THE NEUROPHARMACOLOGICAL EVALUATION OF *Ocimum kenyense, Ocimum kilimandscharicum and Ocimum masaiense* SPECIES FOUND IN KENYA.

By

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A thesis submitted in fulfillment of conditions for the award of the degree of **Doctor of Philosophy (PhD)** of the University of Nairobi

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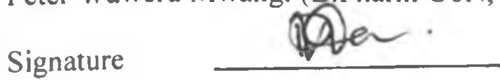
DECLARATION.

This thesis is my original work and has not been presented for examination for a degree in any other university.

Peter Waweru Mwangi (B.Pharm UoN, M.Sc UoN)



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   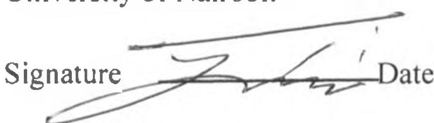

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   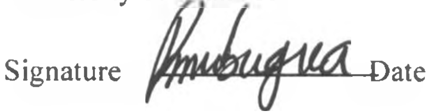

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DEDICATION.

This thesis is dedicated to the following people; My beloved son Sean Murigi who changed my life and inspires everything I do, My late mother Jedidah Njoki whose sacrifice and dedication made all this possible, Larry Kamamia for showing me that a friend in need is a friend indeed and Dr. Simon Gicharu the Chairman Board of Trustees, Mt. Kenya University who daily by word and deed reminds me that one should strive to construct the world as it should be and not wallow and complain of what it currently is.

"There is a tide in the affairs of men,
Which, taken at the flood, leads on to fortune;
Omitted, all the voyage of their life
Is bound in shallows and in miseries.
On such a full sea are we now afloat;
And we must take the current when it serves,
Or lose our ventures."

(Brutus in *Julius Caesar* by W. Shakespeare)

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LIST OF SYMBOLS AND ABBREVIATIONS.

NMDA; N-Methyl, D- Aspartate.

TLC; Thin Layer Chromatography.

NMR; Nuclear Magnetic Resonance

GC-MS; Gas chromatography Mass Spectroscopy

PAG; Periaqueductal Grey Area

RVLM; Rostroventrolateral medulla.

WHO; World Health Organization

HPA; Hypothalamic- Pituitary Adrenal axis.

TRP; Transient Receptor Potential channels.

CRF; Corticotrophin Releasing Factor.

5HT; Serotonin.

NRM; Nucleus Raphe Magnus

COX; Cyclooxygenase enzyme.

BDNF; Brain Derived Neurotrophic Factor.

KOR; Kappa opioid receptor

GABA ; Gamma Amino Butyric Acid

CNS; Central Nervous System

NMDA; N Methyl D- Aspartate

ACTH ; Adrenocorticotrophic Hormone

TRPA1 ; Transient receptor potential ankyrin 1

DEG/ENaC; degenerin/Epithelial Sodium channel

ASIC; Acid Sensing ion channel

RNA; Ribonucleic acid.

WDR; Wide dynamic range

NS; nociception specific

SPR; Substance P receptor

AMPA; Alpha methyl isoxazole

ERK; extracellular signal related kinases

NAM; Negative allosteric modulator.

NE; Noradrenaline (Norepinephrine)

NSTT; Neospinothalamic Tract

PSTT; Paleospinothalamic tract

OM; *Ocimum masaiense*

OKi; *Ocimum kilimandscharicum*

OKe; *Ocimum kenyense.*

CEOMR; Chloroform/ethanol extract of *Ocimum masaiense* roots.

DMOMR; Dichloromethane/methanol extract of *Ocimum masaiense* roots

ABSTRACT

The genus Ocimum consists of 35 species of aromatic annual perennial herbs and shrubs in the family Lamiaceae. The members of this genus find extensive application in traditional medicine systems all over the world. This study screened organic extracts prepared from three species indigenous to Kenya; *Ocimum kilimandscharicum, Ocimum masaiense* and *Ocimum kenyense* for analgesic activity. The most potent extract from the screening experiments then underwent bioassay guided fractionation procedures in an attempt to isolate the chemical moiety (-ies) responsible for the antinociceptive activity.

Organic extracts were prepared from the various plant parts of these species that is the root, stem and leaf by soxhlet extraction using ethanol, chloroform and 1:1 chloroform/ethanol mixture as the extraction solvents. The resulting extracts were then evaporated to dryness using a rotor evaporator. The antinocicptive activities of these extracts were then assayed in the Tail Flick Test (radiant form). The experimental data was analyzed statistically with Kruksall-Wallis non-parametric test using GraphPad Prism® statistical software with $p<0.05$ being set as the level of significance. Extracts with the most potent antinociceptive activity were then slected for evaluation in the Formalin Test and for fractionation. Various receptor agonists/ blockers were administered together with the extract in the Formalin Test in an attempt to elucidate the mechanism(s) of the antinociceptive action of the extract. The extract underwent fractionation using the liquid-solid adsorption column chromatography technique using dichloromethane, ethyl acetate and methanol as the eluting solvents. The resulting fractions were combined on the basis of TLC records. The antinociceptive activity of the resulting fractions was assayed in the Tail Flick Test. The most potent non polar fractions underwent further fractionation using a combination of Preparative TLC and Liquid-solid adsorption chromatography techniques until pure compounds were yielded. The antinociceptive activity of the pure compounds was assayed in the Tail Flick Test and their structure elucidated using a combination of Proton NMR and GC-MS.

Twenty one of the twenty five extracts screened showed significant antinociceptive activity in the Tail Flick Test. The chloroform/ethanol extract of *Ocimum masaiense* roots possessed the most potent analgesic activity at the 100mg/kg dose level (12.6 ± 2.61 seconds) in this test ($p=0.0041$). It also showed significant antinociceptive activity in the Formalin Test ($p<0.0001$). Atropine ($p= 0.0009$) significantly enhanced the antinociceptive effects of the extract in the early phase of the Formalin Test but had no significant effects in the late phase. Ketamine ($p= 0.0005$) and Naloxone ($p=0.005$) significantly inhibited but did not abolish the antinociceptive effects of the extract in the first phase but had no significant effects in the late phase of the Formalin Test. Capsaicin had a significant inhibitory effect but did not abolish the antinociceptive effect of the extract in both phases of the Formalin Test ($p= 0.006$ and $p= 0.0001$). Fractionation of ten (10) grams of dichloromethane/methanol extract of *Ocimum masaiense* roots using Dichloromethane, ethyl acetate and methanol as the successive elution solvents yielded six fractions on the basis of TLC patterns. All the fractions showed significant antinociceptive activity in the Tail Flick Test ($p< 0.05$). Fractionation of fractions I, II and III using Hexane and hexane; dichloromethane mixtures (Hexane 90%, DCM 10%) yielded two compounds (Ia and Ib) in sizable quantities of 3g and 2g respectively. These compounds showed significant antinociceptive activity in the Tail Flick Test ($p<0.0003$ and $p<0.006$ respectively). Compound **Ia** was identified as betulinic acid while the structure of compound **Ib** could not be conclusively determined. It is therefore concluded that;

1. Organic extracts prepared from various plant parts from three Ocimum species indigenous to Kenya that is *Ocimum masaiense*, *Ocimum kilimandscharicum* and *Ocimum kenyense* possessed statistically significant antinociceptive activity in the Tail Flick Test. This validates the traditional uses of *Ocimum kilimandscharicum* and *Ocimum kenyense* as analgesics/antirheumatics.
2. Betulinic acid a compound with known analgesic activity was isolated from *Ocimum masaiense*; the first time this compound has been isolated from an Ocimum species.

CHAPTER 1

1.0. INTRODUCTION

It has been estimated that over 80% of the population in developing countries rely partially or entirely on traditional systems of medicine (WHO, 2003). This is probably an understatement when one considers the situation in rural Africa. The popularity of these traditional remedies is on the rise with the traditional remedies being used concurrently with the allopathic medicines (Chan et al., 2010). The scientific evaluation and validation is therefore of paramount importance so as to ascertain their efficacy and/or biosafety.

The increasing popularity of traditional medicine therapies can be explained by a number of reasons. Currently marketed allopathic remedies for many chronic conditions for example diabetes, arthritis, cancer are plagued by the twin problems of low efficacy and toxicity. This has provoked a reawakening of interest in traditional therapies as possible sources of solutions to these problems. Traditional remedies are also culturally more acceptable to communities due to their additional focus on psychosocial aspects of disease in addition to the therapies administered. The introduction of traditional Aryuvedic and Chinese traditional remedies with their aggressive promotional and marketing tactics has also contributed to this increasing awareness of traditional remedies.

The highly developed traditional medicine practices of the Indian sub-content and China stand in stark contrast to the underdeveloped practices of their counterparts in Africa. In the aforementioned Asian countries traditional remedies constitute a billion dollar industry with wide social acceptability and governmental recognition and acceptability. They therefore represent the benchmark to which African traditional medicine practitioners and natural product scientists should aspire. In particular research efforts should be geared at solving three of the most pressing problems bedeviling African traditional medicine remedies which are; batch to batch variations in formulations, poor presentation (packaging) and poor/erratic posologic recommendations (WHO, 2003).

The twin neurological/neuropsychiatric conditions, depression and chronic pain share a number of common epidemiological, pathological as well as pathophysiological characteristics (Blackburn-Munro and Blackburn-Munro, 2003: Blackburn-Munro, 2004). They have a high incidence in the community (10-20% prevalence for chronic pain and 20% for depression) (WHO, 2003). They are frequently comorbid with many depressed patients complaining of pain as a somatic symptom. Conversely, many chronic pain patients present with mood disturbances and often go on to develop depression (Kinney et al., 1993; Merskey, 1994; Romano and Turner, 1985; Ruoff, 1996). Additionally, neuroimaging studies of depressed patients indicate that the same corticolimbic structures are activated in both depression and chronic pain patients (Drevets, 2001). This further buttresses the notion that mood disturbances are core symptoms of chronic pain syndromes in humans (Price et al., 2001). These common characteristics are elaborated in detail in the succeeding paragraphs below.

The loss of neurochemical homeostasis is a hallmark of both of these disorders. This results in or is the result of maladaptive neuroplasticity (Stahl and Briley, 2004). Various theories have and continue to be advanced as to how the loss of the homeostatic mechanisms arises. These theories can be briefly described as the monoamine deficiency theory and the Hypothalamic-Pituitary –Adrenal (HPA) Axis dysfunction theory (Nestler and Krishnan, 2008). Each of these theories is discussed in greater detail below.

1.1. Monoamine deficiency theory

The monoamine deficiency theory was put forward as a result of the observation that both of these conditions are characterized by relative deficits in the amounts of the monoamine neurotransmitters serotonin and noradrenalin (Woolf and Salter, 2000; Nemeroff and Vale, 2005). The deficits in these modulatory neurotransmitters in turn are thought to lead to the loss of the tonic inhibitory effect of GABA on neural excitation in the CNS (Azami et al., 2001). This results in enhanced neural excitation mediated by the unopposed excitatory action of glutamate. This in turn results in activation of the NMDA receptors and in the release of various neuropeptides such as Substance P, Galanin, Neuropeptide Y (NPY), among others (Wiesenfield-Hallin and Xu, 2001). The activation of the NMDA receptors is considered to be a central event in the neuroplasticity of pain (Woolf and Salter, 2000) and

affective disorders (Mathew et al., 2001; Manji et al., 2001; Waxman and Lynch, 2005). The putative role of the NMDA receptor in the pathogenesis of these two conditions has recently received clinical and experimental validation with the recent publication of data showing the positive antidepressant effects of the non-competitive NMDA antagonist ketamine (Zarate et al., 2006; Garcia et al., 2008; Maeng et al., 2008). These antidepressant effects were in addition to the already well established analgesic effects of ketamine (Clements and Nimmo, 1981; Grant et al., 1981).

1.2. HPA Axis Theory

Hyperactivity of the HPA axis is one of the canonical signs in major depression (Berton and Nestler, 2006: Binder and Nemeroff, 2010). Indeed many patients have elevated Corticotropin Releasing Factor (CRF) levels and reduced feedback inhibition of CRF synthesis/secretion by circulating glucocorticoids and CRF itself (Barden, 2004; Gillespie and Nemeroff, 2005; De Kloet et al., 2005; McEwen, 2005; Pariante, 2006). In addition, the normalization of the HPA axis has been shown to precede the successful therapeutic effects of antidepressants (Arzt and Holsboer, 2006). The injection of algogenic substances for example formalin is associated with elevation in serum levels of ACTH and corticosterone (Taylor et al., 1998). In addition, pain is a potent activator of the HPA axis (Blackburn-Munro and Blackburn-Munro, 2003). Imaging studies have also shown elevations in the activity of CRF-secreting hypothalamic nuclei (Paulson et al., 2002). These results are an indication of the prominent roles played by CRF in both peripheral and central analgesia (Lariviere and Melzack, 2000).

In view of the foregoing, it is therefore not surprising that therapeutic agents targeted for management of one condition commonly show efficacy in the management of the other condition. Antidepressants are extensively used in the management of chronic pain especially chronic neuropathic pain. Indeed tricyclic antidepressants for example Amitryptiline together with anticonvulsants are considered to be the first line drugs for the treatment of neuropathic pain (Mico et al., 2006). The analgesic action seems to be independent of the antidepressant activity. This is because the analgesic effect is observed even in chronic pain patients having no concomitant depression (Saarto and Wiffen, 2005). In addition the doses used for the production of the analgesic effects are usually much lower

than those used in the management of depression (Goldstein et al., 2005). The rapid onset of the analgesic effect as compared to that of the antidepressant effect and the differing analgesic potencies of the different chemical classes of antidepressants provides further validation of this hypothesis (Goldstein et al., 2005).

Many analgesics have been reported to possess antidepressant activity. Opioids are widely accepted to possess mood elevating properties with euphoria being one of the side effects associated with the use of these drugs (Akil and McNally, 2002). Tramadol, a synthetic centrally acting opioid analgesic has been shown to possess antidepressant activity in both laboratory and clinical settings (Rojas-Corrales et al., 1998; 2002; Spencer, 2000; Shapira et al., 2001; Kalra et al., 2008). The Non-Steroidal Anti-inflammatory drugs (NSAIDS) have also been shown to possess antidepressant activity (Dhir and Kulkarni, 2008).

Current therapy of depression and chronic pain is unsatisfactory in terms of efficacy, tolerability and toxicity (Nemeroff and Owens, 2002; Scholz and Woolf, 2002). This makes the development /discovery of more efficacious drugs a priority in view of the disabling effects of these conditions with the attendant socio-economic costs and consequences. Depression causes considerable morbidity in sufferers and is estimated to be the second leading cause of disability worldwide (Klerman and Weissman, 1992). The co-morbidity of depression with anxiety and other psychiatric disorders renders the depressive patient highly susceptible to suicide. Since suicide is one of the top ten causes of death in the world, this therefore makes depression a lethal condition. The fact that depression is a major independent risk factor for the development of coronary artery disease, stroke and other major medical disorders (Nemeroff et al., 1999) suggests that its effective management is imperative. The prevalence of chronic pain conditions seem to be rising inexorably with the attendant costs to the economy in terms of healthcare and productivity losses (Bennett and Xie, 1988). As an illustrative example, low back pain is the single largest cause of absenteeism from work in the UK (Apkarian et al., 2009). The cost to the UK economy due to this absenteeism was estimated at 11 billion pounds in 1993 (Frank, 1993; Maniadakis and Gray, 2000)!

1.3. Plants as a source of Analgesics

Although many of the analgesics in current clinical use were first isolated from natural plant sources for example morphine and aspirin this approach of obtaining novel analgesics has relatively been abandoned. This is because it is believed that with advances in biotechnology as well as combinatorial chemical synthesis, the former approach is wasteful, relatively time consuming and therefore costly. Despite the foregoing, natural plant sources remain attractive sources for novel chemical neuroactive compounds. They also can be used as lead compounds for the synthesis of novel antidepressants, analgesics, anxiolytic, antipsychotic and other classes of neuropharmacological compounds (Prasad et al., 2005; Chen et al., 2007). Most of the new chemical moieties often have unique structures which may be modified and therefore optimized into clinically useful products. These sources may often yield compounds that can act as novel agonists or blockers for specific type of receptors. Salvinorin A which was recently isolated from the Mexican plant species *Salvia divinorum* is an iconic example (Siebert, 1994). Salvinorin A represents the first known non-nitrogenous Kappa opioid receptor (KOR) selective agonist and the first non-alkaloidal hallucinogen (Roth et al., 2002).

The plant species that are the subject of this study *Ocimum kilimandscharicum*, *Ocimum mosaiense* and *Ocimum kenyense* belong to the genus ocimum which is part of the Lamiaceae family of plants. The ocimum genus is made up of 35 species of aromatic annual and perennial herbs and shrubs (Paton et al., 1994). The members of this genus find wide application in traditional medicine systems of the world with *Ocimum sanctum* (Holy Tulsi) being a salutary example (Gupta et al., 2007). Two of the species evaluated in this study, *Ocimum kenyense* and *Ocimum kilimandscharicum* find application in traditional medicine systems of various ethnic communities of Kenya as analgesics (Kanui, 2006; Njoroge and Bussman, 2006; Kokwaro, 2009).

1.4. Objectives

The main objective of this study was to evaluate the analgesic activity of these three ocimum species that are found in Kenya.

The specific objectives of the study were;

- Preparation of organic extracts using chloroform, ethanol and 1:1 mixture of chloroform and ethanol

- Evaluation of the analgesic activity of the extracts prepared using the Tail Flick Test.

- Evaluation of the most potent extract in the Tail Flick Test for analgesic activity testing in the Formalin Test.

- Administration of standard receptor agonists and antagonists together with the extract chosen above in an attempt to determine the possible mechanisms of action of the analgesic activity of the extract.

- Fractionation of the chosen extract using a bioassay guided fractionation strategy.

- Evaluation of the analgesic activity of the resulting fractions using the Tail Flick Test.

- Further fractionation of the most potent analgesic fractions

- Evaluation of the analgesic activity of the compounds obtained using the Tail Flick Test.

- Elucidation of the structure of the compounds obtained using standard chemical spectroscopic techniques for example proton NMR and GC-MS.

CHAPTER TWO

2.0. LITERATURE REVIEW

The three Ocimum species that are the subject of this study that is *Ocimum kilimandscharicum* Baker ex Guerke, *Ocimum masaiense* Ayobangira ex Paton and *Ocimum kenyense* Ayobangira ex Paton grow in the wild in Kenya and the surrounding areas of East and Central Africa (Kokwaro, 1976). The synonyms of the above named plants are listed in the following sentences. *Ocimum kilimandscharicum*- African basil, hoary basil, camphor basil, gacuki (Kikuyu), mukuri (Kimeru), Okita (dholuo) and mwonyi (luhya). The synonyms for *Ocimum kenyense* include; yenye, mutaa (Kamba), ayobangira (amharic) while the synonym for *Ocimum masiense* is lamuran (maa language). In common with Ocimum species from other parts of the world two of these species (*Ocimum kenyense* and *Ocimum kilimandscharicum*) find application in traditional medicine while *Ocimum masaiense* strangely is not used in traditional medicine.

Ocimum kilimandscharicum is traditionally used in the treatment of upper respiratory infections for example colds and flu, coughs, etc. it is also used in the treatment of malaria. It is used in combination with other plants in the treatment of measles (Kokwaro, 1976). The leafy parts of the plants are used as insect repellents to expel mosquitoes and other insects from households after burning. Leaves of the plant are commonly used as grain protectants in East Africa (Jembere et al., 1995; Kokwaro, 1976). It is commonly used in apiculture to attract bees to beehives due to its aromatic smell. *Ocimum kenyense* is traditionally used in Eritrea as fumigant (Obeng-Ofori et al., 1997). The leaves of this plant are used in parts of Ukambani as an analgesic in the management of rheumatism/joint pain (Kanui, 2006).

Most of the phytochemical studies on these species have concentrated on the chemical characterization of the essential oils found in these plants. The essential oil of *Ocimum kilimandscharicum* is rich in camphor (56%), DL-limonene (14%) and Camphene (7%) and has been used as an industrial source of camphor in India (Anand et al., 2011). 1,8 cineole (38%) and methyl chavicol (24%) were reported as being the major constituents of the

essential oil obtained from *Ocimum kenyense* (Mwangi et al., 2004). There are as yet no published findings on the chemical composition of *Ocimum masaiense*.

2.1 Pain and nociception.

The International association for the Study of Pain defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Bonica, 1979). Pain is a complex experience that involves not only the transduction of noxious environmental stimuli, but also of cognitive and emotional processing by the brain (Julius and Basbaum, 2001). Pain is usually classified into various sub-types. The common classification criteria used being either the anatomical site of the pain or the temporal character of the pain. Pain is therefore commonly classified into visceral, somatic or neuropathic pain (anatomical basis of classification) or into acute or chronic pain (temporal classification).

Nociception on the other hand is specifically geared to the detection of noxious stimuli. This ability to detect noxious stimuli is essential for organismal wellbeing and survival. This is dramatically illustrated by the reports in scientific literature of individuals that congenitally are unable to detect/respond to noxious stimuli. These individuals often suffer severe injury and even death due to the lack of this sensory modality (Cox et al., 2006; Dib-Hajj et al., 2008).The nociceptive system can be regarded as an adaptive alarm system that signals potential or continuing tissue damage (Woolf, 2010). The nociceptive system shares many characteristics with other sensory systems. One of these characteristics includes the presence of selectively sensitive receptors (nociceptors) that detect noxious stimuli. Nociceptors are therefore analogous to photoreceptors and odoreceptors which are selectively sensitive to light and odorant stimuli respectively. Other characteristics in common include the presence of dedicated sensory transmission pathways (neospinothalamic and paleospinothalamic pathways) (Giordano, 2005) and the modulation of this transmission via both peripheral and central mechanisms (Woolf et al., 2003). Nociception differs from other sensory modalities in its unique ability to trigger changes in the arousal and affective states of the organism. The adaptive value of this unique property is easily appreciated when one considers the protective roles played by this sensory modality.

2.2 The nociceptor and nociceptive transmission.

The nociceptor has four major functional components; the peripheral terminal, the cell body, the axon and the central terminal (Basbaum, 2000; Woolf, 2010). The peripheral terminal transduces external stimuli and is the site of initiation of action potentials. The cell body on the other hand controls the identity and integrity of the neuron. The axon on the other hand conducts the action potentials whiles the central terminal forms the presynaptic element of the first synapse in the spinal cord dorsal horn. There exists in scientific literature a variety of nociceptor classification schema. The most popular of these are based on the anatomical and functional properties of the nociceptor axon. In this scheme nociceptors are classified either as myelinated (Aδ fibers) or unmyelinated nociceptors (C –fibers) (Ringkamp et al., 2010).

The myelinated nociceptors (Aδ) mediate the acute and well localized "fast pain". They are commonly sub-divided into two main classes type I and type II. Type I are also known as the High threshold mechanonociceptors (HTM) (Meyer and Campbell, 1981). These nociceptors have high heat thresholds (>50°C) but lower mechanical and chemical stimulus thresholds. It should be noted however that the heat thresholds as well the chemical and mechanical thresholds will drop in the setting of tissue injury (sensitization) (Meyer and Campbell, 1981). The heat thresholds will also be reduced in the presence of sustained thermal stimulation. Type II nociceptors on the other hand have much lower thermal thresholds but much higher mechanical stimulus thresholds (Perl, 1968). It can therefore be seen that the type I myelinated nociceptors mediate the "fast" pain associated with mechanical stimuli while the type II nociceptors mediate the "fast "pain associated with thermal nociceptive stimuli (Basbaum et al., 2009).

The unmyelinated nociceptors mediate the slow aching (burning) and poorly localized pain. They are very heterogeneous in nature and are most usefully classified into peptidergic and non-peptidergic nociceptors. Peptidergic nociceptors release the peptidergic neurotransmitters Substance P and Calcitonin Gene Related Peptide (CGRP) in addition to glutamate at their central terminals (Snider and McMahon, 1998). They also express TrkA receptors which are high affinity receptors for Nerve Growth Factor (NGF) (Jing et al.,

1992). The non-peptidergic afferents express c-Ret neurotrophin receptors which are receptors for Glial Derived Neurotrophic Factor (GDNF) as well as neurturin and artemin (Stucky et al., 1999). A large proportion of this type of nociceptor also binds IB_4 isolectin and expresses the Mrg subtype of G-Protein Coupled Receptors (GPCRS) (Stucky et al., 1999; Dong et al., 2001). The non-peptidergic C fiber nociceptors also express specific purinergic receptor subtypes especially the P_2X_3 receptor (Woolf and Salter, 2000). These two classes of nociceptors show marked differences in functional properties (Basbaum et al., 2009; Woolf, 2010).

The mature nociceptor expresses a wide variety of ion channels and receptors which are responsible for its ability to detect specific nociceptive stimuli (Woolf, 2010). Nociceptors are historically functionally classified according to the form of stimulus that they selectively respond to. They are therefore classified into chemonociceptors, mechanonociceptors and thermonociceptors (Burgess and Perl, 1967). The selective sensitivity of the nociceptor will be determined by the complement of receptors and ion channels that it possesses.

The Transient Receptor Potential (TRP) superfamily of ion channels in particular plays a prominent role in nociception. It consists of proteins that each contains 6 transmembrane domains with cytoplasmic C and N termini (Moran et al., 2004; Basbaum et al., 2009). These proteins assemble into homo- or hetero- tetramers to form cation- permeable ion channels (Hellwig et al., 2005). Twenty-eight (28) TRP channels have been discovered to date in mammals and they have been classified into six (6) families according to sequence homology (Moran et al., 2004). The families are TRPC, TRPV, TRPA, TRPP and TRPML (Montell, 2005). Each of these families has been implicated in nociception with the TRPV, TRMP and TRPA families playing particularly prominent roles in thermonociception (Basbaum et al., 2009).

Thermosensitive nociceptors (thermonociceptors) are generally classified into hot and cold receptors. Thermal sensitivity in the warm-hot range is mediated by multiple Transient Receptor Potential (TRP) channels TRPV1, TRPV2, TRPV3 and TRPV4 (Basbaum et al., 2009). All these channels possess a carboxyl terminal domain that confers thermal sensitivity. The capsaicin sensitive thermosensitive nociceptors express the TRPV1 channels and have a stimulus threshold of about 45°C (Julius and Basbaum, 2001). Many lines of

evidence exist in support for the putative role of the TRPV1 as the receptor for noxious heat. The first piece of evidence was provided by studies showing that TRPV1 knockout mice display marked impairments in the detection and response to noxious heat (Birder et al., 2002). Other studies have shown that this channel is mainly expressed in the majority of thermonociceptors (Caterina et al., 1997). The responses of this channel are markedly increased in the settings of tissue injury and inflammation (Tominaga et al., 1998). Indeed proalgesic agents such as protons and proinflammatory agents such as neurotrophins and bradykinin markedly reduce the response threshold of this channel (Tominaga et al., 1998). These characteristics reflect the ability of this channel to act as a molecular integrator of thermal and chemical stimuli (Birder et al., 2002; Davis et al., 2000). The capsaicin-insensitive thermosensitive nociceptors express the TRPV2, TRPV3 and TRPV4 ion channels. The TRPV2 expressing nociceptors have a higher stimulus threshold of about 52°C (Tominaga et al., 1998). Conversely the TRPV3 and TRPV4 expressing nociceptors have a stimulus threshold of between 25°C and 35°C (Lumpkin and Caterina, 2007). It can be clearly seen that the sensitivity profiles of these latter types of TRPV ion channels seem to flank those of the TRPV1 ion channel on either direction that is at lower and higher temperatures than those of TRPV1. Interestingly, the use of high dose capsaicin which results in the ablation of the central terminals of the TRPV1 expressing nociceptors is also associated with complete loss of thermosensitivity (Cavanaugh et al., 2009).

The cold receptor (CM1/ TRPM8), shows maximal responses to noxious cold at between 8° and 25°C (Jordt et al., 2003; McKemy et al., 2002; Peier et al., 2002). It is also strongly activated by menthol and eucalyptol (Hensel and Zotterman, 1951; Reid and Flonta, 2001). The transduction mechanism in these cold receptors is still not known. It is thought that noxious cold causes depolarization in the primary afferent either by inhibiting the Na/K pump or by promoting the influx of calcium and/or sodium ions (Askwith et al., 2001; Reid and Flonta, 2001; Suto and Gotoh, 1999). Melastatin 8 (TRPM8) was first discovered in 2002 (Jordt et al., 2003; McKemy et al., 2002). Experimental data from studies of TRPM8 null mice have helped establish that TRPM8 is the major cold and cooling transduction channel in mammalian sensory neurons (Bautista et al., 2007; Colburn et al., 2007; Dhaka et al., 2007; McKemy et al., 2002).This is illustrated by studies showing that TRPM8 deficient mice show substantial deficits in behavioral responses to noxious cold (Bautista et al., 2007;

Dhaka et al., 2007). Indeed TRPM8 is required for the analgesic effect of cooling compounds such as menthol (Proudfoot et al., 2006; Dhaka et al., 2007). Paradoxically, it is also essential for the development of cold hypersensitivity after tissue injury. It should be noted however that the TRPM8 channels are not involved in the transduction of noxious cold at temperatures below 10C (Story et al., 2003) a role that seem to be played by the TRPA1 channels. it should also be noted that rodents with TRPM8 deficient neurons show no deficits in the detection of noxious heat (Bautista et al., 2007). This is an indication that the TRPV1 and TRPM8 are not co-expressed in nociceptive neurons showing spatial and functional separation in the detection of noxious cold and noxious heat.

TRP Ankyrin 1 (TRPA1) is the only member of the Ankyrin sub-family found in mammals (Dhaka et al., 2007). It has 20% sequence homology to the TRPV1 structure. It is usually co-expressed with the TRPV1 channel in primary afferent nociceptor terminals. It is activated by cold temperature below 15° C and its activation mediates a variety of distinct noxious cold sensations including aching, prickling or burning cold (Bautista et al., 2008; Dhaka et al., 2007; Sawada et al., 2007; Story et al., 2003). It is also activated by menthol and icilin which both have a cooling effect (Bandell et al., 2004; Story et al., 2003). Many studies however seem to indicate that its main contribution to cold nociception is mainly in the setting of tissue injury and not under physiological conditions (Cavanaugh et al., 2009; Karashima et al., 2009; Kwan and Corey, 2009). This role is complementary to its role in chemonociception as explained in the succeeding paragraphs.

2.2.1. Mechanonociceptors

Mechanonociceptors are activated by mechanical stress. This mechanical stress can result from direct pressure, tissue deformation or from osmotic changes. There are two possible transduction mechanisms in mechanociceptors. The mechanical stress activates a mechanically gated protein. This results in an influx of cations resulting in depolarization of the primary afferent. Another mechanism involves a mechano-chemical process where the tissue deformation results in the release of a diffusible chemical messenger. The binding of the chemical messengers to specific receptors results in the opening of ligand-gated ion channels. This results in cation influx leading to the depolarization of the primary afferent

(Nakamura and Strittmatter, 1996). ATP is a good example of a diffusible chemical messenger. The TRPA ion channel may be involved in mechanonociception and also low threshold mechanosensation. This is because studies involving TRPA knockout mutants in a wide variety of animal species ranging from *Caenorhabditis elegans*, Drosophila to mice show deficiencies in mechanonociception at low temperatures (Bautista et al., 2007; Dhaka et al., 2007). In addition studies have shown that blockade of this channel by low molecular weight blockers reduces mechanical hyperalgesia secondary to inflammation in mice (Bandell et al., 2004). Many other ion channels and receptors have been proposed to act as the main mechanociceptors in primary afferents. These include the mec-4 and mec-10 ion channels which are members of the degenerin/Epithelial Sodium channel (DEG/ENaC) family of ion channels (Chalfie, 2009). Other ion channels suggested include the Acid Sensing ion channels (ASICs) ASIC1, ASIC2 and ASIC3 ion channels (Page et al., 2004; Price et al., 2000). It should be noted that much less information is available on mechanonociception compared to chemonociception and thermonociception (Lewin et al., 2008).

2.2.2. Chemonociceptors.

Chemonociceptors are activated by the binding of specific chemical substances to their specific receptors. These substances are usually released in response to tissue injury and/or inflammation. The binding results in the opening of ligand-gated cation channels. This leads to an influx of various cations into the cell and consequently depolarization of the primary afferent. Many types of ion channels have been implicated in chemonociception. The TRPA1 ion channel is activated by a diverse group of compounds. This chemical activation is in addition to its activation by cold temperatures as outlined above. The chemical activator compounds may be of either environmental or endogenous origin. Examples of the endogenous activator compounds include; arachidonic acid, Bradykinin, and oxidative stress by-products for example 15 deoxy Δ 12, 14 Prostaglandin J2 (15d-PgJ2) (Andersson et al., 2008; Bandell et al., 2004). Examples of the environmental activator compounds include those found in; mustard oil (allyl isothiocyanate), Cinnamon oil (cinnaladehyde), gas exhaust (acrolein) raw garlic and onions (allicin) and formaldehyde (Story et al., 2003; Woolf, 2010). This ion channel therefore signals the presence of noxious chemical

substances that are produced either endogenously or emanate from the environment (Basbaum et al., 2009). Further evidence for its role in nociception has come from a variety of studies showing that its messenger RNA is up regulated after tissue inflammation and peripheral nerve injury (Katsura et al., 2006; Obata et al., 2005). Experimental data showing that the subcutaneous injection of 15d-PgJ2 evokes nocifensive behaviors in rodents provides additional evidence that it's an endogenous ligand of these ion channels (Cruz-Orengo et al., 2008). Similarly to the TRPV1 channel with which it's frequently co-expressed, the activation thresholds are reduced in the presence of Bradykinin, phospholipase C and other inflammatory mediators (Bandell et al., 2004; Basbaum et al., 2009). This indicates therefore that the TRPA1 channels also act as molecular integrators of various nociceptive stimuli analogously to the TRPV1 channel. On the other hand the acid sensing ion channel (ASIC) receptor family forms probably the purest examples of chemosensitive nociceptors. This is because they only respond to local changes in hydrogen ion concentration (Waldmann et al., 1997).

2.2.3. Nociceptive transmission in the dorsal horn

Most of the primary afferent fibers terminate in the various laminae of the spinal cord (Basbaum, 2000). A-delta fibers usually synapse on second order neurons located in laminae I and V. C-fibers on the other hand terminate in laminae I and IIa (Snyder and McMahon, 1998; Braz et al., 2005). Glutamate is the primary neurotransmitter at the synapse between the primary afferents and the dorsal horn cells (Giordano, 2005). The primary afferents are capable of synthesizing and releasing other neurotransmitters at the synapse. The neurotransmitter released is dependent on the intensity of stimulation of the primary afferent as well as by injury to the neuron or tissue (Hokfelt et al., 1994; Woolf and Salter, 2000). The second order neurons then ascend in the spinothalamic tracts.

The dorsal horn neurons are anatomically and physiologically classified into nociceptive-specific (NS) and wide-dynamic range neurons (WDR). NS neurons are concentrated in laminae I and II. They receive input solely from nociceptive A-delta and C-fibers. They have small centre-surround receptive fields in which the central region is excited by high intensity stimuli. On the other hand the outer region is inhibited by non-noxious input. These

receptive field properties render the NS neurons maximally responsive to discrete high threshold inputs. This makes the NS cells ideal for their functions in localization and discrimination of noxious stimuli (Dubner and Bennett, 1983). The receptive field properties of the NS cells render them susceptible to sensitization with high frequency stimulation.

WDR neurons are found in progressively increasing numbers from laminae I, II, V to VI. Unlike the NS neurons these neurons receive both nociceptor and non-nociceptor inputs. They also receive both A-delta and C-fiber input. The cells have large receptive fields with limited discrete areas excited by non-noxious input. The receptive fields also contain broad zones that are highly responsive to high threshold stimulation (Dubner and Bennett, 1983). These characteristics make the WDR cells to exhibit a frequency-dependent, progressive increase in neuronal excitability in response to repeated electrical activation of afferent C-fibers ("wind-up") (Herrero et al., 2000; Mendell and Wall, 1965). This ultimately results in amplification of the pain signal.

2.2.4. Ascending nociceptive pathways.

Projection neurons originating from laminae I and V constitute the major output from the dorsal horn to the brain (Basbaum, 2000). These projection neurons form multiple ascending pathways that terminate in various regions of the brain. The ascending nociceptive pathways can be phylogenetically classified into two groups. The first group consists of the paleospinothalamic, spinoreticular and the spinomesencephalic tracts. It is the phylogenetically older one. These tracts run through the medial region of the brain stem. The second group consists of the Neospinothalamic tract, the spinocervical tract and the postsynaptic pathway of the dorsal horn (Millan, 1999). These tracts include the spinothalamic, spinoreticular, spinomesencephalic and the postsynaptic dorsal column.

Most of the projection neurons decussate and ascend in the anterolateral column of the spinal cord to form the spinothalamic tract. The spinothalamic tract is sub-divided into the Neospinothalamic and Paleospinothalamic tracts. Axons from neurons found in laminae I and II form the Neospinothalamic tract (NSTT). The Paleospinothalamic tract (PSTT) is formed by axons from neurons found in laminae IIa and V.

Although both the NSTT and PSTT are involved in conveying nociceptive information, there are distinct differences in the type of information conveyed. The NSTT is composed of NS fibers from laminae I and II that project to the parabrachial nucleus and the ventroposterior nucleus of the thalamus. The parabrachial projection subserves the autonomic and arousal responses to pain. It does this via secondary connections to the amygdala and hypothalamus. The thalamic projections of the NSTT on the other hand convey information about stimulus intensity, modality and localization (Almeida and Pertovaara, 2006; Giordano, 2005).

The PSTT on the other hand, is mainly composed of WDR neurons which originate from laminae IIa and V. It projects to the parabrachial nucleus, rostroventral medulla, thalamus, caudal pons and the mid-brain (periaqueductal grey area and periventricular grey area). The PSTT is mainly involved in the mediation of the perceptual, cognitive and emotional aspects of pain stimuli. It is clear from its anatomical projections to the midbrain it is also involved in the activation of the descending inhibitory pathways involved in the modulation of nociception.

The neurons of the spinoreticular tract originate primarily in Laminae V, VII and VIII of the spinal cord. They terminate in many sites throughout the brain stem reticular formation (Millan, 1999). Neurons from the reticular formation project to many areas of the brain, including the hypothalamus and the thalamus. They also project both directly and indirectly to the limbic forebrain and neocortex. This tract plays a critical role in mediating the motivational, affective, and neuro-vegetative responses to pain (Millan, 1999; Villanueva et al., 1990). This tract also plays an important role in the modulation of nociceptive transmission. It does this by activating the brain stem structures responsible for descending inhibition for example the PAG (Kevetter et al., 1982; Villanueva et al., 1990).

The Spinocervical tract originates mainly from laminae III and IV. It projects to the lateral cervical nucleus and the nucleus tractus solitarius (NTS). These two nuclei in turn project to these laminae. This tract is mainly involved in the mediation of the sensory-discriminative, affective and autonomic characteristics of pain (Almeida et al., 2004). It also plays a major role in the modulation of nociceptive transmission (Blomqvist et al., 1996; Lu, 1989; Millan, 1999; Yerzieski and Schwartz, 1986).

The postsynaptic pathway of the dorsal column mainly originates from laminae III and IV (Willis et al., 1999). The postsynaptic pathway of the dorsal column represents the largest afferent pathway for information of visceral origin. This has been confirmed by studies showing that visceral cancer pain is effectively controlled by midline myelotomy (Willis et al., 1999). This procedure is more effective than the interruption of the anterolateral pathways (Al-Chaer et al., 1996; Millan, 1999).

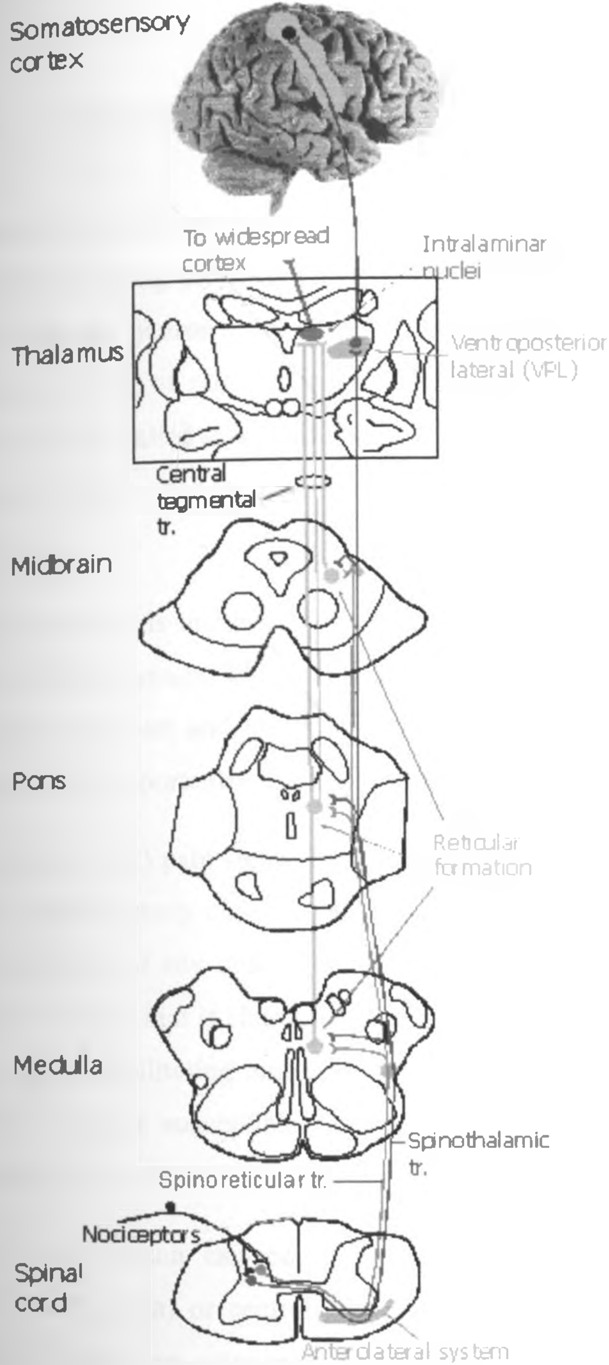

Figure 1. Diagram showing the afferent nociceptive pathways (copied from http://painconsortium.nih.gov/symptomresearch/chapter_25/images/fig1_v2.gif.Acessed on 5/11/2011).

2.3. Pathological pain states.

The nociceptive system is probably unique in its functional plasticity. This plasticity manifests itself all along the nociception neuraxis that is at the nociceptor, at the dorsal horn level, the projection neuron level and even in the higher brain centers. This plasticity can result in the lowering of nociceptor thresholds to the extent that they begin to respond to non-noxious stimuli (allodynia). It can also result in enhanced responses to noxious stimuli (hyperalgesia). These changes in the sensitivity of the nociceptor neuraxis are known as sensitization.

In acute pain states this increased hypersensitivity may actually be beneficial in promoting healing after tissue damage. This is because the tissue hypersensitivity promotes 'guarding' of the injured body part and limits its usage. This in turn promotes tissue healing in the affected part. Most importantly this hypersensitivity resolves with tissue injury.

In chronic (pathologic) pain states this hypersensitivity does not resolve with tissue healing but persists. Indeed many chronic pain states are characterized by hyperalgesia/allodynia even in the absence of any observable tissue damage (Apkarian et al., 2009; Zhuo, 2008). The spontaneous pain that is characteristic of clinical chronic pain states explains why these conditions are so debilitating and cause much morbidity in the community. The chronic pain sufferer becomes susceptible to other comorbid psychiatric and somatic conditions further complicating the condition.

Nociceptive sensitization can occur both peripherally that is at the nociceptor itself (peripheral sensitization) or centrally at the spinal cord and higher CNS centers (central sensitization). These two categories of sensitization may occur concurrently. Indeed one type may lead to the other; for example peripheral sensitization may lead to central sensitization and vice versa (Woolf and Salter, 2000; Woolf, 2010).

2.3.1. Peripheral sensitization

Peripheral sensitization occurs when the sensitivity and excitability of the nociceptor terminal is increased (Julius and Basbaum, 2001). This may occur when inflammatory

mediators such as Prostaglandins, nerve growth factor (NGF), bradykinin and ATP released during tissue injury or inflammation bind to receptors expressed on nociceptor terminals. The binding to the receptors results in the activation of various intracellular metabolic pathways resulting in the phosphorylation of receptors and ion channels in the nociceptor membrane. This results in the lowering of the nociceptor threshold that is increase in sensitivity and excitability of the nociceptor terminal.

The factors released during tissue injury/ inflammation are collectively referred to as the "inflammatory soup". The chemicals that form this soup belong to a wide array of chemical substances including; neurotransmitters, neuropeptides for example Substance P, CGRP, eicosanoids for example prostaglandins, thromboxanes, leukotrienes, neurotrophins, cytokines and chemokines (Basbaum et al., 2009). Examples of ion channels modulated by members of the inflammatory soup include; TRPV1, TRPA1 and ASICS. It is useful to note that the TRPV1 channel is profoundly modulated by elements of the inflammatory soup (Lumpkin and Caterina, 2007). Indeed there is now broad agreement that the modulation of this channel's function plays a vital role in tissue injury evoked hypersensitivity. This particularly happens in the setting of inflammation and even bone cancer (Honore et al., 2009; Lumpkin and Caterina, 2007).

Peripheral sensitization may also occur as a result of damage to the axon of the primary nociceptive afferent (Woolf, 2010). This consequently leads to alterations in the conductive or neurotransmitter properties of the neuron. These changes may lead to spontaneous depolarization of the neuron and changes in the neurotransmitter release patterns respectively. The mechanism by which damage to the primary afferent leads to hypersensitivity is much more complex than that for inflammatory changes. Some of the putative mechanisms involved are outlined.

Physical damage of peripheral afferents is thought to induce the release of specific humoral signals. These signals in turn are detected by, and activate, microglia (Woolf, 2010). Experimental evidence implicates ATP as one of the putative signaling molecules. It binds to P2X4 (Tsuda et al., 2003), P2X7 (Hughes et al., 2007) and P2Y12 (Kobayashi et al., 2008; Haynes et al., 2006) purinergic receptors. Experimental evidence for this putative role of ATP is provided by experiments which show that genetic and pharmacological blockade

of purinergic receptor function prevents or reverses nerve-injury induced mechanical allodynia (Tsuda et al., 2003 Honore et al., 2006; Kobayashi et al., 2008; Tozaki-Saitoh et al., 2008). The binding of ATP to its receptors is then believed to cause the release of Brain Derived neurotrophic factor (BDNF) by the activated microglia (Woolf, 2010).

BDNF then binds to TrkA receptors located on the projection nociceptive afferents located in lamina 1 of the spinal cord dorsal horn. The binding of BDNF to its receptor activates a signaling cascade of events all of which have the eventual effect of elevating neuronal excitability. Examples of these signaling events include; decrease in expression of Potassium Chloride ion co-transporter (KCCNQ) (Milligan et al., 2008).and the release of the chemokine fractalkine.

The Fractalkine receptor (CX3CL1) is expressed by both primary afferents and dorsal horn neurons. The receptor expression is up-regulated after peripheral nerve injury (Watkins et al., 2005; Zhang and An, 2007). Evidence for its involvement in pain hypersensitivity comes from experiments showing that the blockade of CX3CL1 with a neutralizing antibody prevents both the development and maintenance of injury-induced persistent pain (Milligan et al., 2004; Zhang and An, 2007). Cathepsin S is required for the release of fractalkine from the microglia and is released by activated microglia. Inhibitors of Cathepsin S inhibit nerve injury- induced hyperalgesia and allodynia (Clark et al., 2007). Peripheral nerve injury has been shown to activate microglia not only in the spinal cord but also in the brain stem. The activated brain stem microglia have been shown to contribute to the supraspinal facilitatory influences on nociceptive transmission (Gao et al., 2007). Descending facilitation is discussed in greater detail in succeeding paragraphs.

It should be noted that peripheral sensitization secondary to neural injury is associated with changes in transcription of more than 1000 genes (Woolf, 2010). This is in marked contrast to peripheral sensitization secondary to the inflammation that results from tissue injury. This is because in inflammatory sensitization the main mechanism of sensitization is mainly post-translational. The foregoing goes some way in explaining why neuropathic pain is much more refractory to treatment in the clinical setting compared to inflammatory pain. Peripheral sensitization often leads to or occurs concurrently with central sensitization to which attention is now turned.

2.3.2. Central sensitization

Central sensitization is defined as the process through which a state of hyperexcitability is established in the CNS leading to enhanced transmission (processing) of nociceptive impulses (Woolf, 1988). Central sensitization has been shown to occur in rodent, cat and non-human primate dorsal horn neurons. In addition, it can be generated in many parts of the CNS including the Rostroventral medulla, the Anterior Cingulate cortex and Amygdala (Kenshalo et al., 1982; Hentall et al., 1991; Schaible et al., 1991; Dougherty and Willis, 1992; Willis, 2002). This is an indication that central sensitization plays a pivotal role in the establishment of the central pain hypersensitivity characteristic of most clinical pain conditions.

Many mechanistic explanations have been put forward to try and explain central sensitization. However most experimental evidence seems to favor three main mechanisms; alterations in glutaminergic neurotransmission which in turn leads to NMDA receptor mediated hypersensitivity. This then leads to the two related phenomena "wind-up" and dorsal horn Long Term Potentiation (Dorsal LTP).The other two mechanisms proposed include the loss of tonic inhibitory control in the CNS (disinhibition) and the most recent one, glial –neuronal interactions (cross-talk) leading to immune-mediated hypersensitivity.

Wind-up is referred to as the reversible synaptic plasticity that occurs during a train of repeated low frequency C-fiber or nociceptor stimulation (Mendell and Wall, 1965). It is characterized by progressive increases in the amplitude and frequency of action potentials from dorsal horn neurons. It occurs as a result of the various neuropeptides released by the primary afferent neurons due to repeated stimulation. The neuropeptides released include Substance P and Calcitonin Gene Related Peptide (CGRP). The release of these neuropeptides results in the generation of slow synaptic potentials lasting for several hundred milliseconds (Murase et al., 1986; Silvilotti and Woolf, 1994). The temporal and spatial summation of these slow synaptic potentials is thought to result in "wind-up" (Woolf, 1996; Herrero, 2000). It is however a short-term phenomenon that only occurs during the period of nociceptor stimulation.

Dorsal horn LTP is an activity-dependent long lasting (as compared to wind-up) homosynaptic facilitation of Excitatory Post Synaptic Potentials (EPSPS) in response to brief high frequency nociceptor stimulation (Randic et al., 1993; Sandkuhler and Liu, 1995, 1998; Sandkuhler et al., 2000; Ueda et al., 2003). It seems to mainly occur in Substance P-expressing spinoparabrachial lamina I neurons. The induction of Dorsal LTP requires synergistic interaction between NMDA and NK1 receptors as well as the activation of a low-threshold T-type calcium current (Ikeda et al., 2003). The influx of Calcium into the neuron results in the activation of multiple downstream signaling pathways (Latremoliere and Woolf, 2009). Examples of these downstream molecules include the kinases; (mitogen-activated protein kinase (MAPK), protein kinase A (PKA), Protein Kinase C (PKC), Phosphoinositide -3 kinase (PI3K) and Src. These kinases further increase neuronal excitability especially by modulating NMDA receptor function (Latremoliere and Woolf, 2009). Experimental evidence for the involvement of Src in central sensitization is provided by studies that show that Src blockers are able to markedly attenuate the central hypersensitivity secondary to peripheral injury (Liu et al., 2008). Further to this Src null mice show reduced mechanical allodynia after nerve injury (Liu et al., 2008).

The process of dorsal horn LTP shares many electrophysiological characteristics with the more famous hippocampal LTP (Drdla and Sandkuhler, 2008). Drugs that block the development of spinal LTP markedly reduce hyperalgesia secondary to tissue injury (Sandkuhler, 2007). This is a strong indication that this is a possible mechanism of central sensitization (Sandkuhler, 2007). There is also accumulating evidence that metabotropic glutamate receptors in the dorsal horn are also involved in this process (Galik et al., 2008).

The superficial laminae of the spinal cord contain high densities of glycinergic and GABAergic interneurons and form the anatomic correlate of the "pain gate" expounded 40 years ago by Melzack and Wall (1965). Loss of function of these interneurons will therefore result in the establishment of central pain hypersensitivity states (disinhibition). Evidence for this is provided by studies showing that the administration of GABA-A (Bicuculline) and glycine (Strychnine) receptor antagonists results in the production of behavioral pain hypersensitivity (Yaksh, 1989; Silvilotti and Woolf, 1994; Malan et al., 2002). This hypersensitivity closely resembles that observed after peripheral injury (Yaksh, 1989). In

addition, peripheral injury has been shown to result in decreased tonic inhibition of the transmission neurons (Moore et al., 2002; Polgar et al., 2005). This results in enhanced nociceptive transmission to the CNS thereby contributing to the mechanical allodynia seen in central sensitization (Keller et al., 2007).

The mechanisms that underlie this disinhibition are varied. They include the modulation of glycinergic dorsal horn transmission via the spinal actions of Prostaglandin PGE_2 (Harvey et al.. 2004). Prostaglandins which are released in inflammatory states bind to EP2 receptors expressed by both the interneurons and projection neurons. The receptor binding activates intracellular signaling cascades that result in the phosphorylation of the GlyRα3 receptor subunits (Harvey et al., 2004).The phosphorylation of the receptor renders it unresponsive to the glycine leading to the increased excitability of the neuron. Central sensitization may also be accompanied by changes in the GABA-A receptor subunit expression (Knabl et al., 2008; Zeilhofer et al., 2009)

Glial cells especially microglia and astrocytes in addition to their already discussed roles in pathogenesis of peripheral sensitization also participate in the establishment of central sensitization. Microglia have been shown to rapidly cluster in the termination zone of injured peripheral afferents. These microglia then become activated and release a diverse range of inflammatory mediators for example cytokines (Tumor Necrosis Factor-α, Interleukin 1β and Interleukin 6). These cytokines enhance neuronal excitability leading to central sensitization (DeLeo et al., 2007). Indeed studies have shown that microglia are necessary and sufficient to trigger the persistent pain conditions characteristic of central sensitization (Coull et al., 2005; DeLeo et al., 2007; Tsuda et al., 2003).

The changes in the electrophysiological properties in the dorsal horn interneurons are more often accompanied by changes in the electrophysiological characteristics of the projection neurons. The most commonly documented electrophysiological change is in the expression patterns of the K^+Cl^- cotransporter (KCC2) (Coull et al., 2003). This transporter is highly expressed in lamina I projection neurons and plays a pivotal role in the maintenance of K+ and Cl- gradients across the plasma membrane (Coull et al., 2003). Downregulation of the transporter as seen in central sensitization results in the inversion of the Chloride gradient. This has the effect of inverting the response of the neuron to GABA from inhibition to

activation .This will obviously lead to enhanced nociceptive transmission (Coull et al., 2003; Keller et al., 2007).

Central sensitization has been shown to play a role in the pathogenesis of acute post-operative pain, post traumatic pain, migraine and neuropathic pain (Benoliel et al., 2002; Campbell et al., 1988; Koltzenburg and Scadding, 2001; Mannion et al., 1999; Stubhaug et al.. 1997; Woolf et al., 1989). All the above named conditions are characterized by the phenomenon where innocuous stimulation of the uninjured areas surrounding the injury also produces pain. This phenomenon is also known as secondary hyperalgesia. It results from the heterosynaptic facilitation where signals from the Aβ primary afferents which mediate light touch sensations are now routed through pain circuits leading to enhanced nociceptive transmission. It has been shown that these aberrant circuits are commonly established in the listed as well as other clinical pain syndromes (Campbell et al., 1988).

2.4. Algogenic substances

2.4.1. Substance P.

Substance P is released after high-intensity stimulation of and/or prolonged activity of c-fibers (Duggan et al., 1987; McCarthy and Lawson, 1989; Schaible et al., 1990). It initially binds to low affinity Neurokinin-2 (NK-2) receptors. It subsequently binds to the high affinity Neurokinin-1 (NK-1) receptors that are more sensitive to lower concentrations (Nakanishi, 1991).The binding of the substance P to the NK-1 receptors which are G-protein linked receptors leads to alterations in synaptic receptor structure and receptor expression (Giordano, 2005). This leads to changes in nociceptive transmission and consequently, hyperalgesia and allodynia (Scott and Luo, 2001; Thompson et al., 1994). Substance P containing neurons have been shown to be important for the development of hyperalgesia and noficensive behavior produced by capsaicin, inflammation and nerve injury (Mantyh et al., 1997; Nichols et al., 1999; Simone et al., 1999). Neurons possessing the substance P receptor (SPR) play a pivctal role in central sensitization. This is because they are capable of driving the sensitization of other nociceptive neurons after tissue injury (Brown et al., 1995; Littlewood et al., 1995). Interestingly, despite all the foregoing evidence implicating substance P in the pathogenesis of chronic pain, NK-1 receptor antagonists show poor

analgesic potency in clinical trials (De Felipe et al., 1998; Garces et al., 1993; Mansikka et al., 1999; Weng et al., 2001).

2.4.2. Arachidonic acid metabolites

Arachidonic acid and its metabolites occupy a pivotal position in nociception. This is especially true in the inflammation and sensitization present in pathological pain states. The metabolites involved in nociception include; thromboxanes, prostaglandins, leukotrienes, lipoxins, epoxyeicisatetranoic acids, isoprostanes and cyclopentenone prostaglandins (Smith, 2006). The listed metabolites of arachidonic acid are generated as a result of local tissue changes.

The rate limiting step in the generation of these metabolites is the release of arachidonic acid from the plasma membranes of the endoplasmic reticulum and nucleus. This reaction is catalyzed by Phopholipase A2 (Balsinde et al., 2002). Its expression is elevated in the dorsal horn in chronic pain models.

The arachidonic acid produced by the action of Phopholipase A2 can be metabolized via various pathways including; the cyclooxygenase pathway, the lipooxygenase pathway, the cytochrome P450 pathway and non-enzymatic lipid peroxidation pathway.

The cyclooxygenase pathway remains the most intensely studied arachidonic acid metabolic pathway. This is because of the historically important role attached to prostaglandin E in nociception and inflammation. Cyclooxygenase (COX) exists in 3 isoforms, COX-1, COX-2 and COX-3 (Chandrasekharan et al., 2002). COX-1 produces prostaglandins constituitively for secretion as extracellular mediators. In contrast, COX-2 produces prostaglandins predominantly within the nuclear fat. The two COX systems thus can be regarded as two distinct prostanoid biosynthetic systems with separate biological functions for their product (Smith, 2006). Conventional wisdom holds that COX-2 is an inducible enzyme while COX-1 is a constitutive enzyme. This is however an oversimplification.

This is because COX-1 expression can be induced under certain conditions such as neural insult. Indeed it has been found that spinal prostaglandins synthesized by COX-1 are important in the development of allodynia associated with neural insult (Hefferan et al.,

2003; Samad et al., 2002; Schwab et al., 2000; Zhu and Eisenach, 2003). COX-1 indeed plays a major role in the pathogenesis of neuropathic pain (Broom et al., 2004).

COX-2 is expressed constitutively by such tissues such as the kidney and nerve tissue. The expression of COX-2 is up-regulated by various neurotransmitters, lipopolysaccharides, calcium and small peptide hormones (Yermakova and O'Banion, 2000; Samad et al., 2002). Its expression is upregulated in inflammation and therefore plays a predominant role in inflammatory pain (Ma and Eisenach, 2002). The constitutive COX-2 in the spinal cord plays a major role in inflammatory hyperalgesia due to the Prostaglandin E2 it generates (Tanioka et al., 2000).

Prostaglandin E2 has a major functional role in nociception and inflammation. This is in addition to its other many physiological functions for example in vasodilation and in pyrexia (Dinarello et al., 1984; Samuelsson et al., 1987; Williams and Peck, 1977). The binding results in the activation of adenyl cyclase enzyme followed ultimately by the activation of protein kinase A. The protein kinase A phosphorylates the sodium channel NaV 1.8 which leads to enhanced tetrodoxin-resistant sodium current (Vanegas and Schaible, 2001). This leads to increased excitability of the primary nociceptor afferents, the release of substance P and ultimately peripheral sensitization.

The activation of protein kinase A also leads to the phosphorylation of the glycine α3 receptor (Harvey et al., 2004). This has the effect of inactivating the receptor. The glycine α3 is normally found on nociceptive afferents in the dorsal horn and its activation results in inhibition of nociceptive transmission (Marx, 2004). Therefore it can be seen that prostaglandin E2 has a facilitatory action on ascending nociceptive transmission. Other prostaglandins for example prostaglandins I, D2 and F2α may also play a role in nociceptive processing (Vanegas and Schaible, 2001).

2.5. Neurotransmitter systems in nociceptive transmission

2.5.1. Cholinergic system.

It is now known that both muscarinic and nicotinic receptors are involved in antinociception (Pleuvry and Tobias, 1971). The muscarinic and nicotinic receptors show differential

localization in the spinal cord. The muscarinic receptors are mainly found in the superficial and deep laminae of the spinal cord (Eisenach, 1999). The nicotinic receptors on the other hand are mainly found on the small primary terminal afferents (Khan et al., 2003).

The antinociceptive effects of both nicotinic and muscarinic receptor agonists as well as those of neostigmine can be blocked by the intrathecal administration of atropine (Harty et al., 1985; Zhuo and Gebhart, 1991). This indicates that the antinociceptive effects of acetylcholine are mainly mediated by muscarinic receptor mechanisms. It is thought that the nicotinic receptors cause antinociception by stimulating the release of acetylcholine from intrinsic spinal cholinergic neurons. The released acetylcholine then binds on muscarinic receptors to cause analgesia (Prado and Sagalla, 2004). There is evidence that the antinociceptive effects of acetylcholine are mediated by a combination of the M2 and M4 receptors (Wess et al., 2002).

Acetylcholine is released from the spinal cord in response to pain and activation of opiate receptors in the brain. It is also released in response to the activation of spinal α2 adrenergic receptors (Eisenach, 1999). This reflects the integration of the spinal cholinergic antinociceptive system with the descending pain modulating pathways. Neostigmine (a cholinesterase inhibitor) is used clinically for the relief of post-operative and cancer pain (Collins, 1995; Klamt et al., 1996; Prado and Goncalves, 1997).

2.5.2. Glutaminergic system.

Glutamate is the principal excitatory neurotransmitter in nociceptive transmission pathways. It is released from the primary afferent nocicepive terminals. Glutamate exerts its effects via a diverse set of receptors. These receptors are commonly classified either as ionotropic and metabotropic receptors. There are 3 types of ionotropic receptors; alpha methyl isoxazole (AMPA), Kainate and N-methyl D- Aspartate (NMDA) receptors. There are 8 types of metabotropic receptors commonly denoted as mGluR1- 8. The respective roles of the ionotropic and metabotropic receptors in nociception are outlined below.

The AMPA receptor is the main ionotropic receptor involved in normal acute nociceptive processing (Giordano, 2005). The NMDA receptor however is the main predominant

ionotropic receptor involved in nociceptive processing in pathological pain states (Woolf, 2003). This is because NMDA has a higher activation threshold than the AMPA receptor. The activation of the NMDA receptor results in an influx of Calcium ions into the cell. The raised intracellular calcium concentrations result in the activation of a myriad of calcium-dependent enzymes. This results in electrophysiological changes in the primary afferents leading to hyperalgesia.

NMDA receptors play a major role in central and peripheral sensitization (Chatterton et al., 2002). As a result many attempts have been made to synthesize NMDA receptor antagonists which would act as a novel group of analgesics. These attempts have been disappointing due to the unfavorable side-effect profiles of these molecules for example amnesia, hallucinations, etcetera (Chizh et al., 2001). The NR2B subunit of the NMDA receptor however seems to be selectively expressed in nociceptive pathways. Indeed NR2B agonists have shown promise as a novel group of analgesics with much better side-effect profile as compared to non-selective NMDA antagonists (Chizh et al., 2001).

Metabotropic glutamate receptors (mGluRs) belong to the class C of the GPCR superfamily. Eight (8) mGluRs have been cloned so far and divided into three groups; Group1 (mGlu1 and mGlu5), Group II (mGlu2 and mGlu3) and Group III (mGlu4, 6, 7, 8). These receptors are coupled to Gi/o and involve inhibition of adenyl cyclase enzyme and regulate the signaling activity of various ion channels in nociceptor primary afferents. Metabotropic glutamate receptors were once thought to play no role in nociceptive processing. However dorsal horn nociceptive neurons have been found to contain one or more of the eight types of the metabotropic glutamate receptors (Bhave et al., 2001).Their location can be either post-synaptic or pre-synaptic.

The postsynaptic receptors (mainly Group I) play important roles in the up-regulation of neuronal excitability. Since they are often co-expressed with the ionotropic glutamate receptors they also regulate the ionic currents in the ionotropic receptors. The Group I receptors play primarily a pro-nociceptive role. These receptors have been identified in the spinal cord, on the primary afferents, brain stem, thalamus, cortex and amygdala (Varney and Gereau, 2002). The activation Group I receptors in turn leads to the activation of Extracellular signal regulated kinase (ERK) in the spinal dorsal horn. The activation of ERK

leads to the phophorylation of the Kv4.2 Pottasium channels which then leads to the reduction of the A current in nociceptive neurons(Chen and Sandkuhler 2000; Hu et al., 2006; Jung et al., 2006; Karim et al., 2001). This consequently leads to an increase in neuronal excitability. Therefore the activation of these receptors causes hyperalgesia and conversely inhibition of these receptors leads to inhibition of hyperalgesia in inflammatory pain models (Walker et al., 2001).

Type 1 glutamate metabotropic receptors (mGluR1) play major roles in pathological nociceptive processing. They have been implicated in a variety of pain conditions for example inflammation, neuropathy and spinal injury (Dolan and Nolan, 2002; Hudson et al., 2002; Karim et al., 2001; Meller et al., 1993; Mills et al., 2000; Neugebauer, 2002; Walker et al., 2001; Zhang et al., 2002). The co-localization of the metabotropic and the NMDA receptors on the cell membrane of the primary afferent neurons has important pathologic implications (Boxall et al., 1998). This is because the metabolic cascade triggered by the activation of the metabotropic receptors ultimately results in the phosphorylation of the NMDA receptor. This results in both peripheral and central sensitization (Guo et al., 2004).

The type I metabotropic glutamate receptors have also been shown to play an important role in modulating nociceptive transmission in the higher centers. These receptors present a viable drug target in the development of novel analgesics. This is because of the roles played by these receptors in pathological pain processing. Indeed the inhibition of these receptors has been shown to attenuate post-operative pain (Zhang et al., 2002). The presence of ADX 10059 which is a mGluR5 receptor antagonist and which is now in phase II clinical trials vindicates this approach in analgesic drug discovery (Keywood et al., 2009).

The Group II and Group III receptors on the other hand are located presynaptically. Their activation usually results in a reduction in neurotransmitter release at the central terminals of the primary afferents (Yang and Gereau, 2004). Agonists of these classes of receptors are usually analgesic. They can however also be found on GABAergic interneurons in the spinal cord. Their activation causes a reduction in GABA release which results in enhanced ascending nociceptive transmission. The expression of Group II receptors is dramatically upregulated in the spinal cord following inflammation (Dolan et al., 2003). This upregulation has also been observed in a monoarthritic model in rats (Boxall et al., 1998;

Neto et al., 2001). The analgesic action of L-Acetylcarnitine is dependent on the upregulation of Group II receptors (Chiechio et al., 2002; 2006a; 2006b) and its activity is not observable in the absence of upregulation. Tolerance however develops with repeated administration of these Group II/III receptor agonists (Jones et al., 2005). This has dampened expectations that the development of agonists to these receptors constitutes a viable analgesic drug development strategy (Jones et al., 2005).

2.5.3. Noradrenergic system

Noradrenaline (NE) shows widespread distribution in the CNS. Most of the noradrenergic neurons in the CNS originate from the Locus ceruleus/subceruleus (A5 and A6) as well as A7. Indeed supraspinal descending pathways are the only source of noradrenaline in the spinal dorsal horn. Noradrenaline is involved in mediating the descending inhibition of spinal nociceptive transmission (Guo et al., 1996; Pertovaara et al., 1991). It acts via binding to the presynaptic α2 adrenergic receptors with the α2A subtype said to be more important (Kuraishi et al., 1979; Sagen and Proudfit, 1984). Noradrenaline tonically inhibits spinal nociceptive transmission that is even in the absence of any nociceptive stimulus (Reddy et al.. 1980). Noradrenaline interacts with the following neurotransmitters in the spinal cord; serotonin, opioids, substance P, nitric oxide and adenosine.

Noradrenaline blocks the release of substance P and glutamate from primary nociceptive afferents. This is the mechanism of action of the antinociceptive effects of noradrenaline (Eide and Hole, 1993; Jasmin et al., 2002; Olave and Maxwell, 2003). The depletion of spinal noradrenaline abolishes the antinociceptive effect of serotonin in the spinal cord (Minor et al., 1985; 1986). Noradrenaline potentiates the antinociceptive activity of the opioids and adenosine (Aran and Proudfit, 1990; Bohn et al., 2000; Isbrucker et al., 1990). The antinociceptive effects of nitric oxide are mediated via the release of noradrenaline in the spinal cord (Zhang et al., 1999).

There is accumulating evidence that some of the antinociceptive effects of NE may be mediated by the α1receptors (Baba et al., 2000a; 2000b; Gassner et al., 2009). This antinociceptive action may be mediated by the release of GABA and glycine by local

interneurons due to the excitatory effect of NE binding to these receptors (Gassner et al., 2009).

2.5.4. Serotonergic system.

Serotonin has dual effects in nociceptive transmission being pronociceptive in peripheral tissues but having antinociceptive activity centrally. The $5HT_3$ receptor which is a ligand-gated ion channel plays a pivotal role in nociceptive transmission (Sufka et al., 1992). It can be allosterically modulated by local anesthetics, gonadal steroids and neurosteroids (Giordano and Sacks, 1997).

Serotonergic fibers originate from the Rostroventrolateral medulla (RVLM) including the Nucleus Raphe Magnus (NRM) and the Nucleus Reticularis magnocellularis. These fibers project mainly to the superficial and to a lesser extent to the deep dorsal horn of the spinal cord. Serotonin by acting on its different receptor subtypes exerts complex modulatory effects on nociceptive transmission in the dorsal horn. The complex actions of serotonin in nociceptive transmission are amply illustrated by the experimental studies involving LMX mice These mice which lack Serotonergic neurones in the brainstem show less sensitivity to noxious mechanical stimuli but are more sensitive to noxious inflammatory stimuli compared to control mice (Zhao et al., 2007). These results seem to indicate that 5HT is antinociceptive in response to inflammatory stimuli and pronociceptive in response to mechanical stimuli.

$5HT_1$ receptor activation is mainly antinociceptive with the activation of postsynaptic 5HT1A in particular resulting in the inhibition of the excitability of spinothalamic fibers and excitatory interneurons of the spinal dorsal horn (Carmichael et al., 2008). Presynaptic 5HT1B/D receptors inhibit neurotransmitter release from the primary afferents (Granados-Soto et al., 2010). The putative antinociceptive activity is still a controversial subject with some authors claiming that the $5HT_7$ receptor is the one involved and not the $5HT_1$ receptor (Brenchat et al., 2009). Conversely pronociceptive effects are mediated by the $5HT_2$ and $5HT_3$ receptor subtypes (Kjorsvik et al., 2001; Suzuki et al., 2002).

$5HT_3$ receptors are ionotropic receptors whose activation results in depolarization of the neuronal membrane. The activation of presynaptic $5HT_3$ receptors causes an increase in neurotransmitter release and consequently and increase in the excitability of the spinothalamic tract fibers.

In peripheral tissues serotonin is released from peripheral mast cells. The serotonin binds to $5HT_3$ receptors located peripherally and in the dorsal root ganglion. This results in the release of substance P and/or other mediators of inflammation (Inoue et al., 1997). This results in a cascade of events which ultimately lead to neurogenic inflammation and peripheral sensitization.

The activation of the $5HT_3$ receptor also ultimately results in alterations in the sensitivity of the neurokinin receptors (Moore et al., 1999). The persistent activation of $5HT_3$ receptors located on visceral and peripheral efferents appears to sub serve a type of allodynia in conjunction with $5HT_4$ receptors (Smith et al., 1999).

The central $5HT_3$ receptors located on the pools of local and segmental interneurons in the spinal cord in contrast are involved in the mediation of supraspinal analgesia. This is via the serotonergic pathway that emanates from the raphe nucleus magnum of the rostroventral medulla (Martin et al., 1978).

2.5.5. Gaba-ergic system

The control of neuronal excitability has a major impact on nociception. GABA is the predominant inhibitory neurotransmitter in the CNS. Indeed, the attenuation or loss of this inhibitory function has been shown to be important in pathogenesis of chronic pain conditions (Coull et al., 2003; Milligan et al., 2008). GABA-ergic and glycinergic interneurons in the dorsal horn are involved in tonic inhibition of nociceptive transmission (Ishikawa et al., 2000; Millan, 1999; Silvilotti and Woolf, 1994). Two types of GABA receptors, GABA-A and GABA-B are implicated in the inhibition of nociceptive processing. GABA-A receptors are mainly located on the postsynaptic membrane whereas GABA-B receptors on the other hand are mainly located on the presynaptic membrane. The roles

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played by each class of receptor in nociceptive processing are outlined in the following paragraphs.

The ionotropic GABA-A receptors found on lamina II nociceptive dorsal horn neurons play a crucial role in nociceptive processing (Woolf and Salter, 2000). The thermal hyperalgesia and mechanical allodynia associated with inflammatory pain and other chronic pathological pain states has been shown to be accompanied by marked decrease in the inhibitory activity of these receptors (Millan, 1999; Patel, 2001; Poisbeau et al., 2005). The intrathecal administration of Bicuculline a GABA-A receptor antagonist has been shown to induce the appearance of pain behavior (Charlet et al., 2008; Yaksh, 1989). The decrease in receptor activity is accompanied by decreases in the synthesis of 5α neurosteroids which are important allosteric regulators of GABA-A receptor activity (Poisbeau et al., 2005).

The potentiation of GABA-A receptor function therefore provides a potentially viable approach in the development of new analgesic agents especially against neuropathic pain (De Koninck, 2007). In support of this hypothesis, 3α – reduced neuroactive steroids (neurosteroids) which are allosteric modulators of GABA-A receptors have been shown to be potent analgesics (Frye and Duncan, 1994; Pathirathna et al., 2005; Aouad et al., 2009). Etifoxine which is a non-benzodiazepine anxiolytic has been shown to have potent analgesic effects in a vincristine-induced neuropathic pain model (Aouad et al., 2009) further validating this therapeutic approach.

The analgesic activity of baclofen which is a GABA-B receptor agonist was the first indication that this class of receptor had a putative role in nociception (Dirig and Yaskh, 1995; Patel et al., 2001; Potes et al., 2006). The analgesic effects are mediated by both the spinal and supraspinal receptors. The activation of the supraspinal GABA-B receptors inhibits the ascending Dopaminergic and Noradrenergic inflow to the brain (Sawynok, 1984). The activation of the supraspinal GABA-B receptors conversely facilitates inhibitory descending noradrenergic outflow to the spinal cord dorsal horn (Sawynok, 1984). The anatomic location of these receptors at the spinal cord is mainly presynaptic on the peptidergic primary afferents (Price et al., 1984). The mechanism of action is via inhibition of the voltage-gated Ca channels on the presynaptic terminals of the primary nociceptive

afferents which in turn leads to inhibition of glutamate and peptide neurotransmitter release (Ataka et al., 2000; Bowery, 2006; Malcangio and Bowery, 1995; Marvizon et al., 1999).

GABA-B receptors are also involved in the mediation of the inhibitory effects of other neurotransmitters on glutaminergic neurotransmission. These include; cholinergic transmission via M1 receptors, adenosine via A1 receptors and endocannabinoids via CB1 receptors (Chen and Pan, 2004; Naderi et al., 2005; Suzuki et al., 2005). The tonic activation of GABA-B receptors appears to contribute to the establishment of nociceptive thresholds (Schuler et al., 2001). Conversely the inhibition of these receptors leads to the lowering of nociceptive thresholds (hyperalgesia). Indeed, GABA-B receptor knockout mice present with thermal hyperalgesia in the tail flick and hot plate tests (Magnaghi et al., 2008). Interestingly these mice have high thresholds to mechanonociceptive stimuli for example to the Von Frey filaments.

It has been shown that the analgesic action of serotonin in neuropathic pain is partially mediated by spinal GABA-ergic and cholinergic mechanisms related to different sub-types of 5HT receptors (Obata et al., 2002; Okazaki et al., 2008). There is much evidence indicating that the GABAergic, cholinergic and serotoninergic systems interact in antinociception (Furst, 1999).

2.5.6. Opioidergic system

The opioids consist of the most effective class of analgesics in clinical use today (Akil and McNally, 2002). There are more than a dozen endogenous opioids which are classified into 3 main groups; enkephalins, dynorphins and endorphins. They exert their antinociceptive effects by binding to opioid receptors which are all G-protein linked receptors. Three types of opioid receptors have been isolated; μ opioid receptors (MORs), δ opioid receptors (DORs) and κ opioid receptors (KORs) (Wang et al., 1994; Simonin et al., 1995). The opioid receptors show a high degree of structural homology but in their transmembrane and intracellular domains. They however differ widely in their extracellular domains. These differences explain the difference in ligand-sensitivity between the three receptor types (Akil

et al., 1998). The interactions between the opioids and their receptors are very complex with a lot of cross-sensitivity (Mansour et al., 1995).

All the three opioid receptors are coupled to G_i subtype of the G_α subtype. Therefore the activation of all the three types of opioid receptors results in the inhibition of Adenyl cyclase. The net result of opioid binding is a reduction in neuronal excitability (Stein et al., 2009). Various intracellular mechanisms are involved in this reduction of neuronal excitability. The activation of the three types of opioid receptor results in the suppression of the activity of the various types of Ca^{2+} channels (N, T- and P/Q) found on the presynaptic membrane of the primary afferent (Childers, 1991; Kiefer, 1995; Stein et al., 2009). This results in a reduction in the Ca^{2+} influx into the presynaptic neuron. Consequently this leads to a reduction in the excitation and/or neurotransmitter release in many neuronal systems. A prominent example of this is the inhibition of substance P release from central and peripheral terminals of sensory neurons (Yaksh, 1988; Kondo et al., 2005).

Opioid receptors mediate hyperpolarization at the postsynaptic membrane by causing the opening of K+ rectifier ion channel. This has the net effect of reducing neuronal excitabilty thereby preventing excitation and/or propagation of action potentials (Zöllner and Stein, 2007). There is also evidence that these opioid receptors may also be coupled to other second messenger systems (Gutstein et al., 1997).

The analgesic effects of the opioids are exerted both peripherally and centrally. Opioid receptors are expressed peripherally on small, medium size and large-diameter dorsal root ganglion neurons (Buzas and Cox, 1997; Cheng et al., 1997; Coggeshall et al., 1997; Gendron et al., 2006; Mansour et al., 1995; Rau et al., 2005; Silbert et al., 2003; Wang and Wessendorf, 2001). The peripheral antinociceptive effects of opioids are mediated by peripheral opioid receptors (Bartho et al., 1990; Stein et al., 1990; Stein, 1995). The peripheral opioid receptors are often coexpressed with neuropeptides such as substance P (SP) and calcitonin-generelated peptide (CGRP) (Li et al., 1998; Minami et al., 1995; Mousa et al., 2007a, b; Ständer et al., 2002) in a pattern similar to that of the central opioid receptors. As a result of this coexpression, opioid agonists can attenuate inflammation induced increases in the excitability of primary afferent neurons and the release of proinflammatory neuropeptides (SP, CGRP) from central and peripheral terminals (Junger et

al., 2002; Stein et al., 2003). Particularly within injured tissue, these events lead to antinociceptive and anti-inflammatory effects.

The expression of these peripheral opioid receptors is upregulated in the presence of tissue inflammation as well as in the presence of neural damage (Ballet et al., 2003; Ji et al., 1995; Kabli and Cahill, 2007; Mousa et al., 2002; Püehler et al., 2004; Shaqura et al., 2004; Truong et al., 2003; Walczak et al., 2005; Zöllner et al., 2006). The upregulation in receptor expression occurs acutely that is within minutes or hours after tissue damage/ neural damage. The molecular mechanisms that underlie this upregulation are varied and include increased receptor trafficking to the cell membrane as well as increased receptor protein synthesis among others (Stein et al 2009; Zollner et al., 2006). These peripheral opioid receptors therefore mediate the early peripheral antinociceptive response which in most cases precedes the midbrain mediated descending analgesia.

The central opiodergic analgesic effects arise from the activation of the descending inhibitory systems arising from the mid brain structures (Gutstein et al., 1997; Mansour et al., 1995). The first evidence for an endogenous opioid system came from studies showing that microinjections of morphine into the Periaqueductal Grey Area (PAG) caused analgesia (Yaksh et al., 1977). The analgesic effect could be reversed by the administration of naloxone (Akil et al., 1976). Opioids also exert forebrain mechanisms of analgesia in addition to the dorsal horn and mid brain mechanisms described above (Franklin and Mathies, 1992; Manning and Mayer, 1995). This is in addition to evidence implicating the amygdala in morphine analgesia (Harris, 1996). It should be noted that all the three types of opioid receptors participate in the mediation of the descending opioid analgesia in contrast to earlier beliefs which tended to ascribe these effects solely to the μ receptors.

2.5.7. Endocannabinoid system

The endocannabinoid system is made up of the endocannabinoids anandamide (arachidonoylethanolamide) and sn-2-arachidonoylglycerol (2-AG) their receptors (CB1 and CB2) and the metabolic apparatus for the endocannabinoids (Guindon and Hohmann, 2009;

Nyilas et al., 2009; Price et al., 2003).The endocannabinoids are produced in an activity dependent manner and act to modulate synaptic transmission by binding to the aforementioned receptors. It is important to note that these endocannabinoids act in a retrograde manner in the synaptic modulation.

The endocannabinoid system has been implicated in nociceptive processing in addition to its many roles in cognition for example learning, memory and emotional processing (Hohmann and Suplita, 2006). Indeed *Cannabis sativa* (marijuana) has been used since antiquity as an analgesic (DiMarzo et al., 2004; Mechoulam, 1986). Furthermore, marijuana has been licensed in the U.S. and Canada to be used medically for the relief of intractable pain in cancer and multiple sclerosis (Perez and Ribera, 2008). However its use in modern therapeutics is limited due to its multiple psychotropic effects as a result of the binding of the main active ingredient in marijuana delta tetrahydrocannabinol on central CB1 receptors (Hollister, 1974; Jones et al., 1974).

Both anandamide and 2-AG have been shown to exert antinociceptive effects in the Formalin Test (Guindon et al., 2007; Jaggar et al., 1998). These findings were reinforced by results showing that CB1 receptor knockout mice had elevated nociceptive responses to the intraplantar injection of capsaicin and formalin (Agarwal et al., 2007). Interestingly there is increased expression of the CB2 receptors on nociceptive afferents in the chronic constriction injury (CCI) and spinal nerve ligation (SNL) models of neuropathic pain (Beltramo et al., 2006; Zhang et al., 2003). Both classes of receptor seemed to be involved in the mediation of the antinociceptive effect of the endocannabinoids (Nackley et al., 2003). Indeed the non-selective receptor agonist was shown to have analgesic effects in the carrageenan, bone cancer and the burn-induced thermal and mechanical hyperalgesia models (Kehl et al., 2003; Khasabova et al., 2004; Ruiu et al., 2003).

It should be noted that compounds which potentiate the action of the endocannabinoid system via other non-receptor mediated mechanisms have also shown analgesic activity. Some of these mechanisms include; inhibition of breakdown pathways and inhibition of the reuptake of the endocannabinoids among others. Inhibitors of the enzymes Fatty Acid Hyrolase (FAAH) and Monoacylglycerol Lipase (MGL) which are the metabolizing enzymes of the endocannabinoids anandamide and 2-AG have been shown to possess

antinociceptive activity in inflammatory pain models (Chang et al., 2006; Jayamanne et al., 2006; Lichtman et al., 2004; Russo et al., 2007a, b). These compounds however did not have any activity in neuropathic pain models. Indeed Paracetamol is converted by FAAH into AM404 which in turn inhibits the reuptake of anandamide thereby potentiating endocannabinoid system activity (Mallet et al., 2008; Smith, 2009).

In conclusion it is also important to note that the analgesic effects of endocannabinoids seem to be mainly indirect. That is the analgesic effects are mainly mediated by potentiating the activities of other neurotransmitter systems. Experimental evidence has shown antinociceptive effects of cannabinoids involve activation of the opioid system and vice versa (Da Fonseca Pacheco et al., 2008; Ibrahim et al., 2006). Indeed the analgesic effects of paracetamol have been shown to rely on a fully functional descending serotonergic system (Hole et al., 1991). Ablation of these descending serotonergic neurons is associated with the loss of the analgesic activity (Bonnefont et al., 2005; Hole et al., 1991; Pelissier et al., 1996). It is also noteworthy that CB receptor agonists either directly or indirectly increase the analgesic effect of non-steroidal anti-inflammatory drugs (NSAIDs) (Guindon et al., 2006a, b. Naidu et al., 2007; Ulugöl et al., 2006). This further buttresses the notion that the main mode of action of endocannabinoids is mainly indirect.

2.6. Antinociceptive mechanisms.

The modulation of nociceptive transmission is of great clinical and scientific interest. This is because of its application in the management of clinical pain states. The knowledge obtained from studies of nociceptive modulation can be used in the development of novel analgesics

2.6.1. Descending modulation of nociceptive transmission.

It is now a well established fact that brain stem structures play significant roles in modulation of nociceptive transmission especially at the spinal cord level (Basbaum and Fields, 1984; Millan, 2002; Sandkuhler, 1996). It is commonly considered that these brainstem-spinal cord pathways inhibit nociceptive transmission (Basbaum and Fields, 1984). There is however accumulating evidence that these descending pathways also have facilitatory effects on nociceptive transmission (Lima and Almeida, 2002; Pertovaara, 2000;

Porreca et al., 2002; Ren and Dubner, 2002; Urban and Gebhart, 1999; Vanegas and Schaible, 2004).

Most descending pain inhibitory pathways originate in or relay through a number of brain stem nuclei. Each pathway has a different neuroanatomical connection and utilizes a unique neurotransmitter (Pertovaara, 2004; Pertovaara and Almeida, 2006). It should be noted that there is no anatomical separation between the structures involved in the mechanisms of descending inhibition and descending facilitation. The separation is mainly physiological. Indeed, common loci in both the rostroventral medulla and nucleus tractus solitarius (NTS) give rise to pathways mediating descending facilitation and descending inhibition via contrasting mechanisms (Basbaum and Fields, 1984; Fields et al., 1991; Nuseir and Proudfit, 2000).

The descending pathways modulate nociceptive transmission by interacting with various types of neurons found in the dorsal horn. These include primary afferent terminals, projection neurons, excitatory interneurons, inhibitory interneurons and even terminals of other descending pathways (Basbaum and Fields, 1984; Millan, 2002).

2.6.2. Anatomical sites of origin of descending modulatory pathways.

Figure 1 shows the main brain areas that are the origins of descending modulation pathways. These areas are discussed in more detail

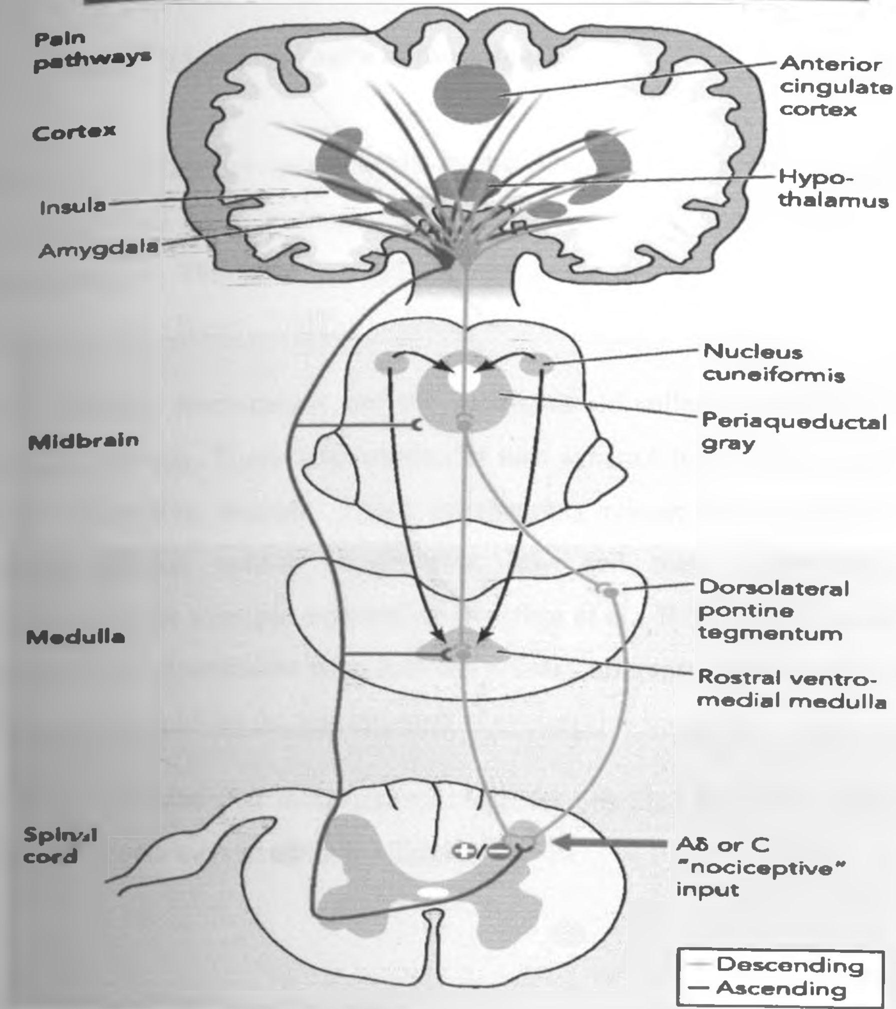


Figure 2. Diagram showing the anatomical sources of descending nociceptive modulation (Adapted from; Bingel U. and Tracey I. Imaging CNS Modulation of Pain in Humans. Physiology. 2008; 23: 371-380).

2.6.3. The dorsal horn of the spinal cord.

The local modulatory mechanisms in the dorsal horn are mainly mediated by intrinsic excitatory and inhibitory interneurons. These modulatory mechanisms are mainly intrasegmental. This is in marked contrast to the descending pathways whose effects are intersegmental (Jessell, 2000).

The inhibitory interneurons are the recipients of collateral input from the nociceptive primary afferents. These interneurons in turn synapse upon primary afferents and second order nociceptive neurons. These interneurons release the inhibitory neurotransmitters GABA, glycine, opioids (dynorphins, leu- and met- enkephalin) and endogenous cannabinoids for example amantadine (Bautista et al., 2007). These interneurons inhibit the release of neurotransmitter from both the primary afferents as well as the projection neurons. This therefore inhibits the transmission of nociceptive impulses to supraspinal structures.

It should be noted that these intrinsic interneurons also form the substrate via which the descending pathways exert their effects.

2.6.4. The hypothalamus.

The hypothalamus plays an important role in nociceptive processing. This is in addition to its roles in the coordination of autonomic and sensory information. Several hypothalamic centers including the paraventricular nucleus, the arcuate nucleus, the tuberomammilary nucleus and the posterior periventricular nucleus provide direct projections to the dorsal horn and other regions of the spinal cord (Porreca et al., 2002). Corticotrophin releasing factor and the ACTH both produce moderate analgesia (Berridge and Dunn, 1990).

The hypothalamus receives a major nociceptive sensory input from the dorsal horn via the Neo spinothalamic tract (NSTT) (Giesler, 1995). The medial pre-optic nucleus, anterior

hypothalamus, lateral hypothalamus, arcuate nucleus and the ventromedial /dorsomedial nuclei of the hypothalamus have all been implicated in nociceptive modulation.

The medial preoptic nucleus has strong connections to the Nucleus Tractus Solitarius (NTS), periaqueductal grey area (PAG) and to the rostroventral medulla (RVM). Glutamate is the neurotransmitter involved (Millan, 1999). The stimulation of this nucleus inhibits the response of the projection neurons following noxious stimuli. This is in addition to being strongly involved in the autonomic responses via its anatomic connections outlined above (Lumb, 1990).

The stimulation of the anterior hypothalamus has been shown to suppress the response of the WDR neurons in the dorsal horn (Carstens, 1996; Workman and Lomb, 1997). The injection of opioids into the posterior, arcuate and pre-optic nuclei has been shown to elicit antinociception (Manning and Franklin, 1998; Yaksh, 1999). Stimulation of the lateral hypothalamus elicits antinociception via the activation of the descending noradrenergic pathways to the dorsal horn via relays to the PAG and RVM (Franco and Prado, 1996; Holden and Naleway, 2001).

2.6.5. Nucleus tractus solitarius (NTS) and parabrachial nucleus.

The NTS receives a major input from the vagus nerves and plays a major role in the processing of visceral nociceptive information (Gamboa-Esteves et al., 2001). The stimulation of the NTS elicits antinociception (Aicher and Randic, 1990; Morgan et al., 1989)

The NSTT projects to the Parabrachial nucleus which plays an important role in the relay and integration of nociceptive and autonomic information (Basbaum and Fields, 1984; Yoshida et al., 1997).Various subdivisions of this nucleus project to the NTS, rostroventral medulla, the trigeminal nucleus and the dorsal horn of the spinal cord. Stimulation of the Parabrachial nucleus suppresses the response of the dorsal horn neurons to both nociceptive and non-nociceptive input (Beitz et al., 1987; Yoshida et al., 1997).

2.6.6. Rostroventral medulla (rvm).

The rostroventral medulla first gained attention as the relay nucleus through which the Periaqueductal grey area exerted its descending inhibitory effects on nociceptive transmission. This is because the periaqueductal grey area lacks a direct anatomical connection to the dorsal horn of the spinal cord (Basbaum and Fields, 1984). Although it receives some direct sensory input the activity of the descending pathways emanating from it is primarily modified by afferents from the Periaqueductal grey (PAG), the Parabrachial nucleus and Nucleus tractus solitarius (NTS) which are involved in the processing of nociceptive information (Basbaum and Fields, 1984; Fields et al, 1991; Zagon, 2001). The RVM is involved in both descending facilitation and inhibition of nociceptive transmission.

Rostroventral medulla sites which act as substrates for descending facilitation and inhibition appear to be intermingled with no obvious sign of topographical separation (Basbaum and Fields, 1984; Zhuo and Gebhart, 1997).

The neurons of the rostroventral medulla can be classified into 3 groups according to their physiological response characteristics (Fields et al, 1991);

a) ON cells that give an excitatory response to a noxious stimulus and are inhibited by opioids. These cells are thought to be involved in the mediation of descending facilitation

b) OFF cells which are indirectly stimulated by opioids via a GABAergic mechanism and inhibited by noxious stimuli. They are thought to be involved in the mediation of descending inhibition (Basbaum and Fields, 1984).

c) NEUTRAL cells on the other hand give variable responses or are unresponsive to noxious stimuli (Fields et al., 1991). The ON and OFF cells play a major role in the modulation of nociceptive processing under conditions of sustained pain due to inflammation and injury to peripheral afferents (Azami et al., 2001; Porreca et al., 2002). The function of the NEUTRAL cells remains unclear although there is speculation that the serotonergic NEUTRAL cells contribute to spinal anti-nociceptive action by modulating effects induced by the ON and OFF cells (Mason, 1999). Both OFF and ON cells are serotonergic in nature.

The anatomical connections to the PAG ensure that the rostroventral medulla is the final relay station for descending antinociceptive action from all the structures of the brain (Gebhart, 2004).The noradrenergic neuron groups A6 (locus ceruleus), A7 and A5 are also found in the rostroventral medulla and are the source of the descending inhibitory noradrenergic pathway (Kwiat and Basbaum, 1992; Proudfit, 1988). These noradrenergic cell groups are connected to other pain control centers including the PAG which projects to them via glutaminergic afferents (Beitz, 1990; Bajic et al., 2001) mediating descending inhibition.

2.6.7. The periaqueductal grey area (PAG).

The Periaqueductal grey area is the midbrain grey matter that is located around the cerebral aquaduct within the midbrain. It plays a pivotal role in the modulation of nociceptive processing (Bajic et al., 2001; Fields and Basbaum, 1978). It is heterogeneous in terms of its cytoarchitecture and in terms of neurotransmitters found within it (Basbaum and Fields, 1984; Fields et al., 1991; Millan, 1982; Ruiz-Torner et al., 2001). It has reciprocal connections with a diverse set of CNS structures including the amygdala, the hippocampus, the hypothalamus and the cerebral cortex. This ensures that it is the efferent arm of the descending inhibition of nociception in the dorsal horn mediated by virtually all the supraspinal CNS structures (Almeida and Pertovaara, 2006). The PAG has no direct anatomical link to the spinal cord. Rather, its anatomical links to the serotonergic neurons of the RVM as well as to the A7 noradrenergic neurons of the medulla are important pathways for the mediation of its descending inhibitory effects on nociceptive transmission (Cameron et al, 1995; Mason, 1999; Odeh and Antal, 2001). Excitatory glutaminergic and neurotensin-containing neurons project to the RVM and mediate the descending inhibition. These neurons are under the tonic inhibitory control of GABA-ergic inhibitory interneurons in the PAG (Basbaum and Fields, 1984). It has been found that the inhibition of these inhibitory interneurons by cannabinoids and μ receptor agonists for example morphine contributes to their induction of analgesia by the PAG (Basbaum and Fields, 1984; Hernandez and Vanegas, 2001).

In contrast, a few inhibitory GABA-ergic and enkephalin-containing neurons project to the rostroventral medulla. These neurons have direct anatomical connections to neurons mediating descending inhibition and descending facilitation. This is thought to contribute to descending facilitation and inhibition respectively (Fang and Proudfit, 1998). These neurons also target inhibitory interneurons in the rostroventral medulla and can therefore indirectly mediate descending inhibition (Bajic et al, 2001).

2.6.8. Medullary reticular nucleus.

A discussion of descending modulation would be incomplete without a discussion of the roles of the medullary reticular nucleus and the cerebral cortex in the modulation of nociception.

The dorsal reticular nucleus receives nociceptive input from both somatic and visceral tissue. It projects directly to both superficial and deep laminae of the spinal cord. These anatomical connections include direct connections to a population of projection neurons. These projection neurons in turn project to the dorsal reticular nucleus. They thereby close a reverberating dorsal horn- dorsal reticular nucleus- dorsal horn loop (Dugast et al., 2003).

Its pattern of brain projections in rats suggests that the nucleus is possibly implicated in the modulation of the ascending nociceptive transmission in a number of ways. It is probably involved in the motivational-affective dimension of pain. It is also probably involved in the modulation of the endogenous supraspinal pain control system centered in the periaqueductal gray area(PAG) and also probably involved in the modulation of the motor reactions associated with pain (Almeida and Pertovaara, 2006).

2.6.9. Cortical and sub-cortical structures.

Cortical structures along with functionally associated subcortical structures such as the amygdala and the cerebellum have been found to play a greater role in the modulation of nociception than has been previously supposed (Bechara et al., 2000; Casey, 1999; Labuda and Fuchs, 2001). Most of these novel insights have been obtained using neuroimaging

techniques especially PET scans and Functional MRI (fMRI) (Akparian, 2005; 2009; Bingel and Tracy, 2008). The concept of a "pain matrix" consisting of brain areas activated by nociceptive stimuli has emerged from these studies. Some of the brain areas that belong to this "pain matrix include; the somatosensory cortices S1 and S2, the insula, the amygdala, the cerebellum, the various regions of the prefrontal cortex such as the Anterior cingulate cortex (ACC), dorsolateral prefrontal cortex and the medial prefrontal cortex as well as the thalamus (Bingel and Tracy, 2008).

The pain matrix consists of areas that subserve the sensory discriminative aspects (S1, S2, thalamus, dorsolateral prefrontal cortex) and the affective (Anterior cingulate cortex, insula, amygdala, medial prefrontal cortex) aspects of pain. The current scientific thinking on the roles of higher brain centers in pain processing is that they play pivotal roles in the establishment of the non-linear relationship between stimulus and pain sensation (Apkarian et al., 2009).

The rich anatomical connections between these forebrain areas and the mid brain and brain stem nociceptive areas underlie the modulatory roles of the cortical areas on nociception. Fibers from the frontal, prefrontal and orbital cortex can engage limbic circuits of the amygdala, septal nuclei and hypothalamus to modulate nociception and pain perception (Petrovic et al., 2002). The prefrontal cortex has strong projections to the Nucleus Raphe Magnus and other regions of the rostroventral medulla (Apkarian et al, 2001). Stimulation of the anterior cingulate cortex elicits descending facilitation in the rat (Calejesan et al., 2000). Activation of the PAG by the rostral anterior cingulate cortex is thought to play a major role in placebo-induced and opioid-induced analgesia (Petrovic et al., 2002). Numerous studies have shown that the ventrolateral orbital cortex (VLO) forms part of an endogenous analgesic system consisting of the PAG, spinal cord dorsal horn and the submedius nucleus of the thalamus (Craig and Burton, 1981; Huo et al., 2008). A more detailed discussion of the mechanisms of placebo analgesia will follow below.

2.7. Mechanisms of action of analgesics.

The notion that there is one class of drug, "the universal analgesic" which can treat all forms of pain is now obsolete. This is because the pain forms differ in terms of etiology,

mechanisms and temporal characteristics (Scholz and Woolf, 2002). That said, the analgesics in clinical use can be classified according to their site of action and/or mechanism of action. Using this method of classification, analgesics can be classified into locally acting and centrally acting analgesics.

Locally acting analgesics usually act by blocking the transmission of nociceptive information from the peripheral nociceptors to the CNS. They do this in various ways. Local anesthetics cause local analgesia by direct interaction with voltage-gated Na+ channels. This blocks the Na+ current ensuring that the generation of active potentials in the primary afferents is prevented (Butterworth and Strichartz, 1990; Catterall et al., 1996). Anti-depressant drugs for example amitryptiline are frequently used to treat diabetic neuropathic pain. One of the ways in which they act is by blocking sodium channels. They therefore prevent the ectopic discharges that cause hyperalgesia and allodynia in this condition (Graves and Hanna, 2005). Capsaicin acts by depleting substance P from the afferent nerves. The area being treated gradually becomes insensitive to pain (Lynn, 1990). The non-steroidal anti-inflammatory drugs (NSAIDs) act by inhibiting cyclooxygenase enzyme (Smith and Willis, 1971; Vane, 1971). They therefore prevent the production of prostaglandins which cause peripheral and central sensitization as outlined above.

Centrally acting analgesics usually prevent nociceptive transmission in CNS structures. They can act at either the dorsal horn or at the supraspinal structures involved in descending modulation of nociception. The opoid drugs act both at the dorsal horn and at the supraspinal structures. They block the release of substance P and other excitatory neurotransmitters in the dorsal horn (Jessell et al., 1978; Kondo et al., 2005; Yaksh et al., 1980). They produce antinociception in the mid brain centers partly by removal of the GABA-ergic inhibition of the rostroventral medulla-projecting neurons of the PAG (Fields et al., 1991; Millan, 1999). Clonidine is used clinically for the relief of migraine and post-operative pain. It is an α_2 adrenergic receptor agonist and acts at the dorsal horn and at the homologous trigeminal nucleus. It is administered intrathecally for the relief of post operative pain. This is an indication that its locus of activity is mainly in the dorsal horn (Lee and Yaksh, 1995; Paalzow and Paalzow, 1976; Paech et al., 2004). Neostigmine which is also administered intrathecally is a cholinesterase inhibitor. It therefore increases the

concentrations of acetylcholine in the dorsal horn. It is used clinically for the relief of post-operative and cancer pain (Collins, 1995; Klamt et al., 1996; Lauretti et al., 1996). The antidepressants may also exert their analgesic activity by inhibiting the reuptake of Serotonin and Noradrenaline in the dorsal horn as well in the supraspinal structures. This has the effect of increasing their local concentrations in the CNS. This leads to antinociceptive effects (Millan, 2002).

Placebos have been shown to activate the opioidergic descending modulatory pathways in brain imaging studies (Petrovic et al., 2002). Placebo analgesic effects can be reversed by administration of naloxone (Levine et al., 1978). Opioid-mediated placebo effects are thought to involve the periaqueductal gray area (PAG), which contains many of the brain's opioid-containing neurons (Oliveira and Prado, 2001; Rosen et al., 2004; Willis and Westlund, 1997). Subsequent studies have shown that the placebo effect also involves non-opioidergic mechanisms (Gracely et al., 1983). The increase in opioid activity in the PAG and other opioid-rich subcortical areas of the brain is usually secondary to activation of the dorsolateral and medial prefrontal lobes (Wager et al., 2007).

2.8. Depression

Depression is a chronic, recurring and potentially life-threatening illness. It affects up to 20% of the global population (Berton and Nestler, 2006; Gillespie and Nemeroff, 2005; Manji et al., 2001). It has been estimated to be the second cause of disability worldwide (Murray and Lopez, 1996). The precise pathophysiology of depression remains obscure. A number of hypotheses on its pathophysiology have been put forward. A brief discussion of these hypotheses follows below.

2.8.1. Monoamine hypothesis of depression.

This was the first major hypothesis of depression formulated about 30 years ago. It proposed that the main symptoms of depression are due to functional deficiency of norepinephrine, serotonin and/or dopamine. These three neurotransmitters are the major monoamine

neurotransmitters in the brain. This hypothesis has received support from recent research findings that show that depression results from the dysfunction of monoamine neurotransmitter circuits in the brain (Nemeroff and Ressler, 1999; Nemeroff and Owens, 2002). Further evidence in support of this hypothesis comes from the fact that all currently available antidepressants act by modulating the function of monoaminergic neurotransmitter systems (Morilak and Frazer, 2004). These antidepressants act in one of three ways. Some act via the blockade of presynaptic monoamine transporter proteins, thereby increasing the local concentrations of serotonin and/or norepinephrine. Examples of this type of drugs are the selective serotonin reuptake inhibitors for example fluoxetine. Some antidepressants inhibit monoamine oxidase which is the main enzyme involved in breaking down of catecholamines for example moclobemide. Some types of anti-depressants act by inhibiting or activating various types of pre- and post-synaptic receptors involved in the regulation of monoamine transmission or neuronal excitation.

The delay in treatment response to antidepressants (3-5weeks) despite the fact that these drugs have immediate pharmacological effects on CNS neurons is quite intriguing. This has led to the almost universal view that monoamine systems are not the final common pathway in terms of the pathogenesis of depression (Nemeroff and Owens, 2002). This has led to a decrease in acceptance of the monoamine hypothesis.

2.8.2. Hypothalamic-pituitary-adrenal (HPA) axis hypothesis of depression.

A substantial percentage of depressed patients show derangements in their HPA axis. Hyperactivity of the HPA axis is observed in the majority of depressed patients (Binder and Nemeroff, 2010). This is manifested by hypercotisolemia. There are also increases in the levels of corticotrophin releasing factor (CRF) in the cerebrospinal fluid. This is accompanied by reduced feedback inhibition of the HPA axis by CRF and glucocorticoids. Clinical studies indicate that normalization of the HPA axis is a necessary step for the remission of depression (DeBellis et al., 1993a,b; Veith et al., 1993).

The above findings have suggested that the HPA axis and CRF in particular play major roles in the pathophysiology of depression. CRF is heterogeneously distributed in the CNS in addition to being released by the hypothalamus. It serves to orchestrate the endocrine, autonomic, behavioral and immune responses to stress (Binder and Nemeroff, 2010). The neural pathways of CRF interact extensively with noradrenergic and serotonergic systems. The antidepressant activity of CRF1 receptor antagonists provides support for the putative role of CRF in the pathophysiology of depression. These drugs have however been plagued by problems of toxicity (Nestler and Krishnan, 2008).

2.9. Methods of preclinical assessment of novel putative analgesic molecules

2.9.1. Nociceptive tests

Various behavioral animal models of pain have been developed and have found extensive use in research (Le Bars et al., 2001).The relevance of these behavioral tests to the actual clinical pain states that they are supposed to represent remains controversial (Besson, 1999). One of the most valid criticisms of these pain models is that they involve the application of noxious stimuli in healthy animals while clinical pain often appears as a symptom of an underlying pathophysiological process for example the neuropathy associated with diabetes or the pain associated with neoplastic disorders. In general behavioral pain models should possess the following attributes; specificity, sensitivity, validity, reliability and reproducibility (Cooper and Vierck, 1986; Gjerstad et al., 1997; Hammond, 1989; Watkins, 1989). There are various classification schemes for these nociceptive tests/models.

The first scheme involves the classification of the tests according to the nature of the nociceptive stimulus used that is into electrical, mechanical and chemical. This classification method is however of limited utility. It has been superseded by the method of classification that is based on the duration of the delivery of noxious stimuli. Using this scheme of classification nociceptive tests are classified into Phasic and tonic pain tests (Le Bar et al., 2001).

2.9.2. Phasic pain tests.

Phasic pain tests are the most commonly used tests due to their convenience, speed and hence the rapidity in obtaining experimental data. They are therefore commonly used in the rapid screening of putative analgesic molecules. They involve a short period of stimulation in the order of seconds and involve small somatic sites of stimulation. They can also be classified according to the nature of the stimulus used into mechanical, thermal and electrical phasic pain tests. In these types of pain tests, the experimental parameter recorded is usually the response time to a stimulus of increasing intensity. This therefore involves the assumption that the reaction time is related to the stimulus intensity. These types of tests suffer from the disadvantage that because they involve measuring threshold responses they do not provide any information about responses to frankly nociceptive stimuli or even different intensities of nociceptive stimuli. They also suffer from the disadvantage that they do not accurately mimic clinical pain states which are mainly chronic. Examples of phasic pain tests include the Tail Flick Test and the hot plate test.

2.9.3. The tail flick test.

The Tail Flick Test is a phasic nociceptive test which uses a thermal stimulus. There are two types of the Tail Flick Test. The first type involves the application of radiant heat to the animal's tail (Hardy et al., 1957) while the second types involves the application of convectional heat in that it involves the immersion of the experimental animal's tail in water heated to a predetermined noxious temperature (Janssen et al., 1963). The immersion type of the Tail Flick Test involves a much larger area of stimulation and results in abrupt movement of the tail and in some case even of the body (Ben-Bassat et al., 1959; Grotto and Sulman, 1967).The reaction times in these experiments are usually between 2 and 10 seconds with common reaction times between 2 and 4 seconds (Raffa et al., 1992). The experiments have to be stopped if the reaction times go beyond 10-20 seconds to avoid tissue damage.

The experimental variable of interest in both variants is the reaction time involved in the withdrawal and movement of the tail respectively. The prolongation of the reaction time is taken as an indicator of analgesic activity (Cargill et al., 1985; Peets and Pomerantz, 1987; Raffa et al., 1992). The advantages of the Tail Flick Test are its simplicity, the speed of data collection and the small inter-animal variability in reaction times under a given set of controlled experimental conditions (Peet and Pomeranz, 1987). It also has the advantage that cold stimuli can also be used in the immersion variant of this experiment (Pizziketi et al., 1985; Wang et al., 1995).The Tail Flick Test is however prone to habituation with the level of habituation being positively correlated with repetitive stimulation and increase in stimulus intensity (Carstens and Wilson, 1999). This test has been said to be efficient only for revealing the activity of opioids and not for the activity of mild analgesics (Grumbach, 1966) although some workers have proposed modifications of the immersion model using lower temperatures to increase the sensitivity to minor analgesics for example NSAIDS (Luttinger, 1985). The sensitivity to mild analgesics can also be increased by using a bath where the temperature of the water is increased slowly (Farre et al., 1989).

2.9.4. Tonic pain tests

Tonic pain tests involve the use of irritant algogenic substances as the nociceptive stimulus. They have the major advantage over the phasic pain tests in that they involve the quantitative determination of behavior after administration of a nociceptive stimulus. They can therefore be thought of as models of tonic pain as their responses are in the orders of tens of minutes. They have the disadvantage of being laborious and time consuming as they involve the intraperitoneal or intradermal administration of algogenic agents. Examples of these tonic pain tests include the Formalin Test and the intradermal administration of either Bradykinin, capsaicin, Freunds adjuvant, hypertonic saline, etc. (Foong et al, 1982; Hwang and Wilcox, 1992; Sakurada et al., 1992; Ueda et al, 2003).

Experimental models of visceral pain that involve the stimulation of hollow organs can be regarded as variants of tonic pain models. These models of visceral pain can be further sub-divided according to stimulus type. The first group includes those involve the administration of algogenic agents for example the writhing test that involves the intraperitoneal

administration of 0.5 % acetic acid (Hammond, 1989; Vyklicky, 1979). The second group involves the distension of hollow organs with or without the administration of algogenic agents (Laird et al., 2001; Ozaki et al., 2002).

2.9.4.1. The formalin test.

The Formalin Test is the most widely used model of tonic pain. 0.5-15% solution of formalin is injected into the dorsal surface on the hind paw of a mouse or rat. It provokes biphasic nociceptive behavior which can then be scored. The biphasic behavior consists of an initial phase occurring in the first five minutes after injection followed by a quiescent period and then a second phase between the 20th and 30th minutes. Various methods for scoring pain behavior have been developed. The first method described by Dubuisson and Dennis (1977) for the rat involves making a continuous record of how the animal treats the injected paw, with full weight bearing scoring `0', light pressure on the floor or limping `1', elevation of the paw `2' and licking or biting the paw `3'. The response is given a mark, and the results are expressed either continuously per unit time or at regular intervals when several animals are observed sequentially (Abbott et al, 1999). The test is optimized by weighting each level on this scale (Coderre et al., 1993; Abbott et al., 1999; Watson et al., 1997). A pain score with a range of 0 to 3 is then computed by using a formula (Dubuisson and Dennis, 1977). This method of scoring has been used in the mouse and in primates (Murray et al., 1988; Tjolsen et al., 1992). Both the weighted score, and a simple sum of time the paw is elevated or licked, are log-linearly related to formalin concentration in rats (Rosland et al., 1990; Aloisi et al., 1995; Clavelou et al., 1995). The other methods of assaying nociceptive behavior are; counting the number of flinches, licks or shakes of the injected limb in unit time (Wheeler-Aceto and Cowan, 1991), the cumulative time spent in biting, licking or biting the paw (Sufka et al., 1998).

All the scoring methods have attracted much criticism due to the fact that they are quite laborious and time-consuming and so researchers are always trying to develop novel and improved scoring methods (Abbot et al., 1999). Some of these novel scoring methods include; time sampling methods (Watson et al., 1997; Abbot et al, 1999). Most of the scoring methods were first developed for use in rats but because of high rates of movement

among mice these scoring methods have had to be modified (Hunskaar et al., 1985; Hunskaar and Hole, 1987; Rosland, 1991; Tjolsen et al., 1992; Gjerstad et al., 1997).The most common method of scoring in the mouse Formalin Test involves the cumulative scoring of the time spent licking and/or biting the injected paw (Saddi and Abbott, 2000).

Various explanations have been put forward to try and explain the biphasic nociceptive response. The first phase is as result of the direct stimulation of the nociceptors whereas the second phase results from a period of sensitization during which inflammatory phenomena occur (Tjolsen et al., 1992).

The Formalin Test is sensitive to opioid drug substances. This is because opioids show antinociceptive activity against both phases of the nociceptive behavior response with the second phase showing greater sensitivity (McCormack et al., 1998). NSAIDS in contrast show activity only against the second phase (Hunskaar and Hole, 1987; Shibata et al., 1989; Yaksh and Malmberg, 1992; Jourdan et al., 1997). It should be also taken into consideration that stress levels in the experimental animal may affect the nociceptive threshold and can therefore confound the experimental results. It is necessary to habituate the animals to the experimental apparatus and environment before performing the experiments (Taylor et al., 1998).

2.10. Animal models of depression.

In common with pain, the wide spectrums of disruptions that characterize depression highlight the difficulty that researchers face in developing valid laboratory animal models for depression (Lucki et al., 2002). Despite these problems a few models that show good predictive validity as well as reproducibility have been developed (Geyer and Markou, 1995).

2.10.1. The forced swim test.

The Forced Swim Test is the most widely used tool for the preclinical assessment of antidepressant activity (Porsolt et al., 1977). The popularity of this method stems from its ease of use, reproducibility, speed and its sensitivity that is its ability to detect a wide

spectrum of antidepressant agents (Borsini and Meli, 1988). Although first developed for use in rats the adaptation of this method for use in mice further increased its utility (Porsolt et al., 1977; Porsolt et al., 2000). The test is based on the fact that rodents acquire a posture of immobility after trying to escape when placed in an inescapable cylinder of water. The development of immobility since it's associated with behavioral despair or disengagement from stressful stimuli can be positively affected by the administration of antidepressant compounds (Lucki, 1997). The antidepressants will therefore increase the duration of escape behavior. A major drawback of this test however, is its relative insensitivity to the effects of the Selective Serotonin Reuptake Inhibitors (SSRI's) the most widely prescribed group of antidepressants (Castagne et al., 2006). This shortcoming led to the development of the Modified Forced Swim Test which is sensitive to the effects of the SSRI's (Castagne et al., 2009). The modifications to the Forced Swim Test are as follows; the depth of water is increased to 30cm as compared to 15-18 cm used in the Forced Swim Test and the scoring of the following pain behaviors using a time sampling technique; climbing, swimming and immobility (Lucki, 1997). The increased sensitivity can be explained by the fact that the increased depth used ensures that the time of immobility is reduced considerably. Catecholaminergic agents decrease immobility with commensurate increases in climbing behavior while serotonergic compounds decrease immobility while increasing swimming behavior (Cryan et al., 2001; Lucki, 1997; Reneric et al., 2001; Welberg et al., 2001).

2.10.2. The tail suspension test.

The tail suspension test is another commonly used animal model of depression. Its popularity is only rivaled by that of the Forced Swim Test. Its popularity stems from its ease of use, reliability, sensitivity and reproducibility (Porsolt, 2000). The test animal is suspended from a horizontal bar and the behaviors scored are the immobility latency and also duration of the immobility during a time period of 300 seconds. As in the forced swim test immobility is taken as an indication of behavioral despair and hence the duration of immobility will be reduced after the administration of the antidepressant agents (Geyer and Markou, 1995). It shows very good predictive validity (Bai et al., 2001). The disadvantage with this test is that some mice strains display tail climbing behavior leading to invalidation of the results (Mayorga and Lucki, 2001).

CHAPTER 3.

3.0. MATERIALS AND METHODS.

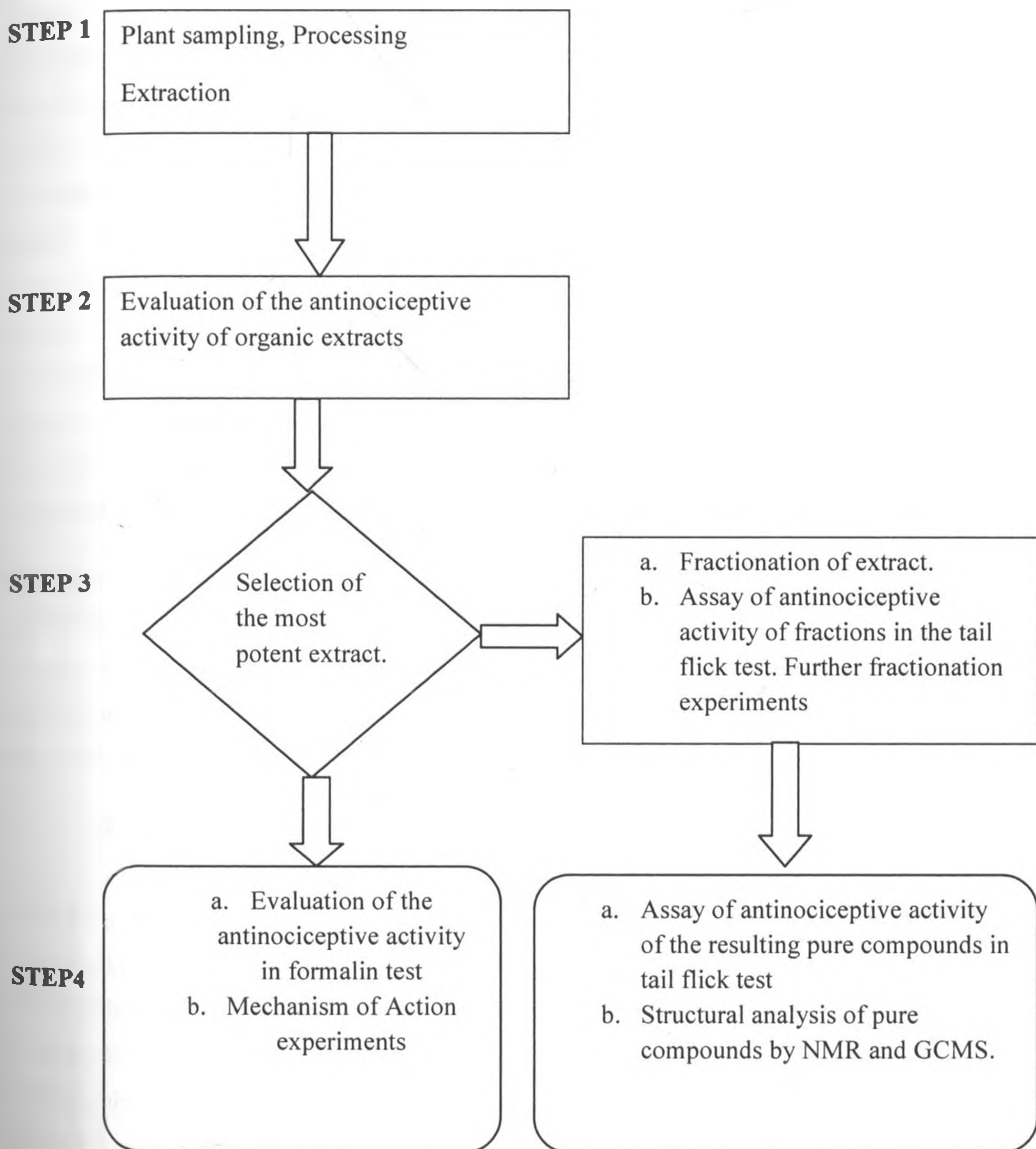


Figure 3. Flow chart depicting steps in the experimental procedure.

The steps undertaken in the experimental procedure are depicted in figure 3.

3.1. Collection of plant materials

The collection of the specimens of the three plant species *Ocimum masaiense* Ayobangira ex Paton, *Ocimum kenyense* Ayobangira ex A.J. Paton and *Ocimum kilimandscharicum* Baker ex Gurke which are all from the Lamiaceae family was carried out between the months of September and October 2007. The three (3) species were selected because of their proximity to the university and so were economical to collect. In addition, *Ocimum kenyense* and *Ocimum kilimandscharicum* were known to be used in traditional medicine as analgesic agents. The *Ocimum kenyense* and *Ocimum kilimandscharicum* were collected at the Lenana area of Nairobi while the *Ocimum masaiense* was collected at the Ngong area of Kajiado district. The whole plant that is, the aerial parts as well as the roots, were collected. Ten (10) kilograms (fresh weight) each of the *Ocimum masaiense* and *Ocimum kilimandscharicum* were collected while five (5) kilograms (fresh weight) of the *Ocimum kenyense* were collected. Lower quantities of the *Ocimum kenyense* were collected compared to those of the other two species in view of its smaller size and relative scarcity.

The identities of the collected plant specimens were identified at the herbarium located within the School of Biological Sciences (SBS), University of Nairobi. A voucher specimen of each of the plant species collected was deposited at the herbarium. The voucher numbers are *Ocimum masaiense* (voucher no. 2309007), *Ocimum kenyense* (voucher no. 2309008) and *Ocimum kilimandscharicum* (voucher no. 2209005).

3.2. Sample preparation

Plant parts used were the roots, leaves and stems of each of the plant species. The leaves were plucked out and air dried at room temperature. The other plant parts were separated and air-dried for one week at ambient room temperature away from direct sunlight. The woody parts that are the roots and stems were cut into small pieces so as to facilitate the drying of the plant parts. The three plant species as well as their respective plant parts were processed and stored separately in order to avoid cross contamination of the plant samples. The dried stems and roots were milled into a fine powder using a standard laboratory scale

mill (Kanchan, India). The dried leaves were ground into a fine powder using a standard kitchen blender (Phillips).

3.3. Extraction

The extracts were prepared by soxhlet extraction for four hours. Fifty (50) grams of the respective plant part powder material was placed in the one liter soxhlet apparatus and extracted with solvent in a ratio of 1:10 w/v (50g: 500ml) to completeness. General Purpose Laboratory grades of pure solvent were used in the extraction process. Three types of extract were prepared for each plant part for each of the species; ethanol, chloroform and chloroform/ethanol (1:1 mixture).

The extracts were evaporated to dryness in a rotary evaporator (Ugo Basile Laborota® 1073) at 40° C and 376 pascals pressure. The extract was then weighed and placed in an airtight amber-colored sample bottle and stored at 4° C in a refrigerator. A total of twenty-seven extracts were prepared.

3.4. Experimental animals

Adult swiss albino mice aged 5-6 weeks and weighing between eighteen and twenty- five grams were used as the experimental animals. The animals were procured from Kenya Agricultural Research Institute (KARI). They were housed in standard animal cages. Care was taken to maintain ambient temperatures of 20° C and 23° C within the animal house. The relative humidity in the animal house was maintained at between 45%- 55%. A twelve (12) hour light-dark cycle was maintained within the animal house. The animals were fed *ad libitum* with food pellets obtained from Unga Feeds (K) Ltd. Water was also provided *ad libitum.*

Ethical approval to conduct the experiments was obtained from the Department of Medical Physiology. All the animal experiments were conducted in accordance with the NIH guide for the care and use of laboratory animals (NIH Publication No. 80-23; revised 1978). More specifically, the pain experiments conformed to the guidelines issued by the International Association for the Study of Pain (IASP) for animal pain experimentation.

3.5. The tail flick test.

The extracts produced above were screened for antinociceptive activity using the radiant tail flick test using an analgesiometer (IITC Model number W33). The mice were acclimatized by habituation to the testing environment for an hour prior to the beginning of testing. The tail flick test was carried out in a room adjacent to the area where the animals were usually kept within the confines of the animal house. This ensured that the animals were exposed to broadly similar environmental conditions in the test room.

The mice received either extract at 100 mg/kg, 200mg/kg, 400mg/kg or 800mg/kg dosage levels or solvent mixture (Normal saline+ DMSO) which acted as the negative control. The extract was prepared for injection by using a solvent mixture of DMSO (Dimethyl sulfoxide) as the suspending agent and normal saline in a ratio of 0.1:0.9 respectively. The randomization of the mice to the various experimental groups was carried out using an online randomization tool obtained at www. randomization.org. there were five (5) experimental groups for each extract (4 experimental and 1 negative controls). Each experimental group contained five (5) animals.

The extract was injected intraperitoneally and the tail flick test was performed one (1) hour later to ensure maximal absorption of the compounds contained in the extract. The beam intensity of the analgesiometer was set at eighty (80) and cut-off point response duration was set at twenty (20) seconds to avoid tissue damage. The experimenter was blind to the treatment group of the experimental mice. The tail flick latencies were determined using a stopwatch and recorded in seconds and rounded off to one decimal place. Paracetamol (200mg/kg) was used as a positive control.

The results were analyzed using the Kruksal-Wallis non-parametric test in view of the small subject numbers and the skewness of the experimental data obtained using GraphPad Prism ™ suite of statistical software. The significance level was set at $P \leq 0.05$. The relative potencies of the extracts were determined by comparing the mean+/- Standard error of the mean (S.E.M.) of the most potent dose in the different experimental groups. The most potent extract which was the chloroform/ethanol extract of *Ocimum masaiense* root (ECOM) was used in the subsequent phases of the project.

3.5.1. The evaluation of the analgesic activity of chloroform/ethanol extract of *Ocimum masaiense* roots in the formalin test

Twelve (12) mice were randomly assigned to either the extract (100mg/kg) or the vehicle group. 50μL of 0.5% w/v formalin solution was then injected into the dorsal surface of the hindpaw of each mouse one hour after the intraperitoneal administration of extract/vehicle. The time the animal demonstrated pain behavior after injection of the formalin was then scored in blocks of five minutes for a total of one hour. The pain behavior was defined as the licking, biting and shaking of the injected paw. The scorer was blind as to the experimental group of the mouse.

The total time spent in pain behavior in the first 10 minutes after formalin injection was recorded as the total time spent in behavior in first phase of the formalin test. The total time spent in pain behavior between 20 and 60 minutes after injection of the formalin was recorded as total time spent in pain behavior in the late phase of the formalin test. Statistical analysis of the experimental data obtained was performed using the unpaired t-test using GraphPad Prism ™statistical software suite. The significance level was set at $P \leq 0.05$.

3.5.2. Mechanism of action experiments.

Various receptor agonists/ blockers were administered together with the extract/ control. This was so as to evaluate the possible mechanism of analgesic action of the extract.The mice were randomized to receive either; (a) the vehicle, (b) extract or (c) the extract+ blocker/agonist before undergoing testing in the formalin test.

The blocker/agonist was administered thirty (30) minutes before the extract in the extract+ blocker/agonist groups. All the drugs were administered intraperitoneally. The doses of blocker/agonist drugs used were obtained from literature. Each experimental group contained six (6) animals.

The blockers/agonists used in the experiments were the following; Ketamine (a non-selective NMDA receptor antagonist), Atropine (a non-selective muscarinic receptor blocker), Capsaicin (a vanilloid receptor agonist) and Naloxone (a μ opioid receptor receptor antagonist).The data was analyzed using ONE-WAY ANOVA using the GraphPad suite of software. The significance level was set at $P \leq 0.05$.

3.5.3. Evaluation of the antidepressant activity of Chloroform/ethanol extract of *Ocimum masaiense* roots

The antidepressant activity of ECOM was evaluated using the modified forced swim and tail suspension tests respectively. The mice were randomized to receive either vehicle or the extract which were administered intraperitoneally. The extract was administered at a dose of 100mg/kg. Each experimental group in each experiment that is control and treatment consisted of 5 (five) animals.

3.5.4. Modified forced swim test

The procedure used was as described by Porsolt (Porsolt *et al*., 1977). The mouse was placed with a cylinder filled with water at a temperature of (23-25) °C. The test was modified by increasing the depth of the water in the cylinder to 15 cm above the bottom of the cylinder (Lucki, 1997; Cryan *et al*., 2002). The animals were placed in the cylinder one hour after extract/saline administration. The times spent in swimming, climbing, and immobile behavior were measured. The experimental sessions were recorded using a video camera. The experimental sessions were then scored by a scorer blind to the types of treatment administered to the experimental treatment. Immobility behavior was defined as the time spent still or only using righting movements to remain afloat. Swimming behavior was defined as any movement horizontal in nature that involved at least two limbs. Climbing behavior was defined as any vertical movement in which the bottom of the front paws touched the sides of the cylinder. A time sampling technique was employed whereby the predominant behavior in each 5-s period of the 300-s test was recorded. Since the experimental animal was the laboratory mouse, the behavior in the first 60 seconds of the

test period was not recorded. The experimental data was analyzed using the unpaired student's t-test method. The significance level was set at $P \leq 0.05$.

3.5.5. Tail suspension test

The mice received either the vehicle or CEOMR in a dose of 100mg/kg. The tail suspension test was then performed thirty (30) minutes after administration of extract/vehicle. The tail suspension test was performed by suspending the mouse upside down by the tail. The animals were individually suspended thirty (30) cm above the laboratory bench surface via adhesive tape attached two (2) cm from the tip of the tail to a horizontal metal bar. The experimental duration of six minutes was recorded using a video camera. The dominant behavior during each 5-second time block was scored. The behavioral parameters of interest were either immobility or climbing behavior. The scoring of behavior was performed by a scorer blind to the experimental group of the mouse. The experimental data was analyzed using the unpaired student's t-test. The level of significance was set at $P \leq 0.05$.

3.6. Fractionation experiments.

Ten (10) grams of dichloromethane/ethanol extract of *Ocimum masaiense* roots were adbsorbed on twenty (20) grams of GRADE 20 silica gel. The sample was then packed into a column containing three hundred (300) g of GRADE 20 Column chromatography silica gel (E Merck). The sample was then fractionated using the adsorption liquid-solid column chromatography technique. The fractionation process used the following successive solvents and solvent mixtures; dichloromethane 100%, followed by a 1:1mixture of dichloromethane and ethyl acetate. This was then followed by ethyl acetate 100% and then a 1;1 mixture of ethyl actetate and methanol. The extract was then finally fractionated using pure methanol. The solvents were added in batches of 200mls. The resulting fractions were finally combined on the basis of Thin Layer Chromatography (TLC) results into six major fractions which were labeled as I-VI.

3.6.1. Tail Flick testing of the fractions obtained

The analgesic activity of each of the fractions was assayed in the tail-flick test in the manner described above. Paracetamol at a dose of 200mg/kg body weight was used as the positive control. The solvent mixture used as the vehicle for the suspension of the extracts (normal saline 0.9ml + 0.1 ml DMSO) was used as the negative control. The dosage levels of 12.5mg/kg, 25mg/kg, 50mg/kg and 100mg/kg of the extract were used in the tail flick experiments. The data were statistically analyzed using the Kruksal-Wallis test using the Graph Pad Prism statistical suite of software. Significance was set at $p < 0.05$.

3.6.2. Isolation of pure compounds and assay of antinociceptive activity.

All the fractions were shown to possess significant analgesic activity. However only Fractions I,II, III, IV underwent further fractionation in the adsorption based liquid-solid chromatography technique since Fractions V and VI were too polar and could therefore not be successfully fractionated using the available equipment. The further fractionation processes are summarized below

Fraction I on TLC analysis using pure dichloromethane as the mobile phase was found to contain more than seven spots so a decision was made to further fractionate the fraction in a smaller column using a solvent mixture of dichloromethane/ ethyl acetate (9:1) as the mobile phase. This yielded twelve (12) compounds but the yields were too low for bioassay and structural elucidation

Fraction II underwent preparative TLC using DCM: Acetone (9.5:0.5) solvent mixture as the mobile phase after TLC analysis using the same solvent mixture. This yielded three (3) pure crystalline compounds on the basis of TLC. Two of the crystalline compounds were in sufficient quantities for bioassay in the tail flick test and were coded compounds **Ia** and **Ib**.

Fraction III was subjected to preparative TLC using DCM; acetone (8:2) solvent mixture as the mobile phase after TLC analysis using the same solvent mixture. This yielded four (4) compounds but whose quantity was insufficient for bioassay.

The fractionation process yielded a total of sixteen (16) pure compounds on the basis of TLC but only two were in sufficient quantities to allow for bioassay and structure determination.

The analgesic activity of the two pure compounds named Ia and Ib was evaluated in the tail-flick test. Paracetamol at a dose of 200mg/kg was used as the positive control. A mixture of normal saline and DMSO used to suspend the extract was used as the negative control. The compounds were administered at dosage levels of 25mg/kg, 50mg/kg and 100mg/kg.

3.6.3. Identification of Pure compounds

The structures/identities of these pure compounds were determined using Proton (^{1}H) and Carbon (^{13}C) Nuclear Magnetic Resonance (NMR) and Gas Chromatography- Mass spectroscopy (GC-MS) spectroscopic techniques.

3.7. GC- MS Procedure

Each sample was separately dissolved in 1st three (3) ml hexane, vortexed, centrifuged at 13,000g for five (5) minutes and passed through glass wool before analysis then in DCM: Acetone (3:1) and prepared as above In both processes the samples were pre-concentrated by passing through a gentle flow of $N_{2\,(g)}$ 500µl. The sample was then transferred into auto sampler vials containing Teflon caps.

The samples were analyzed by GC-MS on a 7890A stand-alone gas chromatograph (Agilent Technologies, Inc., Beijing, China) and a 5975 C mass selective detector (Agilent Technologies, Inc., Santa Clara, CA, USA) by using the following conditions: Inlet temp 270°C, transfer line temp of 280°C, and column oven temperature programmed from 35 to 280°C with the initial temperature maintained for 5 min then 10 °C/min to 280 °C for 10.5 min and the final one for 29.9min 50 °C/min to 285 °C. The GC was fitted with a HP-5 MS low bleed capillary column (30 m × 0.25 mm i.d., 0.25-µm) (Restek, Bellefonte, PA, USA). Helium at a flow rate of 1.25 ml/min served as carrier gas. The Agilent 5973 mass selective detector maintained an ion source temperature of 250°C and a quadruple temperature of 180°C. 230°C was set as the MS ion source temperature. Electron impact (EI) mass spectra were obtained at acceleration energy of 70 eV. A 1.0 µL aliquot of extract was automatically

injected in the split/ splitless mode using an auto sampler 7683 (Agilent Technologies, Inc., Beijing, China). Fragment ions were analyzed over 40-550 m/z mass range in the full scan. The filament delay time was set as 3.3 min.

The injector used was in the splitless mode with the following conditions: the heater was on at 250°C, the pressure was set at 8.8271 psi, total flow was set at 10.2 ml/min, septum purge flow at 3ml/min, gas saver at 20ml/min after and the purge flow to split vent set at 6ml/min for 0.8 minutes.

The column used was HP-5MS, (5% methyl silox), (30 m × 250μm × 0.25μm) .The Compounds identified were generated from computer program which involved calculation by the data system of a similarity index, match factor or purity between the unknown spectrum and library (reference) spectra and for this analysis NIST/EPA/NIH MASS SPECTRAL LIBRARY (NIST 05) and NIST MASS SPECTRAL SEARCH PROGRAM Version 2.0d was used

3.8. NMR procedure

Twenty (20) mg of the sample were weighed into a clean, dry vial. Approximately 0.7 to 0.8 mls of deuteroacetone containing a small amount of tetramethylsilane (TMS) which acted as the internal standard were added to the vial. The vial was swirled gently until the sample dissolved completely. The sample was then transferred to a 5-mm NMR tube using a clean, dry Pasteur pipet. Care was taken to ensure that there was at least five (5) cm of solvent in the tube. The NMR tube was then capped and labeled. ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) were recorded on Varian-Mercury® 200 spectrometer using TMS as internal standard.

CHAPTER FOUR

4.0. RESULTS

Percent yield of root, stem and leaf.
A total of twenty-six extracts were prepared as shown in Table 1

Table 1. Percentage yield of extracts of Ocimum plant parts .

Ocimum species.	Plant Part.	Solvent System used.	Weight of extract	% yield.
O.kilimandscharicum	root	Ethanol	1.08	2.16
O.kilimandscharicum	stem	Chloroform	0.42	0.83
O.kilimandscharicum	root	Chloroform	0.6	1.2
O.kilimandscharicum	stem	Ethanol	1.72	3.43
O. masaiense	root	Ethanol	3.28	6.56
O. masaiense	root	CHCl/EtOH	0.66	1.32
O. masaiense	leaves	Ethanol	10.85	21.70
O. masaiense	stem	Chloroform	0.57	1.14
O. masaiense	stem	Ethanol	2.28	4.56
O. masaiense	stem	CHCl/EtOH	4.42	8.84
O. masaiense	root	Chloroform	0.492	0.985
O. masaiense	leaves	Chloroform	5.97	11.94
O. kenyense	leaves	Ethanol	6.10	12.20
O.kilimandscharicum	root	CHCl/EtOH	1.15	2.30
O. masaiense	leaves	CHCl/EtOH	13.98	27.96
O.kilimandscharicum	stem	CHCl/EtOH	2.97	5.94
O.kilimandscharicum	leaves	CHCl/EtOH	5.43	10.87
O. kenyense	leaves	Chloroform	0.98	1.96
O.kilimandscharicum	leaves	Chloroform	9.47	18.94
O. kilimandscharicum	leaves	Ethanol	14.3	28.6
O. kenyense	stem	CHCl/Ethanol	2	4
O. kenyense	root	Ethanol	1.5	3
O. kenyense	root	CHCl/EtOH	1.75	3.5
O. kenyense	stem	Ethanol	1	2
O. kenyense	root	Chloroform	0.75	1.5
O. masaiense	root	DCM/MeOH	1.25	2.5

DCM= dichloromethane, MeOH= methanol, EtOH= Ethanol, CHCl= chloroform.

It is clear from Table 1 above that the roots generally produced the lowest yields for any particular plant species and for any particular solvent system. The chloroform solvent system was associated with the lowest yield for any particular plant part and/or species. The chloroform/ethanol solvent system on the other hand had the highest yields. This can be easily explained by the fact that the chloroform/ethanol solvent system has the ability to extract compounds with a wide variety of physicochemical characteristics. This is in marked contrast to the chloroform which is only able to extract non-polar compounds and hence the lower product yields.

4.1. Screening experiment results

Twenty-one of the twenty five organic extracts screened in the Tail Flick Test showed significant antinoceptive activity in this experimental pain model. The results of these screening experiments are summarized in table 2 below.

Table 2. The results of the screening tests in the Tail Flick Test.

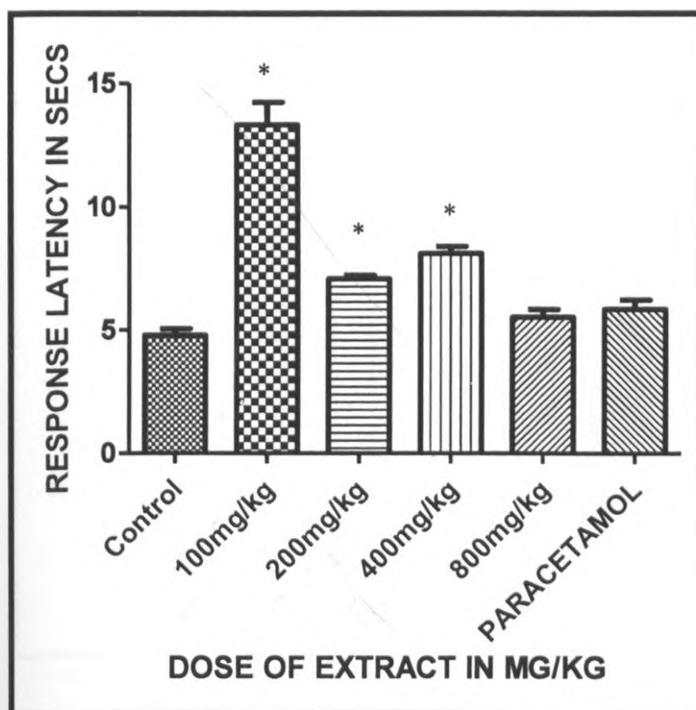
EXTRACT	P value
Chloroform extract of *Ocimum kilimandscharicum* roots	0.5 (ns)
Chloroform/ethanol extract of *Ocimum kilimandscharicum* stems	0.021
Chloroform/ethanol extract of *Ocimum masaiense* stems	0.01
Chloroform extract of *Ocimum kilimandscharicum* stems	0.003
Ethanol extract of *Ocimum kilimandscharicum* stems	0.0015
Chloroform/ ethanol extract of *Ocimum kenyense* leaves	0.003
Ethanol extract of *Ocimum kenyense* leaves	0.336 (ns)
Chloroform/ethanol extract of *Ocimum masaiense* leaves	0.007
Ethanol extract of *Ocimum kenyense* stems	0.008
Chloroform/ethanol extract of *Ocimum kenyense* stems	0.002
Chloroform extract of *Ocimum kenyense* stems	0.014
Ethanol extract of *Ocimum masaiense* stems	0.0111
Chloroform extract of *Ocimum masaiense* stems	0.096 (ns)
Chloroform/ethanol extract of *Ocimum kilimandscharicum* leaves	0.022
Ethanol extract of *Ocimum kilimandscharicum* leaves	0.0011
Chloroform extract of *Ocimum kenyense* leaves	0.0567 (ns)
Ethanol extract of *Ocimum masaiense* leaves	0.953ns
Chloroform extract of *Ocimum masaiense* leaves	0.0029
Chloroform/ethanol extract *Ocimum kilimandscharicum* roots	0.0018
Ethanol extract of *Ocimum kilimandscharicum* roots	0.004
Ethanol extract of *Ocimum kenyense* roots	0.027
Chloroform/ethanol extract of *Ocimum kenyense* roots	0.0084
Chloroform extract of *Ocimum masaiense* roots	0.0076
Chloroform/ethanol extract of *Ocimum masaiense* roots	0.0041
Ethanol extract of *Ocimum masaiense* roots	0.0132
Dichloromethane/methanol of *Ocimum masaiense* roots	0.0001

ns= not significant.

The chloroform/ethanol extract of *Ocimum masaiense* roots was selected for the formalin and the agonist/blocker experiments. The dichloromethane/methanol extract of *Ocimum masaiense* roots was selected for the fractionation experiments. These two extracts were selected for the next phase of experiments since they possessed highly significant antinociceptive activity in the Tail Flick Test and also had the highest latency scores at the 100mg/kg dosage level in the Tail Flick Test. The experimental results for the two extracts in the Tail Flick Test are described in detail below.

4.1.1. Effect of Dichloromethane/Methanol extract of *Ocimum masaiense* roots.

The extract possessed significant antinociceptive activity in the tail-flick test ($p = 0.0001$). Post- hoc statistical analysis using the Wilcoxon- Mann/Whitney statistical test showed that the extract possessed significant antinociceptive activity at the 100mg/kg ($p = 0.012$), 200mg/kg ($p = 0.012$), 400mg/kg ($p = 0.012$) dosage levels. Conversely the extract did not possess significant antinociceptive activity at the 800mg/kg dosage level ($p = 0.296$). The maximal antinociceptive activity of the extract was observed at the 100mg/kg dosage level (13.34± 1.81s vs. 4.79± 0.53s control). A graphical representation of the experimental data is shown in Figure 4 below.

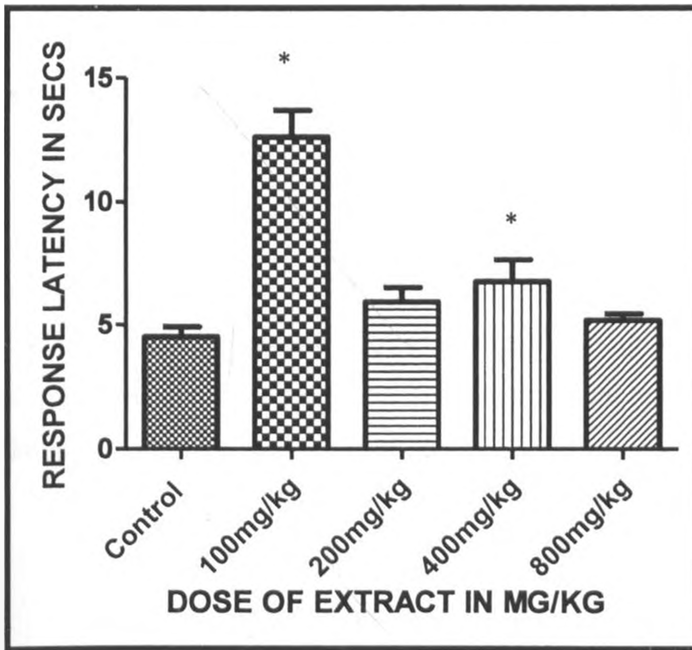


*=p<0.05. Paracetamol dose is 400mg/kg

Figure 4. Effect of Dichloromethane/methanol extracts of *Ocimum masaiense* roots in the tail-flick test.

4.1.2. Effect of Chloroform/ethanol extract of *Ocimum masaiense* roots.

The extract possessed significant antinociceptive activity in the tail-flick test ($p = 0.004$). Post- hoc statistical analysis using the Wilcoxon- Mann/Whitney statistical test showed that the extract possessed significant antinociceptive activity at the 100mg/kg ($p = 0.012$) and 400mg/kg ($p = 0.037$) dosage levels. It did not however possess significant antinociceptive activity at the 200mg/kg ($p = 0.144$) and 800mg/kg ($p = 0.296$) dosage levels. The maximal analgesic activity of the extract was observed at the 100mg/kg dosage level (12.62± 2.16s vs. 4.52± 0.8s control). A graphical representation of the experimental data is shown in Figure 5.

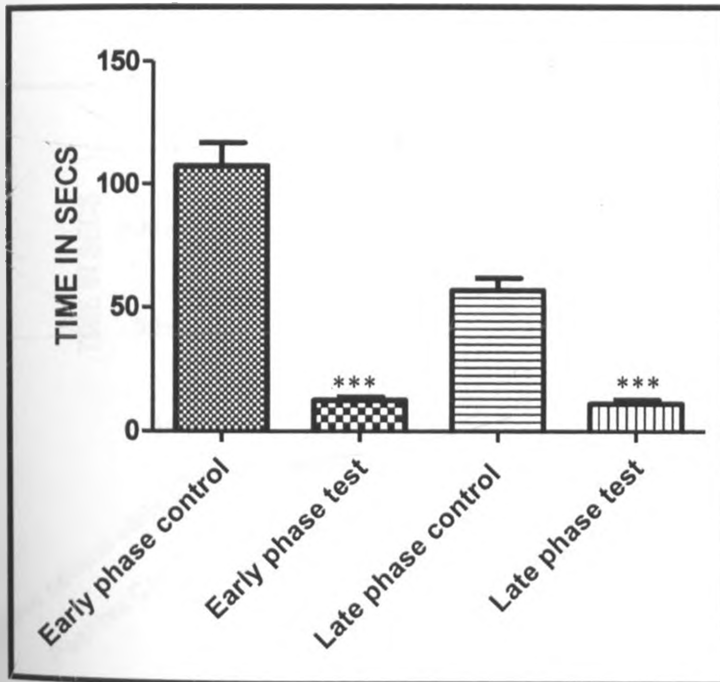


Key;*=p<0.05

Figure 5.Effect of Chloroform/ethanol extracts of *Ocimum masaiense* roots in the Tail Flick Test.

4.2. Effect of Chloroform/ethanol extracts of *Ocimum masaiense* roots in the formalin test.

The extract had significant antinociceptive activity in both the early ($p < 0.0001$) and late ($p < 0.0001$) phases of the Formalin Test. It caused reductions in the duration of time spent in pain behavior in both the early (12.46± 2.41s vs. 107.4± 18.73s control) and late phases (11.9± 2.92s vs. 56.8± 9.70s control). The extract therefore seemed to possess robust antinociceptive activity in the Formalin Test. A graphical representation of the experimental data is shown in Figure 6.



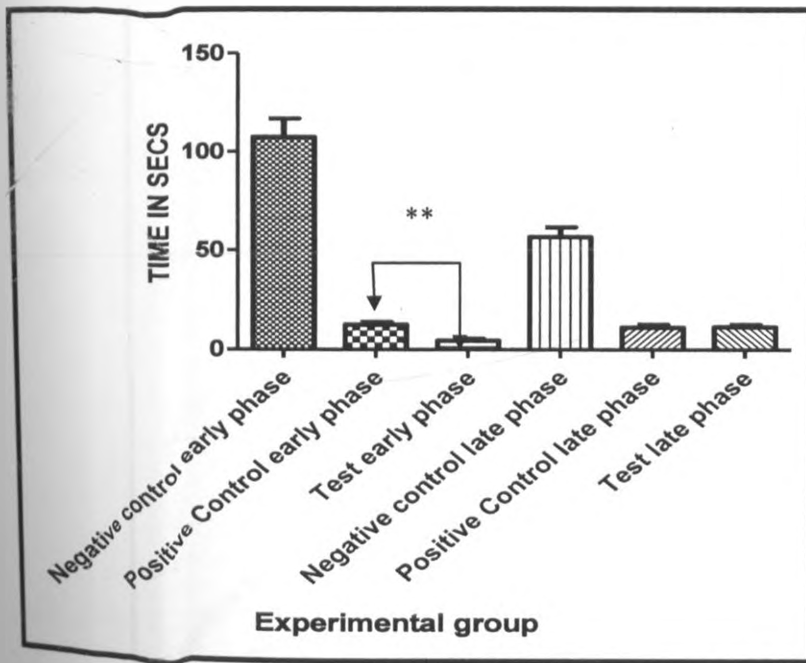
Key; *** = $p < 0.0005$

Figure 6. Effect of 100mg/kg Chloroform/ethanol extracts of *Ocimum masaiense* roots in the Formalin Test.

4.3. Effects of atropine, ketamine, capsaicin and naloxone on the antiniociceptive effects of Chloroform/ethanol extracts of *Ocimum masaiense* roots in the formalin test.

A. ATROPINE

Atropine significantly enhanced the antinociceptive activity of the extract in the early phase of the Formalin Test (4.61± 1.99s test vs. 12.6± 2.42s positive control, p = 0.0009). In contrast, it did not have a significant effect on the antinociceptive activity of the extract in the late phase of the Formalin Test (11.58± 2.27s test vs. 11.2± 2.92s positive control, p= 0.842). A graphical representation of the experimental data is shown in Figure 7.



Key; ** = p< 0.005

Figure 7. Effect of Atropine (4mg/kg) on the antinociceptive effect of 100mg/kg Chloroform/ethanol extract of *Ocimum masaiense* roots in the Formalin Test.

B. KETAMINE.

Ketamine had a significant inhibitory effect on, but did not abolish the antinociceptive effect of the extract in the early phase of the Formalin Test (25.2± 10.46s positive control vs. 62.26± 7.97s test, $p = 0.0005$). However it had no significant effect on the antinociceptive activity of the extract in the late phase of the Formalin Test (5.8± 4.99s positive control vs. 11.87± 6.22s test, $p = 0.167$). A graphical representation of the experimental data is shown in Figure 8.

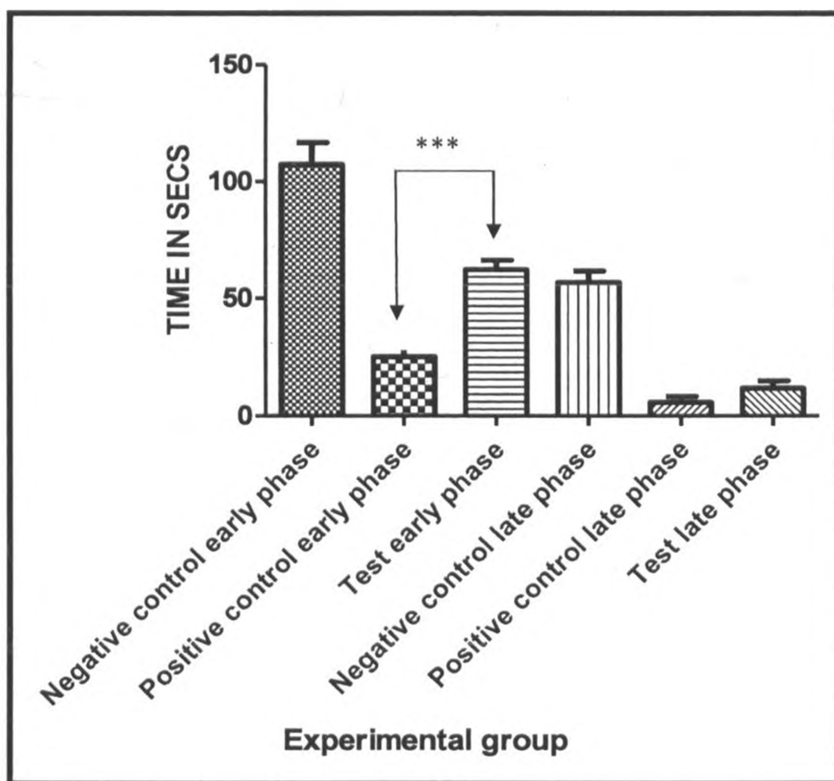
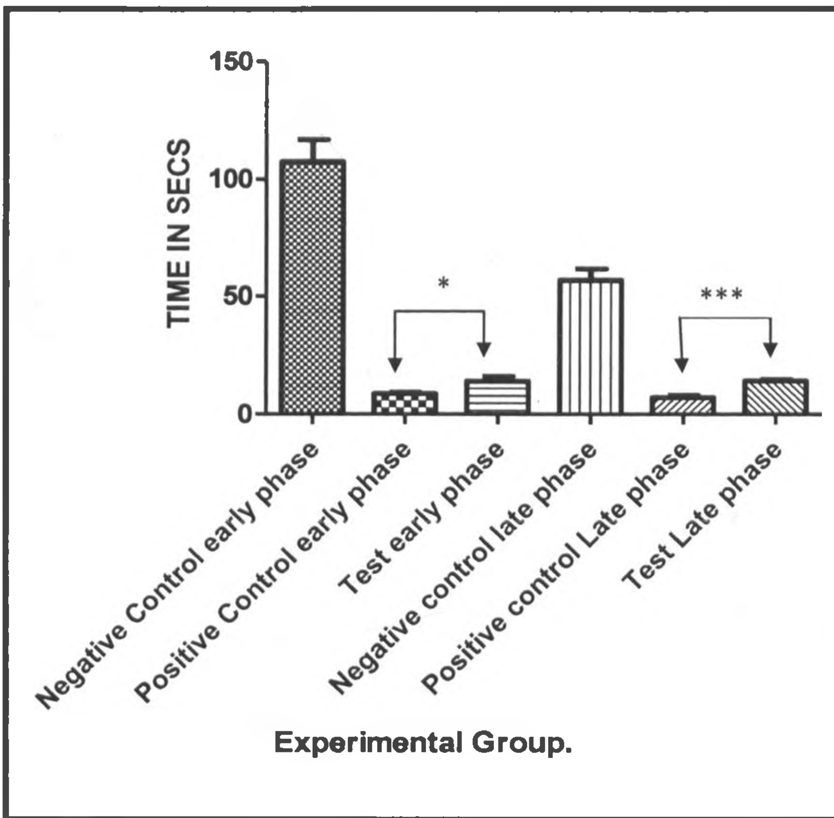


Figure 8. Effect of Ketamine on the antinocieptive effect of 100mg/kg chloroform/ethanol extract in the Formalin Test.

C. CAPSAICIN

Capsaicin had a significant inhibitory effect on but did not abolish the antinociceptive activity of the extract in both the early (8.76± 1.17s positive control vs.14.04± 4.02s test, p = 0.036) and late (7.22± 1.76 s positive control vs. 14.3± 1.08s test, p = 0.0001) phases of the Formalin Test. It caused robust increases in the duration of pain behaviors in both phases of the Formalin Test. A graphical representation of the experimental data is shown in Figure 9.

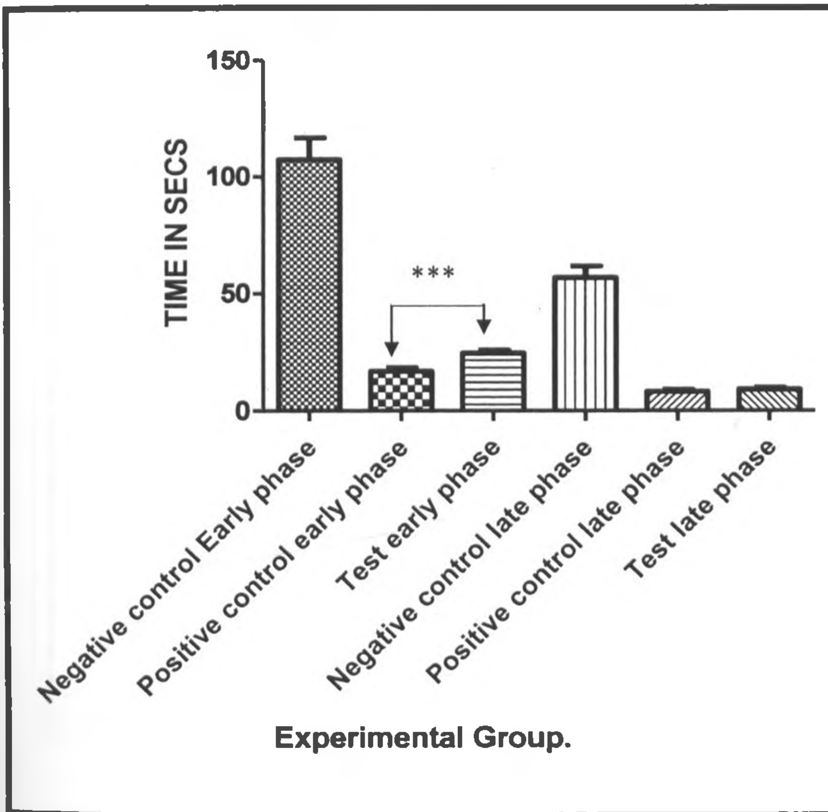


Key; *= p< 0.05, *** = p< 0.0005

Figure 9. Effect of capsaicin on the antinociceptive effect of the chloroform/ethanol extract of *Ocimum masaiense* roots in the Formalin Test.

D. NALOXONE

Naloxone had a significant inhibitory effect on, but did not abolish the antinociceptive activity of the extract in the early phase of the Formalin Test (16.78± 3.16s positive control vs. 24.5± 2.62s test, p = 0.0055). It however did not have any significant effect on the antinociceptive activity of the extract in the late phase of the Formalin Test (8.23± 1.46s positive control vs. 9.04± 1.47s test, p = 0.46). A graphical representation of the experimental data is shown in Figure 10.



Key; *** = p< 0.0005

Figure 10. Effect of naloxone on the antinociceptive effect of the 100mg/kg Chloroform Ethanol extract of *Ocimum masaiense* roots in the Formalin Test.

4.4. Antidepressant activity of Chloroform/ethanol extracts of *Ocimum masaiense* roots.

i. Tail suspension test.

CEOMR did not possess significant antidepressant activity in the tail suspension test ($p = 0.194$) compared to the control. The extract caused a sizeable but non-significant increase in the duration of mobility behavior (92± 5.1s test vs. 75± 23.45s control). A graphical representation of the experimental data is shown in Figure 11.

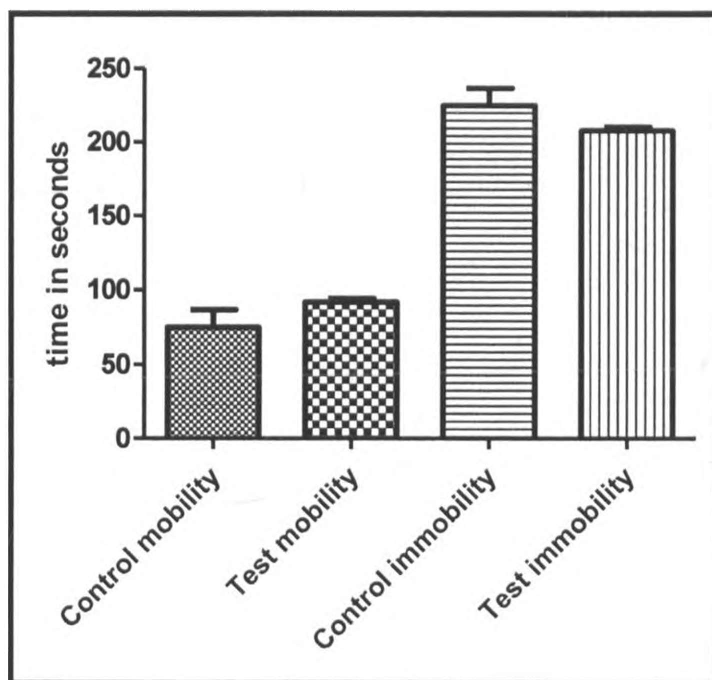
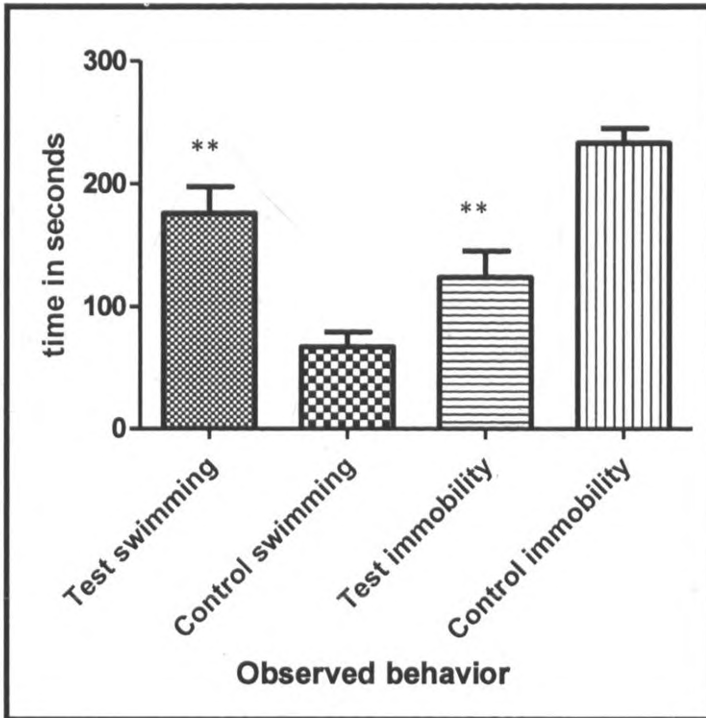


Figure 11. Effect of 100mg/kg Chloroform/ethanol extratcs of *Ocimum masaiense* roots in the tail suspension test.

ii. Forced swim test.

CEOMR had a significant effect on the duration of swimming behavior compared to control in the forced swim test (176± 43.2s test vs. 67± 24.4s control, p= 0.002). This was an indication that the extract possessed significant antidepressant activity in this test. A graphical representation of the experimental data is shown in Figure 12.



Key; **=p<0.005

Figure 12. Effect of 100mg/kg Chloroform/ethanol extract of *Ocimum masaiense* roots in the forced swim test.

4.5. The antinociceptive effect of fractions of Dichloromethane/methanol extracts of *Ocimum masaiense* roots.

The fractionation of the DMOMR resulted in six (6) fractions whose weights are shown in table 3.

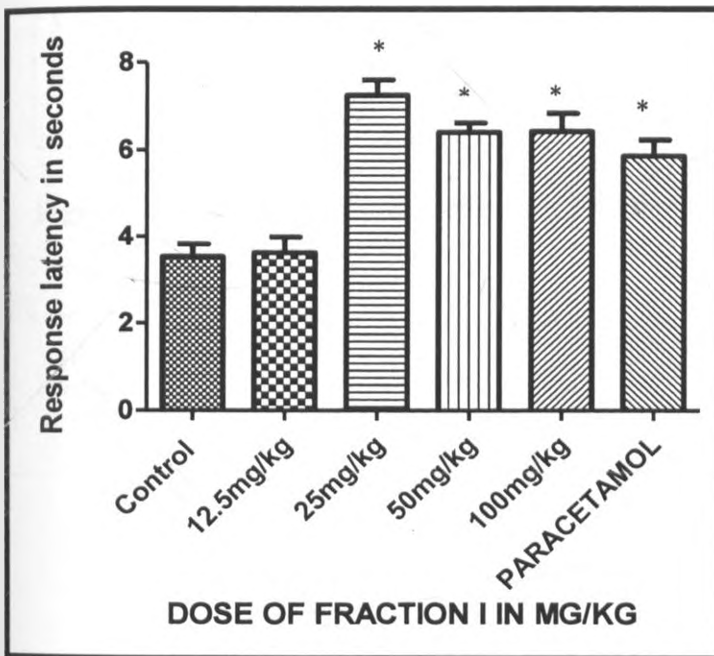
Table 3. Fractions obtained following the fractionation of Dichloromethane/methanol extract of *Ocimum masaiense* roots.

Fraction number (code)	Weight (g)
I(DCMF1)	1.1
II (DCMEAF2)	1.05
III (EAF3)	0.77
IV (EAMF4)	0.52
V (MEF5)	2.94
VI (MEF6)	0.19

The results of the evaluation of the antinociceptive activity of the fractions obtained in the Tail Flick Test are shown in the following pages.

A. DCMF1

DCMF1 possessed significant antinociceptive activity in the Tail Flick Test (p = 0.0005). Post- hoc statistical analysis using the Wilcoxon- Mann/Whitney statistical test showed that the fraction possessed significant antinociceptive effect at the 25mg/kg (p = 0.012), 50mg/kg (p = 0.012) and 100mg/kg (p = 0.012) dosage levels. The fraction did not possess significant antinociceptive activity in the 12.5mg/kg dosage level (p = 0.834). A graphical representation of the experimental data is shown in Figure 13. A fitted dose response curve for the data obtained using Microsoft Excel is shown in Figure 14. The calculated ED_{50} for fraction I was 20mg/kg.



Key; *= p< 0.05. Paracetamol dose =400mg/kg

Figure 13. Effect of Fraction 1 (DCMF1) in the tail-flick test.

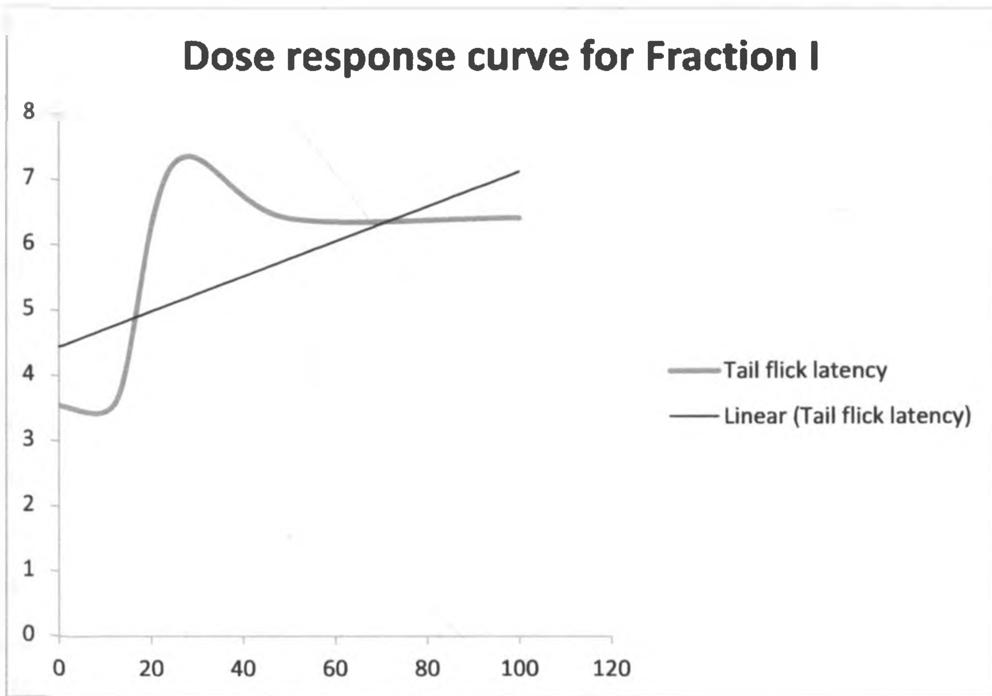
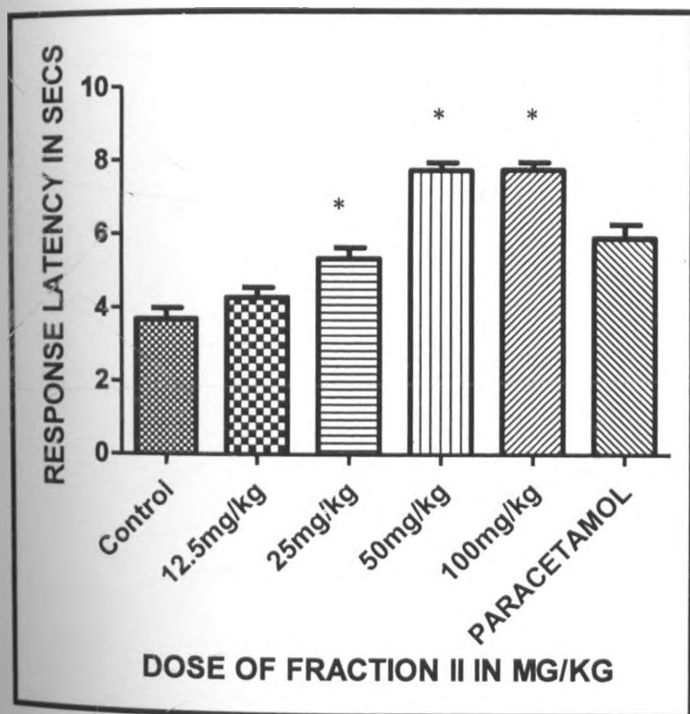


Figure 14. Fitted dose response curve for Fraction I (DCMF1)

B. DCMEF2

DCMEF2 possessed significant antinociceptive activity in the tail-flick test (p = 0.0001). Post- hoc statistical analysis using the Wilcoxon-Mann/Whitney statistical test showed that Fraction II possessed significant antinociceptive activity at the 25mg/kg (p = 0.012), 50mg/kg (p = 0.012) and 100mg/kg (p = 0.012) dosage levels compared to control. In contrast, the fraction did not possess significant antinociceptive activity at the 12.5mg/kg dosage level (p = 0.403). The maximal antinociceptive activity was observed at the 100mg/kg dosage level (7.71± 0.41s vs. 3.69± 0.58s negative control). A graphical representation of the experimental data is shown in Figure 15. A fitted dose- response curve produced using the Microsoft Excel is shown in Figure 16 below. The calculated ED_{50} for this fraction was 25mg/kg.



Key; * = p< 0.05. Paracetamol dose= 400mg/kg

Figure 15. Effect of Fraction II (DCMEF2) in the tail-flick test.

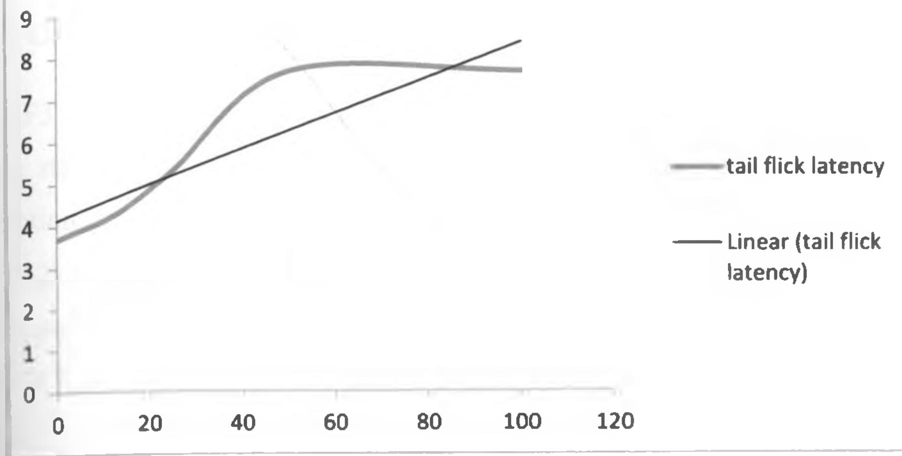
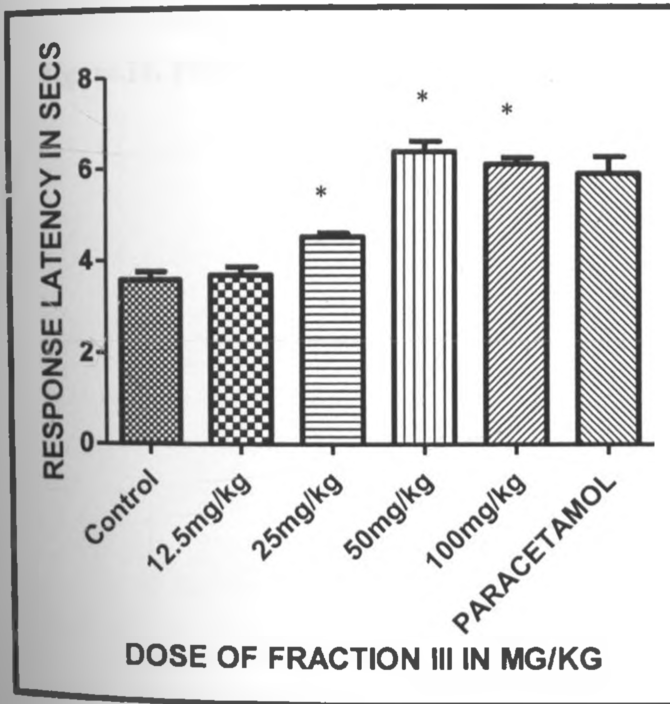


Figure 16. Fitted dose response curve for Fraction II (DCMEF2).

C. EAF3

EAF3 possessed significant antinociceptive activity in the tail-flick test (p = 0.0002). Post-hoc statistical analysis using the Wilcoxon- Mann/Whitney statistical test showed that the fraction possessed significant antinociceptive activity at 25mg/kg (p = 0.012), 50mg/kg (p = 0.012) and 100mg/kg (p = 0.012) dosage levels compared with the negative control. It did not, however possess significant antinociceptive activity at the 12.5 mg/kg dosage level (p = 0.753). The maximal antinociceptive activity was observed at the 50mg/kg dosage level (6.34± 0.44s vs. 3.58± 0.34s negative control). A graphical representation of the experimental data is shown in Figure 17. A fitted dose response curve is shown in Figure 18. The calculated ED_{50} for this fraction was 25mg/kg.



Key; * = $p < 0.05$, Paracetamol dose= 400mg/kg

Figure 17. Effect of Fraction III (EAF3) in the tail flick test.

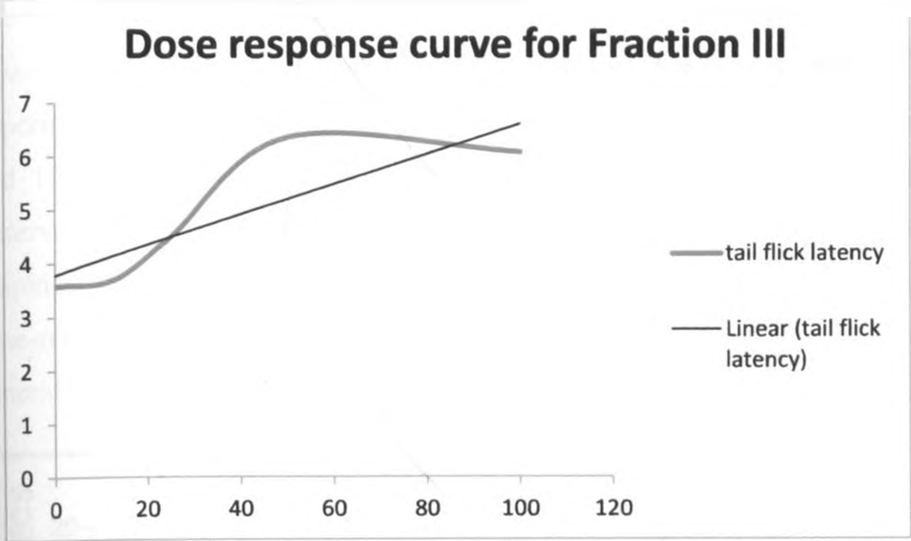
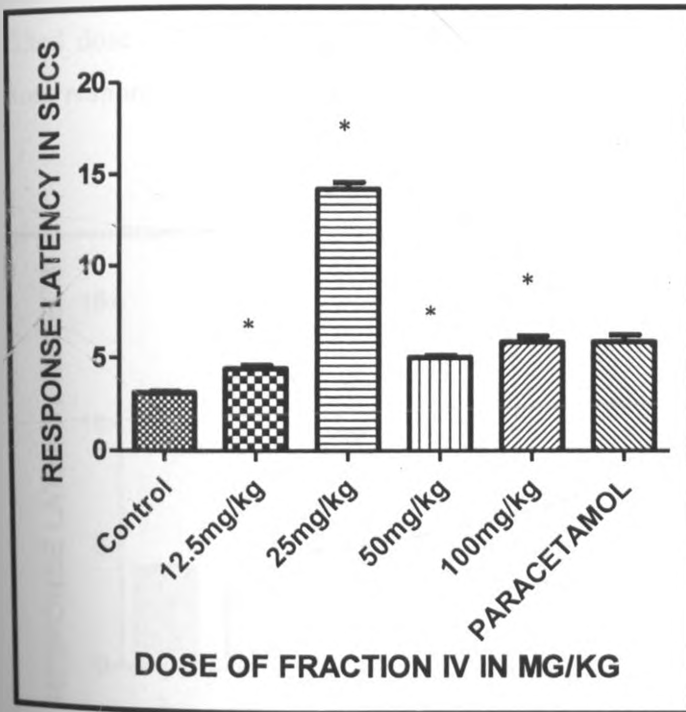


Figure 18. Fitted dose response curve for Fraction III (EAF3).

D. EAMF4.

EAMF4 possessed significant antinociceptive activity in the tail-flick test ($p = 0.0001$). Post-hoc statistical analysis using the Wilcoxon- Mann/Whitney test showed that the extract possessed significant antinociceptive activity at all the dosage levels tested in the experiment; that is at the 12.5mg/kg ($p = 0.012$), 25mg/kg ($p = 0.012$), 50mg/kg ($p = 0.012$) and 100mg/kg ($p = 0.012$). The maximal antinociceptive activity of the fraction was observed at the 25mg/kg dosage level (14.15± 0.72s vs. 3.14± 0.13s negative control). A graphical representation of the experimental data is shown in Figure 19 below. An idealized dose-response curve was not drawn in this case because the dose response curve had a general inverted U- shape curve instead of the normal sigmoid shaped dose response curve.

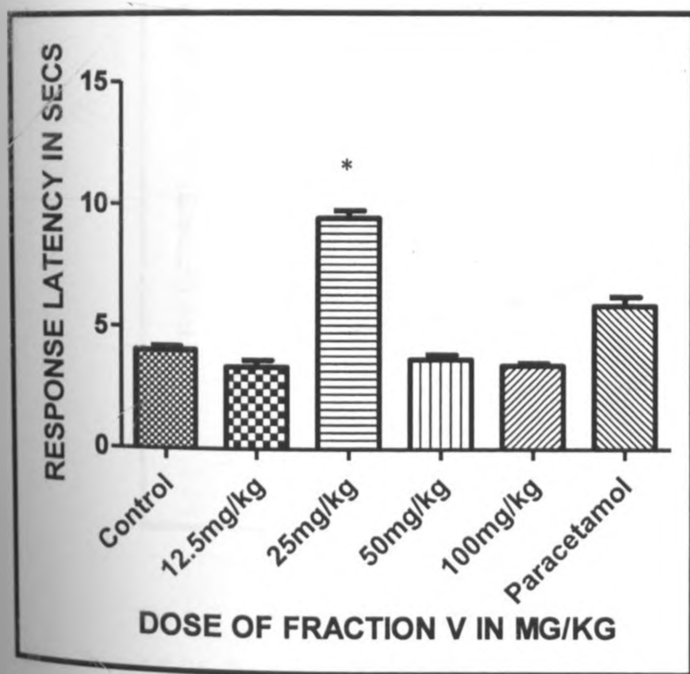


Key; * = $p < 0.05$. Paracetamol dose = 400mg/kg

Figure 19. Effect of Fraction IV (EAMF4) in the tail-flick test.

E. MEF5.

MEF5 possessed significant antinociceptive activity in the tail-flick test ($p = 0.0004$). Post-hoc statistical analysis using the Wilcoxon- Mann/Whitney statistical test showed that the fraction possessed significant antinociceptive activity compared to the negative control at the 25mg/kg dosage level ($p = 0.012$) only. The fraction did not possess significant antinociceptive activity at the other dosage levels tested; that is at the 12.5mg/kg ($p = 0.21$), 50mg/kg ($p = 0.296$) and 100mg/kg ($p = 0.216$) dosage levels. The maximal antinociceptive activity was observed at the 25mg/kg dosage level (9.43± 0.61s vs. 4.05± 0.31s, negative control). A graphical representation of the experimental data is shown in Figure 20 below. A fitted dose-response curve was not drawn because of the general inverted U shape of the dose response curve.

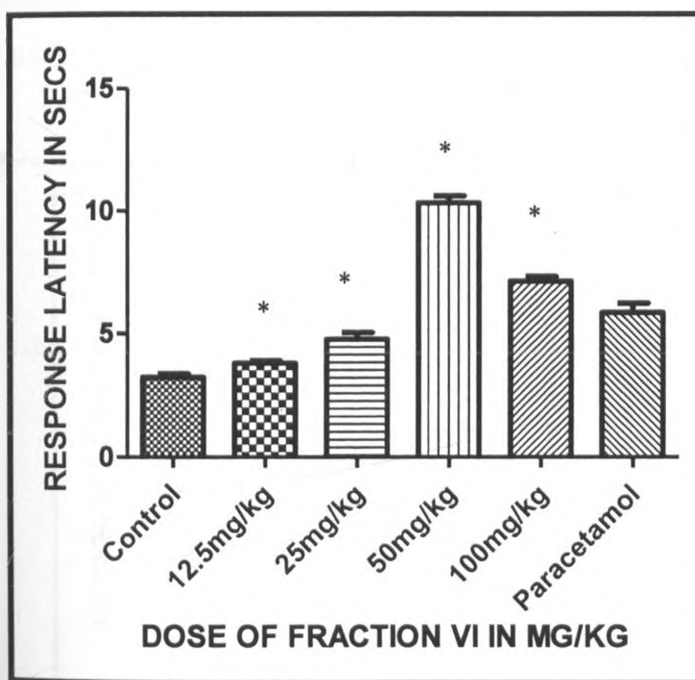


Key; * = $p < 0.05$. Paracetamol dose = 400mg/kg

Figure 20. Effect of Fraction V (MEF5) in the tail-flick test.

F. METF6

METF6 possessed significant antinociceptive activity in the tail-flick test ($p < 0.0001$). Post-hoc statistical analysis using the Wilcoxon-Mann/Whitney statistical test showed that the fraction possessed significant antinociceptive activity compared to the negative control at all the dosage levels tested; that is at the 12.5mg/kg ($p = 0.046$), 25mg/kg ($p = 0.012$), 50mg/kg ($p = 0.012$) and 100mg/kg ($p = 0.012$) dosage levels. The maximal antinociceptive activity was observed at the 50mg/kg dosage level (10.33± 0.58s vs. 3.23± 0.29s, negative control). A graphical representation of the experimental data is shown in Figure 21 below. A fitted dose response curve was not drawn due to the general inverted U shape of the dose response curve of the fraction.



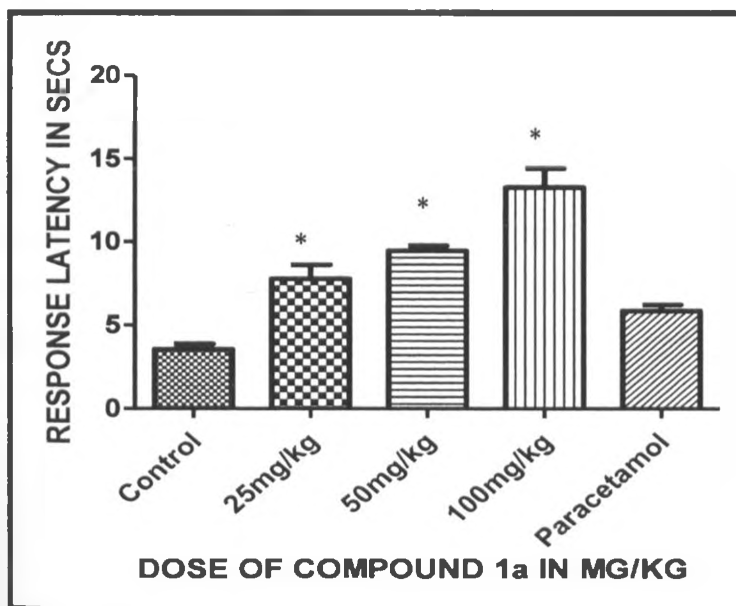
Key; * = $p < 0.05$. Paracetamol dose is 400mg/kg.

Figure 21. Effect of Fraction VI (METF6) in the tail-flick test.

4.6. Antinociceptive activity of the pure compounds obtained.

I. Compound 1a

Compound **Ia** possessed significant antinociceptive activity in the tail-flick test (p = 0.0003). Post- hoc statistical analysis using the Wilcoxon- Mann/Whitney statistical test showed that the compound possessed significant antinociceptive activity compared to control at all the dosage levels tested; that is at 25mg/kg (p = 0.012), 50mg/kg (p = 0.012) and 100mg/kg (p = 0.012) dosage levels. The maximal antinociceptive activity was observed at the 100mg/kg dosage level (13.28±2.28s). A graphical representation of the experimental data is shown in Figure 22. A fitted dose response curve drawn using the Microsoft Excel showed that the ED_{50} was estimated to be at the 25mg/kg dosage level. The fitted dose response curve is shown in Figure 23.



Key; * = p< 0.05

Figure 22. Effect of Compound Ia in the Tail Flick Test.

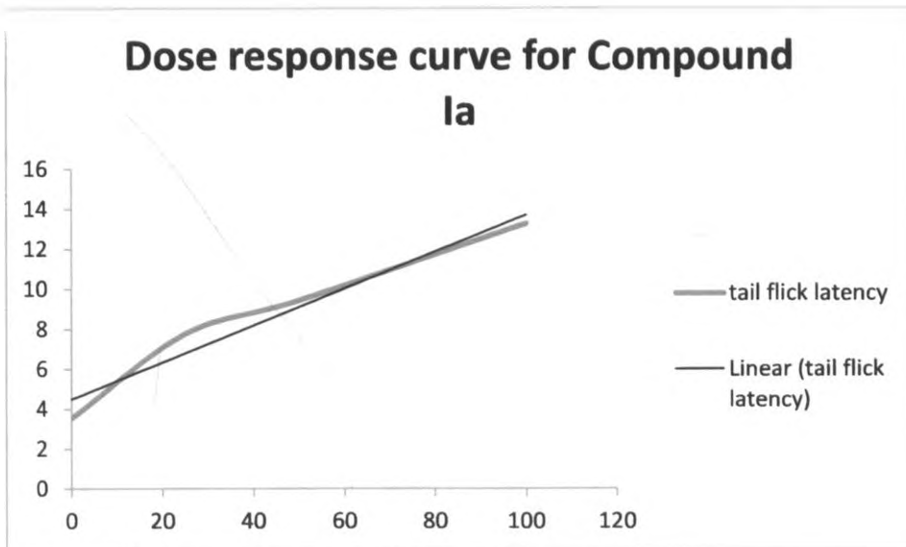
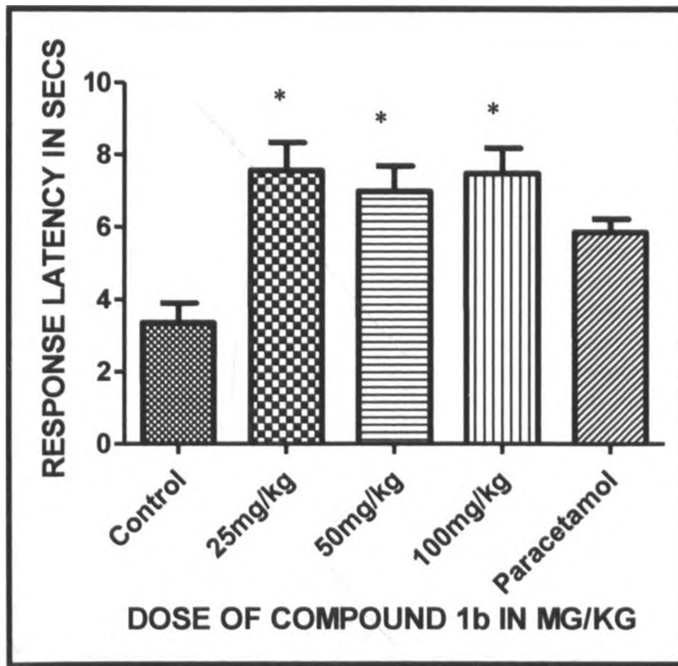


Figure 23. Fitted dose response curve for compound Ia

5. Compound Ib.

Compound Ib possessed significant antinociceptive activity in the tail-flick test (p= 0.006). Post-hoc statistical analysis using the Wilcoxon-Mann/Whitney statistical test showed that the compound possessed significant antinociceptive activity at the 25m/kg (p= 0.012), 50mg/kg (p= 0.012) and 100mg/kg (p= 0.012) dosage levels compared to control. The maximal antinociceptive activity of the compound was observed at the 100mg/kg dosage level. A graphical representation of the experimental results is shown in Figure 24 below. A fitted dose-response curve drawn using Microsoft Excel showed that the ED_{50} of the compound was 25mg/kg. The idealized dose response curve as well as the dose response curve for the observed data is shown in Figure 25.



Key; *=p< 0.05.

Figure 24. Effect of Compound 1b in the tail-flick test.

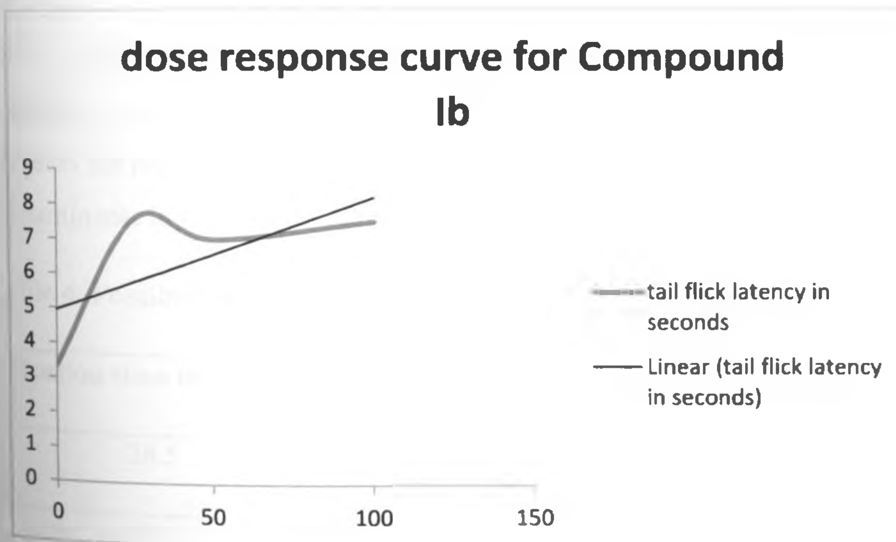


Figure 25. Fitted dose response curve for compound Ib

4.8. Structural determination of the pure compounds.

Compound Ia

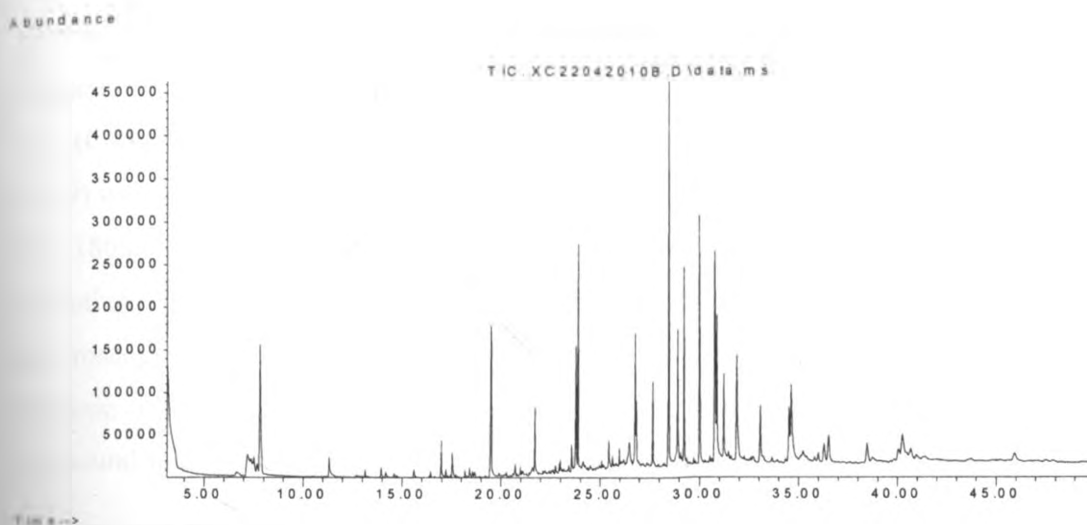


Figure 26. The chromatogram in GC-MS for compound Ia

Compound **Ia** was evaluated for purity using GC-MS. The chromatogram obtained is presented in figure 26. The chromatogram shows that the compound was not pure. Table 4 presents the retention times, m/z ratios and possible identities of the most common contaminants in Compound **Ia.**

Table 4. Possible contaminants of Compound Ia.

Retention time in minutes	m/z value	Possible identity
28.5	352	Pentacosane
19	200	Dodecanoic acid
24	270	Hexadecanoic methyl ester

From the GC-MS chromatogram it was suggested Compound **Ia** was suspected to be betulinic acid methyl ester which had a retention time of 45.9 minutes. It had a molecular ion peak (m/z) of 470 with significant fragment ions at m/z of 189 and 207 which are two known to be significant fragment ions for the lupane-type triterpenes such as betulinic acid (Cichewicz and Kouzi, 2004).

Additional evidence was provided by ^{13}C NMR spectrum data which had the following characteristics; it showed six methyl groups at δC 27.6 (C-23), 15.4 (C-24), 16.2 (C-25), 16.3 (C-26), 14.4 (C-27), 19.6 (C-30) and exomethylene group at δC 150.0 (C-30), 108.8 (C-29) and a secondary carbon bearing hydroxyl at δc 79.0 (C-3) and a carboxyl group at δC= 180.6 (C-28) in addition to ten methylene, five methine and five quaternary carbons indicating that the compound had a total of thirty (30) carbon atoms. The NMR spectroscopic pattern obtained was virtually identical to that in published literature for betulinic acid (Peng et al., 1998; Sholichin et al., 1980). The proposed structure of compound **Ia** is shown

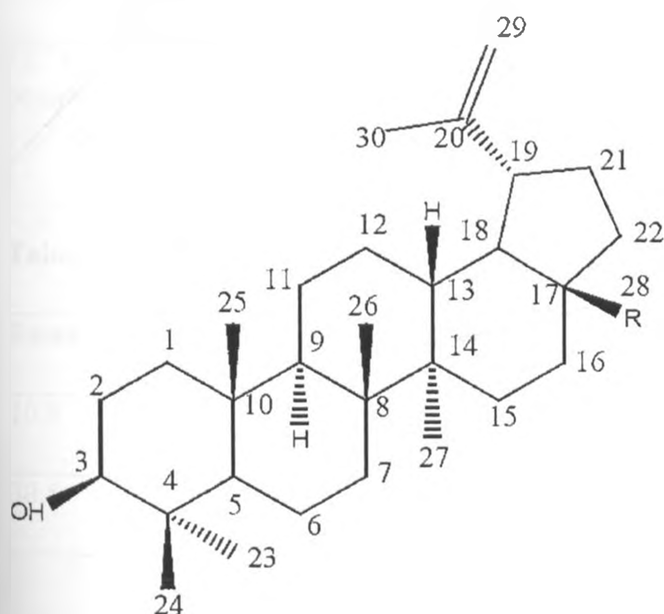


Figure 27. Structure of betulinic acid

Compound Ib

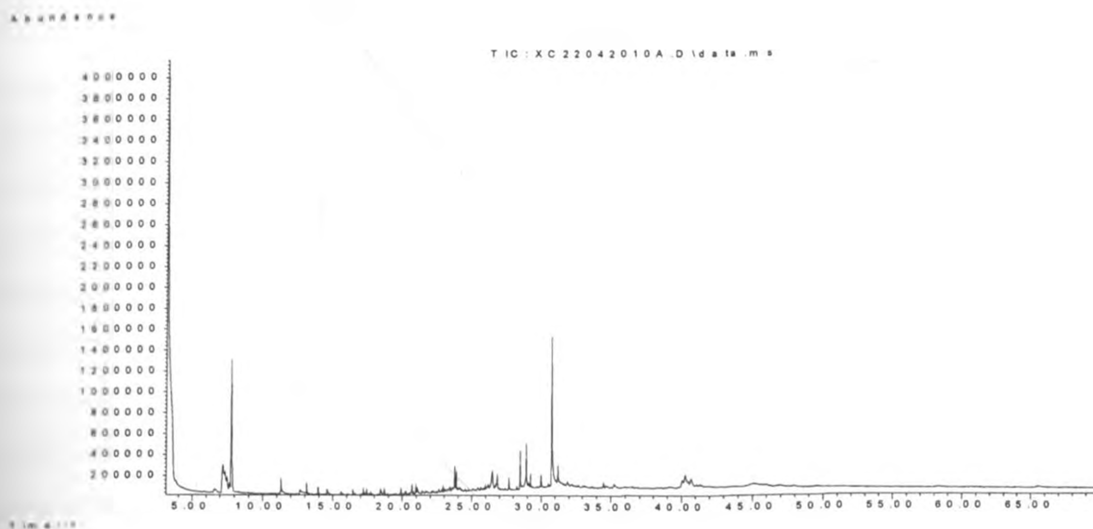


Figure 28. The chromatogram in GC-MS for compound Ib

Compound **Ib** was evaluated for purity using GC-MS. The chromatogram obtained for Compound **Ib** is shown in figure 28. Table 5 presents the retention times, m/z ratios and possible identities of the most common contaminants in Compound **Ib.**

Table 5. Possible contaminants of Compound Ib.

Retention time in minutes	m/z value	Possible identity
10.0	46	Formic acid
30.5	274	Peucenin 7 methyl ether

CHAPTER 5

DISCUSSION

Twenty one (21) out of the twenty five (25) extracts prepared showed significant antinociceptive activity in the Tail Flick Test. These results are line with previous published studies on the analgesic activity of Ocimum plant extracts (Khanna and Bhatia, 2003; Makonnen et al., 2003a, b; Muthuraman et al., 2008). The study results clearly demonstrate that extracts prepared from Ocimum species indigenous and endemic to Kenya also possess antinociceptive activity. This study was more comprehensive compared to those in the published literature in that it investigated the antinociceptive activity of all the possible plant parts except flowers and seeds.This is in contrast to previously published works that mainly concentrated on leaves and sometimes flowers (Gupta et al., 2007; Khanna and Bhatia, 2003).

Most of the previously published work on Ocimum spps. has tended to ascribe the documented biological activity to the essential oils produced by these plants (Obeng-Ofori et al., 1997;Odalo et al., 2005). Indeed many of the industrial projects designed to exploit the analgesic potential of these plants have tended to concentrate on the extraction/ isolation of the essential oils. A case in point is the ICIPE driven project in Western Kenya which concentrates on the isolation of the essential oil from *Ocimum kilimandscharicum*. This oil is rich in camphor. It is formulated into a commercially marketed balm Naturerub™ (Seyoum et al., 2003).The extraction methods used in this study tend to promote the evaporation of these essential oils into the atmosphere. Therefore one can state with a fair amount of confidence that the antinociceptive effects observed in these experiments cannot be ascribed to the presence of the essential oils.

The finding of this project that extracts obtained from *Ocimum masaiense* possessed the most potent antinociceptive activity was unexpected. This is because the plant is currently not used in traditional medicine by local communities. Indeed they tended to dismiss the plant as a weed only good for livestock fodder. This was in contrast to *Ocimum kenyense* and *Ocimum kilimandscharicum* whose use in traditional medicine was already well

established. The potent activity of *Ocimum masaiense* closely parallels that of the closely related species *Ocimum lamiifolium* which finds extensive application in traditional medicine in Ethiopia as an analgesic (Makonnen et al., 2003a,b)

It is of some interest to note that the most potent analgesic extracts were obtained from the root and not as would be expected from the published literature (>3000 papers on ocimum), from the leaves. The root extracts were observed to possess many other neurobehavioral effects including grooming, rearing and vocalization. The results of this study should therefore provide the impetus to carry out more in depth studies on other pharmacological properties possessed by this plant part.

The most potent analgesic root extracts were the chloroform/ethanol, followed by the ethanol and finally the chloroform extracts. This sequence in terms of analgesic potency is what one would expect of CNS- acting compounds. This is because very polar and non polar compounds would have great difficulty crossing the blood-brain barrier and getting absorbed into the blood stream respectively. The compounds present in the ethanol extract are very polar in nature and so would not be able to cross the blood-brain barrier. The very non-polar compounds found in the chloroform extracts on the other hand are poorly absorbed. The greater analgesic potency of the chloroform-ethanol extracts in this model can therefore be explained by their possession of these optimal chemical characteristics. That is, good aqueous and lipid solubility.

Eleven of the twenty one extracts possessing significant antinociceptive activity in the Tail Flick Test had generally inverted U-shaped dose response curves as opposed to the conventional sigmoidal dose response curves. The high frequency of the occurrence of this peculiarly shaped dose response curves rules out the possibility that these curves were as a result of experimental errors (artifacts). There are a number of plausible explanations for the existence of this phenomenon. There are many reports in literature of compounds in scientific literature possessing this type of analgesic dose response curves including some in widespread clinical use (Calabrese, 2008). The most prominent example of this type of compounds is Buprenorphine which is widely used clinically (Cowan et al., 1977; Pick et al., 1997; Rance, 1979). Examples of other compounds showing this pharmacological property include; clonidine (Filos et al., 1994), Apomorphine (Paalzow, 1983),

Promethazine (Paalzow and Paalzow, 1985), Pentazocine (Rance et al., 1979), Nalburphine (Gear et al., 1999) among others.

Many of the compounds possessing this type of dose response curve often have mixed agonist and antagonist properties for example buprenorphine. It is therefore reasonable to expect that an extract containing many different chemical moieties would display similar pharmacological behavior. This is because organic extracts will often contain a wide variety of compounds some of which will exert contradictory pharmacological effects. This explanation is buttressed by the experimental results which show that the pure compounds possess sigmoidal dose response curves. The same also applies to the fractions from which the pure compounds were ultimately isolated that is fractions I, II and III.

The chloroform/ethanol extract of *Ocimum masaiense* roots possessed significant antinociceptive activity in both the early and late phases of the Formalin Test. It is generally accepted that centrally acting analgesics have effects on both phases whereas peripherally acting analgesics will affect only the first phase (Shibata et al., 1989; Tjolsen et al., 1992). This is because the injection of formalin results in the release of glutamate and aspartate in the dorsal horn (Urban and Gebhart, 1998). Therefore the early phase of the Formalin Test represents the transmission of nociceptive impulses. The second phase of the Formalin Test on the other hand represents the events of central sensitization and wind-up (Coderre and Melzack, 1992; Vaccarino et al., 1993). One can therefore state with a fair degree of confidence that the antinociceptive compounds found in the extract mainly act centrally. Blocker and agonist experiments were carried out in an attempt to elucidate the putative mechanisms of the observed antinociceptive action.

Atropine which is a non specific muscarinic acetylcholine receptor blocker enhanced the antinociceptive activity of the chloroform/ethanol extract of the *Ocimum masaiense* roots. This was an unexpected finding since it is generally accepted that muscarinic analgesia is exclusively mediated by M2 and M4 receptors at both spinal and supraspinal sites especially in rats (Wess et al., 2002; 2007). The location of the spinal muscarinic receptors is both pre- and post-synaptic (Wess et al., 2002). The presynaptic muscarinic receptors located on the dorsal horn projection neurons function to inhibit excitatory neurotransmitter release (Bleazard and Morris, 1993; Ribeiro Da Silva and Cuello, 1990). The post-synaptic

muscarinic receptors on the other hand are located on the spinal dorsal horn GABAergic interneurons where they promote the release of GABA (Moore et al., 2002; Urban et al., 1989). One would therefore reasonably expect that atropine would be pronociceptive rather than antinociceptive as shown in this experiment.

There are however published studies showing that atropine is antinociceptive in the hot plate test at very low doses but pronociceptive at higher doses (Ghelardini et al., 1990). Ghelardini proposed that atropine at low doses blocks the presynaptic receptors while blocking the post-synaptic receptors at higher doses (Ghelardini et al., 1990). This explanation has recently gotten further support from studies that show that there are species differences in the anatomical locations of the M2 and M4 receptors between the rat and mouse (Chen et al., 2009). In the mouse the activation of the M2 and M4 receptors will result in the inhibition of GABA release in marked contrast to the effect in the rat (Zhang et al., 2006). This therefore provides a logical explanation for the seemingly paradoxical effects of atropine on nociception in the Formalin Test.

Ketamine which is a non specific NMDA receptor blocker significantly inhibited but did not abolish the antinociceptive activity of the chloroform/ethanol extract of *Ocimum masaiense* roots in the early phase but had no significant effect on the late phase of the Formalin Test. Parenteral as well as oral ketamine has been shown to possess antinociceptive activity at subanesthetic doses in a wide variety of animal pain models as well as clinically (Baumeister and Advokat, 1991; Clark and Kalan, 1995; Eide et al., 1994; Ryder et al., 1978; Shimoyama et al., 1999). The antinociceptive activity of ketamine would not come as a surprise especially when one considers the pivotal roles played by the NMDA receptor in nociceptive transmission. NMDA receptors have been implicated in the pathogenesis of both wind-up and central sensitization which are implicated in the development of chronic pain states (Dubner and Ruda, 1992; Woolf and Thompson, 1991). Indeed pretreatment with NMDA enhances pain behavior in the Formalin Test (Coderre and Melzack, 1992).

Since the second phase of the Formalin Test mainly represents central sensitization events NMDA antagonists preferentially decrease the nociceptive responses in the second phase of the Formalin Test compared with the first phase (Yamamoto, 1992; Coderre and Melzack, 1992; Vaccarino et al., 1993). Most of the published literature shows that ketamine also has

significant effects in the first phase but which are much reduced compared to those in the second phase (Shimoyama et al., 1999; Yamamoto and Yaksh, 1992). The seemingly paradoxical pronociceptive effects of ketamine in the first phase can however be explained by the fact that ketamine inhibits the Hyperpolarization Cyclic Nucleotide Gated (HCN) ion channels which play an important role in the modulation of neuronal excitability (Chen et al., 2005; 2009). This would form a valid explanation of ketamine's effect on the first phase especially in light of the doses of ketamine used in the experiment.

Naloxone significantly inhibited but did not abolish the antinociceptive activity of the chloroform/ethanol extract of *Ocimum masaiense* roots in the first phase of the Formalin Test but had no significant effect in the second phase. Opioid drugs such as morphine have been shown to attenuate and even completely extinguish pain behavior in both phases of the Formalin Test (Yaksh, 1997; Yamamoto and Yaksh, 1992). Naloxone is a μ opioid receptor blocker. It would thus be expected that naloxone would exert some but not complete inhibitory effect on the antinociceptive effect of the extract in the Formalin Test if its mechanism of action were opiodergic. The inhibitory effect would not be total because the antinociceptive activity of the opioids involves all the the three classes of opioid receptor that is μ, κ, δ receptors (Coggeshall et al., 1997; Stein et al., 2009) whereas naloxone only blocks the μ receptors only. In view of the well documented increase in peripheral opioid receptor expression and upregulation in response to tissue injury and inflammation one would expect the effects of naloxone to be manifested minimally in the second phase compared to the first phase (Busch-Diensfertig and Stein, 2010; Stein et al., 2009: Zollner et al., 2006).

Capsaicin had a significant inhibitory effect but did not abolish the antinociceptive effect of the chloroform/ethanol extract of the *Ocimum masaiense* roots in both phases of the Formalin Test. Capsaicin is a known agonist of the TRPV1 (vanilloid) receptor (Julius and Basbaum, 2001). The experimental evidence therefore indicates that the blockade of TRPV1 receptors is a possible mechanism of the antinociceptive activity of the extract. The effect of the extract in the first phase may involve the blockade of the TRPV1 receptors located peripherally and which mediate nociceptive responses to protons, noxious chemicals and heat (Immke and Gavva, 2006; Woolf, 2010). The effects on the second phase on the other

hand may involve the blockade of the central TRPV1 receptors. The central TRPV1 receptors are found in the dorsal horn of the spinal cord, hippocampus, cerebral cortex, PAG as well other CNS areas of the pain neuraxis (Chu et al., 2003; Cristino et al., 2006; De Petrocellis and Di Marzo, 2009; Palazzo et al., 2010). Activation of these central receptors by anandamide and other mediators causes the release of glutamate and Substance P as well other neuropeptides (Fowler 2005; Fowler et al., 2006; Sagar et al., 2009; Singh Tahim et al., 2005). TRPV1 sensitivity to anandamide and other endocannabinoids is elevated in the presence of protons, bradykinin as well as other inflammatory mediators (Sagar et al., 2009; Singh Tahim et al., 2005; Woolf, 2010) further underscoring its roles in the establishment of pain hypersensitivity states. The analgesic activity of TRPV1 receptor antagonists for example capsazepine (Bevan et al., 1992; Walker et al., 2003), AMG-517 (Doherty et al., 2007), SB-705498 (Chizh et al., 2007), AMG-9810 (Gavva et al., 2005) among other chemical moieties undergoing clinical trials is a clear demonstration that blockade of the TRPV1 receptor is a potentially fruitful approach in the discovery of new analgesic drugs (Roberts and Connor, 2006). Indeed it is believed that the TRPV1 antagonists represent the next important class of analgesics (Immke and Gavva, 2006; Wong and Gavva, 2009). The proposed mechanism therefore represents a valid explanation for the extract's antinociceptive activity.

It is interesting to note that all the blockers and agonists administered except capsaicin exerted significant effects on the first phase of the Formalin Test and had no significant effects on the second phase. This indicates that the effects of the blockers/agonists were manifested preferentially on nociceptive transmission as opposed to the central sensitization events of the second phase. The most probable explanation for these experimental results is that descending inhibition is mainly a quantitative event as opposed to nociceptive transmission which is highly specific and therefore qualitative. This is because descending inhibition involves an increase in the activity of intrinsic descending modulatory pathways. The increased activity manifests as a generalized decrease in pain behavior independent of the underlying mechanism. In contrast inhibition of nociceptive transmission as characterized in the first phase of the Formalin Test is much more selective since nociceptive transmission involves specific neurotransmitter systems and receptors. This distinction gains particular importance in experiments involving crude extracts like the one

used in this experiment which often contain many different compounds each with specific and often different mechanisms of action.

The results of the mechanism of action experiments indicate that the antinociceptive effects appear to be mediated via multiple mechanisms. These effects could be mediated through cholinergic, opidergic as well as endocannabinoid neurotransmitter systems. These findings may be explained by the fact that crude extracts such as the one tested in the experiment often contain multiple compounds each of which have unique modes of antinociceptive action. Further, the results obtained indicate that the antinociceptive activity of the extract involve an interplay of both central and peripheral effects. These results are in concurrence with those of Khanna and Bhatia (2003) who in their study of possible antinociceptive mechanisms of action of *Ocimum sanctum* leaves, showed that the analgesic effects were exerted both centrally as well as peripherally, and involved interplay between various neurotransmitter systems.

The chloroform/ethanol extract of *Ocimum masaiense* roots showed significant antidepressant activity in the forced swim test model. The antidepressant activity observed seems to validate one of the principal assumptions in the project proposal, that compounds that show antinociceptive activity would also be expected to possess antidepressant activity. This is because of the common pathophysiologic mechanisms shared by depression and chronic pain. Interestingly these positive antidepressant effects were not observed in the tail suspension test. This can be however be explained by the fact that the forced swim test possesses superior predictive validity as well as sensitivity compared to the tail suspension test (Cryan et al., 2002).

All the fractions obtained after fractionation of dichloromethane/methanol extract of *Ocimum masaiense* root possessed significant antinociceptive activity in the Tail Flick Test. This was a surprising result indeed. This is a strong indication that this extract probably possesses multiple compounds possessing antinociceptive activity over and above those characterized in this project.

3β-Hydroxy-lup-20(29)-en-oic acid (betulinic acid) is a member of the lupane type of triterpenes (Cichewicz and Kouzi, 2004). It has a molecular formula of $C_{30}H_{48}O_3$ and an

exact mass of 456.3603. NMR methods including ^{1}H- and ^{13}C NMR have indisputably become the single most important spectroscopic technique for the identification and structure elucidation of betulinic acid and its analogs (Peng et al., 1998; Sholichin et al., 1980). The NMR spectroscopic data obtained in this study was in concordance with published data for betulinic acid thereby proving the possible identity of the pure compound **Ia**. A noteworthy result of the spectroscopic experiments was the fact that the GC-MS results indicated that the components of compound **Ia** were completely different from those found in compound **Ib**

This is the first published report of betulinic acid being isolated within the genera Ocimum and more generally in the labiatae family. Previous published phytochemical studies on the memebers of this genus have tended to concentrate on the chemical characterization of the essential oils found in this plant (Mwangi et al., 2004). Most of the medicinal properties of *Ocimum sanctum* have been ascribed to Ursolic acid a triterpenoid which has been found to possess anti-inflammatory, anti-stress and antimicrobial activities (Gupta et al., 2007). Gupta (2007) has isolated the following novel compounds from leaves of *Ocimum sanctum*; ocimumosides A and B and ocimarin all of which possessed anti-stress activity.

Betulinic acid showed significant antinociceptive activity in the Tail Flick Test. The antinociceptive activity of this compound at the 100mg/kg dosage level was less than that of the crude extract. This is further indication that the extract may contain other compounds that possessed significant antinociceptive activity.

Betulinic acid (3β-hydroxy-lup-20 (29) –en-28-oic acid) is a pentacyclic lupane type triterpene. it has been isolated in a number of plant species including; *Quisqualis fructus, Coussarea paniculata, Vitex negundo, Uapaca nidida, Syzrgium claviforum* among others (Chandramu et al., 2003; Fujioka et al., 1994; Kwon et al., 2003; Prakash Chaturvedula et al., 2003; Steele et al., 1999). It has been shown to possess a variety of biological activities which include antimalarial, anthlelminthic, antioxidant, antiviral as well as anti-inflammatory properties (Yogeeswari and Sriram, 2005).

Betulinic acid has been shown to posssess anti-inflammatory activity in the Carrageenan and Serotonin paw edema test models (Recio et al., 1995). It was also shown to possess potent

antinociceptive activity in both the acetic acid writhing and Formalin Tests (Krogh et al., 1999). Therefore the results obtained in this study which showed significant antinociceptive activity in the Tail Flick Test are in concordance with those in published literature. It should be noted that although the compound isolated was a methyl ester of betulinic acid, the pharmacologically active form is always the free betulinic acid. This compound is obtained after hydrolysis of the ester by esterase enzymes.

The analgesic activity of betulinic acid can be easily predicted from its structure. It is well established that the NSAIDs which are commonly used analgesics act by inhibiting the cyclooxygenase enzymes (COX-1 and COX-2). All the NSAIDS are highly lipophilic and are also strong acids (Mehanna, 2003). Indeed these dual characteristics must be present for these pharmacological activities. Betulinic acid has a PKa of 4.9 and an octanol-water partition coefficient of 6.86. Both of these properties indicate a highly lipophilic strong organic acid. These chemical properties can adequately explain the antinociceptive activities displayed by this molecule in the Tail Flick Test.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS.

It can be seen that the current ethnobotanical use of plants belonging to the Ocimum genus has scientific basis and should therefore be encouraged. However, one of the key findings of this study was that the roots possess the most potent analgesic activity compared to the leafy parts of the plant. This is a surprising finding given that the leafy parts of the plant are the ones being used ethnobotanically. The use of the leafy parts is more advantageous compared to the use of the roots since it is a more sustainable approach in the utilization of the plant. Hence the current usage methods of the plants should be encouraged.

The results of the screening tests seemed to validate another common practice in traditional medicine; that of using mixture of plants in many herbal remedies. This is a practice that flies in the face of conventional allopathic medicine practices. The herbalists however stand strongly by this practice explaining that multicomponent remedies are often more potent than unicomponent preparations. The results of the screening experiments would seem to validate this hypothesis since they display the so-called "ceiling effect" where further increases in dose will result in a reduction in analgesic effect. This ceiling effect is a common characteristic of mixed opioid receptor agonist/antagonist drugs in clinical use for example nalburphine, pentazocine and buprenorphine (Rance, 1979; Pugh and Drummond, 1987). Indeed this "ceiling effect" is now regarded as advantageous since it greatly reduces the risk of development of tolerance, addiction as well as overdose (Lee, 1997; Gunion et al., 2004). The ceiling effect has necessitated the introduction of clinical analgesic regimens that use more than one opioid drug (Lee, 1997). It has also triggered research into analgesics that have multiple mechanisms of action (Fowler et al., 2009). This is an indication that the traditional medicine practices have much to recommend them and should not be discarded without reason.

The discovery that a previously unutilized plant species that is *Ocimum masaiense* possessed the most potent analgesic activity should prompt enhanced studies on other neglected Ocimum species as this is an indication that they may also possess previously undiscovered

antinociceptive activity. More importantly the crude extracts on fractionation yielded multiple compounds an indication that this genus is a potentially rich source of new bioactive agents.

In conclusion this project was a successful one in that it was able to come up with three main findings; all the species evaluated as well as all the plant parts possessed significant antinociceptive activity , a new medicinal plant species was identified and one (1) novel chemical moiety was identified for the very first time in Ocimum species. This novel compound can act as a lead compound for the development of novel classes of analgesics/ antidepressants. The results of this study should also encourage more detailed chemical studies on commonly used herbal remedies.

REFERENCES.

Abbott F.V., Ocvirk R., Najafee R., Franklin K.B. Improving the efficiency of the Formalin Test. *Pain.* 1999; 83:561-569.

Agarwal N., Pacher P., Tegeder I., Amaya F., Constantin C.E., Brenner G.J., Rubino T., Michalski C.W., Marsicano G., Monory K., Mackie K., Marian C., Batkai S., Parolaro D., Fischer M.J., Reeh P., Kunos G., Kress M., Lutz B., Woolf C.J., Kuner R. Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nature Neuroscience.* 2007; 10(7):870-9.

Aicher S.A., and Randich A. Antinociception and cardiovascular responses produced by electrical stimulation in the nucleus tractus solitarius, nucleus reticularis ventralis, and the caudal medulla. *Pain.* 1990; 42(1):103-19.

Akil H., Mayer D.J., Liebeskind J.C. Antagonism of stimulation-produced analgesia by naloxone, a narcotic antagonist. *Science.* 1976; 191(4230):961-2.

Akil H. and McNally G.P. In *Neuropsychopharmacology .Fifth generation of progress* .2002: New York: Lippincott, Williams, & Wilkins. 35 – 46.

Akil H., Owens C., Gutstein H., Taylor L., Curran E., Watson S. *Drug and Alcohol Dependence.* 1998; 51(1-2):127-40.

Al-Chaer E.D., Lawand N.B., Westlund K.N., Willis W.D. Visceral nociceptive input into the ventral posterolateral nucleus of the thalamus: a new function for the dorsal column pathway. *Journal of Neurophysiology.* 1996; 76(4):2661-74.

Almeida A. and Pertovaara A. Descending inhibitory systems. *Handbook of Clinical Neurology*; Chapter 13. 2006; 81:179-92.

Almeida, T.F. Roizenblatt, S. Tufik, S. Afferent pain pathways: a neuroanatomical review. *Brain Research.* 2004; 1000(1-2):40-56.

Aloisi A.M., Albonetti M.E., Carli G. Behavioural effects of different intensities of formalin pain in rats. *Physiology and Behavior*. 1995; 58: 603-610.

Andersson D.A., Gentry C., Moss S., Bevan S. Transient Receptor Potential A1 Is a Sensory Receptor for Multiple Products of Oxidative Stress. *Journal of Neuroscience.* 2008; 28(10): 2485–2494.

Aouad M., Charlet A., Rodeau J.L., Poisbeau P. Reduction and prevention of vincristine-induced neuropathic pain symptoms by the non-benzodiazepine anxiolytic etifoxine are mediated by 3alpha-reduced neurosteroids. *Pain.* 2009; 147(1-3):54-9.

Apkarian A.V., Baliki M.N., Geha, P.Y. Towards a theory of chronic pain. *Progress in Neurobiology*. 2009, 87 (2): 81-97.

Apkarian A.V., Thomas P.S., Krauss B.R., Szeverenyi N.M. Prefrontal cortical hyperactivity in patients with sympathetically mediated chronic pain. *Neuroscience Letters*. 2001; 311(3):193-7.

Aran S. and Proudfit H.K. Antinociception produced by interactions between intrathecally administered adenosine agonists and norepinephrine. *Brain Research.* 1990; 513(2):255-63.

Arzt E. and Holsboer F. CRF signaling: molecular specificity for drug targeting in the CNS. *Trends in Pharmacological Sciences.* 2006; 27(10):531-8.

Askwith C.C., Benson C.J., Welsh M.J., Snyder P.M. DEG/EnaC ion channels involved in sensory transduction are modulated by cold temperature. *Proceedings of the National Academy of Sciences. USA.*2001; 98: 6459–6463.

Ataka T., Kumamoto E., Shimoji K., Yoshimura M. Baclofen inhibits more effectively C-afferent than Adelta-afferent glutamatergic transmission in substantia gelatinosa neurons of adult rat spinal cord slices. *Pain.* 2000; 86(3):273-82.

Azami J., Green D.L., Roberts M.H.T., Monhemius R. The behavioral importance of dynamically activated descending inhibition from the nucleus reticularis gigantocellularis pars alpha. (University Hospital of Wales, Cardiff, United Kingdom). *Pain* .2001; 92:53-62.

Baba H., Goldstein P.A., Okamoto M., Kohno T., Ataka T., Yoshimura M., Shimoji K. Norepinephrine facilitates inhibitory transmission in substantia gelatinosa of adult rat spinal cord (Part 2): Effects on somatodendritic sites of GABAergic neurons. *Anaesthesiology*.2000; 92(2): 485-492.

Baba H., Shimoji K., Yoshimura M. Norepinephrine facilitates inhibitory transmission in substantia gelatinosa of adult rat spinal cord (part 1): effects on axon terminals of GABAergic and glycinergic neurons.*Anesthesiology*. 2000; 92(2):473-84.

Bai F., Li X., Clay M., Lindstrom T., Skolnick P. Intra- and interstrain differences in models of "behavioral despair". *Pharmacology Biochemistry and Behavior.* 2001; 70(2-3):187-92.

Bajic D., Van Bockstaele E.J., Proudfit H.K. Ultrastructural analysis of ventrolateral periaqueductal gray projections to the A7 catecholamine cell group. *Neuroscience.* 2001; 104(1):181-97.

Ballet S., Conrath M., Fischer J., Kaneko T., Hamon M., Cesselin F. Expression and G-protein coupling of mu-opioid receptors in the spinal cord and dorsal root ganglia of polyarthritic rats. *Neuropeptides.* 2003; 37(4):211-9.

Balsinde J., Winstead M.V., Dennis E.A. Phospholipase A (2) regulation of arachidonic acid mobilization. *FEBS Letters.* 2002; 531(1):2-6.

Bandell M., Story G.M., Hwang S.W., Viswanath V., Eid S.R., Petrus M.J., Earley T.J., Patapoutian A. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron.* 2004; 41(6):849-57.

Barden N. Implication of the hypothalamic–pituitary–adrenal axis in the

physiopathology of depression. *Journal of Psychiatry and Neuroscience*. 2004; 29(3): 185–193.

Bartho L., Stein C., Herz A. Involvement of capsaicin-sensitive neurones in hyperalgesia and enhanced opioid antinociception in inflammation. *Naunyn Schmiedebergs Archives of Pharmacology*. 1990; 342(6):666-70.

Basbaum A.I., Bautista D.M., Scherrer G., Julius, D. Cellular and molecular mechanisms of pain. *Cell. 2009*; 139(2):267-84.

Basbaum A.I. The perception of pain. *Principles of Neural Science*, Edited by Kandel ER, Schwartz JH, Jessell TM - 2000 - New York, McGraw-Hill.

Basbaum A.I. and Fields H.L. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *Annual Review of Neuroscience*. 1984; 7:309-38.

Baumeister A. and Advokat C. Evidence for a supraspinal mechanism in the opioid-mediated antinociceptive effect of ketamine. *Brain Research*. 1991; 566(1-2):351-3.

Bautista D.M., Siemens J., Glazer J.M., Tsuruda P.R., Basbaum A.I., Stucky C.L., Jordt S.E., Julius D. The menthol receptor TRPM8 is the principal detector of environmental cold. *Nature*. 2007; 448(7150):204-8.

Bautista D.M., Sigal Y.M., Milstein A.D., Garrison J.L., Zorn J.A., Tsuruda P.R., Nicoll R.A., Julius D. Pungent agents from Szechuan peppers excite sensory neurons by inhibiting two-pore potassium channels. *Nature Neuroscience*. 2008; 11(7):772-9.

Bechara A., Tranel D., Damasio H. Characterization of the decision-making deficit of patients with ventromedial prefrontal cortex lesions. *Brain*. 2000; 123 (Pt 11):2189-202.

Beitz A.J., Clements J.R., Ecklund L.J., Mullett M.M. The nuclei of origin of brainstem enkephalin and cholecystokinin projections to the spinal trigeminal nucleus of the rat. *Neuroscience*. 1987; 20(2):409-25.

Beitz A.J. Relationship of glutamate and aspartate to the periaqueductal gray-raphe magnus projection: analysis using immunocytochemistry and microdialysis. *Journal of Histochemistry and Cytochemistry.* 1990; 38(12):1755-65.

Beltramo M., Bernardini N., Bertorelli R., Campanella M., Nicolussi E., Fredduzzi S., Reggiani A. CB2 receptor-mediated antihyperalgesia: possible direct involvement of neural mechanisms. *European Journal of Neuroscience.* 2006; 23(6):1530-8.

Ben-Bassat J., Peretz E., Sulman F.G. Analgesimetry and ranking of analgesic drugs by the receptacle method. *Archives Internationale Pharmacodynamie et Therapie.* 1959; 122: 434-447.

Bennett G.J. and Xie Y.K. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain.* 1988; 33:87–107.

Benoliel R., Wilensky A., Tal M., Eliav E. Application of a pro-inflammatory agent to the orbital portion of the rat infraorbital nerve induces changes indicative of ongoing trigeminal pain. *Pain.* 2002; 99(3):567-78.

Berridge C.W. and Dunn A.J. Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? *Brain Research Brain Research Reviews.* 1990; 15(2):71-100.

Berton O. and Nestler E.J. New approaches to antidepressant drug discovery: beyond monoamines. *Nature Reviews Neuroscience.* 2006; 7(2):137-51.

Besson J.M. The neurobiology of pain. *Lancet.* 1999; 353(9164):1610-15.

Bevan S., Hothi S., Hughes G., James I.F., Rang H.P., Shah K., Walpole C.S., Yeats J.C. Capsazepine: a competitive antagonist of the sensory neurone excitant capsaicin. *British Journal of Pharmacology.* 1992; 107(2):544-52.

Bhave G., Karim F., Carlton S.M., Gereau R.W. 4th. Peripheral group I metabotropic glutamate receptors modulate nociception in mice. *Nature Neuroscience.* 2001;

4(4):417-23.

Binder E.B. and Nemeroff C.B. The CRF system, stress, depression and anxiety–insights from human genetic studies. *Molecular Psychiatry* 2010; 15(6):574-588.

Bingel U. and Tracey I. Imaging CNS modulation of pain in humans. *Physiology (Bethesda).* 2008; 23:371-80.

Birder L.A., Nakamura Y., Kiss S., Nealen M.L., Barrick S., Kanai A.J., Wang E., Ruiz G., De Groat W.C., Apodaca G., Watkins S., Caterina M.J. Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. *Nature Neuroscience.* 2002; 5(9):856-60.

Blackburn-Munro G. Hypothalamo-pituitary-adrenal axis dysfunction as a contributory factor to chronic pain and depression. *Current Pain and Headache Reports.* 2004; 8(2):116-24.

Blackburn-Munro G. and Blackburn-Munro R. Pain in the brain: are hormones to blame? *Trends in Endocrinology and Metabolism.* 2003; 14(1):20-7.

Bleazard L. and Morris R. The effects of cholinoceptor agonists and antagonists on C-fibre evoked responses in the substantia gelatinosa of neonatal rat spinal cord slices. *British Journal of Pharmacology.* 1993; 110(3): 1061–1066.

Blomqvist A., Ericson A.C., Craig A.D., Broman J. Evidence for glutamate as a neurotransmitter in spinothalamic tract terminals in the posterior region of owl monkeys. *Experimental Brain Research.* 1996; 108(1):33-44.

Bohn L.M., Xu F., Gainetdinov R.R., Caron M.G. Potentiated opioid analgesia in norepinephrine transporter knock-out mice. *Journal of Neuroscience.* 2000; 20(24):9040-5.

Bonica J.J. Pain; The need of a taxonomy. *Pain.* 1979; 6(3):247–8.

Bonnefont J., Chapuy E., Clottes E., Alloui A., Eschalier A. Spinal 5-HT1A receptors differentially influence nociceptive processing according to the nature of the noxious stimulus in rats: effect of WAY-100635 on the antinociceptive activities of paracetamol, venlafaxine and 5-HT. *Pain.* 2005; 114(3):482-90.

Borsini F. and Meli A. Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacology (Berl.).*1988; 94: 147–160.

Bowery N.G. GABAB receptor: a site of therapeutic benefit. *Current Opinion in Pharmacology.* 2006; 6(1):37-43.

Boxall S.J., Berthele A., Tölle T.R., Zieglgänsberger W., Urban L. mGluR activation reveals a tonic NMDA component in inflammatory hyperalgesia. *Neuroreport.* 1998; 9(6):1201-3.

Braz J.M., Nassar M.A., Wood J.N., Basbaum A.I. Parallel "pain" pathways arise from subpopulations of primary afferent nociceptor. *Neuron.* 2005; 47(6):787-93.

Brenchat A., Romero L., García M., Pujol M., Burgueño J., Torrens A., Hamon M., Baeyens J.M., Buschmann H., Zamanillo D., Vela J.M. 5-HT7 receptor activation inhibits mechanical hypersensitivity secondary to capsaicin sensitization in mice. *Pain.* 2009; 141(3):239-47.

Broom D.C., Samad T.A., Kohno T., Tegeder I., Geisslinger G., Woolf C.J. Cyclooxygenase 2 expression in the spared nerve injury model of neuropathic pain. *Neuroscience.* 2004; 124(4):891-900.

Brown J.L., Liu H., Maggio J.E., Vigna S.R. Morphological characterization of substance P receptor-immunoreactive neurons in the rat spinal cord and trigeminal nucleus caudalis. *Journal of Comparative Neurology.* 1995; 356 (3):327-44.

Burgess P. R. and Perl E. R. Myelinated afferent fibres responding specifically to noxious stimulation of the skin. *Journal of Physiology.* 1967; 190: 541–562.

Butterworth J.F. and Strichartz G.R. Molecular mechanisms of local anesthesia: a review *Anesthesiology* 1990. ; 724: 711-734.

Búzás B. and Cox B.M. Quantitative analysis of mu and delta opioid receptor gene expression in rat brain and peripheral ganglia using competitive polymerase chain reaction. *Neuroscience.* 1997; 76(2):479-89.

Calabrese E.J. Pain and u-shaped dose responses: occurrence, mechanisms, and clinical implications. *Critical Reviews in Toxicology.* 2008; 38 (7):579-90.

Calejesan A.A., Kim S.J., Zhuo M. Descending facilitatory modulation of a behavioral nociceptive response by stimulation in the adult rat anterior cingulate cortex. *European Journal of Pain.* 2000; 4(1):83-96.

Cameron A.A., Khan I.A., Westlund K.N., Willis W.D. The efferent projections of the periaqueductal gray in the rat: a Phaseolus vulgaris-leucoagglutinin study. II. Descending projections. *Journal of Comparative Neurology.* 1995; 351(4):585-601.

Campbell J.N., Raja S.N., Meyer R.A., Mackinnon S.E. Myelinated afferents signal the hyperalgesia associated with nerve injury. *Pain.* 1988; 32(1):89-94.

Cargill C.L., Steinman J.L., Willis W.D. A fictive tail flick reflex in the rat. *Brain Research.* 1985; 345: 45-53.

Carmichael N.M., Charlton M.P., Dostrovsky J.O. Activation of the 5-HT1B/D receptor reduces hindlimb neurogenic inflammation caused by sensory nerve stimulation and capsaicin. *Pain.* 2008; 134(1-2):97-105.

Carstens E. and Wilson C. Rat tail flick reflex: magnitude measurement of stimulus-response function, suppression by morphine and habituation. *Journal of Neurophysiology.* 1999; 70: 630-639.

Carstens E. Quantitative experimental assessment of pain and hyperalgesia in animals and underlying neural mechanisms. *Progress in Brain Research.* 1996; 110:17-31.

Casey K.L. Forebrain mechanisms of nociception and pain: analysis through imaging. *Proceedings of the National Academy of Sciences U S A.* 1999; 96(14):7668-74.

Castagné V., Porsolt R. D., Moser P. Early behavioral screening for antidepressants and anxiolytics. *Drug Development Research.* 2006; 67: 729–742.

Castagné V., Porsolt R.D., Moser P. Use of latency to immobility improves detection of antidepressant-like activity in the behavioral despair test in the mouse. *European Journal of Pharmacology.* 2009; 616(1-3):128-33.

Caterina M.J., Schumacher M.A., Tominaga M., Rosen T.A., Levine J.D., Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature.* 1997; 389(6653):816-24.

Catterall W.A., Ragsdale D.S., McPhee J.C., Scheuer T., Common molecular determinants of local anesthetic, antiarrhythmic, and anticonvulsant block of voltage-gated Na+ channels. *Proceedings of the National Academy of Sciences U S A.* 1996; 93(17):9270-5.

Cavanaugh D.J., Lee H., Lo L., Shields S.D., Zylka M.J., Basbaum A.I., Anderson D.J.Distinct subsets of unmyelinated primary sensory fibers mediate behavioral responses to noxious thermal and mechanical stimuli. *Proceedings of the National Academy of Sciences U S A.* 2009; 106(22):9075-80.

Chalfie M. Neurosensory mechanotransduction. *Nature Reviews Molecular Cell Biology.* 2009; 10(1):44-52.

Chan E., Tan M., Xin J., Sudarsanam S., Johnson D.E. Interactions between traditional Chinese medicines and Western therapeutics. *Current Opinion in Drug Discovery and Development.* 2010; 13(1):50-65.

Chandramu C., Manohar R.D., Krupadanam D.G., Dashavantha R.V.Isolation, characterization and biological activity of betulinic acid and ursolic acid from *Vitex negundo* L.*Phytotherapy Research.* 2003; 17(2):129-34.

Chandrasekharan N.V., Dai H., Roos K.L., Evanson N.K., Tomsik J., Elton T.S., Simmons D.L. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *Proceedings of National Academy of Sciences U S A.* 2002; 99(21):13926-31.

Chang L., Lin L., Palmer J.A., Steven S., Wilson S.J., Barbier A.J., Breitenbucher J.G., Chaplan S.R., Webb M. Inhibition of fatty acid amide hydrolase produces analgesia by multiple mechanisms. *British Journal of Pharmacology.* 2006; 148 (1): 102-113.

Charlet A., Lasbennes F., Darbon P., Poisbeau P. Fast non-genomic effects of progesterone-derived neurosteroids on nociceptive thresholds and pain symptoms. *Pain.* 2008; 139(3):603-9.

Chatterton J.E., Awobuluyi M., Premkumar L.S., Takahashi H., Talantova M., Shin Y., Cui J., Tu S., Sevarino K.A., Nakanishi N., Tong G., Lipton S.A., Zhang D. Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. *Nature.* 2002; 415(6873):793-8.

Chen J. and Sandkühler J. Induction of homosynaptic long-term depression at spinal synapses of sensory a delta-fibers requires activation of metabotropic glutamate receptors. *Neuroscience.* 2000; 98(1):141-8.

Chen S.R. and Pan H.L. Activation of muscarinic receptors inhibits spinal dorsal horn projection neurons: role of GABAB receptors. *Neuroscience.* 2004; 125(1):141-8.

Chen X., Shu S., Bayliss D.A. HCN1 channel subunits are a molecular substrate for hypnotic actions of ketamine. *Journal of Neuroscience.* 2009; 29(3):600-9.

Chen X., Sirois J.E., Lei Q., Talley E.M., Lynch C. 3rd. Bayliss D.A . HCN subunit-specific and cAMP-modulated effects of anesthetics on neuronal pacemaker currents. *Journal of Neuroscience.* 2005; 25:5803–581.

Cheng P.Y., Liu-Chen L.Y., Pickel V.M. Dual ultrastructural immunocytochemical labeling of mu and delta opioid receptors in the superficial layers of the rat cervical

spinal cord. *Brain Research.* 1997; 778(2):367-80.

Chiechio S., Caricasole A., Barletta E., Storto M., Catania M.V., Copani A., Vertechy M., Nicolai R., Calvani M., Melchiorri D., Nicoletti F.L. Acetylcarnitine induces analgesia by selectively up-regulating mGlu2 metabotropic glutamate receptors. *Molecular Pharmacology.* 2002; 61(5):989-96.

Chiechio S., Copani A., De Petris L., Morales M.E., Nicoletti F., Gereau R.W. Transcriptional regulation of metabotropic glutamate receptor 2/3 expression by the NF-kappaB pathway in primary dorsal root ganglia neurons: a possible mechanism for the analgesic effect of L-acetylcarnitine. *Molecular Pain.* 2006 ;2:20-26.

Chiechio S., Copani A., Nicoletti F., Gereau R.W. L-Acetylcarnitine: A Proposed Therapeutic Agent for Painful Peripheral Neuropathies. *Current Neuropharmacology.* 2006; 4(3): 233–237.

Childers S.R.Opioid receptor-coupled second messenger systems. *Life Sciences.* 1991; 48(21):1991-2003.

Chizh B.A., Headley P.M., Tzschentke T.M. NMDA receptor antagonists as analgesics: focus on the NR2B subtype.*Trends in Pharmacological Sciences.* 2001; 22(12):636-42.

Chizh B.A., O'Donnell M.B., Napolitano A., Wang J., Brooke A.C., Aylott M.C., Bullman J.N., Gray E.J., Lai R.Y., Williams P.M., Appleby J.M. The effects of the TRPV1 antagonist SB-705498 on TRPV1 receptor-mediated activity and inflammatory hyperalgesia in humans. *Pain.* 2007; 132(1-2):132-41.

Chu C.J., Huang S.M., De Petrocellis L., Bisogno T., Ewing S.A., Miller J.D., Zipkin R.E., Daddario N., Appendino G., Di Marzo V., Walker J.M. N-oleoyldopamine, a novel endogenous capsaicin-like lipid that produces hyperalgesia. *Journal of Biological Chemistry.* 2003; 278(16):13633-9.

Cichewicz R.H. and Kouzi S.A. Chemistry, biological activity, and chemotherapeutic potential of betulinic acid for the prevention and treatment of cancer and HIV infection.

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Medicinal Research Reviews. 2004; 24(1):90-114.

Clark A.K., Yip P.K., Grist J., Gentry C., Staniland A.A., Marchand F., Dehvari M., Wotherspoon G., Winter J., Ullah J., Bevan S., Malcangio M. Inhibition of spinal microglial cathepsin S for the reversal of neuropathic pain. *Proceedings of the National Academy of Sciences U S A.* 2007; 104(25):10655-60.

Clark J.L. and Kalan G.E. Effective treatment of severe cancer pain of the head using low-dose ketamine in an opioid-tolerant patient. *Journal of Pain and Symptom Management.* 1995; 10(4):310-4.

Clavelou P., Dallel R., Orliaguet T., Woda A., Raboisson P. The orofacial Formalin Test in rats: effects of different formalin concentrations. *Pain.* 1995; 62: 295-301.

Clements J.A. and Nimmo W.S. Pharmacokinetics and analgesic effect of ketamine in man. *British Journal of Anaesthesia.* 1981; 53(1):27-30.

Coderre T.J. and Melzack R. The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. *Journal of Neuroscience.* 1992; 12(9):3665-70.

Coderre T.J., Fundytus M.E., McKenna J.E., Dalal S., Melzack R. The Formalin Test: a validation of the weighted-scores method of behavioural pain rating. *Pain.* 1993; 54:43-50

Coggeshall R.E., Zhou S., Carlton S.M. Opioid receptors on peripheral sensory axons. *Brain Research.* 1997; 764(1-2):126-32.

Colburn R.W., Lubin M.L., Stone D.J. Jr., Wang Y., Lawrence D., D'Andrea M.R., Brandt M.R., Liu Y., Flores C.M., Qin N. Attenuated cold sensitivity in TRPM8 null mice. *Neuron.* 2007; 54(3):379-86.

Collins J.G. Spinally administered neostigmine--something to celebrate. *Anesthesiology.* 1995; 82(2):327-8.

Cooper B.Y. and Vierck C.J. Jr. Measurement of pain and morphine hypalgesia in monkeys. *Pain.* 1986; 26(3):361-92.

Coull J.A., Beggs S., Boudreau D., Boivin D., Tsuda M., Inoue K., Gravel C., Salter M.W., De Koninck Y. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature.* 2005; 438(7070):1017-21.

Coull J.A., Boudreau D., Bachand K., Prescott S.A., Nault F., Sík A., De Koninck P., De Koninck Y. Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. *Nature.* 2003; 424(6951):938-42.

Cowan A., Lewis J.W., MacFarlane I.R. Agonist and antagonist properties of buprenorphine, new antinociceptive agent. *British Journal of Pharmacology.* 1977; 60(19); 537-545.

Cox J.J., Reimann F., Nicholas A.K., Thornton G., Roberts E., Springell K., Karbani G., Jafri H., Mannan J., Raashid Y., Al-Gazali L., Hamamy H., Valente E.M., Gorman S., Williams R., McHale D.P., Wood J.N., Gribble F.M., Woods C.G. An SCN9A channelopathy causes congenital inability to experience pain. *Nature.* 2006; 444(7121):894-8.

Craig A.D. Jr. and Burton H.Spinal and medullary lamina I projection to nucleus submedius in medial thalamus: a possible pain center. *Journal of Neurophysiology.* 1981; 45(3):443-66.

Cristino L., de Petrocellis L., Pryce G., Baker D., Guglielmotti V., Di Marzo V. Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. *Neuroscience.* 2006; 139(4):1405-15.

Cruz-Orengo L., Dhaka A., Heuermann R.J., Young T.J., Montana M.C., Cavanaugh E.J., Kim D. ,Story G.M. Cutaneous nociception evoked by 15-delta PGJ2 via activation of ion channel TRPA1. *Molecular Pain.* 2008; 4:30.

Cryan J.F., Dalvi A., Jin S.H., Hirsch B.R., Lucki I., Thomas S.A. Use of dopamine-beta-hydroxylase-deficient mice to determine the role of norepinephrine in the mechanism of action of antidepressant drugs. *Journal of Pharmacology and Experimental Therapeutics.* 2001; 298(2):651-7.

Cryan J.F., Markou A., Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. *Trends in Pharmacological Sciences.* 2002; 23(5):238-45.

Da Fonseca Pacheco D., Klein A., de Castro Perez A., da Fonseca Pacheco C.M., de Francischi J.N., Duarte D.G. The μ-opioid receptor agonist morphine, but not agonists at δ- or κ-opioid receptors, induces peripheral antinociception mediated by cannabinoid receptors. *British Journal of Pharmacology.* 2008; 154(5): 1143–1149.

Davis J.B., Gray J., Gunthorpe M.J., Hatcher J.P., Davey P.T., Overend P., Harries M.H., Latcham J., Clapham C., Atkinson K., Hughes S.A., Rance K., Grau E., Harper A.J., Pugh P.L., Rogers D.C., Bingham S., Randall A., Sheardown S.A. Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature.* 2000; 405(6783):183-7.

De Bellis M.D., Geracioti T.D. Jr., Altemus M., Kling M.A. Cerebrospinal fluid monoamine metabolites in fluoxetine-treated patients with major depression and in healthy volunteers. *Biological Psychiatry.* 1993; 33(8-9):636-41.

De Bellis M.D., Gold P.W., Geracioti T.D. Jr., Listwak S.J., Kling M.A. Association of fluoxetine treatment with reductions in CSF concentrations of corticotropin-releasing hormone and arginine vasopressin in patients with major depression. *American Journal of Psychiatry.* 1993 ;150(4):656-7.

De Felipe C., Herrero J.F., O'Brien J.A., Palmer J.A. Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. *Nature.* 1998; 392(6674):394-7.

De Kloet E.R., Joëls M., Holsboer F. Stress and the brain: from adaptation to disease.

Nature Reviews Neuroscience. 2005; 6(6):463-75.

De Koninck Y. Altered chloride homeostasis in neurological disorders: a new target. *Current Opinion in Pharmacology.* 2007; 7(1):93-9.

De Petrocellis L. and Di Marzo V. An introduction to the endocannabinoid system: from the early to the latest concepts. *Best Practice Research in Clinical Endocrinology and Metabolism.* 2009; 23(1):1-15.

DeLeo J.A. and Tawfik V. Modulating glial activation in opioid tolerance and neuropathic pain: A role for glutamate transporters. In: Watkins LR, DeLeo JA, Sorkin L (Eds) *Progress in Pain Research and Management:* IASP Press, Seattle, 2007.23-30.

Dhaka A., Murray A.N., Mathur J., Earley T.J., Petrus M.J., Patapoutian A. TRPM8 is required for cold sensation in mice. *Neuron.* 2007; 54(3):371-8.

Dhir A. and Kulkarni S.K. New therapeutic promises in the treatment of depression and schizophrenia. *Drug News Perspectives.* 2008; 21(10):581-92.

Dib-Hajj S.D., Estacion M., Jarecki B.W., Tyrrell L., Fischer T.Z., Lawden M., Cummins T.R., Waxman S.G. Paroxysmal extreme pain disorder M1627K mutation in human Nav1.7 renders DRG neurons hyperexcitable. *Molecular Pain.* 2008; 4:37.

Dinarello C.A., Bernheim H.A., Duff G.W., Le H.V., Nagabhushan T.L., Hamilton N.C., Coceani F. Mechanisms of fever induced by recombinant human interferon. *Journal of Clinical Investigation.* 1984; 74(3):906-13.

Dirig D.M. and Yaksh T.L. Intrathecal baclofen and muscimol, but not midazolam, are antinociceptive using the rat-formalin model. *Journal of Pharmacology and Experimental Therapeutics.* 1995; 275(1):219-27.

Doherty E.M., Fotsch C., Bannon A.W., Bo Y., Chen N., Dominguez C., Falsey J., Gavva N.R., Katon J., Nixey T., Ognyanov V.I., Pettus L., Rzasa R.M., Stec M., Surapaneni S., Tamir R., Zhu J., Treanor J.J., Norman M.H. Novel vanilloid receptor-1

antagonists: 2. Structure-activity relationships of 4-oxopyrimidines leading to the selection of a clinical candidate. *Journal of Medicinal Chemistry.* 2007; 50(15):3515-27.

Dolan S. and Nolan A.M. Behavioral evidence supporting a differential role for spinal group I and II metabotropic glutamate receptors in inflammatory hyperalgesia in sheep. *Neuropharmacology.* 2002; 43(3):319-26.

Dolan S., Kelly J.G., Monteiro A.M., Nolan A.M. Up-regulation of metabotropic glutamate receptor subtypes 3 and 5 in spinal cord in a clinical model of persistent inflammation and hyperalgesia. *Pain.* 2003; 106(3):501-12.

Dong X., Han S., Zylka M.J., Simon M.I., Anderson D.J. A diverse family of GPCRs expressed in specific subsets of nociceptive sensory neurons. *Cell.* 2001; 106(5):619-32.

Dougherty P.M. and Willis W.D. Enhanced responses of spinothalamic tract neurons to excitatory amino acids accompany capsaicin-induced sensitization in the monkey. *Journal of Neuroscience.* 1992; 12:883–894.

Drdla R. and Sandkühler J. Long-term potentiation at C-fibre synapses by low-level presynaptic activity in vivo. *Molecular Pain.* 2008; 4:18.

Drevets W.C. Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. *Current Opinion in Neurobiology.* 2001; 11(2):240-9.

Dubner R. and Bennett G.J. Spinal and trigeminal mechanisms of nociception. *Annual Review of Neuroscience.* 1983; 6:381-418.

Dubner R. and Ruda M.A. Activity-dependent neuronal plasticity following tissue injury and inflammation. *Trends in Neurosciences.* 1992; 15(3):96-103.

Dubuisson D. and Dennis S.G. The Formalin Test: a quantitative study of the analgesic effects of morphine, meperidine and brain stem stimulation in rats and cats. *Pain.* 1977; 4:161-174.

Dugast C., Almeida A., Lima D. The medullary dorsal reticular nucleus enhances the responsiveness of spinal nociceptive neurons to peripheral stimulation in the rat. *European Journal of Neuroscience.* 2003; 18(3):580-8.

Duggan A.W., Morton C.R., Zhao Z.Q., Hendry I.A. Noxious heating of the skin releases immunoreactive substance P in the substantia gelatinosa of the cat: a study with antibody microprobes. *Brain Research.* 1987 ;403(2):345-9.

Eide P.K., Jørum E., Stubhaug A., Bremnes J., Breivik H. Relief of post-herpetic neuralgia with the N-methyl-D-aspartic acid receptor antagonist ketamine: a double-blind, cross-over comparison with morphine and placebo. *Pain.* 1994; 58(3):347-54.

Eide P.K. and Hole K. The role of 5-hydroxytryptamine (5-HT) receptor subtypes and plasticity in the 5-HT systems in the regulation of nociceptive sensitivity. *Cephalalgia.* 1993; 13(2):75-85.

Eisenach J.C. Muscarinic-mediated analgesia. *Life Science.* 1999; 64(6-7):549-54.

Fang F. and Proudfit H.K. Antinociception produced by microinjection of morphine in the rat periaqueductal gray is enhanced in the foot, but not the tail, by intrathecal injection of alpha1-adrenoceptor antagonists. *Brain Research.* 1998; 790(1-2):14-24.

Farré A.J., Colombo M., Gutiérrez B. Maximum tolerated temperature in the rat tail: a broadly sensitive test of analgesic activity. *Methods and Findings in Experimental Clinical Pharmacology.* 1989; 11(5):303-7.

Fields H.L. and Basbaum A.I. Brainstem control of spinal pain-transmission neurons. *Annual Review of Physiology.* 1978; 40:217-48.

Fields H.L., Heinricher M.M., Mason P. Neurotransmitters in nociceptive modulatory circuits. *Annual Reviews of Neuroscience.* 1991; 14: 219–245.

Filos K.S., Goudas L.C., Patroni O., Polyzou V. Hemodynamic and analgesic profile after intrathecal clonidine in humans. A dose-response study. *Anesthesiology.* 1994 ;81(3):591-601; discussion 27A-28A.

Foong F.W., Satoh M., Takagi H. A newly devised reliable method for evaluating analgesic potencies of drugs on trigeminal pain. *Journal of Pharmacological Methods.* 1982; 7: 271-278.

Fowler C.J., Holt S., Nilsson O., Jonsson K.O., Tiger G., Jacobsson S. O. The endocannabinoid signaling system: pharmacological and therapeutic aspects. *Pharmacology, Biochemistry and Behavior.* 2005; 81(2):248-62.

Fowler C.J., Naidu P.S., Lichtman A., Onnis V. The case for the development of novel analgesic agents targeting both fatty acid amide hydrolase and either cyclooxygenase or TRPV1. *British Journal of Pharmacology.* 2009; 156(3):412-9.

Fowler C.J. The cannabinoid system and its pharmacological manipulation--a review, with emphasis upon the uptake and hydrolysis of anandamide. *Fundamental Clinical Pharmacology.* 2006; 20(6):549-62.

Franco A.C. and Prado W. A. Antinociceptive effects of stimulation of discrete sites in the rat hypothalamus: evidence for the participation of the lateral hypothalamus area in descending pain suppression mechanisms. *Brazilian Jorunal of Medical and Biological Research.* 1996; 29(11):1531-41.

Frank A. Low back pain. *British Medical Journal.* 1993; 306:901–909

Franklin K.B. and Matthies B.K. Formalin pain is expressed in decerebrate rats but not attenuated by morphine. *Pain.* 1992; 51(2):199-206.

Frye C.A. and Duncan J.E. Progesterone metabolites, effective at the GABAA receptor

complex, attenuate pain sensitivity in rats. *Brain Research.* 1994; 643(1-2):194-203.

Fujioka T., Kashiwada Y., Kilkuskie R.E., Cosentino L.M., Ballas L.M., Jiang J.B., Janzen W.P., Chen I.S., Lee K.H. Betulinic acid and platanic acid as anti-HIV principles from Syzigium claviflorum, and the anti-HIV activity of structurally related triterpenoids. *Journal of Natural Products.* 1994; 57(2):243-7.

Fürst S. Transmitters involved in antinociception in the spinal cord. *Brain Research Bulletin.* 1999; 48(2):129-41.

Galik J., Youn D.H., Kolaj M., Randić M. Involvement of group I metabotropic glutamate receptors and glutamate transporters in the slow excitatory synaptic transmission in the spinal cord dorsal horn. *Neuroscience.* 2008; 154(4):1372-87.

Gamboa-Esteves F.O., Tavares I., Almeida A., Batten T.F. Projection sites of superficial and deep spinal dorsal horn cells in the nucleus tractus solitarii of the rat. *Brain Research.* 2001; 921(1-2):195-205.

Garces Y.I., Rabito S.F., Minshall R.D., Sagen J. Lack of potent antinociceptive activity by substance P antagonist CP-96,345 in the rat spinal cord. *Life Sciences.* 1993; 52(4):353-60

Garcia L.S., Comim C.M., Valvassori S.S., Réus G.Z., Barbosa L.M., Andreazza A.C., Stertz L., Fries G.R., Gavioli E.C., Kapczinski F., Quevedo J. Acute administration of ketamine induces antidepressant-like effects in the forced swimming test and increases BDNF levels in the rat hippocampus. *Progress in Neuropsychopharmacology and Biological Psychiatry.* 2008; 32(1):140-4.

Gassner M., Ruscheweyh R., Sandkühler J. Direct excitation of spinal GABAergic interneurons by noradrenaline. *Pain.* 2009; 145(1-2):204-10.

Gavva N.R., Tamir R., Qu Y., Klionsky L., Zhang T.J., Immke D.,Wang J., Zhu D., Vanderah T.W., Porreca F., Doherty E.M., Norman M.H., Wild K.D., Bannon A.W., Louis J.C., Treanor J.J. AMG 9810 [(E)-3-(4-t-butylphenyl)-N-(2,3-

dihydrobenzo[b][1,4] dioxin-6-yl)acrylamide], a novel vanilloid receptor 1 (TRPV1) antagonist with antihyperalgesic properties. *Journal of Pharmacology and Experimental Therapeutics.* 2005; 313(1):474-84.

Gear R.W., Miaskowski C., Gordon N.C., Paul S.M., Heller P.H., Levine J.D. The kappa opioid nalbuphine produces gender- and dose-dependent analgesia and antianalgesia in patients with postoperative pain. *Pain.* 1999; 83(2):339-45.

Gebhart G.F. Descending modulation of pain. *Neuroscience Biobehavioral Reviews.* 2004; 27(8):729-37.

Gendron L., Lucido A.L., Mennicken F., O'Donnell D., Vincent J.P., Stroh T., Beaudet A. Morphine and pain-related stimuli enhance cell surface availability of somatic delta-opioid receptors in rat dorsal root ganglia. *Journal of Neuroscience.* 2006; 26(3):953-62.

Geyer M.A. and Markou A. Animal models of psychiatric disorders. In Psychopharmacology: *The Fourth Generation of Progress.* (Bloom, F.E.and Kupfer, D.J., eds); 1995: 787–798, Raven Press.

Ghelardini C., Malmberg-Aiello P., Giotti A., Malcangio M., Bartolini A. Investigation into atropine-induced antinociception. *British Journal of Pharmacology.* 1990; 101(1):49-54.

Giesler Jr G.J. Evidence of direct nociceptive projections from the spinal cord to the hypothalamus and telencephalon. *Seminars in Neuroscience.* 1995; 7(4): 253-261(9).

Gillespie C.F. and Nemeroff, C.B. Hypercortisolemia and depression. *Psychosomatic Medicine.* 2005;67 Suppl 1:S26-8.

Giordano J. and Sacks S.M. Sub-anesthetic doses of bupivacaine or lidocaine increase peripheral ICS-205 930-induced analgesia against inflammatory pain in rats. *European Journal of Pharmacology.* 1997; 334(1):39-41.

Giordano J. The neurobiology of nociceptive and anti-nociceptive systems. *Pain*

Physician. 2005; 8(3):277-90.

Gjerstad J., Tjølsen A., Hole K. A dual effect of 5-HT1B receptor stimulation on nociceptive dorsal horn neurones in rats. *European Journal of Pharmacology.* 1997; 335(2-3):127-32.

Goldstein D.J., Lu Y., Detke M.J., Lee T.C., Iyengar S. Duloxetine vs. placebo in patients with painful diabetic neuropathy. *Pain.* 2005; 116(1-2):109-18.

Gracely R.H., Dubner R., Wolskee P.J., Deeter W.R. Placebo and naloxone can alter post-surgical pain by separate mechanisms. *Nature.* 1983; 306(5940):264-5.

Granados-Soto V., Argüelles C.F., Rocha-González H.I., Godínez-Chaparro B., Flores-Murrieta F.J., Villalón C.M. The role of peripheral 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E and 5-HT1F serotonergic receptors in the reduction of nociception in rats. *Neuroscience.* 2010; 165(2):561-8.

Grant I.S., Nimmo W.S., Clements J.A. Pharmacokinetics and analgesic effects of i.m. and oral ketamine. *British Journal of Anaesthesia.* 1981; 53(8):805-10.

Graves T.D. and Hanna M.G. Neurological channelopathies. *Postgraduate Medical Journal.* 2005; 81(951):20-32.

Grotto M. and Sulman F.G. Modified receptacle method for animal analgesimetry. *Archives of. International Pharmacodynamic Therapy.* 1967; 165: 152-159.

Grumbach L. The prediction of analgesic activity in man by animal testing. In **Pain, 15th International Symposium, Detroit, 1964 (Knighton RS and Dumke PR eds). 1966; 163-182.Little Brown, Boston**.

Guindon J., Desroches J., Beaulieu P. The antinociceptive effects of intraplantar injections of 2-arachidonoyl glycerol are mediated by cannabinoid CB2 receptors. *British Journal of Pharmacology.* 2007; 150(6): 693–701.

Guindon J. and Hohmann A.G. The endocannabinoid system and pain. *CNS and Neurological Disorders Drug Targets.* 2009 ;8(6):403-21.

Guindon J., De Léan A., Beaulieu P. Local interactions between anandamide, an endocannabinoid, and ibuprofen, a nonsteroidal anti-inflammatory drug, in acute and inflammatory pain. *Pain.* 2006; 121(1-2):85-93.

Guindon J., LoVerme J., De Léan A., Piomelli D., Beaulieu P. Synergistic antinociceptive effects of anandamide, an endocannabinoid, and nonsteroidal anti-inflammatory drugs in peripheral tissue: a role for endogenous fatty-acid ethanolamides? *European Journal of Pharmacology.* 2006; 550(1-3):68-77.

Gunion M.W., Marchionne A.M., Anderson C.T.M. Use of the mixed agonist–antagonist nalbuphine in opioid based analgesia. *Acute Pain* .2004; 6(1): 29-39.

Guo T.Z., Jiang J.Y., Buttermann A.E., Maze M. Dexmedetomidine injection into the locus ceruleus produces antinociception. *Anesthesiology.* 1996; 84(4):873-81.

Guo W., Wei F., Zou S., Robbins M.T. Group I metabotropic glutamate receptor NMDA receptor coupling and signaling cascade mediate spinal dorsal horn NMDA receptor 2B tyrosine phosphorylation associated with inflammatory hyperalgesia. *Journal of Neuroscience.* 2004; 24(41):9161-73.

Gupta P., Yadav D.K., Siripurapu K.B., Palit G., Maurya R. Constituents of *Ocimum sanctum* with antistress activity. *Journal of Natural Products.* 2007; 70(9):1410-6.

Gutstein H.B., Rubie E.A., Mansour A., Akil H., Woodgett J.R. Opioid effects on mitogen-activated protein kinase signaling cascades. *Anesthesiology.* 1997; 87(5):1118-26.

Hammond D.L. Inference of pain and its modulation from simple behaviors, in *Issues in Pain Measurement: Advances in Pain Research and Therapy* (Chapman CR and Loeser JD Eds). 1989; 12: 69-91, Raven Press, New York.

Hardy J.D., Stoll A.M., Cunningham D., Benson W.M., Greene L. Responses of the rat to thermal radiation. *American Journal of Physiology*. 1957; 189: 1-5.

Harris J.A. Descending antinociceptive mechanisms in the brainstem: their role in the animal's defensive system. *Journal of Physiology (Paris)*. 1996; 90(1):15-25.

Harty G.J., Dirksen R., Yaksh T.L. Antinociceptive effects of intrathecally injected cholinomimetic drugs in the rat and cat. *European Journal of Pharmacology*. 1985;117(1):81-8.

Harvey R.J., Depner U.B., Wässle H., Ahmadi S., Heindl C., Reinold H., Smart T.G., Harvey K., Schütz B., Abo-Salem O.M., Zimmer A., Poisbeau P., Welzl H., Wolfer D.P., Betz H., Zeilhofer H.U., Müller U. GlyR alpha3: an essential target for spinal PGE2-mediated inflammatory pain sensitization. *Science*. 2004; 304(5672):884-7.

Haynes S.E., Hollopeter G., Yang G., Kurpius D., Dailey M.E., Gan W.B., Julius D. The P2Y12 receptor regulates microglial activation by extracellular nucleotides. *Nature Neuroscience*. 2006; 9(12):1512-9.

Hefferan M.P., Carter P., Haley M., Loomis C.W. Spinal nerve injury activates prostaglandin synthesis in the spinal cord that contributes to early maintenance of tactile allodynia. *Pain*. 2003; 101(1-2):139-47.

Hellwig N., Albrecht N., Harteneck C., Schultz G., Schaefer M. Homo- and heteromeric assembly of TRPV channel subunits. *Journal of Cell Science*. 2005; 118(Pt 5):917-28.

Hensel H. and Zotterman Y. The response of mechanoreceptors to thermal stimulation. *Journal of Physiology*. 1951 ;115(1):16-24.

Hentall I.D., Barbaro N.M., Fields H.L. Spatial and temporal variation of microstimulation thresholds for inhibiting the tail-flick reflex from the rat's rostral medial medulla. *Brain Research*. 1991; 548(1-2):156-62.

Hernandez N. and Vanegas H. Antinociception induced by PAG-microinjected dipyrone (metamizol) in rats: involvement of spinal endogenous opioids. *Brain Research.* 2001; 896(1-2):175-8.

Herrero J.F., Laird J.M., López-García J.A. Wind-up of spinal cord neurones and pain sensation: much ado about something? *Progress in Neurobiology.* 2000; 61(2):169-203.

Hohmann A.G. and Suplita R.L. Endocannabinoid mechanisms of pain modulation. *AAPS Journal.* 2006; 8(4): E693–E708.

Hokfelt T., Zhang X., Wiesenfeld-Hallin Z. Messenger plasticity in primary sensory neurons following axotomy and its functional implications. *Trends in Neuroscience.* 1994; 17(1):22-30.

Holden J.E. and Naleway E. Microinjection of carbachol in the lateral hypothalamus produces opposing actions on nociception mediated by alpha (1) - and alpha (2)-adrenoceptors. *Brain Research.* 2001; 911(1):27-36.

Hole K., Lund A., Tjølsen, A. Antinociceptive effect of paracetamol in rats is partly dependent on spinal serotonergic systems. *European Journal of Pharmacology.* 1991 ;193(2):193-201.

Hollister L.E. Structure-activity relationships in man of cannabis constituents, and homologs and metabolites of delta9-tetrahydrocannabinol. *Pharmacology*. 1974;11(1):3-11.

Honore P., Chandran P., Hernandez G., Gauvin D.M., Mikusa J.P., Zhong C., Joshi S.K., Ghilardi J.R., Sevcik M.A., Fryer R.M., Segreti J.A., Banfor P.N., Marsh K., Neelands T., Bayburt E., Daanen J.F., Gomtsyan A., Lee C.H., Kort M.E., Reilly R.M., Surowy C.S., Kym P.R., Mantyh P.W., Sullivan J.P., Jarvis M.F., Faltynek C.R. Repeated dosing of ABT-102, a potent and selective TRPV1 antagonist, enhances TRPV1-mediated analgesic activity in rodents, but attenuates antagonist-induced hyperthermia. *Pain.* 2009; 142(1-2):27-35.

Honore P., Donnelly-Roberts D., Namovic M.T., Hsieh G., Zhu C.Z., Mikusa J.P., Hernandez G., Zhong C., Gauvin D.M., Chandran P., Harris R., Medrano A.P., Carroll W., Marsh K., Sullivan J.P., Faltynek C.R., Jarvis M.F. A-740003 [N-(1-{[(cyanoimino)(5-quinolinylamino) methyl]amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide], a novel and selective P2X7 receptor antagonist, dose-dependently reduces neuropathic pain in the rat. *Journal of Pharmacology and Experimental Therapeutics*. 2006; 319(3):1376-85.

Hu H.J., Carrasquillo Y., Karim F., Jung W.E., Nerbonne J.M., Schwarz T.L., Gereau R.W 4th. The kv4.2 potassium channel subunit is required for pain plasticity. *Neuron*. 2006; 50(1):89-100.

Hudson L.J., Bevan S., McNair K., Gentry C., Fox A., Kuhn R., Winter J. Metabotropic glutamate receptor 5 upregulation in A-fibers after spinal nerve injury: 2-methyl-6-(phenylethynyl)-pyridine (MPEP) reverses the induced thermal hyperalgesia. *Journal of Neuroscience*. 2002; 22(7):2660-8.

Hughes J.P., Hatcher J.P., Chessell I.P. The role of P2X (7) in pain and inflammation. *Purinergic Signalling*. 2007; 3(1-2):163-9.

Hunskaar H.S., Fasmer O.B., Hole K. Formalin Test in mice, a useful technique for evaluating mild analgesics. *Journal of Neuroscience Methods*. 1985; 14: 69-76.

Hunskaar S. and Hole K. The Formalin Test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain*. 1987; 30: 103-114.

Huo F.Q., Qu C.L., Li Y.Q., Tang J.S., Jia H. GABAergic modulation is involved in the ventrolateral orbital cortex 5-HT 1A receptor activation-induced antinociception in the rat. *Pain*. 2008; 139(2):398-405.

Hwang A.S. and Wilcox G.L. Intradermal hypertonic saline-induced behavior as a nociceptive test in mice. *Life Sciences*.1992; 38: 2389-2396.

Ibrahim M.M., Rude M.L., Stagg N.J., Mata H.P., Lai J., Vanderah T.W., Porreca F.,

Buckley N.E., Makriyannis A., Malan T.P. Jr. CB2 cannabinoid receptor mediation of antinociception. *Pain.* 2006; 122(1-2):36-42.

Ikeda H., Heinke B., Ruscheweyh R., Sandkühler J. Synaptic plasticity in spinal lamina I projection neurons that mediate hyperalgesia. *Science.* 2003; 299(5610):1237-40.

Immke D.C., and Gavva N.R. The TRPV1 receptor and nociception. *Seminars in Cell and Developmental Biology.* 2006 ;17(5):582-91.

Inoue A., Hashimoto T., Hide I., Nishio H., Nakata Y. 5-Hydroxytryptamine-facilitated release of substance P from rat spinal cord slices is mediated by nitric oxide and cyclic GMP. *Journal of Neurochemistry.* 1997; 68(1):128-33.

Isbrucker R., Reid A., Sawynok J. Adenosine mediates calcium-induced antinociception and potentiation of noradrenergic antinociception in the spinal cord. *Brain Research.* 1990; 524(2):187-95.

Ishikawa T., Marsala M., Sakabe T., Yaksh T.L. Characterization of spinal amino acid release and touch-evoked allodynia produced by spinal glycine or GABA (A) receptor antagonist. *Neuroscience.* 2000; 95(3):781-6.

Jaggar S.I., Sellaturay S., Rice A.S. The endogenous cannabinoid anandamide, but not the CB2 ligand palmitoylethanolamide, prevents the viscero-visceral hyper-reflexia associated with inflammation of the rat urinary bladder. *Neuroscience Letters.* 1998; 253(2):123-6.

Janssen P.A.J., Niemegeers C.J.E., Dony J.G.H. The inhibitory effect of fentanyl and other morphine-like analgesics on the warm induced tail withdrawal reflex in rat. *Arzneim Forsch.* 1963; 13: 502-507.

Jasmin L., Tien D., Weinshenker D., Palmiter R.D. The NK1 receptor mediates both the hyperalgesia and the resistance to morphine in mice lacking noradrenaline. *Proceedings of the National Academy of Sciences U S A.* 2002; 99(2):549-51.

Jayamanne A., Greenwood R., Mitchell V.A., Aslan S., Piomelli D., Vaughan C.W. Actions of the FAAH inhibitor URB597 in neuropathic and inflammatory chronic pain models. *British Journal of Pharmacology.* 2006; 147(3): 281–288.

Jembere B., Obeng-Ofori D., Hassanali A., Nyamasyo G.N.N. Products derived from the leaves of *Ocimum kilimandscharicum* (Labiatae) as post-harvest grain protectants against the infestation of three major stored product insect pests. *Bulletin of entomological research.* 1995; 85(3): 361-367.

Jessell T.M. Neuronal specification in the spinal cord: inductive signals and transcriptional codes. *Nature Reviews Genetics.* 2000;1(1):20-9.

Jessell T.M., Iversen L.L., Cuello A.C. Capsaicin-induced depletion of substance P from primary sensory neurones. *Brain Research.* 1978; 152(1):183-8.

Ji R.R., Zhang Q., Law P.Y., Low H.H., Elde R., Hökfelt T. Expression of mu-, delta-, and kappa-opioid receptor-like immunoreactivities in rat dorsal root ganglia after carrageenan-induced inflammation. *Journal of Neuroscience.* 1995; 15(12):8156-66.

Jing S., Tapley P., Barbacid M. Nerve growth factor mediates signal transduction through trk homodimer receptors. *Neuron.* 1992; 9(6):1067-79.

Jones C.K., Eberle E.L., Peters S.C., Monn J.A., Shannon H.E. Analgesic effects of the selective group II (mGlu2/3) metabotropic glutamate receptor agonists LY379268 and LY389795 in persistent and inflammatory pain models after acute and repeated dosing. *Neuropharmacology.* 2005; 49 Suppl 1:206-18.

Jones G., Pertwee R.G., Gill E.W., Paton W.D., Nilsson I.M., Widman M., Agurell S. Relative pharmacological potency in mice of optical isomers of delta 1-tetrahydrocannabinol. *Biochemical Pharmacology.* 1974; 23(2):439-46.

Jordt S.E., McKemy D.D., Julius D. Lessons from peppers and peppermint: the molecular logic of thermosensation. *Current Opinion in Neurobiology.* 2003; 13(4):487-92.

Jourdan D., Ardid D., Bardin L., Bardin M.A. A new automated method of pain scoring in the Formalin Test in rats. *Pain.* 1997; 71: 265-270.

Julius D. and Basbaum A.I. Molecular mechanisms of nociception. *Nature.* 2001; 413(6852):203-10.

Jung C.Y., Lee S.Y., Choi H.S., Lim E.J., Lee M.K., Yang G.Y., Han S.R., Youn D.H., Ahn D.K. Participation of peripheral group I and II metabotropic glutamate receptors in the development or maintenance of IL-1beta-induced mechanical allodynia in the orofacial area of conscious rats. *Neuroscience Letters.* 2006; 409(3):173-8.

Junger H., Moore A.C., Sorkin L.S. Effects of full-thickness burns on nociceptor sensitization in anesthetized rats. *Burns.* 2002; 28(8):772-7.

Kabli N. and Cahill C.M. Anti-allodynic effects of peripheral delta opioid receptors in neuropathic pain. *Pain.* 2007; 127(1-2):84-93.

Kalra B.S., Tayal V., Chawla S. Antidepressant-like activity of tramadol in mice. *Indian Journal of Psychiatry.* 2008; 50(1):51-3.

Kanui T.I. Multipurpose shrubs used in the management of arthritis. *Proceedings of the Annual DAAD Scholars Conference Kenya.* 2006; 26-32.

Karashima Y., Talavera K., Everaerts W., Janssens A., Kwan K.Y., Vennekens R., Nilius B., Voets T. TRPA1 acts as a cold sensor in vitro and in vivo. *Proceedings of the National Academy of Sciences U S A.* 2009; 106(4):1273-8.

Karim F., Bhave G., Gereau R.W. 4th. Metabotropic glutamate receptors on peripheral sensory neuron terminals as targets for the development of novel analgesics. *Molecular Psychiatry.* 2001; 6(6):615-7.

Katsura H., Obata K., Mizushima T., Yamanaka H., Kobayashi K., Dai Y., Fukuoka T., Tokunaga A., Sakagami M., Noguchi K. Antisense knock down of TRPA1, but not TRPM8, alleviates cold hyperalgesia after spinal nerve ligation in rats. *Experimental*

Neurology. 2006; 200(1):112-23.

Kehl L.J., Hamamoto D.T., Wacnik P.W., Croft D.L., Norsted B.D., Wilcox G.L., Simone D.A. A cannabinoid agonist differentially attenuates deep tissue hyperalgesia in animal models of cancer and inflammatory muscle pain. *Pain.* 2003; 103(1-2):175-86.

Keller A.F., Beggs S., Salter M.W., De Koninck Y. Transformation of the output of spinal lamina I neurons after nerve injury and microglia stimulation underlying neuropathic pain. *Molecular Pain*. 2007; 3:27.

Kenshalo D.R., Leonard R.B., Chung J.M., Willis W.D. Facilitation of the responses of primate spinothalamic cells to cold and mechanical stimuli by noxious heating of the skin. *Pain* .1982; 12: 141–152.

Kevetter G.A., Haber L.H., Yezierski R.P., Chung J.M., Martin R.F., Willis W.D. Cells of origin of the spinoreticular tract in the monkey. *Journal of Comparative Neurology*. 1982; 207(1):61-74.

Keywood C., Wakefield M., Tack J. A proof-of-concept study evaluating the effect of ADX10059, a metabotropic glutamate receptor-5 negative allosteric modulator, on acid exposure and symptoms in gastro-oesophageal reflux disease. *Gut*. 2009; 58(9): 1192–1199

Khan I., Osaka H., Stanislaus S., Calvo R.M., Deerinck T., Yaksh T.L., Taylor P. Nicotinic acetylcholine receptor distribution in relation to spinal neurotransmission pathways. *Journal of Comparative Neurology*. 2003; 467(1):44-59.

Khanna N. and Bhatia J. Antinociceptive action of *Ocimum sanctum* (Tulsi) in mice: possible mechanisms involved. *Journal of Ethnopharmacology*. 2003; 88(2-3):293-6.

Khasabova I.A., Harding-Rose C., Simone D.A., Seybold V.S. Differential effects of CB1 and opioid agonists on two populations of adult rat dorsal root ganglion neurons. *Journal of Neuroscience*. 2004; 24(7):1744-53.

Kieffer B.L. Recent advances in molecular recognition and signal transduction of active peptides: receptors for opioid peptides. *Cellular and Molecular Neurobiology*. 1995; 15(6):615-35.

Kinney R.K., Gatchel R.J., Polatin P. B., Fogarty W. T., Mayer T. G. Prevalence of psychopathology in acute and chronic low back pain patients. *Journal of Occupational Rehabilitation*.1993; 3 (2): 95-103.

Kjørsvik A., Tjølsen A., Hole K. Activation of spinal serotonin (2A/2C) receptors augments nociceptive responses in the rat. *Brain Research*. 2001; 910(1-2):179-81.

Klamt J.G., Dos Reis M.P., Barbieri Neto J., Prado W.A. Analgesic effect of subarachnoid neostigmine in two patients with cancer pain. *Pain*. 1996; 66(2-3):389-91.

Klerman G.L. and Weissman M.M. The course, morbidity, and costs of depression. *Archives of General Psychiatry*. 1992; 49(10):831-4.

Knabl J., Witschi R., Hösl K., Reinold H., Zeilhofer U.B., Ahmadi S., Brockhaus J., Sergejeva M., Hess A., Brune K., Fritschy J.M., Rudolph U., Möhler H., Zeilhofer H.U. Reversal of pathological pain through specific spinal GABAA receptor subtypes. *Nature*. 2008; 451(7176):330-4.

Kobayashi, K. Yamanaka, H. Fukuoka, T. Dai, Y. Obata, K. Noguchi K. P2Y12 receptor upregulation in activated microglia is a gateway of p38 signaling and neuropathic pain. *Journal of Neuroscience*. 2008; 28(11):2892-902.

Kokwaro J. Medicinal Plants of East Africa 3rd Edition. 2009: East African Literature Bureau.

Kokwaro J. O. Medicinal plants of East Africa. 1976: East African Literature Bureau, General printers Ltd, Nairobi.

Koltzenburg M. and Scadding J. Neuropathic pain. *Current Opinion in Neurology*. 2001; 14(5):641-7.

Kondo I., Marvizon J.C., Song B., Salgado F., Codeluppi S., Hua X.Y., Yaksh T.L. Inhibition by spinal mu- and delta-opioid agonists of afferent-evoked substance P release. *Journal of Neuroscience*. 2005; 25(14):3651-60.

Krogh R., Kroth R., Berti C., Madeira A.O., Souza M.M., Cechinel-Filho V., Delle-Monache F., Yunes R.A. Isolation and identification of compounds with antinociceptive action from *Ipomoea pes-caprae (L.)* R. *Br.Pharmazie.* 1999; 54(6):464-6.

Kuraishi Y., Harada Y., Takagi H. Noradrenaline regulation of pain-transmission in the spinal cord mediated by alpha-adrenoceptors. *Brain Research.* 1979; 174(2):333-6.

Kwan K.Y. and Corey D.P. Burning cold: involvement of TRPA1 in noxious cold sensation. *Journal of General Physiology*. 2009; 133(3):251-6.

Kwiat G.C. and Basbaum A.I. The origin of brainstem noradrenergic and serotonergic projections to the spinal cord dorsal horn in the rat. *Somatosensory and Motor Research.* 1992; 9(2):157-73.

Kwon H.C., Min Y.D., Kim K.R., Bang E.J., Lee C.S., Lee K.R. A new acylglycosyl sterol from Quisqualis Fructus. *Archives of Pharmaceutical Research.* 2003; 26(4):275-8.

LaBuda C.J. and Fuchs P.N. Low dose aspirin attenuates escape/avoidance behavior, but does not reduce mechanical hyperalgesia in a rodent model of inflammatory pain. *Neuroscience Letters*. 2001; 304(3):137-40.

Laird J.M., Martinez-Caro L., Garcia-Nicas E., Cervero F. A new model of visceral pain and referred hyperalgesia in the mouse. *Pain.* 2001; 92(3):335-42.

Lariviere W.R. and Melzack R. The role of corticotropin-releasing factor in pain and analgesia. *Pain*. 2000; 84(1):1-12.

Latremoliere A. and Woolf C.J. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. *Pain*. 2009; 10(9):895-926.

Lauretti G.R., Reis M.P., Prado W.A., Klamt J.G. Dose-response study of intrathecal morphine versus intrathecal neostigmine, their combination, or placebo for postoperative analgesia in patients undergoing anterior and posterior vaginoplasty. *Anesthesia and Analgesia.* 1996; 82(6):1182-7.

Le Bars D., Gozariu M., Cadden S.W. Animal models of nociception. *Pharmacological Reviews.* 2001; 53(4):597-652.

Lee S.C., Wang J.J., Ho S.T., Tao P.L. Nalbuphine coadministered with morphine prevents tolerance and dependence. *Anesthesia and Analgesia.* 1997; 84(4):810-5.

Lee Y.W. and Yaksh T.L. Analysis of drug interaction between intrathecal clonidine and MK-801 in peripheral neuropathic pain rat model. *Anesthesiology.* 1995; 82(3):741-8.

Levine J.D., Gordon N.C., Jones R.T., Fields H.L. The narcotic antagonist naloxone enhances clinical pain. *Nature.* 1978; 272(5656):826-7.

Lewin G.R. Stretching it for pain. *Pain.* 2008; 137(1):3-4.

Li J.L., Ding Y.Q., Li Y.Q., Li J.S., Nomura S., Kaneko T., Mizuno N. Immunocytochemical localization of mu-opioid receptor in primary afferent neurons containing substance P or calcitonin gene-related peptide. A light and electron microscope study in the rat. *Brain Research.* 1998; 794(2):347-52.

Lichtman A.H., Leung D., Shelton C.C., Saghatelian A., Hardouin C., Boger D.L., Cravatt B.F. Reversible inhibitors of fatty acid amide hydrolase that promote analgesia: evidence for an unprecedented combination of potency and selectivity. *Journal of Pharmacology and Experimental Therapeutics.* 2004; 311(2):441-8.

Lima D. and Almeida, A. The medullary dorsal reticular nucleus as a pronociceptive centre of the pain control system. *Progress in Neurobiology.* 2002; 66(2):81-108.

Littlewood N.K., Todd A.J., Spike R.C., Watt C., Shehab S.A. The types of neuron in spinal dorsal horn which possess neurokinin-1 receptors. *Neuroscience.* 1995; 66(3):597-608.

Liu X.J., Gingrich J.R., Vargas-Caballero M., Dong Y.N., Sengar A., Beggs S., Wang S.H., Ding H.K., Frankland P.W., Salter M.W. Treatment of inflammatory and neuropathic pain by uncoupling Src from the NMDA receptor complex. *Nature Medicine.* 2008; 14(12):1325-32.

Lu G.W. Spinocervical tract-dorsal column postsynaptic neurons: a double-projection neuronal system. *Somatosensory and Motor Research.* 1989; 6(5-6):445-54.

Lucki I., Markou A., Cryan J.F. Assessing antidepressant activity in rodents: recent developments and future needs. *Trends in Pharmacological Sciences.* 2002; 23(5):238-45.

Lucki I. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behavioral Pharmacology.*1997; 8:523–532.

Lumb B.M. Hypothalamic influences on viscero-somatic neurones in the lower thoracic spinal cord of the anaesthetized rat. *Journal of Physiology.* 1990; 424:427-44.

Lumpkin E.A. and Caterina M.J. Mechanisms of Sensory Transduction in the Skin. *Nature.* 2007; 445(7130):858-65.

Luttinger D. Determination of antinociceptive efficacy of drugs in mice using different water temperatures in a tail immersion test. *Journal of Pharmacological Methods.* 1985; 13: 351-357.

Lynn B. Capsaicin: actions on nociceptive C-fibres and therapeutic potential. *Pain.* 1990; 41(1):61-9.

Ma W. and Eisenach J.C. Morphological and pharmacological evidence for the role of peripheral prostaglandins in the pathogenesis of neuropathic pain. *European Journal of Neuroscience.* 2002; 15(6):1037-47.

Maeng S., Zarate C.A. Jr, Du J., Schloesser R.J., McCammon J., Chen G., Manji H.K. Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biological Psychiatry.* 2008; 63(4):349-52.

Magnaghi V., Ballabio M., Camozzi F., Colleoni M., Consoli A., Gassmann M., Lauria G., Motta M., Procacci P., Trovato A.E., Bettler B. Altered peripheral myelination in mice lacking GABAB receptors. *Molecular and Cellular Neuroscience.* 2008; 37(3):599-609.

Makonnen E., Debella A., Abebe D., Teka F. Analgesic properties of some Ethiopian medicinal plants in different models of nociception in mice. *Phytotherapy Research.* 2003; 17(9):1108-12.

Makonnen E., Debella A., Zerihun L., Abebe D., Teka F. Antipyretic properties the aqueous and ethanol extracts of the leaves of *Ocimum suave* and *Ocimum lamiifolium* in mice. *Journal of Ethnopharmacology.*2003; 88(1): 85-91.

Malan T.P., Mata H.P. Porreca F. Spinal GABA (A) and GABA (B) receptor pharmacology in a rat model of neuropathic pain. *Anesthesiology.* 2002; 96(5):1161-7.

Malcangio M. and Bowery N.G. Possible therapeutic application of GABAB receptor agonists and antagonists. *Clinical Neuropharmacology.* 1995 ;18(4):285-305.

Mallet C., Daulhac L., Bonnefont J., Ledent C., Etienne M., Chapuy E., Libert F., Eschalier A. Endocannabinoid and serotonergic systems are needed for acetaminophen-induced analgesia. *Pain.* 2008; 139(1):190-200.

Maniadakis N. and Gray A. The economic burden of back pain in the UK. *Pain.* 2000; 84:95–103.

Manji H.K, Drevets W.C., Charney D.S. The cellular neurobiology of depression. *Nature Medicine.* 2001; 7(5):541-7.

Manning B.H. and Franklin K.B. Morphine analgesia in the Formalin Test: reversal by microinjection of quaternary naloxone into the posterior hypothalamic area or periaqueductal gray. *Behavioral Brain Research.* 1998; 92(1):97-102.

Manning B.H. and Mayer D.J. The central nucleus of the amygdala contributes to the production of morphine antinociception in the Formalin Test. *Pain.* 1995; 63(2):141-52.

Mannion R.J., Costigan M., Decosterd I., Amaya F., Ma Q.P., Holstege J.C., Ji R.R., Acheson A., Lindsay R.M., Wilkinson G.A., Woolf C.J. Neurotrophins: peripherally and centrally acting modulators of tactile stimulus-induced inflammatory pain hypersensitivity. *Proceedings of the National Academy of Sciences U S A.* 1999; 96(16):9385-90.

Mansikka H., Shiotani M., Winchurch R., Raja S.N. Neurokinin-1 receptors are involved in behavioral responses to high-intensity heat stimuli and capsaicin-induced hyperalgesia in mice. *Anesthesiology.* 1999; 90(6):1643-9.

Mansour A., Watson S.J., Akil H. Opioid receptors: past, present and future. *Trends in Neurosciences.* 1995; 18(2):69-70.

Mantyh P.W., Rogers S.D., Honore P., Allen B.J. Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor. *Science.* 1997; 278(5336):275-9.

Martin R.F., Jordan L.M., Willis W.D. Differential projections of cat medullary raphe neurons demonstrated by retrograde labelling following spinal cord lesions. *Journal of Comparative Neurology.* 1978; 182(1):77-88.

Marvizón J.C., Grady E.F., Stefani E., Bunnett N.W., Mayer E.A. Substance P release in the dorsal horn assessed by receptor internalization: NMDA receptors counteract a tonic inhibition by GABA (B) receptors. *European Journal of Neuroscience.* 1999;

11(2):417-26.

Marx J. Locating a new step in pain's pathway. *Science.* 2004; 304(5672):811.

Mason P. Central mechanisms of pain modulation. *Current Opinion in Neurobiology.* 1999; 9(4):436-41.

Mathew S.J., Coplan J.D., Schoepp D.D., Smith E.L., Rosenblum L.A., Gorman J.M. Glutamate-hypothalamic-pituitary-adrenal axis interactions: implications for mood and anxiety disorders. *CNS Spectrum.* 2001; 6(7):555-6, 561-4.

Mayorga A.J. and Lucki, I. Limitations on the use of the C57BL/6 mouse in the tail suspension test. *Psychopharmacology (Berl.).* 2001; 155: 110–112.

McCarthy P.W. and Lawson S.N. Cell type and conduction velocity of rat primary sensory neurons with substance P-like immunoreactivity. *Neuroscience.* 1989; 28(3):745-53

McCormack K., Prather P., Chapleo C. Some new insights into the effects of opioids in phasic and tonic nociceptive tests. *Pain.* 1998; 78(2):79-98.

McEwen B.S. Stressed or stressed out: what is the difference? *Journal of Psychiatry and Neuroscience.* 2005; 30(5):315-8.

McKemy D.D., Neuhausser W.M., Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature.* 2002; 416(6876):52-8.

Mechoulam R. Cannabis as therapeutic Agent. *Substance Use & Misuse.* 1986; 21 (4): 579 – 587.

Mehanna A.S. NSAIDs: Chemistry and Pharmacological Actions. *American Journal of Pharmaceutical Education.* 2003; 67 (2):1-7.

Meller S.T., Dykstra C.L., Gebhart G.F. Acute mechanical hyperalgesia is produced by coactivation of AMPA and metabotropic glutamate receptors. *Neuroreport.* 1993;

11(2):417-26.

Marx J. Locating a new step in pain's pathway. *Science.* 2004; 304(5672):811.

Mason P. Central mechanisms of pain modulation. *Current Opinion in Neurobiology.* 1999; 9(4):436-41.

Mathew S.J., Coplan J.D., Schoepp D.D., Smith E.L., Rosenblum L.A., Gorman J.M. Glutamate-hypothalamic-pituitary-adrenal axis interactions: implications for mood and anxiety disorders. *CNS Spectrum.* 2001; 6(7):555-6, 561-4.

Mayorga A.J. and Lucki, I. Limitations on the use of the C57BL/6 mouse in the tail suspension test. *Psychopharmacology (Berl.).* 2001; 155: 110–112.

McCarthy P.W. and Lawson S.N. Cell type and conduction velocity of rat primary sensory neurons with substance P-like immunoreactivity. *Neuroscience.* 1989; 28(3):745-53.

McCormack K., Prather P., Chapleo C. Some new insights into the effects of opioids in phasic and tonic nociceptive tests. *Pain.* 1998; 78(2):79-98.

McEwen B.S. Stressed or stressed out: what is the difference? *Journal of Psychiatry and Neuroscience.* 2005; 30(5):315-8.

McKemy D.D., Neuhausser W.M., Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature.* 2002; 416(6876):52-8.

Mechoulam R. Cannabis as therapeutic Agent. *Substance Use & Misuse.* 1986; 21 (4): 579 – 587.

Mehanna A.S. NSAIDs: Chemistry and Pharmacological Actions. *American Journal of Pharmaceutical Education.* 2003; 67 (2):1-7.

Meller S.T., Dykstra C.L., Gebhart G.F. Acute mechanical hyperalgesia is produced by coactivation of AMPA and metabotropic glutamate receptors. *Neuroreport.* 1993;

4(7):879-82.

Melzack R. and Wall P. D. Pain mechanisms - A new theory (Gate control system role in pain mechanism, noting specificity and pattern theories). *Science.* 1965; 150:971-979.

Mendell L.M. and Wall P.D. Responses of single dorsal cord cells to peripheral cutaneous unmyelinated fibres. *Nature.* 1965; 206:97-9.

Merskey H. Logic, truth and language in concepts of pain. *Quality of Life Research.* 1994; 3 Suppl 1:S69-76.

Meyer R.A. and Campbell J.N. Evidence for two distinct classes of unmyelinated nociceptive afferents in monkey. *Brain Research.* 1981; 224(1):149-52.

Micó J.A., Ardid D., Berrocoso E., Eschalier A. Antidepressants and pain. *Trends in Pharmacological Sciences.* 2006; 27(7):348-54.

Millan M.J. Descending control of pain. *Progress in Neurobiology.* 2002; 66(6):355-474.

Millan M.J. The induction of pain: an integrative review. *Progress in Neurobiology.* 1999; 57(1):1-164.

Milligan E.D., Sloane E.M., Watkins L.R. Glia in pathological pain: a role for fractalkine. *Journal of Neuroimmunology.* 2008; 198(1-2):113-20.

Milligan E.D., Zapata V., Chacur M., Schoeniger D., Biedenkapp J., O'Connor K.A., Verge G.M., Chapman G., Green P., Foster A.C., Naeve G.S., Maier S.F., Watkins L.R. Evidence that exogenous and endogenous fractalkine can induce spinal nociceptive facilitation in rats.

Mills C.D., Xu G.Y., Johnson K.M., McAdoo D.J., Hulsebosch C.E. AIDA reduces glutamate release and attenuates mechanical allodynia after spinal cord injury. *Neuroreport.* 2000; 11(14):3067-70.

Minami M., Maekawa K., Yabuuchi K., Satoh M. Double in situ hybridization study on coexistence of mu-, delta- and kappa-opioid receptor mRNAs with preprotachykinin A mRNA in the rat dorsal root ganglia. *Brain Research, Molecular Brain Research.* 1995; 30(2):203-10.

Minor B.G., Archer T., Post C., Jonsson G., Mohammed A.K. 5-HT agonist induced analgesia modulated by central but not peripheral noradrenaline depletion in rats. *Journal of Neural Transmission.* 1986; 66(3-4):243-59.

Minor B.G., Post C., Archer T. Blockade of intrathecal 5-hydroxytryptamine-induced antinociception in rats by noradrenaline depletion. *Neuroscience Letters.* 1985; 54(1):39-44.

Montell C. The TRP superfamily of cation channels. *Science's Signal Transduction Knowledge Environment.* 2005; 2005(272):re3.

Moore K.A., Kohno T., Karchewski L.A., Scholz J., Baba H. Woolf C.J. Partial peripheral nerve injury promotes a selective loss of GABAergic inhibition in the superficial dorsal horn of the spinal cord. *Journal of Neuroscience.* 2002; 22(15):6724-31.

Moore K.A., Taylor G.E., Weinreich D. Serotonin unmasks functional NK-2 receptors in vagal sensory neurones of the guinea-pig. *Journal of Physiology.* 1999; 514 (Pt 1):111-24.

Moran M.M., Xu H., Clapham D.E. TRP ion channels in the nervous system. *Current Opinion in Neurobiology.* 2004; 14(3):362-9.

Morgan M.M., Sohn J.H., Lohof A.M., Ben-Eliyahu S. Characterization of stimulation-produced analgesia from the nucleus tractus solitarius in the rat. *Brain Research.* 1989; 486(1):175-80.

Morilak, D.A. and Frazer A. Antidepressants and brain monoaminergic systems: a dimensional approach to understanding their behavioural effects in depression and

anxiety disorders. *International Journal of Neuropsychopharmacology*. 2004; 7(2):193-218.

Mousa S.A., Machelska H., Schäfer M., Stein C. Immunohistochemical localization of endomorphin-1 and endomorphin-2 in immune cells and spinal cord in a model of inflammatory pain. *Journal of Neuroimmunology*. 2002; 126(1-2):5-15.

Mousa S.A., Bopaiah C.P., Richter J.F., Yamdeu R.S., Schäfer M. Inhibition of inflammatory pain by CRF at peripheral, spinal and supraspinal sites: involvement of areas coexpressing CRF receptors and opioid peptides. *Neuropsychopharmacology*. 2007; 32(12):2530-42.

Mousa S.A., Straub R.H., Schäfer M., Stein C. Beta-endorphin, Met-enkephalin and corresponding opioid receptors within synovium of patients with joint trauma, osteoarthritis and rheumatoid arthritis. *Annals of Rheumatic Disease*. 2007; 66(7):871-9.

Murase K., Ryu P.D., Randić M. Substance P augments a persistent slow inward calcium-sensitive current in voltage-clamped spinal dorsal horn neurons of the rat. *Brain Research*. 1986; 365(2):369-76.

Murray C.J.L. and Lopez A.D. Evidence-Based Health Policy---Lessons from the Global Burden of Disease Study. *Science* 1996: 274; (5288): 740 – 743.

Murray C.W., Porreca F., Cowan A. Methodological refinements to the mouse paw Formalin Test. An animal model of tonic pain. *Journal of Pharmacological Methods*. 1988; 20(2):175-86.

Muthuraman A., Diwan V., Jaggi A.S., Singh N., Singh D. Ameliorative effects of *Ocimum sanctum* in sciatic nerve transection-induced neuropathy in rats. *Journal of Ethnopharmacology*. 2008; 120(1):56-62.

Nackley A.G., Makriyannis A., Hohmann A.G. Selective activation of cannabinoid CB (2) receptors suppresses spinal for protein expression and pain behavior in a rat model of inflammation. *Neuroscience*. 2003; 119(3):747-57.

Naderi N., Shafaghi B., Khodayar M.J., Zarindast M.R. Interaction between gamma-aminobutyric acid GABAB and cannabinoid CB1 receptors in spinal pain pathways in rat. *European Journal of Pharmacology*. 2005; 514(2-3):159-64.

Naidu P.S., Varvel S.A., Ahn K., Cravatt B.F., Martin B.R., Lichtman A.H. Evaluation of fatty acid amide hydrolase inhibition in murine models of emotionality. *Psychopharmacology* (Berl). 2007; 192(1):61-70.

Nakamura F. and Strittmatter S.M. P2Y1 purinergic receptors in sensory neurons: contribution to touch-induced impulse generation. *Proceedings of the National Academy of Sciences U S A*. 1996. 17; 93 (19):10465-70.

Nakanishi S. Mammalian tachykinin receptors. *Annual Review of Neuroscience*. 1991; 14:123-36.

Nemeroff C.B. and Owens M.J. Treatment of mood disorders. *Nature Reviews Neuroscience*. 2002; 5, Suppl: 1068-70.

Nemeroff C.B. and Ressler K.J. Role of norepinephrine in the pathophysiology and treatment of mood disorders. *Biological Psychiatry*. 1999; 46(9):1219-33.

Nemeroff C.B. and Vale W.W. The neurobiology of depression: inroads to treatment and new drug discovery. *Journal of Clinical Psychiatry*. 2005; 66 Suppl 7:5-13.

Nestler E.J. and Krishnan V. The molecular neurobiology of depression. *Nature*. 2008; 455 (7215): 894-902.

Neto F.L., Schadrack J., Platzer S., Zieglgänsberger W., Tölle T.R., Castro-Lopes J.M. Up-regulation of metabotropic glutamate receptor 3 mRNA expression in the cerebral cortex of monoarthritic rats. *Journal of Neuroscience Research*. 2001; 63(4):356-67.

Neugebauer V. Metabotropic glutamate receptors--important modulators of nociception and pain behavior. *Pain*. 2002; 98(1-2):1-8.

Nichols M.L., Allen B.J., Rogers S.D., Ghilardi J.R. Transmission of chronic nociception by spinal neurons expressing the substance P receptor. *Science.* 1999; 286(5444):1558-61.

Njoroge G.N. and Bussmann R.W. Diversity and utilization of antimalarial ethnophytotherapeutic remedies among the Kikuyus (Central Kenya). *Journal of Ethnobiology and Ethnomedicine*. 2006; 2:8.

Nuseir K. and Proudfit H.K. Bidirectional modulation of nociception by GABA neurons in the dorsolateral pontine tegmentum that tonically inhibit spinally projecting noradrenergic A7 neurons. *Neuroscience.* 2000; 96(4):773-83.

Nyilas R., Gregg L.C., Mackie K., Watanabe M., Zimmer A., Hohmann A.G., Katona I. Molecular architecture of endocannabinoid signaling at nociceptive synapses mediating analgesia. *European Journal of Neuroscience.* 2009; 29(10):1964-78.

Obata H., Saito S., Sasaki M., Goto F. Possible involvement of a muscarinic receptor in the anti-allodynic action of a 5-HT2 receptor agonist in rats with nerve ligation injury. *Brain Research.* 2002; 932(1-2):124-8.

Obata K., Katsura H., Mizushima T., Yamanaka H., Kobayashi K., Dai Y., Fukuoka T., Tokunaga A., Tominaga M., Noguchi K. TRPA1 induced in sensory neurons contributes to cold hyperalgesia after inflammation and nerve injury. *Journal of Clinical Investigation.* 2005; 115(9):2393-401.

Obeng-Ofori D., Reichmuth C., Bekele J., Hassanali A. Biological activity of 1,8 cineole, a major component of essential oil of *Ocimum kenyense* (Ayobangira) against stored product beetles. *Journal of Applied Entomology.* 1997; 121: 237–243.

Odalo J.O., Omolo M.O., Malebo H., Angira J., Njeru P.M., Ndiege I.O., Hassanali A. Repellency of essential oils of some plants from the Kenyan coast against Anopheles gambiae. *Acta Tropica.* 2005; 95(3):210-8

Odeh F. and Antal M. The projections of the midbrain periaqueductal grey to the pons and medulla oblongata in rats. *European Journal of Neuroscience.* 2001; 14(8):1275-86.

Okazaki R., Namba H., Yoshida H., Okai H., Miura T., Kawamura M. The antiallodynic effect of Neurotropin is mediated via activation of descending pain inhibitory systems in rats with spinal nerve ligation. *Anaesthesia and Analgesia.* 2008; 107(3):1064-9.

Olave M.J. and Maxwell D. J. Neurokinin-1 projection cells in the rat dorsal horn receive synaptic contacts from axons that possess alpha2C-adrenergic receptors. *Journal of Neuroscience.* 2003; 23(17):6837-46.

Oliveira M.A. and Prado W.A. Role of PAG in the antinociception evoked from the medial or central amygdala in rats. *Brain Research Bulletin.* 2001; 54(1):55-63.

Ozaki N., Bielefeldt K., Sengupta J.N., Gebhart G.F. Models of gastric hyperalgesia in the rat. *American Journal of Physiology Gastrointestinal and Liver Physiology.* 2002; 283(3):G666-76.

Paalzow G. and Paalzow L. Clonidine antinociceptive activity: effects of drugs influencing central monoaminergic and cholinergic mechanisms in the rat. *Naunyn Schmiedebergs Archives of Pharmacology.* 1976; 292(2):119-26.

Paalzow G.H. and Paalzow L.K. Opposing effects of apomorphine on pain in rats. Evaluation of the dose-response curve. *European Journal of Pharmacology.* 1983.18; 88(1):27-35.

Paalzow G.H. and Paaizow L.K. Promethazine both facilitates and inhibits nociception in rats: effect of the testing procedure. *Psychopharmacology (Berl).* 1985; 85(1):31-6.

Paech M.J., Pavy T.J., Orlikowski C.E., Yeo S.T. Postcesarean analgesia with spinal morphine, clonidine, or their combination. *Anaesthesia and Analgesia.* 2004; 98(5):1460-6.

Page A.J., Brierley S.M., Martin C.M., Martinez-Salgado C., Wemmie J.A., Brennan

T.J., Symonds E., Omari T., Lewin G.R., Welsh M.J., Blackshaw L.A. The ion channel ASIC1 contributes to visceral but not cutaneous mechanoreceptor function. *Gastroenterology.* 2004; 127(6):1739-47.

Palazzo E., Starowicz K., Maione S., Di Marzo V. TRPV1 in Neuropathic Pain and Neurological and Neuropsychiatric Disorders. *In Vanilloid Receptor TRPV1 in Drug Discovery: Targeting Pain and Other Pathological Disorders* (Eds A. Gomtsyan and C. R. Faltynek). 2010. John Wiley & Sons, Inc., Hoboken, NJ, USA.

Pariante C.M. The glucocorticoid receptor: part of the solution or part of the problem? *Journal of Psychopharmacology.* 2006; 20(4 Suppl):79-84.

Patel M.K., Pinnock R.D., Lee K. Adenosine exerts multiple effects in dorsal horn neurones of the adult rat. *Spinal cord.* 2001; 920: 19–26.

Pathirathna S., Brimelow B.C., Jagodic M.M., Krishnan K., Jiang X., Zorumski C.F., Mennerick S., Covey D.F., Todorovic S.M., Jevtovic-Todorovic V. New evidence that both T-type calcium channels and GABAA channels are responsible for the potent peripheral analgesic effects of 5alpha-reduced neuroactive steroids. *Pain.* 2005;114(3):429-43

Paton A. M., Harley M., Harley R. M., Weeks S. A Revision of Endostemon (Labiatae). *Kew Bulletin.*1994.49; (4): 673-716

Paulson P.E., Casey K.L., Morrow T.J. Long-term changes in behavior and regional cerebral blood flow associated with painful peripheral mononeuropathy in the rat. *Pain.* 2002; 95(1-2):31-40.

Peets J.M. and Pomeranz B. Studies in suppression of nocifensive reflexes measured with tail flick electromyograms and using intrathecal drugs barbiturate anesthetized rats. *Brain Research.* 1987; 416: 301-307.

Peier A.M., Moqrich A., Hergarden A.C., Reeve A.J. A TRP channel that senses cold stimuli and menthol. *Cell.* 2002. 8; 108(5):705-15.

Pelissier T., Alloui A., Caussade F., Dubray C., Cloarec A., Lavarenne J., Eschalier A. Paracetamol exerts a spinal antinociceptive effect involving an indirect interaction with 5-hydroxytryptamine3 receptors: in vivo and in vitro evidence. *Journal of Pharmacology and Experimental Therapy*. 1996; 278(1):8-14.

Peng C., Bodenhausen G., Qiu S.X., Fong H.H., Farnsworth N.R., Yuan S.G. Computer-assisted structure elucidation: Application of CISOC-SES to the resonance assignment and structure generation of betulinic acid. *Magnetic Resonance Chemistry*. 1998; 36:267–78.

Perez J. and Ribera M.V. Managing neuropathic pain with Sativex: a review of its pros and cons. *Expert Opinion in Pharmacotherapy*. 2008; 9(7):1189-95.

Perl E.R. Myelinated afferent fibres innervating the primate skin and their response to noxious stimuli. *Journal of Physiology*. 1968; 197(3):593-615.

Pertovaara A. and Almeida A. Descending inhibitory systems. *Handbook of Clinical Neurology*. 2006; 81:179-92.

Pertovaara A., Kauppila T., Jyvasjarvi E., Kalso E. Involvement of supraspinal and spinal segmental alpha-2-adrenergic mechanisms in the medetomidine-induced antinociception. *Neuroscience*. 1991; 44(3):705-14.

Pertovaara A. Plasticity in descending pain modulatory systems. *Progress in Brain Research*. 2000; 129:231-42.

Petrovic P., Kalso E., Petersson K.M., Ingvar M. Placebo and opioid analgesia-- imaging a shared neuronal network. *Science*. 2002; 295(5560):1737-40.

Pick C.G., Peter Y., Schreiber S., Weizman R. Pharmacological characterization of buprenorphine, a mixed agonist-antagonist with kappa 3 analgesia. *Brain Research*. 1997; 744(1):41-6

Pizziketti R.J., Pressman N.S., Geller E.B., Cowan A., Adler M.W. Rat cold water tail flick: a novel analgesic test that distinguishes opioid agonists from mixed agonist-antagonists. *European Journal of Pharmacology*. 1985; 119: 23-29.

Pleuvry B.J. and Tobias M.A. Comparison of the antinociceptive activities of physostigmine, oxotremorine and morphine in the mouse. *British Journal of Pharmacology*. 1971; 43(4):706-14.

Poisbeau P., Patte-Mensah C., Keller A.F., Barrot M., Breton J.D. Inflammatory pain upregulates spinal inhibition via endogenous neurosteroid production. *Journal of Neuroscience*. 2005; 25(50):11768-76.

Polgár E., Hughes D.I., Arham A.Z., Todd A.J. Loss of neurons from laminas I-III of the spinal dorsal horn is not required for development of tactile allodynia in the spared nerve injury model of neuropathic pain. *Journal of Neuroscience*. 2005; 25(28):6658-66.

Porreca F., Ossipov M.H., Gebhart G.F. Chronic pain and medullary descending facilitation. *Trends in Neuroscience*.2002; 25: 319–325.

Porsolt R.D., Le Pichon M., Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature*. 1977; 266: 730–732.

Porsolt R.D. Animal models of depression: utility for transgenic research. *Nature Reviews Neuroscience*. 2000; 11:53–58

Potes C.S., Neto F.L., Castro-Lopes J.M. Inhibition of pain behavior by GABA (B) receptors in the thalamic ventrobasal complex: effect on normal rats subjected to the Formalin Test of nociception. *Brain Research*. 2006; 1115(1):37-47.

Prado W.A. and Goncalves A.S. Antinociceptive effect of intrathecal neostigmine evaluated in rats by two different pain models. *Brazilian Journal of Medical and Biological Research*. 1997; 30(10):1225-31.

Prado W.A. and Segalla D.K. Antinociceptive effects of bethanechol or dimethylphenylpiperazinium in models of phasic or incisional pain in rats. *Brain Research.* 2004; 1018(2):272-82.

Prakash Chaturvedula V.S., Schilling J.K., Johnson R.K., Kingston D.G. New cytotoxic lupane triterpenoids from the twigs of *Coussarea paniculata. Journal of Natural Products.* 2003; 66(3):419-22.

Prasad A.K., Kumar V., Arya P., Kumar S., Dabur R., Singh N., Chhillar A.K., Sharma G. L., Ghosh B., Wengel J., Olsen C. E., Parmar V. S. Investigations toward new lead compounds from medicinally important plants. *Pure and applied chemistry.* 2005; 77 (1):25-40

Price D.D., Riley J.L., Wade J.B., Turk D.C. *Handbook of pain assessment* (2nd ed.), (Ed); Melzack, Ronald (Ed), (2001).New York, NY, US: Guilford Press. 53-75.

Price G.W., Wilkin G.P., Turnbull M.J., Bowery N.G. Are baclofen-sensitive GABAB receptors present on primary afferent terminals of the spinal cord? *Nature.* 1984; 307(5946):71-4.

Price M.P., Lewin G.R., McIlwrath S.L., Cheng C., Xie J., Heppenstall P.A., Stucky C.L., Mannsfeldt A.G., Brennan T.J., Drummond H.A., Qiao J., Benson C.J., Tarr D.E., Hrstka R.F., Yang B., Williamson R.A. Welsh M.J. The mammalian sodium channel BNC1 is required for normal touch sensation. *Nature.* 2000; 407(6807):1007-11.

Price T.J., Helesic G., Parghi D., Hargreaves K.M., Flores C.M. The neuronal distribution of cannabinoid receptor type 1 in the trigeminal ganglion of the rat. *Neuroscience.* 2003; 120(1):155-62.

Proudfit H.K. Pharmacologic evidence for the modulation of nociception by noradrenergic neurons. *Progress in Brain Research.* 1988; 77:357-70.

Proudfoot C.J., Garry E.M., Cottrell D.F., Rosie R., Anderson H., Robertson D.C., Fleetwood-Walker S.M., Mitchell R. Analgesia mediated by the TRPM8 cold receptor in

chronic neuropathic pain. *Current Biology*. 2006; 16(16):1591-605.

Pugh G.C., Drummond G.B., Elton R.A., Macintyre C.C. Constant i.v. infusions of nalbuphine or buprenorphine for pain after abdominal surgery. *British Journal of Anaesthesia*. 1987; 59(11):1364-74.

Raffa R.B., Friderichs E., Reimann W., Shank R.P., Codd E.E., Vaught J.L. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an "atypical" opioid analgesic. *Journal of Pharmacology and Experimental Therapeutics*. 1992; 260: 275-285.

Rance J.M. Animal and molecular pharmacology of mixed agonist-antagonist analgesic drugs. *British Journal of Clinical Pharmacology*. 1979; 7(Suppl 3): 281S–286S.

Randić M., Jiang M.C., Cerne R. Long-term potentiation and long-term depression of primary afferent neurotransmission in the rat spinal cord. *Journal of Neuroscience*. 1993; 13(12):5228-41.

Rau K.K., Caudle R.M., Cooper B.Y., Johnson R.D. Diverse immunocytochemical expression of opioid receptors in electrophysiologically defined cells of rat dorsal root ganglia. *Journal of Chemical Neuroanatomy*. 2005; 29(4):255-64.

Recio M.C., Giner R.M., Máñez S., Gueho J., Julien H.R., Hostettmann K., Ríos J.L. Investigations on the steroidal anti-inflammatory activity of triterpenoids from *Diospyros leucomelas*. *Planta Medica*. 1995; 61(1):9-12.

Reddy S.V., Maderdrut J.L., Yaksh T.L. Spinal cord pharmacology of adrenergic agonist-mediated antinociception. *Journal of Pharmacology and Experimental Therapeutics*. 1980; 213(3):525-33.

Reid G. and Flonta M.L. Cold current in thermoreceptive neurons. *Nature*. 2001 ; 413(6855):480.

Ren K. and Dubner R. Descending modulation in persistent pain: an update. *Pain*. 2002;

100(1-2):1-6.

Reneric J.P., Bouvard M., Stinus L. Idazoxan and 8-OHDPAT modify the behavioral effects induced by either NA, or 5-HT, or dual NA/5-HT reuptake inhibition in the rat forced swimming test. *Neuropsychopharmacology*. 2001; 24: 379–390.

Ribeiro-da-Silva A. and Cuello A.C. Choline acetyltransferase-immunoreactive profiles are presynaptic to primary sensory fibers in the rat superficial dorsal horn. *Journal of Comparative Neurology*. 1990; 295(3):370-84.

Ringkamp M., Johanek L.M., Borzan J., Hartke T.V., Wu G., Pogatzki-Zahn E.M., Campbell J.N., Shim B., Schepers R.J., Meyer R.A. Conduction properties distinguish unmyelinated sympathetic efferent fibers and unmyelinated primary afferent fibers in the monkey. *PLoS One*. 2010; 5(2):e9076.

Roberts L.A. and Connor M. TRPV1 antagonists as a potential treatment for hyperalgesia. *Recent Patents on CNS Drug Discovery*. 2006; 1(1):65-76.

Rojas-Corrales M.O., Gibert-Rahola J., Micó J.A. Tramadol induces antidepressant-type effects in mice. *Life Sciences*. 1998; 63(12):PL175-80.

Romano J.M. and Turner J.A. Chronic pain and depression: does the evidence support a relationship? *Psychological Bulletin*. 1985 ;97(1):18-34.

Rosén A., Zhang Y.X., Lund I., Lundeberg T., Yu L.C. Substance P microinjected into the periaqueductal gray matter induces antinociception and is released following morphine administration. *Brain Research*. 2004 ;1001(1-2):87-94.

Rosland J.H. The Formalin Test in mice: the influence of ambient temperature. *Pain*. 1991; 45: 211-216.

Rosland J.H., Tjølsen J.H., Mæhle B., Hole K. The Formalin Test in mice: effects of formalin concentration. *Pain*. 1990; 42: 235-242.

Roth B.L., Baner K., Westkaemper R., Siebert D., Rice K.C., Steinberg S., Ernsberger P., Rothman R.B. Salvinorin A: a potent naturally occurring nonnitrogenous kappa opioid selective agonist. *Proceedings of the National Academy of Sciences U S A*. 2002; 99(18):11934-9.

Ruiu S., Pinna G.A., Marchese G., Mussinu J.M., Saba P., Tambaro S., Casti P., Vargiu R., Pani L. Synthesis and characterization of NESS 0327: a novel putative antagonist of the CB1 cannabinoid receptor. *Journal of Pharmacology and Experimental Therapeutics.* 2003; 306(1):363-70.

Ruiz-Torner A., Olucha-Bordonau F., Valverde-Navarro A.A., Martinez-Soriano F. The chemical architecture of the rat's periaqueductal gray based on acetylcholinesterase histochemistry: a quantitative and qualitative study. *Journal of Chemical Neuroanatomy.* 2001; 21(4):295-312.

Ruoff G.E. Depression in the patient with chronic pain. *Journal of Family Practice.* 1996; 43(6 Suppl):S25-33

Russo R., Loverme J., La Rana G., Compton T.R., Parrott J., Duranti A., Tontini A., Mor M., Tarzia G., Calignano A., Piomelli D. The fatty acid amide hydrolase inhibitor URB597 (cyclohexylcarbamic acid 3'-carbamoylbiphenyl-3-yl ester) reduces neuropathic pain after oral administration in mice. *Journal of Pharmacology and Experimental Therapeutics*. 2007; 322(1):236-42.

Russo R., Loverme J., La Rana G., D'Agostino G., Sasso O., Calignano A., Piomelli D. Synergistic antinociception by the cannabinoid receptor agonist anandamide and the PPAR-alpha receptor agonist GW7647. *European Journal of Pharmacology*. 2007; 566(1-3):117-9.

Ryder S., Way W.L., Trevor A.J. Comparative pharmacology of the optical isomers of ketamine in mice. *European Journal of Pharmacology.* 1978; 49(1):15-23.

Saarto T. and Wiffen P.J. Antidepressants for neuropathic pain. *Cochrane Database*

Systematic Reviews. 2005;(3):CD005454.

Saddi G. and Abbott F.V. The Formalin Test in the mouse: a parametric analysis of scoring properties. *Pain.* 2000; 89(1):53-63.

Sagar D.R., Gaw A.G., Okine B.N., Woodhams S.G., Wong A., Kendall D.A., Chapman V. Dynamic regulation of the endocannabinoid system: implications for analgesia. *Molecular Pain.* 2009 ;5:59.

Sagen J. and Proudfit H.K. Effect of intrathecally administered noradrenergic antagonists on nociception in the rat. *Brain Research.* 1984; 310(2):295-301.

Sakurada T., Katsumata K., Tan-No K., Sakurada S., Kisara K. The capsaicin test in mice for evaluating tachykinin antagonists in the spinal cord. *Neuropharmacology.*1992; 31:1279-1285.

Samad T.A., Sapirstein A., Woolf C.J. Prostanoids and pain: unraveling mechanisms and revealing therapeutic targets. *Trends in Molecular Medicine.* 2002; 8(8):390-6.

Samuelsson B., Dahlen S.E., Lindgren J.A., Rouzer C.A., Serhan C.N. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science.* 1987; 237(4819):1171-6.

Sandkühler J. and Liu X.G. Long-term potentiation of C-fiber-evoked potentials in the rat spinal dorsal horn is prevented by spinal N-methyl-D-aspartic acid receptor blockage. *Neuroscience Letters.* 1995;191(1-2):43-6.

Sandkühler J. The organization and function of endogenous antinociceptive systems. *Progress in Neurobiology.* 1996; 50(1):49-81.

Sandkühler J. and Liu X.G. Activation of spinal N-methyl-D-aspartate or neurokinin receptors induces long-term potentiation of spinal C-fibre-evoked potentials. *Neuroscience.* 1998; 86(4):1209-16.

Sandkühler J., Benrath J., Brechtel C., Ruscheweyh R., Heinke B. Synaptic mechanisms of hyperalgesia. *Progress in Brain Research.* 2000; 129:81-100.

Sandkühler J. Understanding LTP in pain pathways. *Molecular Pain.* 2007; 3:9.

Sawada Y., Hosokawa H., Hori A., Matsumura K., Kobayashi S. Cold sensitivity of recombinant TRPA1 channels. *Brain Research.* 2007; 1160:39-46.

Sawynok J., Moochhala S.M., Pillay D.J. Substance P, injected intrathecally, antagonizes the spinal antinociceptive effect of morphine, baclofen and noradrenaline. *Neuropharmacology.* 1984; 23(7A):741-7.

Schaible H.G., Grubb B.D., Neugebauer V., Oppmann M. The effects of NMDA antagonists on neuronal activity in cat spinal cord evoked by acute inflammation in the knee joint. *European Journal of Neuroscience.*1991; 3: 981–991.

Schaible H.G., Jarrott B., Hope P.J., Duggan A.W. Release of immunoreactive substance P in the spinal cord during development of acute arthritis in the knee joint of the cat: a study with antibody microprobes. *Brain Research.* 1990; 529(1-2):214-23.

Scholz J. and Woolf C.J. Can we conquer pain? *Nature Reviews Neuroscience.* 2002; 5 Suppl: 1062-7.

Schuler V., Lüscher C., Blanchet C., Klix N., Sansig G., Klebs K., Schmutz M., Heid J., Gentry C., Urban L., Fox A., Spooren W., Jaton A.L., Vigouret J., Pozza M., Kelly P.H., Mosbacher J., Froestl W., Käslin E., Korn R., Bischoff S., Kaupmann K., van der Putten H., Bettler B. Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic GABA(B) responses in mice lacking $GABA_B$. *Neuron.* 2001; 31(1):47-58.

Schwab J.M., Brechtel K., Nguyen T.D., Schluesener H.J. Persistent accumulation of cyclooxygenase-1 (COX-1) expressing microglia/macrophages and upregulation by endothelium following spinal cord injury. *Journal of Neuroimmunology.* 2000; 111(1-2):122-30.

Scott E.K. and Luo L. How do dendrites take their shape? *Nature Neuroscience.* 2001; 4(4):359-65.

Seyoum A., Killeen G.F., Kabiru E.W., Knols B.G., Hassanali A. Field efficacy of thermally expelled or live potted repellent plants against African malaria vectors in western Kenya. *Tropical Medicine and International Health.* 2003; 8(11):1005-11.

Shapira N.A., Verduin M.L., DeGraw J.D. Treatment of refractory major depression with tramadol monotherapy. *Journal of Clinical Psychiatry.* 2001; 62(3):205-6.

Shaqura M.A., Zöllner C., Mousa S.A., Stein C., Schäfer M. Characterization of mu opioid receptor binding and G protein coupling in rat hypothalamus, spinal cord, and primary afferent neurons during inflammatory pain. *Journal of Pharmacology and Experimental Therapeutics.* 2004; 308(2):712-8.

Shibata M., Ohkubo T., Takahashi H., Inoki R. Modified Formalin Test: characteristic biphasic pain response. *Pain.* 1989; 38: 347-352.

Shimoyama M., Shimoyama N., Gorman A.L., Elliott K.J., Inturrisi C.E. Oral ketamine is antinociceptive in the rat Formalin Test: role of the metabolite, norketamine. *Pain.* 1999; 81(1-2):85-93.

Sholichin M., Yamasaki K., Kasal R., Tanako O. Carbon 13- nuclear magnetic resonance of lupine-type triterpene, lupeol, betulinic acid. *Chemical and Pharmaceutical Bulletin.* 1980; 28:1006–8.

Siebert, D.J. *Salvia divinorum* and salvinorin A: new pharmacologic findings. *Journal of Ethnopharmacology*. 1994; 43(1):53-6.

Silbert S.C., Beacham D.W., McCleskey E.W. Quantitative single-cell differences in mu-opioid receptor mRNA distinguish myelinated and unmyelinated nociceptors. *Journal of Neuroscience*. 2003;23(1):34-42.

Scott E.K. and Luo L. How do dendrites take their shape? *Nature Neuroscience.* 2001; 4(4):359-65.

Seyoum A., Killeen G.F., Kabiru E.W., Knols B.G., Hassanali A. Field efficacy of thermally expelled or live potted repellent plants against African malaria vectors in western Kenya. *Tropical Medicine and International Health.* 2003; 8(11):1005-11.

Shapira N.A., Verduin M.L., DeGraw J.D. Treatment of refractory major depression with tramadol monotherapy. *Journal of Clinical Psychiatry.* 2001; 62(3):205-6.

Shaqura M.A., Zöllner C., Mousa S.A., Stein C., Schäfer M. Characterization of mu opioid receptor binding and G protein coupling in rat hypothalamus, spinal cord, and primary afferent neurons during inflammatory pain. *Journal of Pharmacology and Experimental Therapeutics.* 2004; 308(2):712-8.

Shibata M., Ohkubo T., Takahashi H., Inoki R. Modified Formalin Test: characteristic biphasic pain response. *Pain.* 1989; 38: 347-352.

Shimoyama M., Shimoyama N., Gorman A.L., Elliott K.J., Inturrisi C.E. Oral ketamine is antinociceptive in the rat Formalin Test: role of the metabolite, norketamine. *Pain.* 1999; 81(1-2):85-93.

Sholichin M., Yamasaki K., Kasal R., Tanako O. Carbon 13- nuclear magnetic resonance of lupine-type triterpene, lupeol, betulinic acid. *Chemical and Pharmaceutical Bulletin.* 1980; 28:1006–8.

Siebert, D.J. *Salvia divinorum* and salvinorin A: new pharmacologic findings. *Journal of Ethnopharmacology.* 1994; 43(1):53-6.

Silbert S.C., Beacham D.W., McCleskey E.W. Quantitative single-cell differences in mu-opioid receptor mRNA distinguish myelinated and unmyelinated nociceptors. *Journal of Neuroscience.* 2003;23(1):34-42.

Silvilotti L. and Woolf C.J. The contribution of GABAA and glycine receptors to central sensitization: disinhibition and touch-evoked allodynia in the spinal cord. *Journal of Neurophysiology*. 1994; 72(1):169-79.

Simone D.A., Li J., Menning P.M., Rogers S.D. Primary afferent fibers that contribute to increased substance P receptor internalization in the spinal cord after injury. *Journal of Neurophysiology*. 1999; 81(3):1379-90.

Simonin F., Gaveriaux-Ruff C., Befort K., Matthes H., Lannes B. kappa-Opioid receptor in humans: cDNA and genomic cloning, chromosomal assignment, functional expression, pharmacology, and expression pattern in the central nervous system. *Proceedings of the National Academy of Sciences U S A*. 1995; 92(15):7006-10.

Singh Tahim A., Sántha P., Nagy I. Inflammatory mediators convert anandamide into a potent activator of the vanilloid type 1 transient receptor potential receptor in nociceptive primary sensory neurons. *Neuroscience*. 2005;136(2):539-48.

Smith H.S. Arachidonic acid pathways in nociception. *Journal of Supportive Oncology*. 2006; 4(6):277-87.

Smith H.S. Potential analgesic mechanisms of acetaminophen. *Pain Physician*. 2009; 12(1):269-80.

Smith J.B. and Willis A.L. Aspirin selectively inhibits prostaglandin production in human platelets. *Nature New Biology*. 1971; 231(25):235-7.

Smith M.I., Banner S.E., Sanger G.J. 5-HT4 receptor antagonism potentiates inhibition of intestinal allodynia by 5-HT3 receptor antagonism in conscious rats. *Neuroscience Letters*. 1999; 271(1):61-4.

Snider W.D. and McMahon S.B. Tackling pain at the source: new ideas about nociceptors. *Neuron*. 1998;20(4):629-32.

Spencer C. The efficacy of intramuscular tramadol as a rapid-onset antidepressant.

Australia and New Zealand Journal of Psychiatry. 2000; 34(6):1032-3.

Stahl S. and Briley M. Understanding pain in depression. *Human Psychopharmacology*. 2004; 19 Suppl 1:S9-S13.

Steele J.C., Warhurst D.C., Kirby G.C., Simmonds M.S. In vitro and in vivo evaluation of betulinic acid as an antimalarial. *Phytotherapy Research*. 1999; 13(2):115-9.

Stein C., Clark J.D., Oh U., Vasko M.R., Wilcox G.L., Overland A.C., Vanderah T.W., Spencer R.H. Peripheral mechanisms of pain and analgesia. *Brain Research Reviews*. 2009; 60(1):90-113.

Stein C., Gramsch C., Herz A. Intrinsic mechanisms of antinociception in inflammation: local opioid receptors and beta-endorphin. *Journal of Neuroscience*. 1990; 10(4):1292-8.

Stein C., Schäfer M., Machelska H. Attacking pain at its source: new perspectives on opioids. *Nature Medicine*. 2003; 9(8):1003-8.

Stein C. The control of pain in peripheral tissue by opioids. *New England Journal of Medicine*. 1995;332(25):1685-90.

Story G.M., Peier A.M., Reeve A.J., Eid S.R., Mosbacher J., Hricik T.R., Earley T.J., Hergarden A.C., Andersson D.A., Hwang S.W., McIntyre P., Jegla T., Bevan S., Patapoutian A. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell*. 2003; 112(6):819-29.

Stubhaug A., Breivik H., Eide P.K., Kreunen M., Foss A. Mapping of punctuate hyperalgesia around a surgical incision demonstrates that ketamine is a powerful suppressor of central sensitization to pain following surgery. *Acta Anaesthesiology Scandinavia*. 1997; 41(9):1124-32.

Stucky C.L. and Lewin G.R. Isolectin B(4)-positive and -negative nociceptors are functionally distinct. *Journal of Neuroscience*. 1999; 19(15):6497-505.

Sufka K.J., Schomburg F.M., Giordano J. Receptor mediation of 5-HT-induced inflammation and nociception in rats. *Pharmacology and Biochemistry of Behavior.* 1992; 41(1):53-6.

Sufka K.J., Watson G.S., Nothdurft R.E., Mogil J.S. Scoring the mouse Formalin Test: validation study. *European Journal of Pain.* 1998; 2(4):351-358.

Suto K. and Gotoh H. Calcium signaling in cold cells studied in cultured dorsal root ganglion neurons. *Neuroscience.* 1999; 92(3):1131–35.

Suzuki R., Morcuende S., Webber M., Hunt S.P., Dickenson A.H. Superficial NK1-expressing neurons control spinal excitability through activation of descending pathways. *Nature Neuroscience.* 2002; 5(12):1319-26.

Suzuki T., Nurrochmad A., Ozaki M., Khotib J., Nakamura A., Imai S., Shibasaki M., Yajima Y., Narita M. Effect of a selective GABA(B) receptor agonist baclofen on the mu-opioid receptor agonist-induced antinociceptive, emetic and rewarding effects. *Neuropharmacology.* 2005; 49(8):1121-31.

Tanioka T., Nakatani Y., Semmyo N., Murakami M., Kudo I. Molecular identification of cytosolic prostaglandin E2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E2 biosynthesis. *Journal of Biological Chemistry.* 2000; 275(42):32775-82.

Taylor B.K., Akana S.F., Peterson M.A., Dallman M.F., Basbaum A.I. Pituitary-adrenocortical responses to persistent noxious stimuli in the awake rat: endogenous corticosterone does not reduce nociception in the Formalin Test. *Endocrinology.* 1998; 139(5):2407-13.

Thompson S.W., Dray A., Urban L. Injury-induced plasticity of spinal reflex activity: NK1 neurokinin receptor activation and enhanced A- and C-fiber mediated responses in the rat spinal cord in vitro. *Journal of Neuroscience.* 1994; 14(6):3672-87.

Tjølsen A., Berge O.G., Hunskaar S., Rosland J.H., Hole K. The Formalin Test: an evaluation of the method. *Pain.* 1992; 51: 5-17.

Tominaga M., Caterina M.J., Malmberg A.B., Rosen T.A., Gilbert H., Skinner K., Raumann B.E., Basbaum A.I., Julius D. The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron.* 1998; 21(3):531-43.

Tozaki-Saitoh H., Tsuda M., Miyata H., Ueda K., Kohsaka S., Inoue K. P2Y12 receptors in spinal microglia are required for neuropathic pain after peripheral nerve injury. *Journal of Neuroscience.* 2008; 28(19):4949-56.

Truong W., Cheng C., Xu Q.G., Li X.Q., Zochodne D.W. Mu opioid receptors and analgesia at the site of a peripheral nerve injury. *Annals of Neurology.* 2003; 53(3):366-75.

Tsuda M., Shigemoto-Mogami Y., Koizumi S., Mizokoshi A., Kohsaka S., Salter M.W., Inoue K. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature.* 2003; 424(6950):778-83.

Ueda H. and Rashid M.H. Molecular mechanism of neuropathic pain. *Drug News and Perspectives.* 2003; 16(9):605-13.

Urban L. Willetts J., Murase K., Randić M. Cholinergic effects on spinal dorsal horn neurons in vitro: an intracellular study. *Brain Research.* 1989; 500(1-2):12-20.

Urban M.O. and Gebhart G.F. Supraspinal contributions to hyperalgesia. *Proceedings of the National Academy of Sciences U S A.* 1999; 96(14):7687-92.

Urban M.O. and Gebhart G.F. The glutamate synapse: a target in the pharmacological management of hyperalgesic pain states. *Progress in Brain Research.* 1998; 116:407-20.

Vaccarino A.L., Marek P., Kest B., Weber E., Keana J.F., Liebeskind J.C. NMDA receptor antagonists, MK-801 and ACEA-1011, prevent the development of tonic pain following subcutaneous formalin. *Brain Research.* 1993. 2; 615(2):331-4.

Vane J.R. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Biology.* 1971;231(25):232-5.

Vanegas H. and Schaible H.G. Descending control of persistent pain: inhibitory or facilitatory? *Brain Research Brain Research Reviews.* 2004; 46(3):295-309.

Vanegas H. and Schaible H.G. Prostaglandins and cyclooxygenases [correction of cycloxygenases] in the spinal cord. *Progress in Neurobiology.* 2001; 64(4):327-63.

Varney M.A. and Gereau R.W. 4th. Metabotropic glutamate receptor involvement in models of acute and persistent pain: prospects for the development of novel analgesics. *Current Drug Targets CNS Neurological Disorders.* 2002; 1(3):283-96.

Veith R.C., Lewis N., Langohr J.I., Murburg M.M., Ashleigh E.A., Castillo S., Peskind E.R., Pascualy M., Bissette G., Nemeroff C.B. Effect of desipramine on cerebrospinal fluid concentrations of corticotropin-releasing factor in human subjects. *Psychiatry Research.* 1993; 46(1):1-8.

Villanueva L., Cliffer K.D., Sorkin L.S., Le Bars D., Willis W.D. Jr. Convergence of heterotopic nociceptive information onto neurons of caudal medullary reticular formation in monkey (Macaca fascicularis). *Journal of Neurophysiology.* 1990; 63(5):1118-27.

Vyklicky L. Techniques for the study of pain in animals, *in Advances in Pain Research and Therapy (Bonica JJ, Liebeskind JC and Albe-Fessard DG eds*). 1979; 3: 727-745, Raven Press, New York.

Wager T.D., Scott D.J., Zubieta J.K. Placebo effects on human mu-opioid activity during pain. *Proceedings of the National Academy of Sciences U S A.* 2007;104(26):11056-61.

Waldmann R., Champigny G., Bassilana F., Heurteaux C., Lazdunski M. A proton-gated cation channel involved in acid-sensing. *Nature.* 1997; 386(6621):173-7.

Walker K., Bowes M., Panesar M., Davis A., Gentry C., Kesingland A. Metabotropic glutamate receptor subtype 5 (mGlu5) and nociceptive function. I. Selective blockade of mGlu5 receptors in models of acute, persistent and chronic pain. *Neuropharmacology.* 2001; 40(1):1-9.

Walker K., Reeve A., Bowes M., Winter J., Wotherspoon G., Davis A., Schmid P., Gasparini F., Kuhn R., Urban L. mGlu5 receptors and nociceptive function II. mGlu5 receptors functionally expressed on peripheral sensory neurones mediate inflammatory hyperalgesia. *Neuropharmacology.* 2001; 40(1):10-19.

Walker K.M., Urban L., Medhurst S.J., Patel S., Panesar M., Fox A.J., McIntyre P. The VR1 antagonist capsazepine reverses mechanical hyperalgesia in models of inflammatory and neuropathic pain. *Journal of Pharmacology and Experimental Therapeutics.* 2003 ;304(1):56-62.

Wang H. and Wessendorf M.W. Equal proportions of small and large DRG neurons express opioid receptor mRNAs. *Journal of Comparative Neurology.* 2001 22; 429(4):590-600.

Wang J.B., Johnson P.S., Persico A.M., Hawkins A.L., Griffin C.A., Uhl G.R. Human mu opiate receptor. cDNA and genomic clones, pharmacologic characterization and chromosomal assignment. *FEBS Letters.* 1994; 338(2):217-22.

Wang J.J., Ho S.T., Hu O.Y., Chu K.M. An innovative cold tail-flick test: the cold ethanol tail-flick test. *Anaesthesia and Analgesia.*1995; 80: 102-107.

Watkins L.R. Algesiometry in laboratory and man: current concepts and future directions, in Issues in Pain Measurement: *Advances in Pain Research and Therapy (Chapman CR and Loeser JD Eds).* Raven Press, New York .1989; 12: 249-265.

Watkins L.R., Hutchinson M.R., Johnston I.N., Maier S.F. Glia: novel counter-regulators of opioid analgesia. *Trends in Neuroscience.* 2005; 28(12):661-9.

Watson G.S., Sufka K.J., Coderre T.J. Optimal scoring strategies and weights for the Formalin Test in rats. *Pain.* 1997; 70: 53-58.

Waxman E.A. and Lynch D.R. N-methyl-D-aspartate receptor subtypes: multiple roles in excitotoxicity and neurological disease. *Neuroscientist.* 2005; 11(1):37-49.

Welberg L.A., Seckl J.R., Holmes M.C. Prenatal glucocorticoid programming of brain corticosteroid receptors and corticotrophin-releasing hormone: possible implications for behaviour. *Neuroscience.* 2001; 104(1):71-9.

Weng H.R., Mansikka H., Winchurch R., Raja S.N., Dougherty P.M. Sensory processing in the deep spinal dorsal horn of neurokinin-1 receptor knockout mice. *Anesthesiology.* 2001; 94(6):1105-12.

Wess J., Duttaroy A., Gomeza J., Gan J.W., Siddiqui N. Evaluation of muscarinic agonist-induced analgesia in muscarinic acetylcholine receptor knockout mice. *Molecular Pharmacology.* 2002; 62(5):1084-93.

Wess J., Eglen R.M., Gautam D. Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. *Nature Reviews Drug Discovery.* 2007;6(9):721-33.

Wheeler-Aceto H. and Cowan A. Standardization of the rat paw Formalin Test for the evaluation of analgesics. *Psychopharmacology (Berlin).* 1991; 104: 35-44.

WHO. Traditional medicine. *Fact sheet N°134.* Revised May 2003

Wiesenfeld-Hallin Z. and Xu X.J. Neuropeptides in neuropathic and inflammatory pain with special emphasis on cholecystokinin and galanin. *European Journal of Pharmacology.* 2001; 429(1-3):49-59.

Williams T.J. and Peck M.J. Role of prostaglandin-mediated vasodilation in inflammation. *Nature* 1977; 270:530–532.39.

Willis W.D., Al-Chaer E.D., Quast M.J., Westlund K.N. A visceral pain pathway in the dorsal column of the spinal cord. *Proceedings of the National Academy of Sciences U S A*. 1999;96(14):7675-9.

Willis W.D. Long-term potentiation in spinothalamic neurons. *Brain Research Reviews*. 2002; 40: 202–214.

Willis W.D. and Westlund K.N. Neuroanatomy of the pain system and of the pathways that modulate pain. *Journal of Clinical Neurophysiology*. 1997; 14(1):2-31.

Wong G.Y., and Gavva N.R. Therapeutic potential of vanilloid receptor TRPV1 agonists and antagonists as analgesics: Recent advances and setbacks. *Brain Research Reviews*. 2009; 60(1):267-77.

Woolf C.J. and Salter M.W. Neuronal plasticity: increasing the gain in pain. *Science*. 2000; 288(5472):1765-9.

Woolf C.J. and Thompson S.W. The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. *Pain*. 1991; 44(3):293-9.

Woolf C.J., Moore K.A., Baba H., Kohno T. Peripheral nerve injury alters excitatory synaptic transmission in lamina II of the rat dorsal horn. *Journal of Physiology*. 2003; 548(Pt 1):131-8.

Woolf C.J., Thompson S.W., King A.E. Prolonged primary afferent induced alterations in dorsal horn neurones, an intracellular analysis in vivo and in vitro. *Journal of Physiology (Paris)*. 1988-1989; 83(3):255-66.

Woolf C.J. What is this thing called pain? *Journal of Clinical Investigation*. 2010; 120(11):3742-4.

Woolf C.J. Windup and central sensitization are not equivalent. *Pain*. 1996; 66(2-3):105-8.

Workman B.J. and Lomb B.M. Inhibitory effects evoked from the anterior hypothalamus are selective for the nociceptive responses of dorsal horn neurons with high- and low-threshold inputs. *Journal of Neurophysiology.* 1997; 77(5):2831-5.

Yaksh T. L. and Malmberg A. B. Antinociceptive actions of spinal nonsteroidal anti-inflammatory agents on the Formalin Test in the rat. *Journal of Pharmacology and Experimental Therapeutics.* 1992; 263(1):136-46.

Yaksh T. L. Behavioral and autonomic correlates of the tactile evoked allodynia produced by spinal glycine inhibition: effects of modulatory receptor systems and excitatory amino acid antagonists. *Pain.* 1989; 37(1):111-23.

Yaksh T. L., Jessell T. M., Gamse R., Mudge A. W., Leeman S. E. Intrathecal morphine inhibits substance P release from mammalian spinal cord in vivo. *Nature.* 1980; 286(5769):155-7.

Yaksh T. L. Pharmacology and mechanisms of opioid analgesic activity. *Acta Anaesthesiologica Scandinavia.* 1997; 41(1 Pt 2):94-111.

Yaksh T. L., Plant R. L., Rudy T. A. Studies on the antagonism by raphe lesions of the antinociceptive action of systemic morphine. *European Journal of Pharmacology.* 1977; 41(4):399-408.

Yaksh T. L. Regulation of spinal nociceptive processing: where we went when we wandered onto the path marked by the gate. *Pain.* 1999; Supplement 6:S149-52.

Yaksh T. L. Substance P release from knee joint afferent terminals: modulation by opioids. *Brain Research.* 1988; 458(2):319-24.

Yamamoto T. and Yaksh T. L. Comparison of the antinociceptive effects of pre- and post treatment with intrathecal morphine and MK801, an NMDA antagonist, on the Formalin Test in the rat. *Anesthesiology.* 1992; 77(4):757-63.

Yang D. and Gereau R.W. 4th. Group II metabotropic glutamate receptors inhibit

cAMP-dependent protein kinase-mediated enhancemednt of tetrodotoxin-resistant sodium currents in mouse dorsal root ganglion neurons. *Neuroscience Letters.* 2004; 357(3):159-62.

Yermakova A. and O'Banion M.K. Cyclooxygenases in the central nervous system: implications for treatment of neurological disorders. *Current Pharmaceutical Design.* 2000; 6(17):1755-76.

Yezierski R.P. and Schwartz R.H. Response and receptive-field properties of spinomesencephalic tract cells in the cat. *Journal of Neurophysiology.* 1986; 55(1):76-96.

Yogeeswari P. and Sriram D. Betulinic acid and its derivatives: a review on their biological properties. *Current Medicinal Chemistry.* 2005; 12(6):657-66.

Yoshida A., Chen K., Maritain M., Yakutat N., Nagase Y., Takemura M., Shigenaga Y. Organization of the descending projections from the parabrachial nucleus to the trigeminal sensory nuclear complex and spinal dorsal horn in the rat. *Journal of Comparative Neurology.* 1997; 383(1):94-111.

Zagon A. Synaptic interactions in neurones of the rat rostral ventrolateral medulla oblongata. *Brain Research Brain Research Protocols.* 2001; 7(1):21-9.

Zarate C.A. Jr., Singh J.B., Carlson P.J., Brutsche N.E., Ameli R., Luckenbaugh D.A., Charney D.S., Manji H.K.A. Randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Archives of General Psychiatry.* 2006; 63(8):856-64.

Zeilhofer H.U., Witschi R., Hösl K. Subtype-selective GABAA receptor mimetics--novel antihyperalgesic agents? *Journal of Molecular Medicine.* 2009;87(5):465-9.

Zhang C., Davies M.F., Guo T.Z., Maze M. The analgesic action of nitrous oxide is dependent on the release of norepinephrine in the dorsal horn of the spinal cord. *Anesthesiology.* 1999; 91(5):1401-7

Zhang H.M., Chen S.R., Matsui M., Gautam D., Wess J., Pan H.L. Opposing functions of spinal M2, M3, and M4 receptor subtypes in regulation of GABA-ergic inputs to dorsal horn neurons revealed by muscarinic receptor knockout mice. *Molecular Pharmacology.* 2006; 69(3):1048-55.

Zhang J., Hoffert C., Vu H.K., Groblewski T., Ahmad S., O'Donnell D. Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not inflammatory chronic pain models. *European Journal of Neuroscience*. 2003; 17(12):2750-4.

Zhang J.M. and An J. Cytokines, inflammation, and pain. *International Anesthesiology Clinics.* 2007; 45(2):27-37.

Zhang L., Lu Y., Chen Y., Westlund K.N. Group I metabotropic glutamate receptor antagonists block secondary thermal hyperalgesia in rats with knee joint inflammation. *Journal of Pharmacology and Experimental Therapeutics*. 2002; 300(1):149-56.

Zhao Z.Q., Chiechio S., Sun Y.G., Zhang K.H., Zhao C.S., Scott M., Johnson R.L., Deneris E.S., Renner K.J., Gereau R.W. 4th., Chen Z.F. Mice lacking central serotonergic neurons show enhanced inflammatory pain and an impaired analgesic response to antidepressant drugs. *Journal of Neuroscience.* 2007; 27(22):6045-53

Zhu X., Conklin D., Eisenach J.C. Cyclooxygenase-1 in the spinal cord plays an important role in postoperative pain. *Pain.* 2003; 104(1-2):15-23.

Zhuo M., and Gebhart G.F. Tonic cholinergic inhibition of spinal mechanical transmission. *Pain.* 1991; 46(2):211-22.

Zhuo M. Cortical excitation and chronic pain. *Trends in Neuroscience*. 2008; 31(4):199-207.

Zollner C. and Stein C.Opioids. *Handbook of Experimental Pharmacology*. 2007 ;(177):31-63.

Zollner C., Shaqura M.A., Bopaiah C.P., Mousa S., Stein C., Schafer M. Painful

inflammation-induced increase in mu-opioid receptor binding and G-protein coupling in primary afferent neurons. *Molecular Pharmacology.* 2006; 64(2):202-10.

APPENDIX

APPENDIX

APPENDIX 1;

APPENDIX 1. RAW EXPERIMENTAL DATA

Ethanol extract of *Ocimum kenyense* stems. 4
Chloroform/ethanol extract of *Ocimum kenyense* stems. 4
Chloroform extract of *Ocimum kenyense* stems. 5
Ethanol extract of *Ocimum masaiense* stems. 5
Chloroform extract of *Ocimum masaiense* stems. 6
Chloroform/ethanol extract of *Ocimum masaiense* stems. 6
... 6
Chloroform/ethanol extract of *Ocimum kilimandscharicum* stems 7
Chloroform extract of *Ocimum kilimandscharicum* stems. 7
Ethanol extract of *Ocimum kilimandscharicum* stems 7
Ethanol extract of *Ocimum kilimandscharicum* leaves. 8
Chloroform extract of *Ocimum masaiense* leaves. 8
Ethanol extract of *Ocimum masaiense* leaves 9
Chloroform/ethanol extract of *Ocimum masaiense* leaves. 9
Chloroform extract of *Ocimum kenyense* leaves. 10
Ethanol extract of *Ocimum kenyense* leaves. 10
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Ethanol extract of *Ocimum kenyense* stems.

The raw experimental data from the Tail Flick Test are shown in table 1 below.

Dose of extract in mg/kg and Tail flick latency in seconds.				
CONTROL	**100MG/KG**	**200MG/KG**	**400MG/KG**	**800MG/KG**
4.20	10.20	8.86	7.69	7.63
4.43	9.72	9.20	8.11	7.47
4.51	7.59	7.65	7.32	7.01
5.19	7.79	7.79	7.61	6.97
4.65	6.69	9.15	7.43	10.62

Table 1. Raw Experimental data from Tail Flick test

Chloroform/ethanol extract of *Ocimum kenyense* stems.

The raw experimental data from the Tail Flick Test are shown in Table 2 below.

Dose in mg/kg and tail flick latency in seconds.				
Control	**100mg/Kg**	**200mg/Kg**	**400mg/Kg**	**800mg/Kg**
5.50	6.75	5.40	5.16	9.00
3.96	6.50	6.07	4.78	8.05
4.40	5.80	6.44	4.75	7.95
4.80	4.98	5.22	5.31	8.97
3.75	7.66	4.79	7.00	11.54

Table 2. Raw experimental data from the Tail Flick test

Chloroform extract of *Ocimum kenyense* stems.

The raw experimental data from the Tail Flick test are shown in Table 3 below

Dose in mg/kg and tail flick latency in seconds				
Control	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**
5.50	4.08	4.04	5.13	5.38
3.96	4.62	5.13	5.14	6.14
4.40	4.58	5.18	4.93	5.94
4.80	5.41	5.68	4.92	6.00
3.75	4.58	6.00	4.63	6.80

Table 3. Raw experimental data from the Tail Flick test.

Ethanol extract of *Ocimum masaiense* stems.

The raw experimental data from the Tail Flick test are shown in Table 4 below.

Dose in mg/kg and tail flick latency in seconds				
Control	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**
4.44	10.20	5.29	3.87	3.29
5.55	8.75	4.78	3.14	4.67
3.96	11.30	4.77	4.23	3.57
4.70	13.80	4.10	4.33	3.23
3.75	11.84	4.13	5.78	4.87

Table 4. Raw experimental data from the Tail Flick test

Chloroform extract of *Ocimum masaiense* stems.

The raw experimental data from the Tail Flick test are shown in Table 5.

Dose in mg/kg and tail flick latency in seconds				
Control	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**
5.83	6.62	6.12	7.10	6.59
7.51	7.02	9.43	6.75	6.28
5.53	6.96	6.56	7.48	7.29
6.75	8.94	6.32	8.33	7.19
5.80	11.26	6.33	8.68	6.20

Table 5. Raw experimental data from the Tail Flick test.

Chloroform/ethanol extract of *Ocimum masaiense* stems.

The raw experimental data from the Tail Flick test are shown in table 6.

Dose in mg/kg and tail flick latency in seconds				
Control	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**
3.96	4.62	5.92	9.16	4.96
4.40	4.70	4.67	8.84	4.45
4.80	4.10	4.43	8.12	4.09
5.50	5.16	5.92	7.09	4.12
3.75	4.08	4.60	12.60	5.01

Table 6. Raw experimental data from the Tail Flick test

Chloroform/ethanol extract of *Ocimum kilimandscharicum* stems

The experimental data from the Tail Flick test are shown in table 7 below.

Dose in mg/kg and tail flick latency in seconds				
Control	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**
5.50	4.66	4.54	6.47	3.78
4.80	6.12	4.87	6.28	4.42
3.75	4.84	4.33	5.35	3.93
4.40	7.25	4.93	7.14	5.16
3.96	4.93	4.74	5.78	5.63

Table 7. Raw experimental data from the Tail Flick test

Chloroform extract of *Ocimum kilimandscharicum* stems.

The raw experimental data from the Tail Flick test are shown in table 8

Dose in mg/kg and tail flick latency in seconds.				
Control	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**
4.40	4.30	8.60	3.35	3.64
5.50	3.92	7.20	4.31	3.20
3.96	4.53	8.34	4.03	3.49
3.75	5.33	7.60	4.15	3.74
4.80	4.35	5.01	4.00	4.11

Table 8. Raw experimental data from the Tail Flick test

Ethanol extract of *Ocimum kilimandscharicum* stems.

The raw experimental data from the Tail Flick test are shown in table 9.

Dose in mg/kg and Tail flick latency in seconds				
Control	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**
5.50	8.13	5.46	7.05	10.40
3.96	7.12	6.50	7.80	7.97
4.80	6.99	6.88	6.18	10.67
3.75	8.02	6.87	6.82	7.79
4.40	7.68	9.52	6.50	10.10

Table 9. Raw experimental data from the Tail Flick test

Ethanol extract of *Ocimum kilimandscharicum* leaves.

The raw experimental from the Tail Flick test are shown in table 10.

Dose in mg/kg and tail flick latency in seconds.			
Control	**200mg/kg**	**400mg/kg**	**800mg/kg**
6.78	7.71	13.93	3.29
7.46	6.36	11.10	4.26
6.18	8.55	11.76	4.51
6.69	5.88	15.51	4.67
5.11	5.76	14.87	2.80

Table 10. Raw experimental data from the Tail Flick test

Chloroform extract of *Ocimum masaiense* leaves.

The raw experimental data from the Tail Flick test are shown in table 11 below.

Dose in mg/kg and tail flick latency in seconds			
Control	**200mg/kg**	**400mg/kg**	**800mg/kg**
3.08	16.99	4.45	5.11
5.04	19.25	4.88	6.83
4.77	14.86	5.77	3.80
3.54	12.43	4.78	8.11
2.72	12.22	4.41	6.03

Table 11. Raw experimental data from the Tail Flick test

Ethanol extract of *Ocimum masaiense* leaves

The raw experimental data from the Tail Flick test are shown in table 12.

Dose in mg/kg and tail flick latency in seconds			
Control	**200mg/kg**	**400mg/kg**	**800mg/kg**
6.78	6.15	8.38	4.79
7.46	6.88	6.51	9.00
6.18	5.54	4.78	5.02
6.69	9.10	4.68	8.75
5.11	6.83	9.59	6.23

Table 12 Raw experimental data from the Tail Flick test

Chloroform/ethanol extract of *Ocimum masaiense* leaves.

The raw experimental data from the Tail Flick are shown in Table 13 below.

Dose in mg/kg and tail flick latency in seconds.			
Control	**200mg/kg**	**400mg/kg**	**800mg/kg**
3.08	4.58	7.16	6.93
3.54	4.56	7.22	6.20
5.04	6.16	8.16	7.50
4.77	3.70	6.22	8.48
2.72	7.40	5.86	8.07

Table 13 Raw experimental data from the Tail Flick test

Chloroform extract of *Ocimum kenyense* leaves.

The raw experimental data from the Tail Flick test are shown in table 14.

Dose in mg/kg and tail flick latency in seconds				
Control	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**
3.96	5.09	5.73	5.47	4.98
4.80	5.83	6.25	5.00	4.49
3.75	6.13	5.12	5.78	6.54
4.40	6.50	5.35	6.12	6.31
5.50	7.39	4.38	4.98	6.75

Table 14.Raw experimental data from the Tail Flick test

Ethanol extract of *Ocimum kenyense* leaves.

The raw experimental data from the Tail Flick test are shown in table 15 below

Dose in mg/kg and tail flick latency in seconds				
Control	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**
8.22	11.71	6.56	7.71	6.79
6.80	7.49	9.64	6.50	7.32
5.90	6.34	9.39	9.90	10.54
4.02	6.04	8.49	13.70	5.87
7.65	5.60	7.12	9.65	10.19

Table 15. Raw experimental data from the Tail Flick test

Chloroform/ethanol extract of *Ocimum kenyense* leaves.

The raw experimental data from the Tail Flick test are shown in table 16

Dose in mg/kg and tail flick latency in seconds				
Control	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**
4.40	6.78	6.22	9.89	8.28
5.50	7.20	9.93	9.05	8.38
3.96	6.49	6.23	10.12	8.73
4.80	8.73	6.44	9.89	7.93
3.75	11.47	7.06	8.73	8.14

Table 16. Raw experimental data from the Tail Flick test

Chloroform extract of *Ocimum kilimandscharicum* roots.

The raw experimental data from the Tail Flick test are shown in table 17.

Dose in mg/kg and tail flick latency in seconds.				
Control	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**
6.70	7.62	6.14	6.35	6.16
6.42	6.77	7.04	6.22	6.00
5.59	6.34	6.25	5.42	8.15
6.75	8.21	6.58	5.50	6.67
5.86	5.82	8.27	7.48	9.87

Table 17. Raw experimental data from the Tail Flick test.

Chloroform/ethanol extract of *Ocimum kilimandscharicum* roots.

The raw experimental data from the Tail Flick test are shown in table 18 below.

Dose in mg/kg and tail flick latency in seconds.				
Control	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**
4.95	16.40	10.35	6.85	9.42
5.65	14.37	5.75	5.75	5.63
4.00	16.36	5.26	8.00	7.10
3.65	15.30	5.35	7.30	5.13
4.70	18.49	7.50	6.27	8.55

Table 18. Raw experimental data from the Tail Flick test

Ethanol extract of *Ocimum kilimandscharicum* roots.

The raw experimental data from the Tail Flick test are shown in table 19 below.

Dose in mg/kg and tail flick latency in seconds			
Control	**200mg/kg**	**400mg/kg**	**800mg/kg**
3.08	6.56	2.85	8.14
5.04	6.63	5.40	8.51
4.77	7.55	3.85	7.42
3.54	6.49	3.37	12.65
8.07	8.76	3.40	13.10

Table 19 . Raw experimental data from the Tail Flick test

Ethanol extract of *Ocimum kenyense* roots

The raw experimental data from the Tail Flick test are shown in table 20 below.

Dose in mg/kg and tail flick latency in seconds				
Control	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**
4.20	7.91	10.78	9.90	7.97
4.43	6.82	8.41	7.98	10.18
4.51	10.38	8.71	9.91	8.02
5.19	5.95	9.02	9.13	9.37
5.33	8.54	8.91	6.75	9.85

Table 20. Raw experimental data from the Tail Flick test

Chloroform/ethanol extract of *Ocimum kenyense* roots.

The raw experimental data from the Tail Flick test are shown in Table 21.

Dose in mg/kg and tail flick latency in seconds				
Control	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**
4.20	6.78	6.22	9.89	8.28
4.43	7.20	9.93	9.05	8.38
5.19	11.47	6.23	8.97	8.75
4.51	6.49	6.44	10.30	9.40
5.03	8.73	7.35	8.30	7.38

Table 21. Raw experimental data from the Tail Flick test

Chloroform extract of *Ocimum masaiense* roots.

The raw experimental data from the Tail Flick test are shown in Table 22.

Dose in mg/kg and tail flick latency in seconds				
Control	100mg/kg	200mg/kg	400mg/kg	800mg/kg
6.80	7.16	9.15	7.90	7.71
8.22	7.02	11.35	9.02	8.80
5.90	7.95	8.39	7.30	7.03
4.02	6.82	11.30	6.62	8.17
7.65	6.70	11.09	8.24	10.74

Table 22. Raw experimental data from the Tail Flick test

Chloroform/ethanol extract of *Ocimum masaiense* roots.

The raw experimental data from the Tail Flick test are shown in table 23.

Dosage in mg/kg and tail flick latency in seconds				
Control	100mg/kg	200mg/kg	400mg/kg	800mg/kg
4.00	14.20	6.70	6.88	5.03
3.32	11.00	7.78	10.05	5.45
4.95	13.63	5.26	6.39	4.45
5.65	15.02	5.53	5.00	6.03
4.70	9.24	4.41	5.43	4.93

Table 23. Raw experimental data obtained from the Tail Flick test

Ethanol extract of *Ocimum masaiense* roots.

The raw experimental data from the Tail Flick test are shown in table 24 below.

Dosage in mg/kg and tail flick latency in seconds				
Control	100mg/kg	200mg/kg	400mg/kg	800mg/kg
3.96	6.60	6.16	6.98	6.35
5.50	6.48	7.24	6.88	5.45
4.80	7.20	6.68	6.93	5.77
3.75	6.31	5.77	7.05	8.61
4.40	7.60	7.80	7.10	6.49

Table 24. Raw experimental data from the Tail Flick test

Dichloromethane/methanol extract of *Ocimum masaiense* roots

The raw experimental data from the Tail Flick test are shown in table 25

Dose in mg/kg and tail flick latency in seconds					
Control	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**	**Paracetamol 400mg/kg**
4.11	13.3	6.58	8.30	5.21	6.65
5.46	14.9	7.47	9.10	6.17	6.68
4.24	13.9	6.93	7.77	6.42	5.62
4.93	9.9	7.21	8.02	5.03	4.70
5.23	14.7	7.19	7.41	4.77	5.65

Table 25. Raw experimental data from the Tail Flick test

FORMALIN TEST RESULTS FOR CHLOROFORM/ETHANOL EXTRACT OF *Ocimum masaiense* ROOTS

TIME IN SECONDS.			
Early phase control	**Early phase test**	**Late phase control**	**Late phase test**
98.	16.	48.	6.
83.	9.	58.	13.
110.	13.	47.	10.
106.	11.	74.	14.
140.	14.	57.	13.

Table 26. Results of the Formalin test for chloroform/ethanol extract of *Ocimum masaiense* roots.

AGONIST/BLOCKER EXPERIMENTS IN THE FORMALIN TEST.

ATROPINE

The raw experimental data for the Formalin test in the Atropine blocker test are shown in Table 27 below.

TIME IN SECONDS.					
Negative control early phase	**Positive control early phase**	**Test early phase**	**Negative control Late phase**	**Positive control late phase**	**Test late phase**
98	16	4.02	47	6	9
83	9	1.2	48	13	8.9
110	13	7.02	57	14	12
106	11	6	58	10	14
140	14	4.8	74	13	14

Table 27. Raw experimental data for Atropine in the Formalin test

KETAMINE TEST.

The raw experimental data for the Ketamine blocker test are shown in Table 28.

TIME IN SECONDS.					
Negative control early phase	**Positive control early phase**	**Test early phase**	**Negative control Late phase**	**Positive control late phase**	**Test late phase**
98	10	57	47	3	17.6
83	16	75	48	3	0
110	29	52	57	1	14.6
106	37	64	58	7	14.2
140	34	62	74	15	11.9

Table 28. Raw experimental data in the Ketamine test

CAPSAICIN TEST.

The raw experimental data obtained in the Capsaicin blocker experiment are shown in Table 29;

TIME IN SECONDS.					
Negative control early phase	**Positive control early phase**	**Test early phase**	**Negative control Late phase**	**Positive control late phase**	**Test late phase**
98	8.3	12.7	47	6.0	15.0
83	11.1	8.5	48	6.9	13.5
110	8.2	20	57	6.3	16
106	8.0	17	58	10.7	14
140	9.8	12	74	6.2	13

Table 29. Raw experimental data obtained in the Capsaicin experiment.

NALOXONE

The raw experimental data for the naloxone experiment are shown in Table 30

TIME IN SECONDS.					
Negative control early phase	**Positive control early phase**	**Test early phase**	**Negative control Late phase**	**Positive control late phase**	**Test late phase**
98	12.3	20.2	47	6.9	8.0
83	15	26.9	48	6.9	6.7
110	18.6	25.0	57	9.9	10.6
106	16.4	27.3	58	10.1	10.2
140	21.6	23.1	74	7.35	9.7

Table 30. Raw experimental data from the Naloxone experiment

RESULTS FROM THE TAIL FLICK TESTS FOR THE FRACTIONS OBTAINED

FRACTION I

The raw experimental data from the Tail Flick test for Fraction I is shown in Table 31 below.

Dose in mg/kg and tail flick latency in seconds					
Control	**12.5mg/kg**	**25mg/kg**	**50mg/kg**	**100mg/kg**	**PARACETAMOL**
4.09	2.35	8.17	5.66	6.45	6.65
3.66	4.43	6.27	6.71	5.95	6.68
2.70	3.40	7.02	6.87	6.37	5.62
4.20	4.11	7.92	6.32	5.43	4.70
3.04	3.85	6.87	6.47	7.92	5.65

Table 31.Raw experimental data from Tail Flick test

FRACTION II

The raw experimental data obtained in the Tail Flick test for fraction II are shown in Table 32 below.

Dose in mg/kg and tail flick latency in seconds					
Control	**12.5mg/kg**	**25mg/kg**	**50mg/kg**	**100mg/kg**	**PARACETAMOL**
4.23	5.01	5.34	6.99	7.11	6.65
3.40	4.03	6.23	8.16	7.81	6.68
2.70	3.78	5.20	7.57	8.35	5.62
4.20	3.73	4.42	8.07	7.52	4.70
3.92	4.78	5.39	7.69	7.78	5.65

Table 32 Raw experimental data from Tail Flick test

FRACTION III

The raw experimental data from the Tail Flick test are shown in Table 33.

Dose in mg/kg and tail flick latency in seconds					
Control	**12.5mg/kg**	**25mg/kg**	**50mg/kg**	**100mg/kg**	**PARACETAMOL**
3.94	3.16	4.62	5.66	5.80	6.65
3.36	3.76	4.35	6.84	6.30	6.68
3.04	3.92	4.70	6.03	6.01	5.62
3.66	4.12	4.26	6.47	5.77	4.70
3.92	3.43	4.54	6.72	6.47	5.65

Table 33. Raw experimental data from the Tail Flick test

FRACTION IV

The raw experimental data obtained in the Tail Flick test for fraction IV are shown in Table 34

Dose in mg/kg and tail flick latency in seconds					
Control	**12.5mg/kg**	**25mg/kg**	**50mg/kg**	**100mg/kg**	**PARACETAMOL**
3.23	4.42	15.40	4.83	6.71	6.65
3.14	4.53	14.30	5.27	5.20	6.68
2.98	4.35	13.78	4.76	5.03	5.62
3.32	3.81	14.03	5.17	6.21	4.70
3.01	4.93	13.24	5.03	6.04	5.65

Table 34. Raw experimental data from the Tail Flick test.

FRACTION V.

The raw experimental data obtained in the Tail Flick test for fraction V are shown in Table 35 below

Dose in mg/kg and tail flick latency in seconds					
Control	12.5mg/kg	25mg/kg	50mg/kg	100mg/kg	Paracetamol
3.94	2.65	9.41	3.77	3.49	6.65
4.51	4.07	8.96	4.05	3.40	6.68
4.31	3.13	9.97	2.99	3.67	5.62
3.62	2.93	10.23	3.53	3.04	4.70
3.87	3.97	8.57	4.01	3.47	5.65

Table 35 Raw experimental data from the Tail Flick test

FRACTION VI

The raw experimental data obtained in the Tail Flick test for fraction VI are shown in Table 36.

Dose in mg/kg and tail flick latency in seconds					
Control	12.5mg/kg	25mg/kg	50mg/kg	100mg/kg	Paracetamol
3.23	3.57	4.27	10.01	7.84	6.65
2.98	3.77	5.46	10.02	6.70	6.68
3.14	3.89	4.03	11.37	7.02	5.62
3.77	4.13	5.12	10.50	6.97	4.70
3.01	3.67	4.97	9.73	7.11	5.65

Table 36 . Raw experimental data from the Tail Flick test

RESULTS OBTAINED IN THE TAIL FLICK TEST FOR THE PURE COMPOUNDS OBTAINED

COMPOUND Ia

The raw experimental data obtained in the Tail Flick test for the pure compound Ia are shown in Table 37.

Dose in mg/kg and tail flick latency in seconds				
Control	**25mg/kg**	**50mg/kg**	**100mg/kg**	**Paracetamol**
3.37	7.75	10.40	16.69	6.65
4.74	6.75	9.71	10.28	6.68
3.00	10.98	9.07	14.93	5.62
3.20	6.38	8.64	11.76	4.70
3.50	7.02	9.46	12.75	5.65

Table 37. Raw experimental data from Tail Flick test

COMPOUND Ib

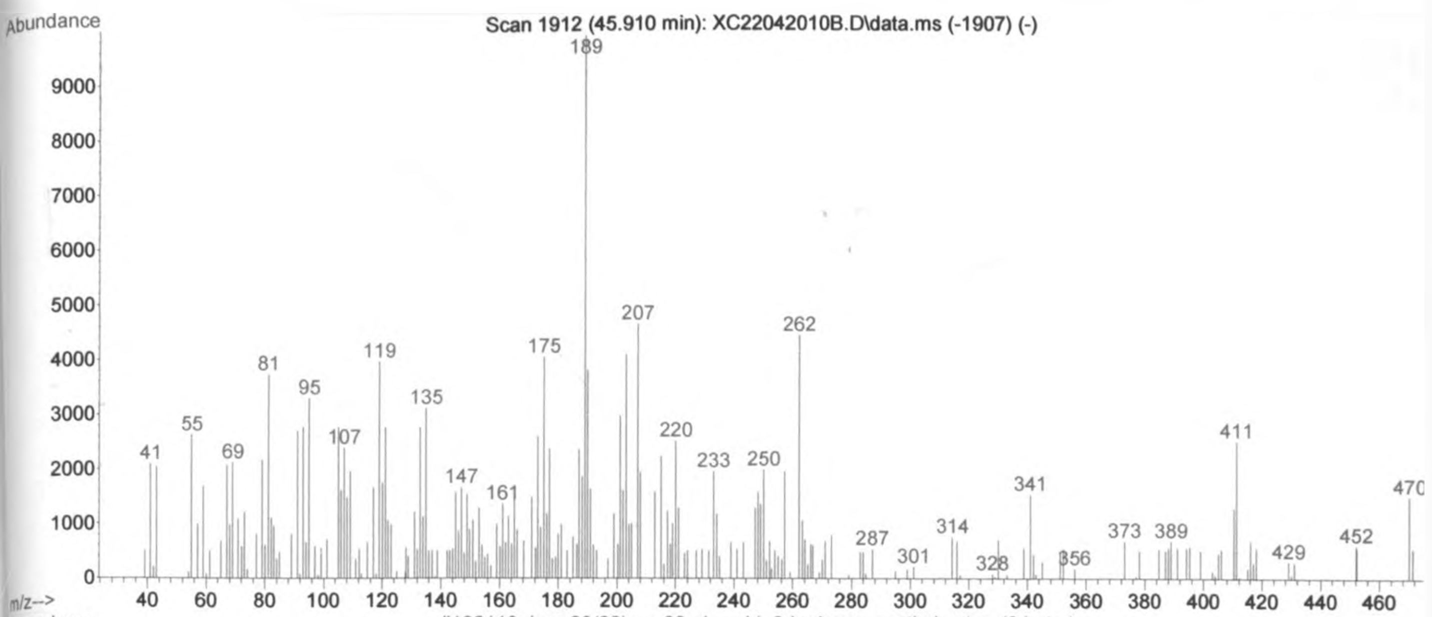
The raw experimental data obtained in the Tail Flick test for compound Ib are shown in Table 38.

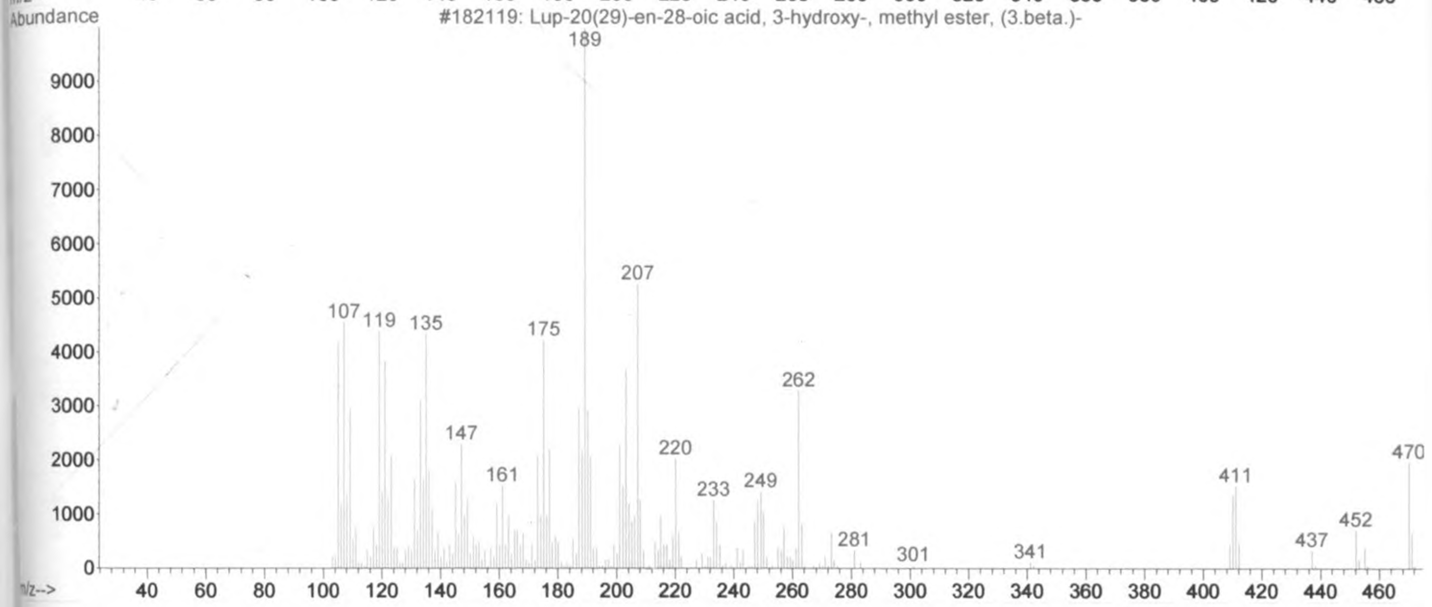
Dose in mg/kg and tail flick latency in seconds				
Control	**25mg/kg**	**50mg/kg**	**100mg/kg**	**Paracetamol**
2.54	5.71	5.76	9.71	6.65
2.76	9.34	8.69	6.14	6.68
5.41	6.76	6.02	8.61	5.62
3.50	6.55	5.74	6.74	4.70
2.60	9.49	8.74	6.24	5.65

Table 38. Raw experimental data from the Tail Flick test

APPENDIX 2;

Library Searched : C:\Database\NIST05a.L
Quality : 98
ID : Lup-20(29)-en-28-oic acid, 3-hydroxy-, methyl ester, (3.beta.)-





O

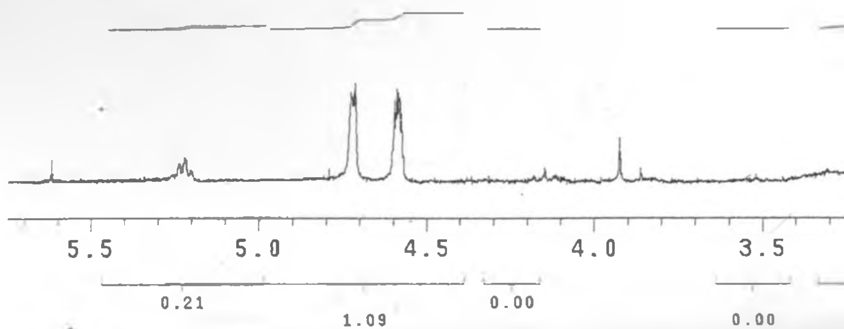
O

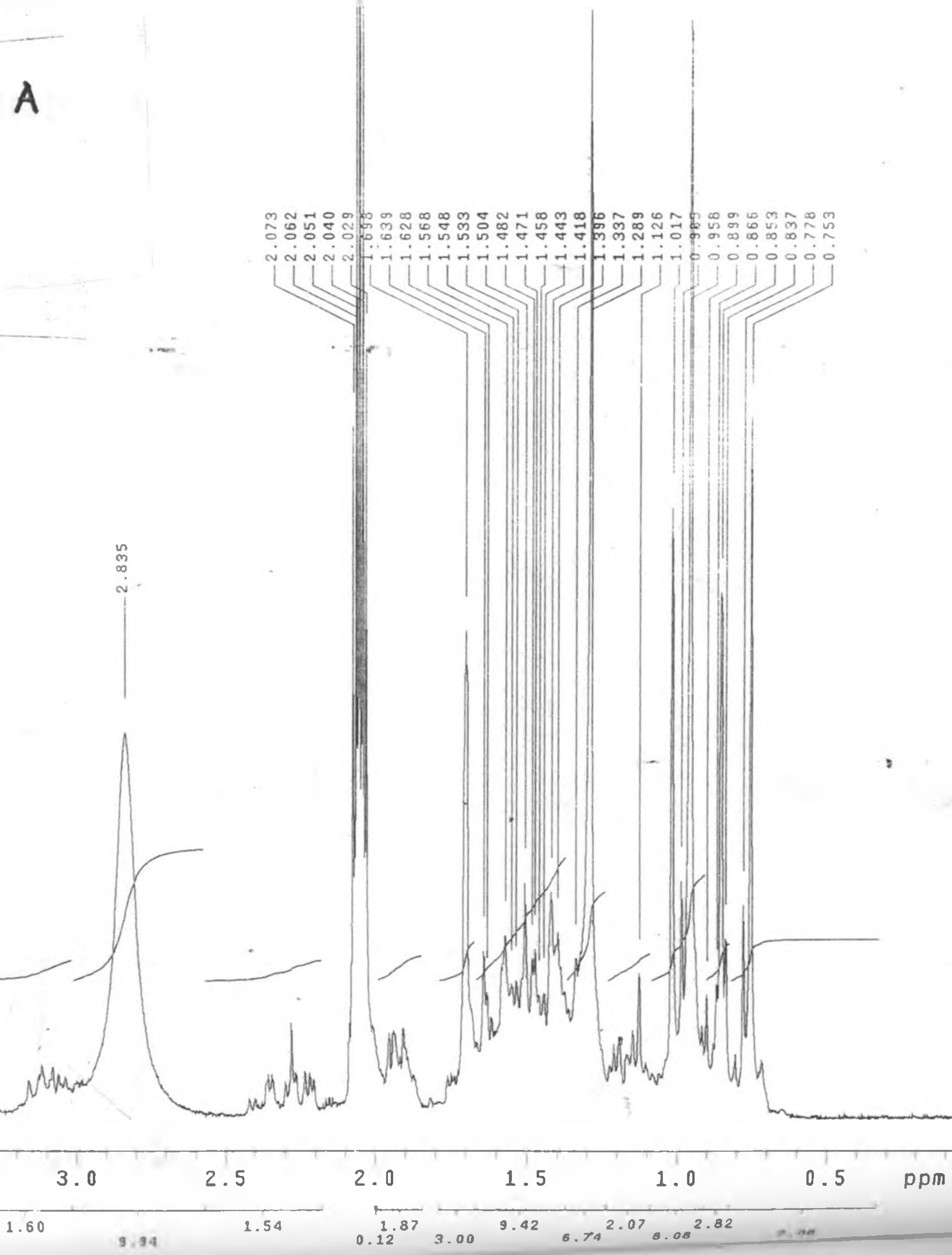
HO

WAWERU
PMW-1A
1H NMR, 200 MHz
acetone-d6
08-11-09

Pulse Sequence: s2pul

COMPOUND I




A
2.073
2.062
2.051
2.040
2.029
1.639
1.628
1.568
1.548
1.533
1.504
1.482
1.471
1.458
1.443
1.418
1.396
1.337
1.289
1.126
1.017
0.958
0.899
0.866
0.853
0.837
0.778
0.753
2.835
3.0
2.5
2.0
1.5
1.0
0.5
ppm
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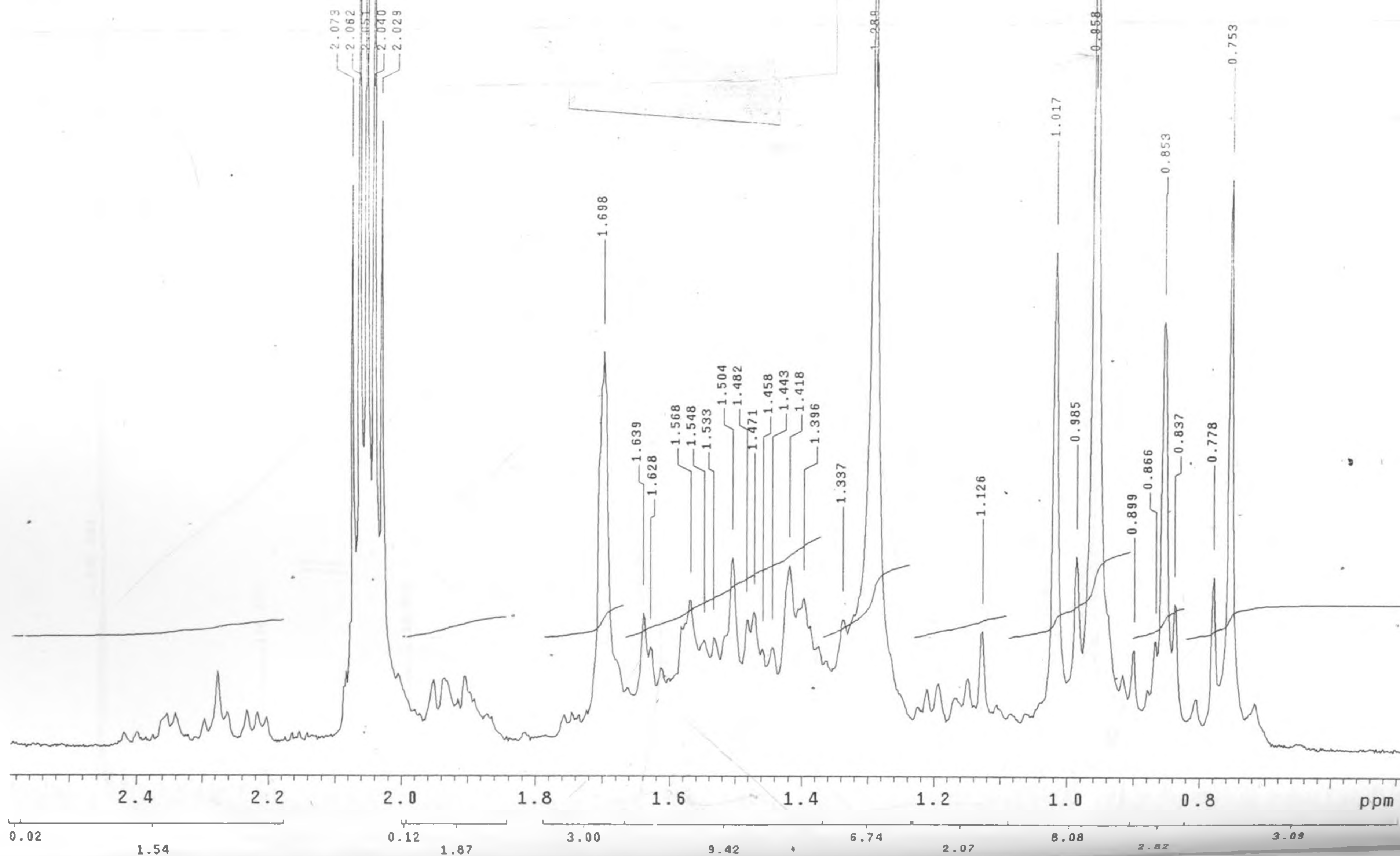
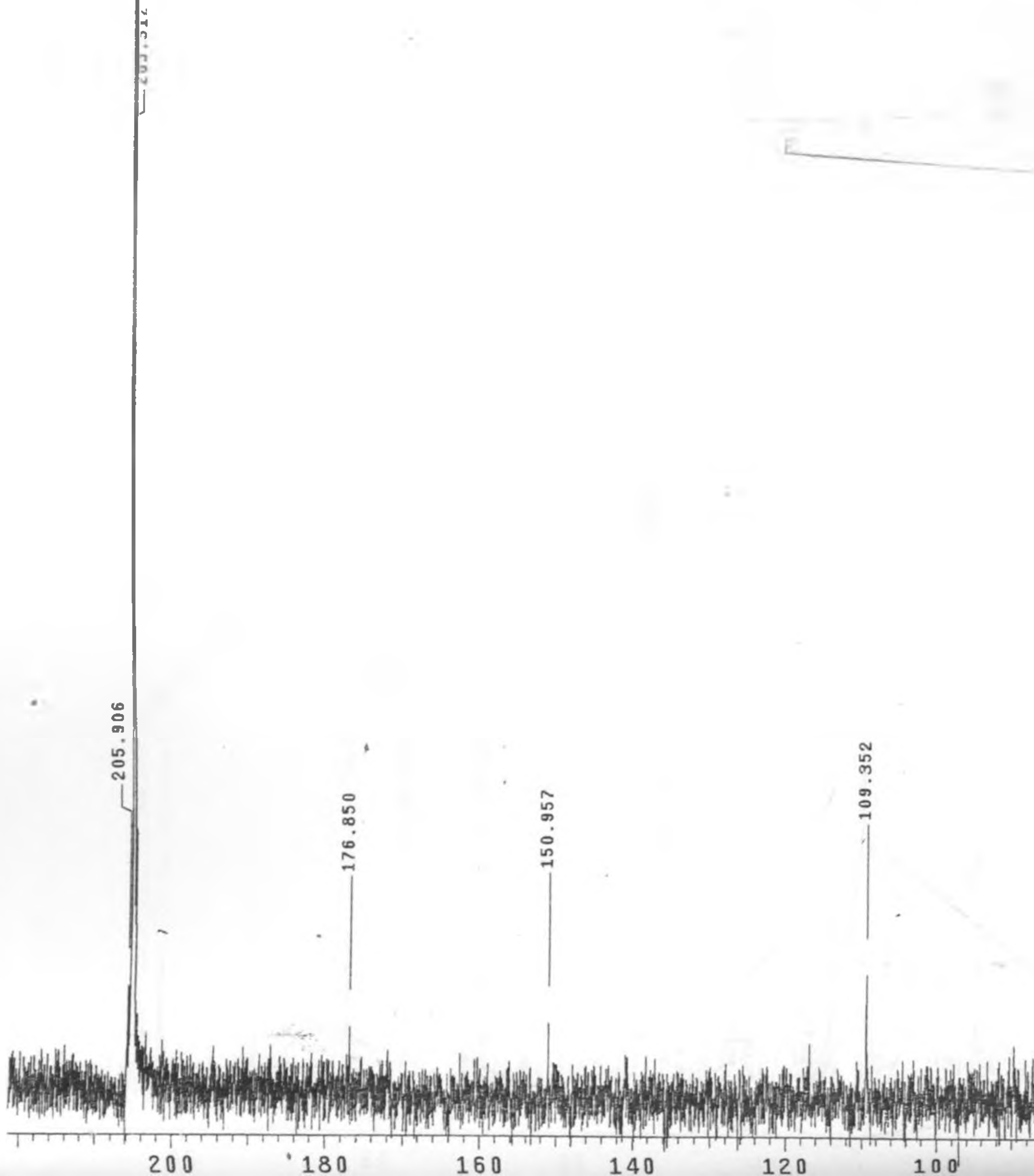
9.94
1.54
1.87
0.12
3.00
9.42
6.74
2.07
8.08
2.82


PMW-1A
1H NMR, 200 MHz
acetone-d6
08-11-09
Pulse Sequence: s2pul
COMPOUND 1 A
2.073
2.062
2.051
2.040
2.029
1.698
1.639
1.628
1.568
1.548
1.533
1.504
1.482
1.471
1.458
1.443
1.418
1.396
1.337
1.288
1.126
1.017
0.985
0.958
0.899
0.866
0.853
0.837
0.778
0.753
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
ppm
0.02
1.54
0.12
1.87
3.00
9.42
6.74
2.07
8.08
2.82
3.09

WAWERU
PMW-1A
13C NMR, 50 MHz
acetone-d6
08-11-09

Pulse Sequence: s2pul

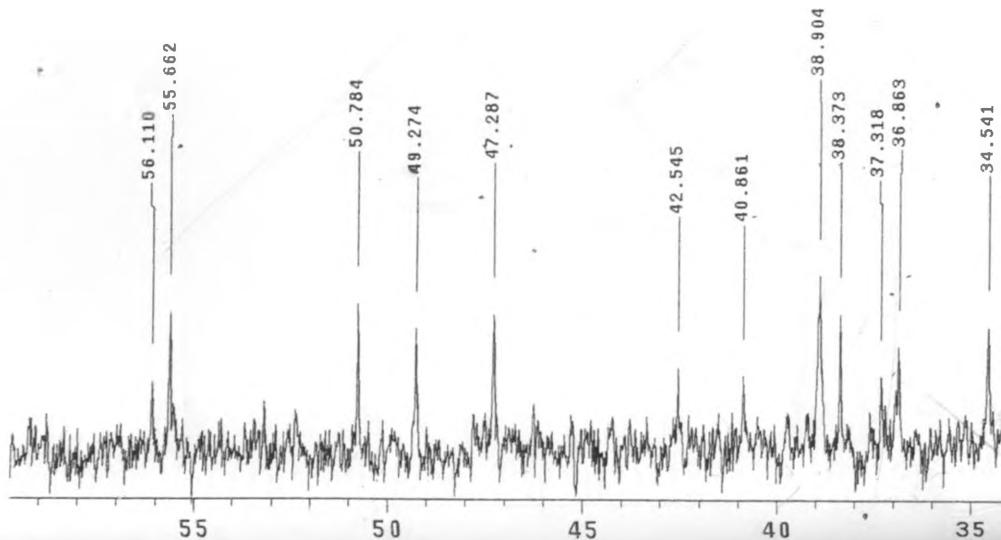


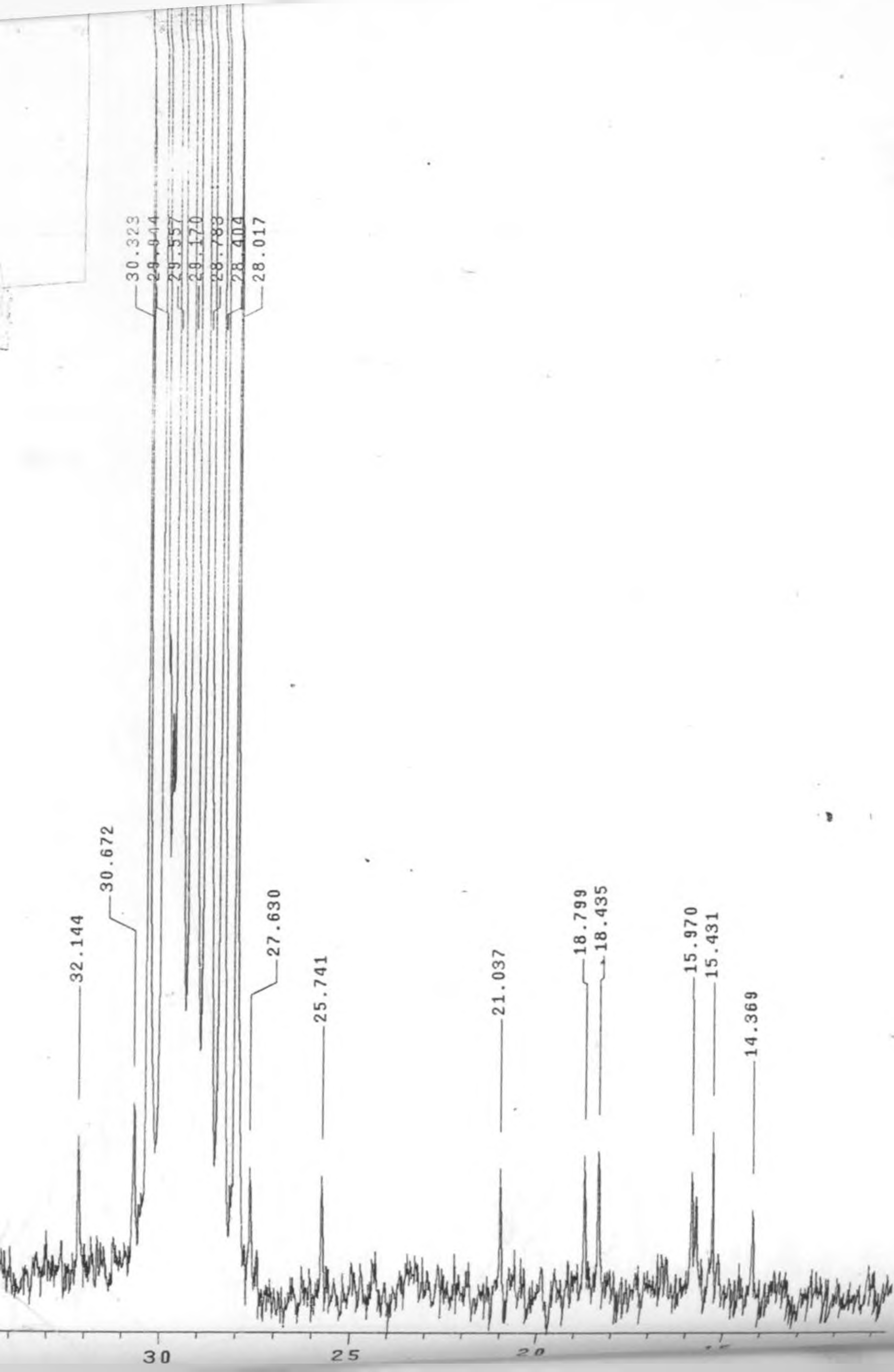
77.876
56.110
55.662
50.784
49.274
47.287
42.545
40.861
38.904
38.373
37.318
36.863
34.541
32.144
30.672
30.323
29.944
29.557
29.170
28.783
28.404
28.017
27.630
25.741
21.037
18.799
18.435
15.970
15.431
14.369

80
60
40
20
0

Pulse Seq ?pul

Compou... 1 A



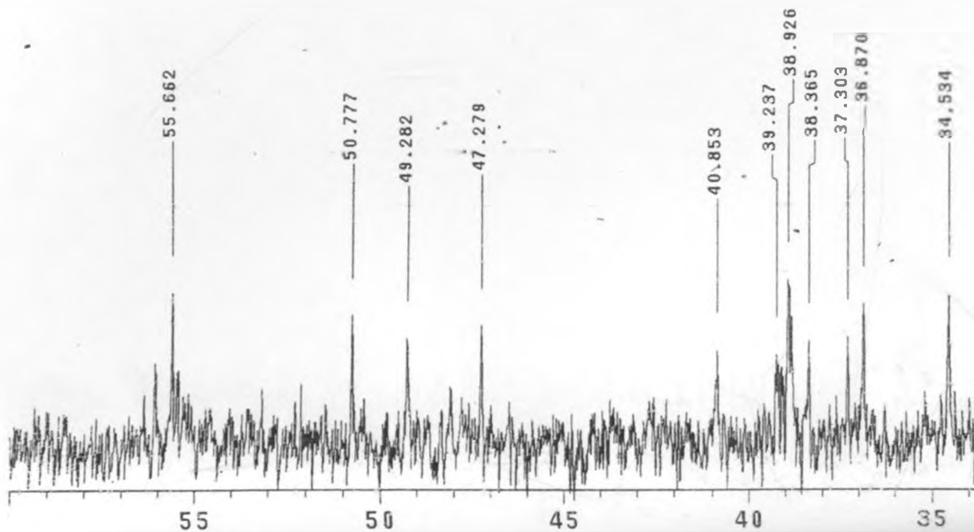


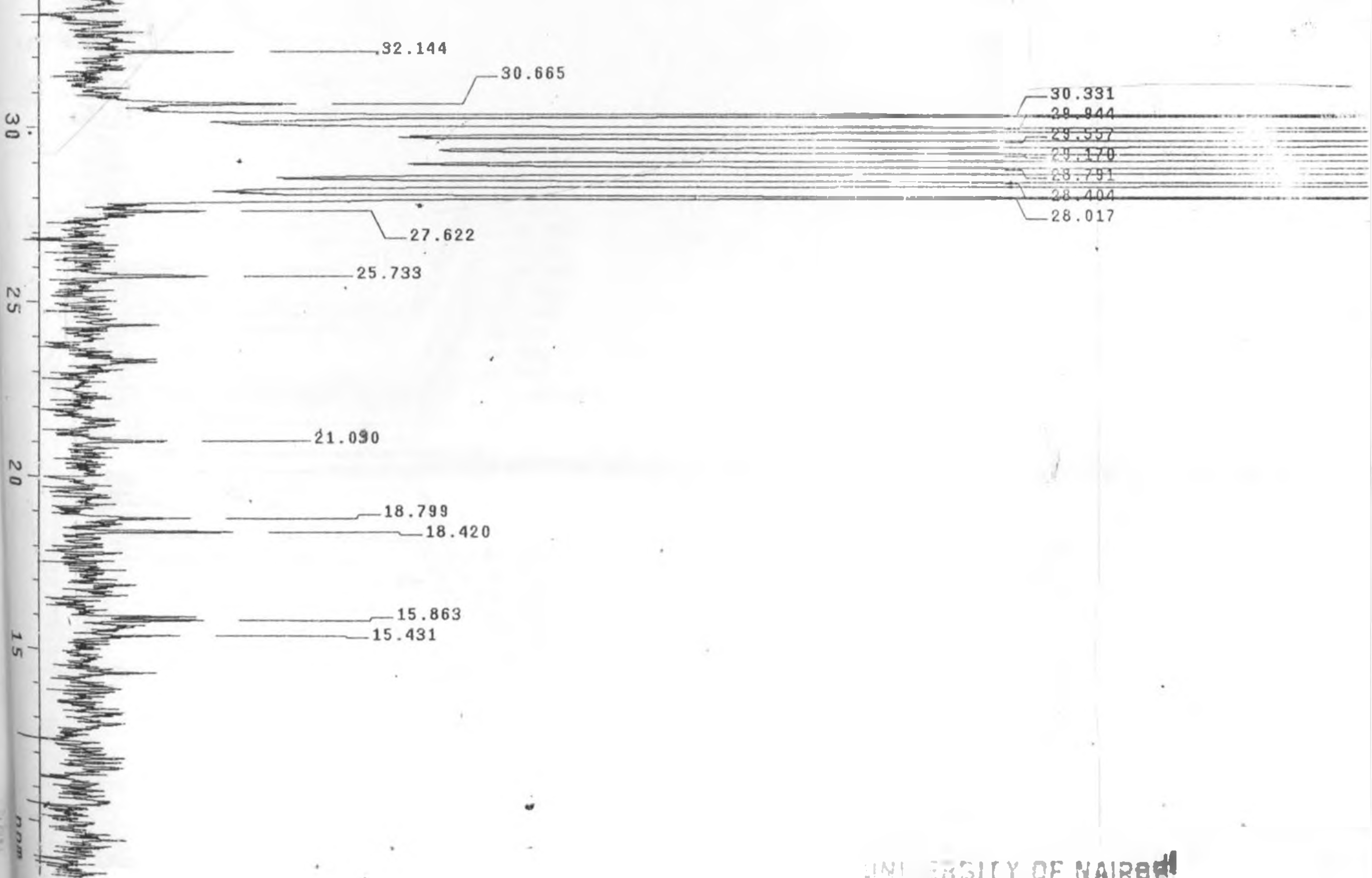
32.144
30.672
30.323
29.944
29.557
29.170
28.783
28.404
28.017
27.630
25.741
21.037
18.799
18.435
15.970
15.431
14.369
30
25
20

WAWERU
PMW-1B
13C NMR, 50 MHz
acetone-d6
09-11-09

Pulse Sequence: s2pul

COMPOUND 1B





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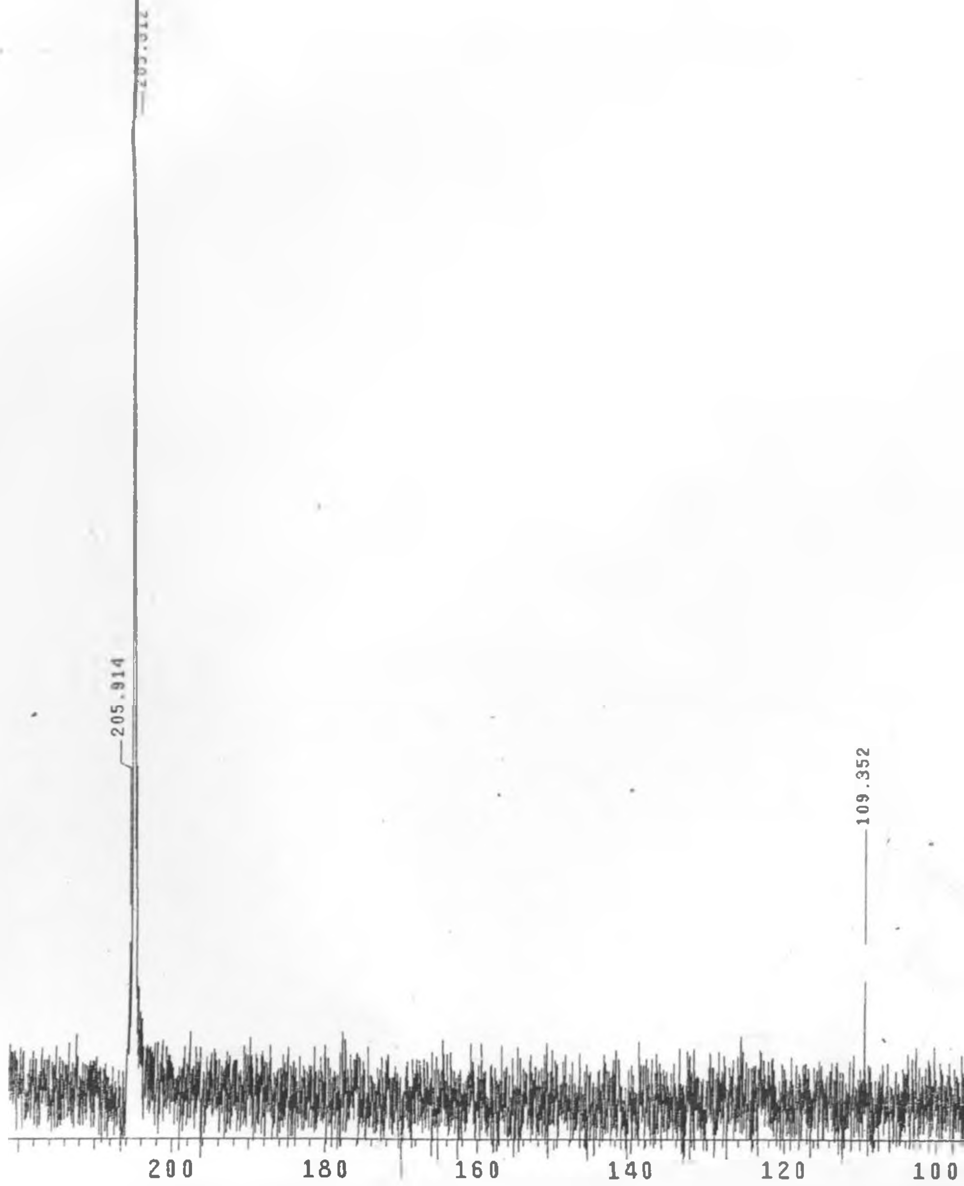
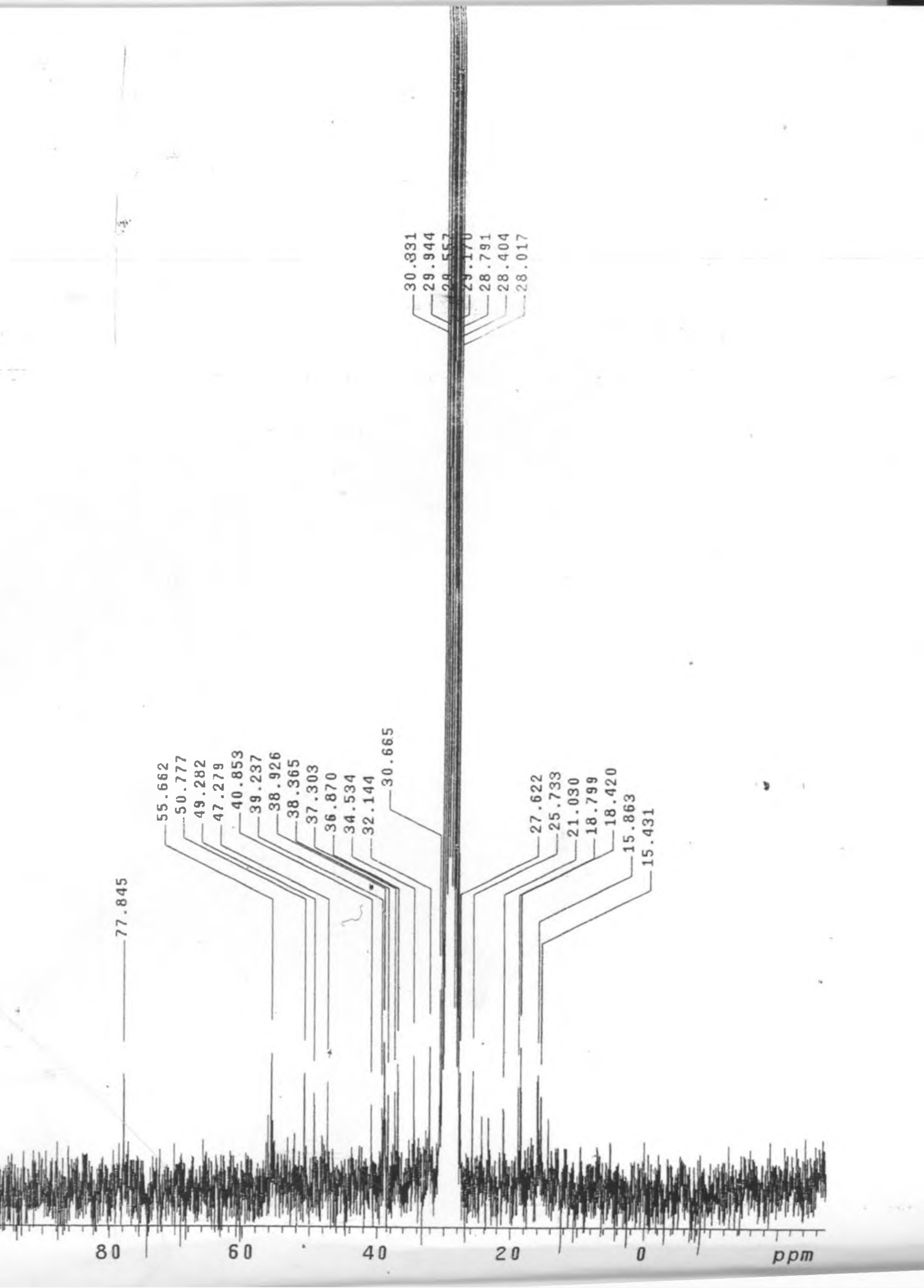
LIBRARY


WAWERU
PMW-1B
13C NMR, 50 MHz
acetone-d6
09-11-09
Pulse Sequence:
COMPOUND 1B
205.914
109.352
200
180
160
140
120
100



77.845
55.662
50.777
49.282
47.279
40.853
39.237
38.926
38.365
37.303
36.870
34.534
32.144
30.665
30.331
29.944
29.170
28.791
28.404
28.017
27.622
25.733
21.030
18.799
18.420
15.863
15.431
80
60
40
20
0
ppm