* **140**:441-451.

Wright KL, Duncan M, Sharkey KA (2008). Cannabinoid CB_2 receptors in the gastrointestinal tract: a regulatory system in states of inflammation**.** *Br J Pharmacol*. 153(2): 263–270.

Xu Z, Zhou J, Cai J, Zhu Z, Sun X, Jiang C (2013). Anti-inflammation effects of hydrogen saline in LPS activated macrophages and carrageenan induced paw oedema. *J of Inf* **9**:2.

Yineger H, Yewhalaw D, Teketay D (2008). Ethnomedicinal plant knowledge and practice of the Oromo ethnicgroup in southwestern Ethiopia. *J Ethnobio Ethnomed* **4**:11.

Zeilhofer HU,(2005). Synaptic modulation in pain pathways. *Rev Physiol Biochem Pharmacol* **154**:73-100.