ANALGESIC, ANTI-INFLAMMATORY AND MECHANISTIC STUDIES OF ETHANOL LEAF EXTRACT OF *HYMENODICTYON FLORIBUNDUM* (Hochst. & Steud) B. L. Rob IN MICE AND RATS

BY

RABI'U NUHU DANRAKA

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,
FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

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Rabi'u Nuhu DANRAKA,

B. Pharm (A.B.U) 2015

P17PHPG8011

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,
AHMADU BELLO UNIVERSITY, ZARIA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER DEGREE IN PHARMACOLOGY

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,
FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

MAY, 2021

DECLARATION

I declare that the work in this thesis entitled: “Analgesic, Anti-inflammatory and Mechanistic studies of ethanol leaf extract of *Hymenodictyon floribundum*(Hochst. & Steud) B. L. Rob in Mice and Rats” has been performed by me in the Department of Pharmacology and Therapeutics. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

Rabi’u Nuhu DANRAKA		
Name of the student	Signature	Date

CERTIFICATION

This Thesis entitled "ANALGESIC, ANTI-INFLAMMATORY AND MECHANISTIC STUDIES OF ETHANOL LEAF EXTRACT OF *HYMENODICTYON FLORIBUNDUM*(Hochst. & Steud) B. L. Rob IN MICE AND RATS" by Rabi'u Nuhu DANRAKA meets the regulations governing the award of the degree of Master of Science in Pharmacology of the Ahmadu Bello University, and is approved for its' contribution to knowledge and literary presentation.

Dr. I.M. Maje

_____	_____	_____
Chairman, Supervisory Committee	Signature	Date

Professor J.I. Ejiofor

_____	_____	_____
Member, Supervisory Committee	Signature	Date

Dr. M.G. Magaji

_____	_____	_____
Head of Department	Signature	Date

Professor S. Abdullahi

_____	_____	_____
Dean, School of Postgraduate Studies	Signature	Date

ACKNOWLEDGEMENT

All praise and gratitude are to Almighty Allah the Most Beneficent, the Most Compassionate, the Most Merciful and the Sustainer of this world who gave me the chance to complete this work. May the blessings of Allah be upon the Noble Messenger, his companions, members of his household and all those that follow his path up to the last day.

My special gratitude and respect go to my supervisors; Dr. I.M. Maje and professor J.I. Ejiofor for their tireless support, guidance, motivation, encouragement, sacrifice, generosity and patience throughout the period of this work, may Almighty Allah reward them abundantly. Also, my sincere appreciation goes to all my lecturers in the Department of Pharmacology and Therapeutics for their advice and contributions towards the successful completion of this study. I specially send my special acknowledgement to Dr Magaji Garba Muhammad and Maryam Salaudeen for continuously motivating and moving me towards the success of the study.

I send my thankful and sincere appreciation to my parent, Alhaji Nuhu Mustapha and Hajiya Mariya Abdulsalam for their unconditional love and moral support throughout my life. May Almighty Allah reward them in this life and hereafter.

I also appreciate all my classmates, friends and siblings for always being there for me whenever i need their presence and assistance particularly Maryam Salauddeen, Nura Bello and Mubarak Senata. May Almighty assist them in all their endeavours.

I owe my sincere thanks to Dr. Bashir, Dr. Aisha Shehu, Dr. Ferhart Khan, Dr Amina Olurokoba and My two great Ogas; Oga Abdulhakim and Oga Mu`Azzamu.

My special appreciation to all the technical staff of the Department of Pharmacology and Therapeutics, Mallam Abubakar Balarabe, Mallam Aliyu, Mallam Muazu, Mallam Idris, Mallam Bashir, Mallam Salihu for their contributions towards the success of this work.

ABSTRACT

The use of complementary medicine has increased greatly in both developing and developed countries due to less adverse effect and relatively safer therapeutic profile. *Hymenodictyon floribundum*is a common shrub in Africa that has been used traditionally in the treatment of pain and inflammatory diseases.This study aims at establishing the analgesic and anti-inflammatory potentials of ethanol leaf extract of *Hymenodictyon floribundum* (HF) and elucidating its possible mechanism of actions. In this study, the analgesic and anti-inflammatory activity of the ethanol leaf extract of *Hymenodictyon floribundum* in mice and ratswere studied after phytochemical screening and acute toxicity tests to determine its LD_{50}. EEHF (375, 750 and 1500 mg/kg *p.o*) was evaluated for analgesic activity using acetic acid induced writhing and hot-plate methods in mice. While the evaluation of anti-inflammatory activity was done using carrageenan-induced hind paw oedema model in rats. The probable mechanism of analgesic activity was also studied by evaluating the activity on opioidergic, adrenergic alpha and beta (α_1, α_2 and β)-receptors, and ATP-sensitive potassium channels pathways. The relationship between the anti-inflammatory activity of the extract and the level of inflammatory biomarkers such as prostaglandins ($PGE_{2)}$, tumor necrotic factor alpha (TNF-α), and interleukins (IL-1β and IL-6) was also assayed using ELISA kits according to manufacturer's guidelines. The ethanol leaves extract exhibited significant ($p<0.05$) and dose-dependent analgesic activity comparable to that of the reference drug morphine (10 mg/kg body weight, *p.o*) in both acetic acid and hot plate methods. The extract also significantly ($p<0.05$) reduced the paw oedema at 1500 mg/kg. The pretreatment of mice with naloxone, yohimbine and propranolol significantly ($p<0.05$) decreased the analgesic effect of the ethanol leaves extract while pretreatment with prazosin and glibenclamide had no significant effect on its

analgesic activity. This is a pointer to that fact that the analgesic activity of the extract may be via opioidergic, α_2 and β-adrenergic receptors pathways. The levels of inflammatory cytokines were unaffected by the extract at all doses tested, except at 375 mg/kg, where there was a significantly high level of IL-6, an indication that the anti-inflammatory activity is unlikely to involve inflammatory biomarkers. The preliminary phytochemical screening of the extract revealed the presence of flavonoids, tannins, saponins, steroids, cardiac glycosides, anthraquinones and phenols. The oral median lethal dose (LD_{50}) of the extract in both mice and rats were found to be greater than 5,000 mg/kg body weight. These findings suggest that the ethanol extract of HF possesses analgesic and anti-inflammatory activity which further supports the ethnomedicinal claims of the plant for the management of pain and inflammation.

Keywords: *Hymenodictyon floribundum*, analgesic, inflammation, writhes, acetic acid, hot plate, carrageenan, inflammatory biomarkers.

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LIST OF SYMBOLS AND ABBREVIATIONS

α_1	Alpha-1
α_2	Alpha-2
β	Beta
%	Percentage
>	Greater than
±	Plus or minus
≤	Less than or equal to
°C	Degree Centigrade
ABUCAUC	Ahmadu Bello university committee on animal use and care
AIDS	Acquired immune deficiency syndrome
ANOVA	Analysis of variance
ASA	Acetylsalicylic acid
ATP	Adenosine triphosphate
Cm	Centimeter
CNS	Central nervous system
COX	Cyclooxygenase
DALYs	Disability adjusted life years
DRG	Dorsal root ganglion
EDTA	Ethylenediamine tetraacetic acid
EEHF	Ethanol Leaf Extract of *Hymenodictyon Floribundum*
$FeCL_3$	Ferric chloride
G	Grams
GLB	Glibenclamide

H_2SO_4	Sulphuric oxide
HF	*Hymenodictyon Floribundum*
HIV	Human immune deficiency virus
i.p	Intraperitonealy
IASP	International Association for the Study 0f Pain
IL	Interleukin
IL-1ra	Interleukin-1 receptor antagonist
Kg	Kilogram
LD_{50}	Median lethal dose
mg	Milligram
Mg/kg	Milligram per kilogram
Ml	Millilitre
Ml/kg	Millilitre per kilogram
MPE	Maximum possible effect
n	Number of animals (per group)
NF-κB	Nuclear factor-kappa B
NIH	National institute of health
NLX	Naloxone
NO	Nitric oxide
NSAIDs	Non-steroidal anti-inflammatory drugs
OECD	Organization of Economic Co-operation and Development
p.o	Per oral
PAMPs	Pathogen-associated molecular patterns

PG	Prostaglandin
PGE	Prostaglandin E
PGE2	Prostaglandin E2
PMNs	Polymorphonuclear leukocytes
PRN	Propranolol
PRZ	Prazosin
Rf	Retardation factor
SEM	Standard error of mean
SPSS	Statistical package for the social sciences
Sub-P	Substance-P
TLC	Thin layer chromatography
TNF- α	Tumour necrosis factor - alpha
TNFR1	Tumor necrosis factor receptor 1
TNFR2	Tumor necrosis factor receptor 2
WHO	World Health Organization
YLD	Year lived with disability
YOH	Yohimbine

CHAPTER ONE

1.0INTRODUCTION

Pain is an indication of severity of illness as many diagnose diseases have underlying features of pain. (Thakur*et al.,* 2021). The International Association for the Study of Pain and the World Health Organization define pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (WHO, 2017; IASP, 2018). Pain remains one of the oldest challenges in medical history (Raffaeli and Arnaudo, 2017). Asides existing as a symptom of an underlying medical condition, pain also often present as a symptom of inflammation. The term inflammation is taken from the Latin word "inflammare" (to burn) and it is a defense mechanism of animal cells against certain painful injuries or microbial infections (Abdulkhaleq*et al.,* 2018). Inflammation may be an acute acute (Serhan *et al.,* 2015) or chronic process (Isailovic *et al.,* 2015).

Pain management involves the employment of both pharmacological and non-pharmacological strategies. The pharmacological management of pain includes the use of opioid analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), Cyclooxygenase (COX) inhibitors and anticonvulsant agents. Pain is also managed with non-drug therapies such as acupuncture, yoga, spinal manipulations, massage, lightning, etc.Despite the availability of these pain medications, many individuals still suffer from refractory pain. Undertreated or unrelieved pain places a high burden on individuals due to decreased productivity, and medical expenses (Gereau*et al*., 2014).

Work disability is one of the major problems in the society that is often linked to chronic pain. Chronic pain is one of the main reasons for work absenteeism. Tackling chronic pain

is a problem which not only affects the clinical settings but also because it reduces working ability and productivity, it affects society in its entirety (Dorner, 2018) resulting to low education, unemployment status and lack of social support (Baillyet *al*., 2015). The main anti-inflammatory drugs are either steroidal e.g., betamethasone, prednisolone, and dexamethasone (Mosa, 2014) or nonsteroidal e.g., aspirin, diclofenac, ibuprofen, indomethacin. naproxen, and celecoxib (Van Furth *et al.,*2013) used to treat inflammatory diseases such as osteoarthritis and rheumatoid arthritis (Van Laar*et al.,* 2012).

However, their prolonged use is associated with various side effects; for example, steroidal drug causes adrenal atrophy (Phalitakul *et al.*, 2011), osteoporosis, immunosuppression, euphoria, cataracts, glaucoma, and non-steroidal drug cause peptic ulcers and bronchospasm due to blockade of both the physiological and inflammatory prostaglandins and concurrent production of leukotrienes (Craig *et al.,* 2004). Thus, taking into account of the adverse effects (Burchum *et al.,* 2014) and high cost of available steroidal or non-steroidal drugs (Izuhara *et al*., 2011). The search for new anti-inflammatory agents from herbal sources is getting popular with the objective to obtain greater safety, better efficacy, and a more provident way to treat inflammation. First, concerns have been raised that modern pharmaceutical practice too often involves costly drugs that produce unacceptable side effects (Dhami, 2013); second, natural substance can address several modern health concerns (Benzie *et al*.,2011); and third, experience shows that modern medicine and traditional herbal medicine can be combined (Wee *et al.,* 2011). Moreover, there is a popular believe that natural is better (Zhang *et al.,* 2011).

Most drugs are naturally derived eg., atropine from *Atropa belladonna* (Solanaceae), reserpine from *Rauwolfia serpentina* (Apocynaceae), digoxin from *Digitalis*

purpurea(Scrophulariaceae), theophylline from *Camellia sinensis* (Theaceae), morphine and codeine from *Papaver somnifera* (Papaveraceae), quinine from *Cinchona officinalis* (Rubiaceae), and vincristine and vinblastine from *Vinca rosea* (Apocynaceae) (Qi *et al*., 2014). Interest in herbal products is increasing day by day, and this calls attention of many researchers and governments because the sales of natural products in the world have exceeded 0.1 trillion US dollars yearly (WHO, 2014). Over a century, the medications were totally of natural origin and extracted from inorganic materials, plant and animal products (Vane *et al.*, 2012).

Phytomedicine has gained recognition as the epitome of alternative medicine and have been utilized for the search of many bioactive substances used as medicines (Sen and Samantha, 2014).There are over 30 species of Hymenodictyonand several ethnobotanical claims have pointed to the use of *H. floribundum* as an effective anti-inflammatory and analgesic plants. *H. excelsum* for example has been scientifically proven to possess significant anti-inflammatory and analgesic properties (Kar *et al*., 2013).

1.1Statement of Research Problem

The prevalence of self-reported chronic pain in the adult general population is approximately 20% in developed countries with all ages being affected, women and the elderly being over-represented (Goldberg *et al.,* 2011). This may be higher in developing countries due to higher prevalence of pain-related diseases, such as diabetes, cancer, HIV/AIDS and arthritis. The prevalence of self-reported chronic pain is even more in developing countries like Nigeria due to inadequate pain management of which attention is often not on the pain itself but on the diseases emanating the pain. Evidence suggests that common barriers to effective pain management in developing countries include the low

priority given to pain management by government agencies, a lack of education in pain management, restriction of drug availability as a result of cost implications, poor patient compliance and a fear of addiction in relation to opioids (Colloca *et al.,* 2017). There is also a high tendency for healthcare professionals in developing countries to pay more attention to treating diseases causing pain, rather than relieving pain itself, and this may contribute to inadequate pain management (Rawal, 2016). Whether inadequate pain management affects prevalence is not known.

Studies of pain in children and adolescents have found that boys generally experience less pain than girls (King *et al*., 2011). Researchers suggest that lower income, lower level of education and unemployment are all associated with an increased prevalence of pain (McBeth and Jones, 2007). Several studies conducted in Africa have found a mean of one-year prevalence of low back pain of 50 % in adults and 33.0% in adolescents (Louw *et al.*, 2007). This appears to be slightly higher than the one-year prevalence for adults in studies conducted in mostly Western countries (mean 1-year prevalence of 38.1%) (Hoy, 2010). Approximately 75% of the population of the world relies on traditional medications of herbal origin for health care purpose as reported by the World Health Organization (Li *et al.,* 2014). They are not the only source of food (or shelter) but have also help the humankind to cure several diseases (Cseke *et al.,* 2016). There is also restriction in the use of available pain relievers related to cost and additional tendencies to opioids drugs.

1.2Justification

Pain is a pathological condition which negatively impacts the quality of life of individuals with an enormous economic implication (Uritu *et al.,* 2018). Pain conditions, especially chronic pain, place a significant burden on patients and patient care-givers. Pain is a

common symptom of many diseases and may also result from surgical interventions or trauma (Mohammadi, 2018). In most patients, it negatively influences the total knowledge of general health, interferes substantially with everyday activities, presently pain is not only a major problem globally, it is also responsible for the increasing number of disabilities worldwide (Gedin *et al*., 2017). It is also associated with depressive symptoms, and considerably and negatively affects relationships and interactions with others (Reid *et al.*, 2011). The interference with functioning and well-being tends to be greatly associated with increasing pain severity (Reid *et al.*, 2011).

In a research sponsored by World Health Organization to study the global burden of disease, it was found that conditions characterized or defined by the presence of pain (low back pain, neck pain, other musculoskeletal disorders and migraine) accounted for 5 of the top 10 conditions responsible for the most year lived with disability (YLD) globally (Vos *et al.*, 2017). Low back pain was responsible for 83 million disability-adjusted life years (DALYs) and was the greatest contributor of years lived with disability (YLD) of all conditions, accounting for 10.7% of all YLD (Hartvigsen,*et al.*, 2018).

In addition to the undeniable impact on a patient's quality of life, chronic pain also has accompanying undesirable financial consequences. Caring for those with chronic pain can also lead to financial costs, with the mean cost per adolescent experiencing chronic pain in the United Kingdom being approximately £8000 per year, including direct and indirect costs (Mahrer, *et al.*, 2018). It has been estimated that individuals with moderate to severe chronic pain lose an average of 8 days of work every 6 months, and 22% lose at least 10 working days (Reid *et al.*, 2011).

Treatment of pain and inflammation is largely achieved by the use of opioids and non-opioid analgesics such as the NSAIDs and COX-Inhibitors. However, the cheap, readily accessible and available analgesic like the NSAIDs have deleterious side-effects such as gastrointestinal bleeding and immunosuppression (Hougee, 2008), the relative safe COX-Inhibitors are often not affordable, whereas the opioids analgesics increase the susceptibility of patients to respiratory depression as well as abuse and addiction (Tick *et al.*, 2018). These entire unwanted phenomena have led to the shifting of attention towards complementary and alternative medicines for the management of pain.

Medicinal plants such as the willow plant have long been recognized as vital sources of therapeutically active compounds related to pain. Evidence-based research supports the medical

and pharmacological benefits of plant-derived compounds, with growing interest in the identification and characterization of bioactive compounds from natural sources (Sandoval *et al.*, 2002).

Though not documented, the leaves of *Hymenodictyonfloribundum* is claimed to possess analgesic and anti-inflammatory properties by the ethnic people of Zaria. However, there is no any documented scientific investigation done to ascertain its analgesic and anti-inflammatory activities.

1.5Aim of Research

The main aim of this work is to evaluate the analgesic and anti-inflammatory properties of *Hymenodictyon floribundum.*

1.6Specific Objectives

The specific objectives of the study are to;

1. Conduct acute toxicity study on the ethanol leaves extract of *H. floribundum*

2. Evaluate the analgesic activity of the ethanol leaves extract of *H. floribundum* in mice

3. Evaluate the anti-inflammatory activity of the ethanol leaves extract of *H. floribundum*

4. Determine the effect of the ethanol leaves extract of *H. floribundum* on the levels of inflammatory biomarkers (1L-β, IL-6, TNF-α, and PgE).

1.7Research Hypothesis

The ethanol leaves extract of *H. floribundum* possess significant analgesic and anti-inflammatory activities.

CHAPTERTWO

2.0 LITERATURE REVIEW

2.1 Pain

Conditions associated with pain are the biggest cause of disability globally (Vos *et al.*, 2017). Pain happens to be a complex experience, initiated by sensory information from an unpleasant stimulus, cultural and cognitive perspectives (Goebel and Molloy, 2021). It is also a designatecharacteristic that serves as a guide to the severity of numerous disease conditions, a prognostic measure, and a decisive factorused in health service (McBeth and Jones 2007).Poor quality healthcare system is a major contributor to the societal burden of pain (Buchbinde *et al.*,2018).

Inflammation is a compositeillness that is associated to inflammatory bowel condition, arthritis, neurodegenerative disorder, and cancer (Calixto *et al.*, 2004). It can be categorized into acute and chronic inflammation. The former occurs in response to trauma, infection (Ariel and Serhan, 2007). Inflammatory mediators including the vasoactive amines, histamine and serotonin, peptides, excitatory amino acids, nitric oxide (NO), cytokines; tumor necrosis factor-a (TNF-a), the interleukins IL-1 and IL-6, and eicosanoids (PGE_2 and the leukotrienes) causes inflammatory episodessuch as pain, swelling, and redness (Van *et al.*, 2000).

Mismanagement of the acute inflammatory response can lead to chronic inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed treatment for many inflammatory conditions. However, long-term use of NSAIDs such as ibuprofen, naproxen, diclofenac, celecoxib etc. are associated with gastrointestinal lesion, bleeding, peptic ulcer, and renal dysfunction. As a results complementary medicine approaches are

gaining in popularity among consumers in developing and developed countries due to less adverse effects and a relatively safer therapeutic profile (Wang *et al.,* 2014).

2.2 Risk Factors for Pain

2.2.1 Age and sex

Studies of pain in children and adolescents shows girls to undergo more pain than boys (King *et al.,* 2011; Wain *et al.,* 2014). Pain is more severe, more frequent and last longer in women than men (Unruh *et al.,* 1996; Macfarlane *et al.,* 2001; Thomas *et al.,* 2011). Prevalence in older people is inconsistent with some studies reporting an increase in prevalence with age and others reporting a decrease in prevalence with age (Abdullah *et al.,* 2013). It was widely believed that adults of working age are most likely to experience pain particularly musculoskeletal pain (Dionne *et al.,*2006). However, studies have found that pain remains serious problem in older age with a prevalence that ranges from 83% to 93% (Abdulla *et al.,* 2013).

2.2.2 Social (group) factors

Social factors are now recognized to play an important role on health care system (Viner *et al.,* 2012). Socioeconomic level is achieved by determining education, income or occupation. Studies on pain in children and adolescents have examined the association between socioeconomic status and pain (Jimenez *et al.,* 2011). In those studies, there has been conflicting evidence of a relationship (King *et al.,* 2011). In adults, there is a direct relationship between socioeconomic status and the prevalence of pain. Data suggest that lower levels of education, lower income and being unemployed are all related with an accelerated prevalence of pain (McBethand Jones,2007).

2.2.3 Individual factors

Individual risk factors are commonly probe in single studies, and most reviews find this challengingdue to lack of harmony across studies. (McBeth and Jones 2007; Taylo *et al.,* 2014). Various occupational factors such as high job demands, job insecurity, sedentary work, job dissatisfaction, and low levels of social support in the workplace have been linked with the onset of musculoskeletal pain (Côté *et al.*,2008; Hoy *et al.,* 2010).Psychosocial factors including stress, anxiety, depression and low self-esteem are directly associated with the prevalence of pain (Hoy *et al.,* 2010; King *et al.,* 2011). Inflammatory processes or nerve injuries are regarded to be the first trigger of chronic pain syndromes (Mogil *et al.,* 2012).

2.3 Epidemiology of Pain

Epidemiology, as defined by the World Health Organization is: "the study of the distribution and determinants of health-related states or events (including disease), and the applications of this study to the control of diseases and other health problems" (WHO, 2017).

Work disability is one of the major consequences associated with chronic pain in the society that is linked to the social dimension of pain. Chronic pain is one of the main reasons for work absenteeism, in form of sickness absences. However, many people tend to stay at their workplace despite chronic pain, with their working ability being strongly affected. (Lin*et al.,* 2020). Whether inadequate pain management affects prevalence is not really known but Interestingly, there is a lack of reliable statistics on the magnitude of chronic pain in both developed and developing countries, making economic planning even more challenging. The majority of population-based surveys estimating the prevalence of

chronic pain have been conducted by non-governmental research programs, implying that governments do not consider epidemiological data on pain as a public health priority (Dueñas *et al.*,2016)

In addition, most epidemiological research is funded by the developed world and accepts investigating teams from affluent countries, resulting in larger sample population sizes. By contrast, studies estimating prevalence in developing countries are rare and tend to be part of multinational surveys. Gureje *et al*., 1998 found that the prevalence of chronic pain ranged from 11.8 (Nagasaki, Japan) to 32.8% (Berlin, Germany) in developed countries and from 5.5 (Ibadan, Nigeria) to 33.0% (Santiago, Chile) in developing countries (Gureje *et al.,* 1998). Tsang and colleagues found that pain prevalence ranged from 38.4 to 49.6% in developed countries and from 24.1 to 60.4% in developing countries (Tsang *et al*., 2008). It is likely that the determinants of chronic pain will differ between developing and developed countries, with a probability that the prevalence of neuropathic pain will be higher in developing countries owing to the predominance of etiologies that damage the somato sensory nervous system. Expert panels have suggested that neuropathic pain is under diagnosed and undertreated in the Middle East (Bohlega *et al.,* 2010) and Asia Pacific (Rosales *et al.,* 2009).

Furthermore, differences in prevalence between developed and developing countries may be exacerbated when comparing urban and rural populations. In 1997, Volinn did a review that found that high-income countries havehigher prevalence than rural-based populations of low-income countries, so also the prevalence was also higher in urban populations of low-income countries than rural populations (Volinn *et al.,* 1997).The prevalence of pain in Nigeria ranged from 33% to 74% (Bello and Bello, 2017).

2.4 Classification of Pain

There are several variables for the classification of pain but the commonly used system includes, the pathophysiological mechanism of pain, duration of pain, the etiology of pain and the anatomic location of pain (Vadivelu *et al*., 2009; WHO, 2012).

2.4.1 Pathophysiological classification of pain

Pathophysiological classification of pain falls into two major types;

2.4.1.1 Nociceptive and neuropathic

The clinical discrepancyamong these pains is practicablesince the treatment modalities are different (WHO, 2012). Nociceptive pain is defined as noxious perception resulting from cellular damage following surgical, traumatic or disease-related injuries. It arises when tissue injury activates specific pain receptors called nociceptors which are sensitive to noxious stimuli. Nociceptors can respond to heat, cold, vibration, stretch stimuli and chemical substances released from tissues in response to oxygen deprivation, tissue disruption or inflammation (Fein, 2012).

Nociceptive pain has also been termed inflammatory pain because peripheral inflammation and inflammatory mediators play major roles in its initiation and development.

In general, the intensity of nociceptive pain is proportional to the magnitude of tissue damage and release of inflammatory mediators (Vadivelu *et al.*, 2009). Scientists distinguish between pain and nociception; where nociception refers to signals arriving in the CNS resulting from activation of specialized sensory receptors called nociceptors that provide information about tissue damage.

Pain then is the unpleasant emotional experience that usually accompanies nociception (Fein, 2012). Nociceptive pain can be subdivided into somatic and visceral pain depending on the location of activated nociceptors.

Somatic pain is caused by the activation of nociceptors in surface tissues (skin, mucosa of mouth, nose, urethra, anus, etc.) or deep tissues such as bone, joint, muscle or connective tissue. It is well localized and generally follows a dermatomal pattern. It is usually described as sharp, crushing, or tearing in character (Vadivelu *et al.*, 2009; WHO, 2012).

Visceral pain is caused by the activation of nociceptors located in the viscera (the internal organs of the body that are enclosed within a cavity, such as thoracic and abdominal organs). It can occur due to infection, distension from fluid or gas, stretching or compression, usually from solid tumours. It is generally poorly localized and non-dermatoma and is described as cramping or colicky. Moderate to severe visceral pain is observed in patients presenting with bowel or ureteral obstructions, as well as peritonitis and appendicitis (WHO, 2012).

Neuropathic pain on the other hand is defined as pain initiated or caused by a pathologic lesion or dysfunction in the peripheral nerves and CNS. It is caused by structural damage and nerve cell dysfunction (WHO, 2012). It is often intense and unrelenting and resistant to relief by available therapies (Fein, 2012). It is usually constant and described as burning, electrical, lancinating, and shooting. Disease states associated with classic neuropathic symptoms include infection (e.g., herpes zoster), metabolic derangements (e.g., diabetic neuropathy), toxicity (e.g., chemotherapy), and Wallerian degeneration secondary to trauma or nerve compression (Fein, 2012).

Clinical distinction between nociceptive and neuropathic pain is based on the anatomic origin of the stimulus, whether it is well-localized or diffuse, and the character of the pain (e.g., sharp, dull or burning). In some types of painful conditions, the pathophysiological mechanisms of pain are not well understood and/or cannot be demonstrated (WHO, 2012).

2.4.2Pain classification based on duration

Pain classification based on duration can be classified into two major types; acute and chronic pain. The acute pain according to IASP 2014, is pain of recent onset and probable limited duration; it usually has an identifiable temporal and causal relationship to injury or disease. It is of sudden onset, is felt immediately following injury, is severe in intensity, but is usually short-lasting (less than 30 days). It arises as a result of tissue injury stimulating nociceptors and generally disappears when the injury heals (WHO, 2012).

Although acute pain and associated responses can be unpleasant and often debilitating, they serve important adaptive purposes. They identify and localize noxious stimuli, initiate withdrawal responses that limit tissue injury, inhibit mobility thereby enhancing wound healing, and initiate motivational and affective responses that modify future behavior (Vadivelu *et al.,* 2009).

Acute pain generally comprises two phases; the first phase (lasting seconds) alerts the individual potentially dangerous stimuli and the second phase (sub-chronic phase, lasting hours to days) may be regarded as a protective mechanism characterized by guarding of the injured tissue as a means of promoting healing and recovery (Smith and South, 2008).

The chronic pain is defined as pain lasting for long period of time (Smith and South, 2008). It persists 3 to 6 months (or more) beyond the expected period of healing (Vadivelu

et al., 2009; WHO, 2012). It may begin as acute pain and persist for long periods or may recur due to persistence of noxious stimuli or repeated exacerbation of an injury. It may also arise and persist in the absence of identifiable pathophysiology or medical illness (Smith and South, 2008; WHO, 2012).

Chronic pain is often regarded as a maladaptive response that confers no physiological advantage, such that the pain state itself has become the disease, requiring treatment (Smith and South, 2008). It can negatively affect all aspects of daily life, including physical activities, school attendance, sleep patterns, family interactions and social relationships and can lead to distress, anxiety, depression, insomnia, fatigue or mood changes, such as irritability and negative coping behavior (WHO, 2012).

2.4.4 Anatomical classification of pain

Anatomical classification is the classification based often on the body location (e.g., head, back or neck) or the anatomic function of the affected tissue (e.g., myofascial, rheumatic, skeletal, neurological and vascular). However, location and function solely address the physical dimension and do not include the underlying mechanism. As such, although anatomical classifications can be useful for differential diagnoses, these classifications (WHO, 2012).

2.5 Management of Pain

Management of pain is categorized into a multidisciplinary approach that is essential to investigate all possible options for optimal management, which include Pharmacotherapy (most commonly employed), psychological support, physical rehabilitation, and interventional procedures.

2.5.1 Pharmacotherapy

Drug treatment is generally the first and most widely used treatment modality to control pain. It is relatively simple to implement and consists of NSAIDs, muscle relaxants, opioids, and other adjuvant therapy. Prescribing these medications is not without risks. However, the patient's cognitive, physiological, and functional status may be affected (Chamberloin *et al.,* 2007). Non-opioid analgesics include acetaminophen, NSAIDs such as ibuprofen, meloxicam, celecoxib and many more are used with caution because of the risk of developing gastrointestinal and renal toxicity, hypertension, heart failure, and other drug-drug and drug-disease interactions.

Opioids analgesics on the other hand, have potential to cause addiction, dependence and tolerance in the users. Not all pain management requires medication. Some pain medications, specifically opioids are high-risk medications that can put patients at risk for respiratory depression as well as abuse and addiction. Also, as more patients present with additional comorbidities, the risk that they may experience side effects or adverse events from opioids is increased (Tick *et al.*, 2018).

2.5.2 Non-pharmacotherapy

2.5.2.1 Psychological support

Because pain is a complex sensory and emotional experience, psychological modalities should be employed in the pain management model. The psychological branch of pain also explains why some patients with minimal disease may have excruciating pain, whereas others with severe disease may have minimal complaints. Pain-coping strategies may include relaxation, prayer, and attention-diversion techniques. Depression and anxiety in the geriatric patient must be addressed with psychotherapy and medication. Furthermore,

the socio-environmental variables of each patient should be adjusted to help the patient cope with pain. A solid support system including relatives and care givers should be established.

2.5.2.2 Physical rehabilitation

The rehabilitative aspect of pain management may help the patient live a more independent and functional life. Rehabilitation may involve adapting to loss of physical, psychological, or social skills. The objectives of rehabilitation include stabilizing the primary disorder, preventing secondary injuries, decreasing pain perception via a multidisciplinary approach, treating functional deficits, and promoting adaptations to current disabilities (Brumme *et al.*,1997).

2.5.2.3 Interventional modalities

Interventional pain modalities may help to determine the underlying cause of pain and help to arrive at a precise diagnosis. It often alleviates the need for heavy medication use, thereby sparing the patient from unwanted side effects associated with larger doses of drugs. Nerve blocks are some of the most commonly used interventional procedures employed by pain physicians; these help not only with diagnosis but also prognosis, preemptive analgesia, and sometimes definitive therapy. Other interventions that maybe used include chemical neurolysis, radio frequency lesioning, neuro-augmentation, and neuraxial drug delivery.

2.6 Barriers to Effective Pain Management

Barriers to effective management include challenges to proper pain assessment, underreporting of pain by patients, atypical manifestations of pain in the elderly, and a

need for increased appreciation of the pharmacokinetic and pharmacodynamic changes of aging (Kaye *et al.*,2010) Physicians can provide appropriate analgesia in patients through proper assessment, a multidisciplinary approach, and appropriate use of treatment modalities.

2.7 Inflammation

Inflammation is a salutary host response against invading pathogens or following sterile tissue injury. It is a biological reaction to a disrupted tissue homeostasis (Mescher *et al* 2017). Basically, it is a tissue-destroying process that involves the recruitment of blood-derived products, such as plasma proteins, fluid and leukocytes, into perturbed tissue (Tay *et al.*,2020). This migration is facilitated by alterations in the local vasculature that lead to vasodilation, increased vascular permeability, and increased blood flow (Medzhitov, 2008; Ashley *et al.*,2012).

The inflammatory response is a spatially and temporally orchestrated event in which cells and mediators collaborate to neutralize and eliminate the damaging stimuli to allow maintenance of homeostasis. Although the primary functions of inflammation are to rapidly destroy or isolate the underlying source of the disturbance, remove damaged tissue, and then restore tissue homeostasis which is a physiological and beneficial process. However, non-resolving inflammatory processes may be involved in the pathogenesis and progression of many inflammatory diseases, including asthma, atherosclerosis, rheumatoid arthritis, multiple sclerosis, rhinitis and ischemia-reperfusion injury (Medzhitov, 2008; Medzhitov, 2010; Alessandri *et al.*, 2013).

2.7.1 Inflammatory pathway

The inflammatory pathway (or mechanism of inflammation) consists of a tightly regulated cascade of immunological, physiological and behavioral processes that are orchestrated by soluble immune signaling molecules called cytokines (Ashley et al., 2012). The inflammatory pathway is classified into five, they are; inducers, sensors, mediators, effectors and resolution of inflammation (Medzhitov, 2008; Medzhitov, 2010; Ashley *et al.*, 2012).

2.7.2 Inducers of inflammation

Inducers of inflammation are defined as the signals that initiate the inflammatory response. They activate specialized sensors, which then elicit the production of specific sets of mediators. These inflammatory inducers may either be exogenous or endogenous (Medzhitov, 2008).

The inducer of inflammation is classified exogenous if the signals that initiate the inflammatory response originate from outside the organism or system. Exogenous inducers are further classified into two groups: microbial and nonmicrobial (e.g., allergens, irritants and toxic compounds).

The microbial inducers are further classified into two: pathogen-associated molecular patterns (PAMPs) and virulence factors. The first class of microbial inducer, PAMPs, is a limited and defined set of conserved molecular patterns that is carried by all microorganisms of a given class (whether pathogenic or commensal). PAMPs are defined in the sense that the host has evolved a corresponding set of receptors (known as pattern-recognition receptors) that detect their presence.

The second class of microbial inducer comprises a variety of virulence factors and is therefore restricted to pathogens. In contrast to PAMPs, they are not sensed directly by dedicated receptors. Instead, the effects of their activity, particularly their adverse effects on host tissues, are responsible for triggering the inflammatory response (Medzhitov, 2008).

The inducer of inflammation is classified endogenous if the signals that initiate the inflammatory response originate from within the organism or system (example include stressed or malfunction tissues) (Jiang *et al.*,2011*).*

2.7.3 Sensors of inflammation

The inflammatory inducers activate specialized sensors which detects and recognize invading pathogens of tissues damage. There is no clear-cut definition for inflammatory sensors. The sensors may sometimes be receptors or messengers. The inducers in most cases determine what sensor to respond. For example, the pore-forming exotoxins produced by Gram positive bacteria are detected by the NALP3 (NACHT-, leucine rich repeat- and pyrin-domain-containing protein) inflammasome, which is sensitive to the efflux of K+ ions that results from pore formation. Similarly, the proteolytic activity of proteases produced by helminthes is sensed by basophils by an unknown sensor.

Many damage signals are recognized by germ-line encoded receptors, such as transmembrane Toll-like receptors and intracellular nucleotide binding domain and leucine-rich-repeatcontaining receptors (NOD-like receptors) (Medzhitov, 2008; Proell *et al.,* 2008; Ashley *et al.,*2012).

2.7.4 Mediators of inflammation

Inducers of inflammation trigger the production of numerous inflammatory mediators, which in turn alter the functionality of many tissues and organs. Many of these inflammatory mediators have effects in common on the vasculature and on the recruitment of leukocytes. Inflammatory mediators can be classified into seven groups according to their biochemical properties; they are: vasoactive amines (e.g. histamine and serotonin), vasoactive peptides (e.g. sub-P), fragments of complement components (anaphylatoxins), lipid mediators (e.g. eicosanoids and platelet-activating factors), cytokines (e.g. tumour necrosis factor-α (TNF-α), interleukin (IL)-1 and IL-6), chemokines and proteolytic enzymes (e.g. elastin, cathepsins and matrix metalloproteinases) (Medzhitov, 2008).

2.7.5 Effectors of inflammation

The effectors of an inflammatory response are the tissues and cells, the functional states of which are specifically affected by the inflammatory mediators. The inflammatory mediators facilitate the recruitment of effector cells, such as monocytes and neutrophils, to the site of disturbance. The net effect of these interactions culminates in the stereotypical cardinal signs of local inflammation: heat, swelling, redness, pain, and loss of function (Medzhitov 2008; Medzhitov 2010; Ashley *et al.*, 2012).

2.7.6 Resolution of inflammation

Resolution is the last phase of inflammation; it is critical for limiting collateral damage to the host. After the first few hours of inflammation, a coordinated program of resolution is set into motion by tissue-resident and recruited macrophages. During acute inflammation, these cells produce pro-inflammatory prostaglandins and leukotrienes, but rapidly switch

to lipoxins, which block further neutrophil recruitment and instead favor enhanced infiltration of monocytes important for wound healing.

The resolution leads to the followings: 1) termination of the inflammatory response (mainly by diminishing granulocyte recruitment and reversing vasodilatation and vascular permeability); 2) switching from pro-inflammatory mediator generation to production of pro-resolution mediators; 3) turning off signaling pathways associated with cytokine production and leukocyte survival; 4) apoptosis of recruited inflammatory cells; 5) phagocyte clearance of apoptotic cells (especially by macrophages in a non-phlogistic process) and; 6) switching from pro-inflammatory cell phenotypes to pro-resolution phenotypes (especially relevant to macrophages) (Serhan and Savill 2005; Medzhitov 2008; Medzhitov, 2010; Ashley et al., 2012; Alessandri e*t al.,* 2013).

Summarily, Inducers are the signals that initiate the inflammatory response. They activate specialized sensors, which then elicit the production of specific sets of mediators. The mediators, in turn, alter the functional states of tissues and organs (which are the effectors of inflammation) in a way that allows them to adapt to the conditions indicated by the particular inducer of inflammation. After few hours of inflammation, macrophages will set in a coordinated program of resolution, in which there will be transformation from proinflammatory to anti-inflammatory mediators, from prostaglandins to lipoxins and finally from inflammation to resolution (Fullerton *et al*., 2016).

2.8 Cytokines

Cytokines are a family of glycosylated or non- glycosylated polypeptides and proteins. They are soluble hormone-like proteins that allow for communication between cells and

the external environment (Tayal and Kalra, 2008). Cytokines include lymphokine (cytokines made by lymphocytes), monokine (cytokines made by monocytes), chemokine (cytokines with chemotactic activities) and interleukin (cytokines made by one leukocyte and acting on other leukocytes). Their actions may include acting on the cells that secrete them (autocrine action), on nearby cells (paracrine action) or in some instances on distant cells (endocrine action) (Zhang and An, 2007). Changes in the circulating levels of these proteins have been linked to many disease states, making them valuable functional biomarkers. Excessive or diminished cytokine levels are associated with many clinical conditions and diseases such as CNS disorders, autoimmunity, cardiac diseases, fibromyalgia, toxicity, diabetes, bacterial infections, viral infections, tumours, allergies and asthma (Khare and Khare, 2014).

Pro-inflammatory cytokines are produced predominantly by activated macrophages and are involved in the up-regulation of inflammatory reactions. Various evidences have shown that certain pro-inflammatory cytokines (e.g., IL-1β and TNF-α) are involved in the process of pathological pain (Zhang and An, 2007).

IL-1β is released primarily by monocytes and macrophages as well as by non-immune cells, such as fibroblasts and endothelial cells, during cell injury, infection, invasion and inflammation. It has also been found that IL-1β is expressed in nociceptive dorsal root ganglion (DRG) neurons (Copray *et al.,* 2001). IL-1β has been found to increase the production of sub-P and prostaglandin E2 (PGE2) in a number of neuronal and glial cells Jeanjean *et al.,* 1995. IL-1 receptor antagonist (IL-1ra), a specific IL-1ra, competitively binds to the same receptor as IL-1β but does not transduce a cellular signal, thereby blocking IL-1β-mediated cellular changes. Administrations of IL-1ra and other anti-

inflammatory cytokines have been demonstrated to prevent or attenuate cytokine mediated inflammatory hyperalgesia (Maier *et al.,* 1993). Increased IL-1β production is associated with sepsis, type-2 diabetes, leukemia, atherosclerosis, schizophrenia, depression, sleep disorders, colitis, periodontitis, rheumatoid arthritis, myasthenia gravis and other inflammatory disease (Khare and Khare, 2014).

IL-6 has both pro and anti-inflammatory properties (Matthews *et al.,* 2010; Shaikh, 2011; Scheller *et al.,* 2011). Its pro-inflammatory activities include contributing to the development of neuropathic pain behavior (Ramer *et al.,* 1998). In addition, intrathecal infusion of IL-6 induces tactile allodynia and thermal hyperalgesia in intact and nerve injured rats, respectively (Zhang and An 2007). But it is said to possess more of anti-inflammatory properties (Matthews *et al*., 2010; Shaikh, 2011).

TNF-α, plays a well-established key role in some pain models; TNF acts on several different signaling pathways through two cell surface receptors, TNFR1 and TNFR2 to regulate apoptotic pathways, nuclear factor-κB (NF-κB) activation of inflammation, and activate stress-activated protein kinases. Intraplantar injection of TNF-α also produces mechanical (Cunha *et al.,* 1992) and thermal hyperalgesia (Perkins and Kelly 1994). TNFα plays a pivotal role in the pathogenesis of various diseases which include bacterial infection, viral replication, septic shock, rheumatoid arthritis, multiple sclerosis, celiac disease, type 1 and type 2 diabetes, crohn‘s disease, systemic lupus erythematosus, depression - schizophrenia - other inflammatory autoimmune and diseases (Khare and Khare, 2014).

2.9 Traditional Medicine

Traditional medicine as defined by the World Health Organization (WHO) is the sum total of all the knowledge, beliefs and practices that are used in diagnosis, prevention and elimination of physical, mental or social imbalance and rely exclusively on practical experiences and observation handed down from generation to generation (WHO, 2002). The elements of traditional medicines include therapies such as herbal medicine, massage, homeopathy, mud bath, music therapy, wax bath, reflexology, dance therapy, hydrotherapy, mind and spirit therapies, self-exercise therapies radiation and vibration, osteopathy, chiropractic, aromatherapy, preventive medicine, radiant heat therapy, therapeutic fasting and dieting spinal manipulation, psychotherapy, etc. (Adeshina, 2008).

Medicinal plants have been used by mankind for their therapeutic value (Aibinu *et al.*, 2007), as they enjoy wide acceptability by the population and serve as cheaper alternatives to orthodox medicine. Plant derived natural products such as flavonoids, terpenes and alkaloids have received considerable attention due to their diverse pharmacological properties including anti-inflammatory, antipyretic and analgesic activities (Shukla *et al.*,2010).

Orthodox medicine somewhat minimized the use of herbs but the development of resistance against orthodox medicine by pathogens, high costs as well as the lack of availability of some of these drugs has in recent times, begun to reverse this trend (Awodele *et al.*,2012). This development is fortified by the notion that all herbal products are safe, effective and have minimal or no side effects (Adam *et al.*, 2011).

2.9.1 Medicinal plants with analgesic and anti-inflammatory activities

Many plants have been scientifically evaluated to possess analgesic and anti-inflammatory properties. These plants include; *Newbouldia laevis* (Usman *et al*., 2008), *Tacazzea apiculata*, Ganodermaap planatum (Ede *et al*., 2012), *Tamarindusindica* (Ukwuani and Hassan, 2014), Vitis vinifera (Singh *et al*., 2009, *Kalanchoe pinnata*(Matthew *et al.*,2013), *Carissa edulis* (Hassan *et al*., 2010), *Cassia occidentalis* (Vijayabhaskar *et al.,* 2013), *Securinega virosa* (Yerima *et al.,* 2009), Ficus ingens (Aiyelero *et al*., 2009), *Argyreia argentea* (Rahman *et a*l., 2010), *Zingiber officinal*e, *Ananas comosus*, *Calotropis procera, Mangifera indica* and *Sidacordifolia* (Anilkumar, 2010), *Dalbergia saxatilis* (Ismail *et al.,* 2015) and more.

Plants have been contributing to the development of modern analgesic and anti-inflammatory drugs. Over the years, natural products have contributed enormously to the discovery of drugs for use in modern medicine. It is estimated that about 40% of all medicines in the market today have been derived from natural sources, 25% being from plants, 13% from microorganisms and 3% from the animals. Some of the principal plants that have contributed to the development of modern analgesic and anti-inflammatory drugs are; *Papaver seminiferum*, salix specie (with over 350 species among which is Salix alba), *cannabis sativa* (with over 60 cannabinoids; some of which are analgesic and anti-inflammatory agents), Capsicum specie (e.g., C. plaster, C. annuum), *Panax ginseng*, *tanacetum parthenium*, Acotinum specie, *Hedyosmum brasiliense*, Phyllanthus species, Protium species etc. (Calixto *et al.*,2009).

There are over 120 distinct chemical substances derived from plants that are considered important drugs currently in use in various countries in the world. Among these drugs are

potent analgesic and anti-inflammatory agents; they include aesin (derived from *Aesculushippocastanum*), borneol (from several Artemisia species), bromelain (from Ananascomosus), codeine and morphine (both are from *Papaver somniferum*), rotundine (from Stephania sinica) and tetrahydropalmatine (from Corydalis ambigua) (Calixto *et al.*,2009).

2.10 Hymenodictyonfloribundum

The Hymenodictyongenus comprises 22 species and of these, 11 are endemic to Madagascar, 4 to Asia and 7 to Tropical Africa (Razafimandimbison and Bremer *2006).* *Hymenodictyonfloribundum* B.L. Rob. (Rubiaceae), endemic to Tropical Africa, is a small tree that grows in the mountains of the Huíla province and its traditional name is NDambiYov`olwi, (omu)Lia-tyimeme. Its trunk bark is used in Angola folk medicine to treat fever (Bossard, 1996). A previous study has shown that the trunk bark of this tree contains scopoletin, hymeselsin, scopolin and 3-O-βD-glucopyranosyl-β-sitosterol (Mitaine *et al.,* 2003).

Although trunk bark components of *H. floribundum* have been well studied, there has as yet been no study which focuses solely on the leaves of this medicinal tree. The major compound trunk bark extract is scopoletin (Borges *et al.,* 2010).

2.10.1 Economic uses

Hymenodictyonspecies have various local uses. For example, the leaves of *H. obovatum* and *H. orixense* are used for dye, the bark as febrifuge, and the inner bark and roots for treating fever in India. The bark of *H. parvifolium* is used in Kenyan folk medicine as a remedy for skin diseases, veneral diseases, and dysentery (Mathias, 1982). In Tanzania, an

infusion from the plant in combination with other plants is commonly used for treatment of insanity when the patient is noisy, abusive, and suicidal (Mathias, 1982).

Kariba (2002) revealed that the extracts from H. parvifolium had antifungal and antibacterial properties. Furthermore, hymexelsin (also called xeroboside), which is a glycoside derivative of scopoletin and β-sitosterol, has been found in both H. floribundum (Mitain *et al.*, 2003) and H. orixense (Rao *et al.*, 1988). In contrast, no local uses of any Malagasy Hymenodictyonand Paracorynanthe species have been recorded in Madagascar.

2.10.2 Habitat

The plants of Hymenodictyonare generally medium sized to emergent trees up to 35 m tall, while the two Paracorynanthe species are medium-sized trees up to 15 m tall. H. epiphyticum is an obligate epiphyte up to 3 m tall, whereas both *H. biafranum* and *H. flaccidum* are facultative epiphytes, which occasionally grow on rocky substrates and become tall trees (up to 15 m) (Razafimandimbison and Bremer 2006).

2.10.3 Leaves

The leaves of Hymenodictyonare simple, opposite, decussate, subcoriaceous or coriaceous, and sometimes membranaceous. There is great variation in the length of petioles and the size of leaf blades. The shortest petioles are found in *H. epiphyticum* and the longest ones in *H. embergeri Cavaco* and H. *occidentale Homolle*. Most Hymenodictyonspecies have smallto medium-sized leaves, but the West African H. pachyantha has the largest leaf blades, 8–31 × 5– 11 cm. The leaf blades are mostly glabrous but can be scabrous (e.g.,*H. parvifolium* ssp. scabrum and all studied collections of *H. occidentale* from the Ankazoabo District, Toliara Province, Madagascar received from P herbarium), puberulous or

pubescent (e.g.,*H. berivotrense*, *H. louhavate*, H. *septentrionaleCavaco)*, or even tomentose (some individuals of *H. perrieriDrake and H. floribundum*).

Therefore, leaf indumentum is useful for species recognition in Hymenodictyon. In general, the secondary and tertiary veins of the leaf blades are adaxially prominulous but abaxially prominent (Jacobs, 1966).

2.10.4 Flowers

The flowers in Hymenodictyonare hermaphroditic, protandrous, typically pedicellate, rarely non pedicellate (e.g., *H. decaryi*, *H. obovatum),* and typically fivemerous, rarely six- or seven-merous. Calyx tubes are absent, but the calyx lobes are well developed and show great variation in indumentums length, shape, and size, useful for species recognition. Corollas of most Hymenodictyonspecies are narrowly tubular up to the midpoint and abruptly open out into cups Corollas are, however, progressively widened (Razafimandimbison and Bremer 2006).

2.10.5 Fruits

The fruits in Hymenodictyonspecies are broadly to narrowly elliptic, lenticulate, grey-brown-tinged, and typically pedicellate. The pedicels, absent in *H. obovatum*, are woody, typically coarse and lenticulate but rather slender and non-lenticulate in both *H. biafranum* and *H.epiphyticum*. The lenticels on the fruits can be elongate or spherical and are typically not elevated, except in *H. perrieri*. Both the pedicels and placentae continue to grow during the fructification time. When the fruits reach maturity the two locular capsules dehisce along the inserting point of the placentae and release the accrescent placentae and seeds at the same time. The fruits of Paracorynanthe are relatively similar to those of Hymenodictyon, except they are grey-redtinged and slightly bilaterally flattened, and

contain two or one seeds per locule. The pedicels are also woody but slender(Razafimandimbison and Bremer 2006).

2.10.6 Seeds

The seeds of Hymenodictyonare reticulate, bilaterally flattened, mostly broadly winged all around, and deeply bifid at the base. In *H. biafranum* and *H. epiphyticum*, they are broadly winged at both ends but narrowly winged along both lateral sides. The shape and size of the wings vary among the species and thus can be useful for species recognition. The smallest seeds are found in *H. biafranum* and *H. epiphyticum* and the largest seeds in some of the Malagasy species (e.g.,*H. louvahate*, *H. occidentale*). The seeds of Paracorynanthe species are also reticulate, bilaterally flattened but narrowly winged along the lateral sides and the lower part of the seeds, and broadly winged in the upper part (Razafimandimbison and Bremer 2006).

2.10.7 Chromosome number

The chromosome number of Hymenodictyonisusually eleven in number (Kiehn, 1986).

2.10.8 Ecology

Sixteen of the 22 Hymenodictyonspecies are restricted exclusively to dry, deciduous forests. In contrast, two African species, *H. biafranum* and *H. epiphyticum*, grow in evergreen rainforests, and two Malagasy species, *H. embergeri* and *H. perrieri*, are confined, respectively, to the sub-humid forests of the Andohahela and Sambirano regions, and the Marojejy and Sambirano regions (Razafimandimbison and Bremer 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Animals

Adult male Wistar rats (150-200 g) and male Swiss Albino mice (18-25 g) obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria were used for the study. The animals were housed in a well-ventilated room in their cages and provided with a normal rodent feed and water *ad libitum* until the end of the study. All the experiments (with the exception of the hot plate pain model) were carried out in the main laboratory of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria while the hot plate pain model was carried out in the Research laboratory of the Department of Pharmacology and Therapeutics, Faculty of Pharmacy, Bayero University, Kano, Kano state, Nigeria. All

experiments were conducted in accordance with the guideline of National Institute of Health (NIH, 1998). Ethical approval number of ABUCAUC/2020/011 for the use of animals was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC).

3.1.2 Equipment

The equipment used for the studies include digital weighing balance (AE240 dual range, Mettlerinstrument corporation, *USA*), digital Vernier caliper 52 *(*Battenfeld Technologies Inc. USA), Ugo Basile hot plate *(*Model 7280, Germany), animal cages, dissecting kits, stop watch, observation chamber, funnel, cotton wool, desiccators, water bath, mortar and pestle, scissors, syringes (1ml, 2ml and 5ml), Petri dishes, cannula, hand gloves, EDTA tubes, sample bottles and laboratory record book.

3.1.3 Drugs, Chemicals and Reagents

Drugs and chemicals used for the studies include glacial acetic acid (May and Baker limited,Dagenham, England), ethanol, carrageenan, chloroform (Sigma Aldrich, St. Louis Mo, USA), naloxone hydrochloride, prazosin hydrochloride, glibenclamide, yohimbine hydrochloride, propranolol hydrochloride (Abcam Plc, Cambridge, UK), morphine sulphate (Martindale Pharmaceuticals, U.K), piroxicam (Rotex Medica, Germany), hydrochloric acid, sulphuric acid (May and Baker, UK), ferric chloride anhydrous (Avishkar, India), ammonia (Lobachemie, India), all the reagents were of analytical standard grade.

3.2Methods

3.2.1 Collection and Identification of Plant Material

The plant material was collected from Kargi Hill along *Birnin Gwari*Road, Zaria Local Government Area, Kaduna state in October 2019. The plant was identified andauthenticated by a taxonomist, Malam Sanusi Namadi of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, with a voucher number (ABU900124).

3.2.2 Extraction of the plant material

The extraction was carried out according to the method described by Kupchan *et al.* (1973). The leaves of constant *Hymenodictyonfloribundum* were dried in a shaded environment and intermittently weighed until weight was obtained and then sized reduced into fine powder with the aid of a mortar and pestle. The powdered leaf material was soaked in (70%$^{w}/_{v}$) ethanol continuously for 72 hours for exhaustive extraction using soxlet apparatus. The extracted material was put on a water bath set at 50°C to remove the solvent, and the obtained ethanol leaf extract of *Hymenodictyon floribundum* (EEHF) was stored. Solutions of the extract were prepared freshly with distilled water for each study. The percentage yield of the extract was calculated using the formula:

$$\%\, Yield = \frac{weight\ of\ the\ extract}{weight\ of\ the\ powdered\ material} \times 100$$

3.2.3 Qualitative phytochemical analysis

The EEHF was subjected to phytochemical screening in accordance with standard protocol as described by Evans (2009). Simple chemical tests were used to detect the presence of

secondary metabolites such as alkaloids, flavonoids, saponins, cardiac glycosides, tannins, anthraquinones, carbohydrates, steroids and triterpenes.

3.2.3.1 Test for alkaloids

- ✓ Wagner test:

  Two drops of Wagner's reagent added to a portion of the extract and which gives a white precipitate indicates the presence of alkaloids.

- ✓ Dragendoff's test:

  Two drops of Dragendoff's reagent were added to a portion of the extract and which gave a reddish-brown precipitate also indicates the presence of alkaloids.

3.2.3.2Test for flavonoids

- ✓ Shinoda test:

  Few magnesium chips and 3 drops of hydrochloric acid added to the extract and which reveals pink color within few minutes is an indicative of the presence of flavonoids.

- ✓ sodium hydroxide tests:

  Two drops of aqueous sodium hydroxide added to the extract and which yields a yellow coloration indicates the presence of flavonoids.

3.2.3.3 Test for saponins

- ✓ frothing test:

  About 5 ml of distilled water added to a portion of the extract in a test tube and shaken vigorously for 30 seconds and allowed to stand for 30 minutes for the occurrence of persistent honey comb-like column indicating the presence of saponins

3.2.3.4. Test for cardiac glycosides

- ✓ Keller-Killiani test:

Extract in a test tube was dissolved with glacial acetic acid containing 5% ferric chloride (FeCl3) held at 45 degrees and 1 ml of sulphuric acid (H_2SO_4) added to the solution down the side and observed for formation of purple ring colour at interphase indicating the presence of cardiac glycoside.

3.2.3.5 Test for tannins

- ✓ Ferric chloride test:

Two drops of $FeCl_3$ solution added to a portion of the extract and observed for a blue or green precipitate indicating the presence of tannins.

3.2.3.6 Test for triterpenes

- ✓ Lieberman-Burchard test:

One milliliter (1 ml) of acetic anhydride and 1ml of chloroform were mixed with the extract and a few drops of sulphuric acid added along the side of the test tube that shows a violent color which gradually changed to blue green ring indicating the presence of triterpenes.

3.2.3.7 Test for steroids

- ✓ Salkowski test:

About two milliliters (2 ml) of chloroform and few drops of sulphuric acid carefully added to a portion of the extract from the test tube and which form a lower layer of reddish-brown ring indicating the presence of steroids.

3.6.8. Test for anthraquinones

- ✓ Bontrager test

About two milliliters (2 ml) of chloroform added to a portion of the extract and shaken for at least 5 minutes was filtered and the filtrate shaken with equal volume of 10% ammonia

solution to observed for a bright pink color in the aqueous (upper) layer indicating the presence of anthraquinones.

3.2.3.9 Test for carbohydrates

- ✓ Molish test:

Two drops of molish reagent added to a portion of the extract and then concentrated sulphuric acid was added down the side of the test tube to observe for a lower layer of reddish colored ring at the interphase that signifies the presence of carbohydrates.

3.2.3.10 Thin layer chromatography

Thin layer chromatographic profile of the extracts of *H. floribundum* was conducted using one-way ascending technique to provide an idea of the quantities of the phytochemicals present in the extract usingTLC plates (20 x 20 cm) coated with silica gel 60 F254 (Stahl, 2005). The extract was dissolved in the initial extraction solvent. The plates were cut into sizes of 5 x 10 cm well and spots of the extraction solvent dissolved extract were applied manually on the wells using capillary tube. The plates were then dried and developed in the chromatographic tank. Developed plates were sprayed with both general detection reagent (p-anisaldehyde/H_2S0_4) and specific detecting reagent (ferric chloride, Lieberman-Buchard and aluminum chloride) and heated at 110°C.

3.7 Acute Toxicity Studies

LD_{50} determination was conducted using Organization for Economic Co-operation and Development (OECD) 423 guidelines in rats and mice. In this method, two groups each of three animals were fasted prior to dosing (food but not water was withheld overnight for rats and 3 hours for mice). The fasted body weights of the animals were used to administer the extract and food was then further withheld for 3-4 hours in rats and 1-2 hours in mice

after the extract had been administered. The test substance was administered in a single oral dose using oral canula. A dose of 2000 mg/kg was used for each animal in the first phase and observed for 48 hours for any clinical sign and symptom of toxicity including death. A dose of 5000 mg/kg was used for the second face and observed for signs and symptoms of toxicity at least once every 30 minutes for the first 4 hours and then daily for 14 days. After the 14 days, the study was terminated. At the end of the test, surviving animals were weighed and sacrificed using chloroform.

3.3 Evaluation of Analgesic Activities in Mice

3.3.1 Acetic acid-induced writhing in mice

The method previously described by Koster *et al.*(1959) was adopted for this study. Thirty (30) male Swiss albino mice were divided into 5 groups of six (6) mice. Mice in group I were administered with distilled water (10 ml/kg, *p.o*) and served as a negative control, while mice in group V received morphine (10 mg/kg, *p.o.*) and served as a positive control. Group II, III and IV received graded doses of EEHF (375, 750 and 1500 mg/kg respectively) via oral route. Sixty (60) minutes after oral administration, acetic acid 0.6% v/v (10 ml/kg) was administered to each mouse via intraperitoneal route and was placed in an observation cage. Five (5) minutes after acetic acid injection, the number of writhes was counted for each mouse for a period of 10 minutes. A reduction in the number of writhes as compared to the vehicle treated control animals was considered as evidence for the presence of analgesia and expressed as percent inhibition of writhes.

$$\% \, Inhibition \; = \frac{Mean\ No.of\ writhes\ (control) - \ Mean\ No.of\ writhes\ (test)}{Mean\ No.of\ writhes\ (control)} \times 100$$

3.3.2 Hot plate test in mice

The method of Eddy and Leimbach (1953) was adopted.Ugo Basile hot plate (model 7280, Germany) at the Research laboratory of the Department of Pharmacology, Faculty of Pharmacy, Bayero University Kano, Nigeria was used for the study. Mice were individually placed on a hot plate (55±1°C), before drug treatment so that each animal served as its own control. The time taken for the animal to either lick the paw, withdraw the paws or jump off the hot plate was taken as reaction time and recorded. A cut-off time of 30 sec was used to avoid damage to the paw. Male Swiss albino mice (30) were divided into 5 groups of six (6) mice each. Mice in group I were administered distilled water (10 ml/kg, *p.o*) and served as a negative control, while group V mice received morphine (10 mg/kg, *i.p*) and served as a positive control. Group II, III and IV received graded doses of EEHF (375, 750 and 1500 mg/kg respectively) via oral route. The latency was observed and recorded after 0, 60, 90, 120 and 150 minutes. The prolongation of the latency times was taken as an analgesic response (percentage maximum possible effect MPE).

$$\%\, MPE\ = \frac{Test\ - Baseline}{Cut\ off\ - Baseline} \times 100$$

Where;

Test = latency response after treatment

Baseline = latency response prior to treatment

Cut – off = latency time at 30 seconds

3.8.3 Investigation of probable mechanisms of analgesic activities

The participation of various pain pathways in the analgesic activities of the extract was investigated using mouse model of acetic acid induced writhing as previously described by

Rangel *et al*., 2012. The most active dose of *Hymenodictyon floribundum* extract was subjected to mechanistic studies. The pain pathways investigated and the receptor antagonist/blocker used for the studies were as follows:

a) Opioidergic, using naloxone (a non-selective opioid receptor antagonist, 2 mg/kg, *i.p*),

b) α_1-adrenergic, using prazosin (an α_1-adrenoceptor antagonist, 1 mg/kg, *i.p*),

c) α2-adrenergic, using yohimbine (an α_2-adrenoceptor antagonist, 1 mg/kg, *i.p*),

d) β-adrenergic, using propranolol (a non-selective β-adrenergic blocker, 20 mg/kg, *i.p*),

e) Potassium ATP, using glibenclamide (a *K*ATP channel blocker, 5 mg/kg, *i.p*),

For each of the pathway, thirty (30) mice were used and randomly divided into 6 groups (n=5). All the groups were treated as follows:

Group I: Distilled water (10 ml/kg, p.o) alone

Group II: EEHF (1,500 mg/kg, p.o) alone

Group III: Morphine (10 mg/kg, p.o) alone

Group IV: Receptor antagonist/blocker (As mentioned above depending on the pathway involved).

Groups V: Pretreated with receptor antagonist/blocker 30 minutes before administering EEHF (1,500 mg/kg, oral).

Group VI: Pretreated with receptor antagonist/blocker 30 minutes before administering morphine (10 mg/kg, oral).

Sixty minutes post treatment (30 minutes for groups IV), mice were challenged with acetic acid induced writhing test adopting the method previously described by Koster *et al.,1*959.

3.4 Evaluation of Anti-inflammatory Activity

3.4.1 Carrageenan-induced paw oedema in rats

The anti-inflammatory study was carried out using the carrageenan-induced paw oedema in rats according to the method previously described by Winter *et al*. (1962). Thirty male rats were divided into 5 groups each containing 6 mice. Group I was orally administered with distilled water (10 ml/kg) which served as a negative control, while group V received piroxicam (10 mg/kg) which served as a positive control. Group II, III and IV received graded doses of EEHF (375, 750 and 1,500 mg/kg) respectively. Sixty minutes post treatment, each rat was injected with 0.1 ml of 1% carrageenan into sub-plantar surface of the right hind paw. The hind paw oedema was measured and recorded at times 0, 1, 2, 3, 4 and 5 hours using vernier caliper to determine the diameter of the oedema. The increase in paw diameter (oedema index) for each rat was calculated as the difference in paw diameter before carrageenan injection and after carrageenan injection at each time interval, while the percentage inhibition of oedema was calculated for each group using the following relationship;

$$\% \text{ Inhibition } = \frac{\text{m} - \text{n}}{z} \times 100$$

Where;

m = Mean increase in paw volume of control group

n = Mean increase in paw volume of treated group

z = Mean control increase in paw volume of control group

3.4.2 Investigating the involvement of inflammatory cytokines of the anti-inflammatory activities of *Hymenodictyonfloribundum*

The method previously described by Santos *et al*., 2003 was used to investigate the role of inflammatory cytokines. Thirty male rats were divided into 5 groups each containing 6 rats. Group I was administered distilled water (10 ml/kg) orally which served as a negative control, while group V received piroxicam (10 mg/kg) and served as a positive control. Group II, III and IV received graded doses of EEHF (375, 750 and 1,500 mg/kg) respectively. Sixty minutes post treatment, each rat was injected with 0.1 ml of 1% carrageenan into the sub-plantar surface of the right hind paw (Winter *et al*., 1962). The hind paw oedema was measured and recorded at times 0,1, 2and 3hours using Vernier caliper to determine the diameter of the oedema. Three hours after carrageenan injection, the rats were anaesthetized using chloroform and blood was collected from orbital sinus into the ethylenediamine tetra-acetic acid (EDTA) bottle. The blood was centrifuged at 3000 rpm for 20 minutes and the plasma was stored at -20 °C for the estimation of plasma pro-inflammatory biomarkers. The levels of PGE_2, IL-1β, IL-6, and TNF-α were determined according to the manufacturer's instructions on the test kids used.

3.5 Statistical Analysis

Results were expressed as mean ± standard error of mean (SEM) and presented as graphs, figures and tables where appropriate. Data obtained were analyzed using Independent Student t-test, one-way analysis of variance (ANOVA) and Mixed design ANOVA in SPSS version 25, followed by Dunnett and Bonferroni's post- hoc test for multiple comparison where appropriate Results were considered significant at $p \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage Yield of the Ethanol Leaf Extract of *HymenodictyonFloribundum*

The percentage yield of a dark brown ethanol Leaf Extract of Hymenodictyonfloribundum was 10.4%.

4.2 Phytochemical Constituents

The preliminary phytochemical screening of the ethanol leaf extract of *H. floribundum* revealed the presence of several phytochemicals as shown in Table 4.1.

Table 4.1: Phytochemical Constituents Present in the ethanol Leaf Extract of *Hymenodictyonfloribundum*

Phytochemical constituents	Inference
Alkaloids	present
Cardiac glycosides	present
Saponins	present
Tannins	present
Triterpenes/Steroids	present

Terpenoids	present
Carbohydrates	present
Anthraquinones	present
Flavonoids	present

SOLVENT-I	**SOLVENT-II**	**SOLVENT-III**	**SOLVENT-IV**

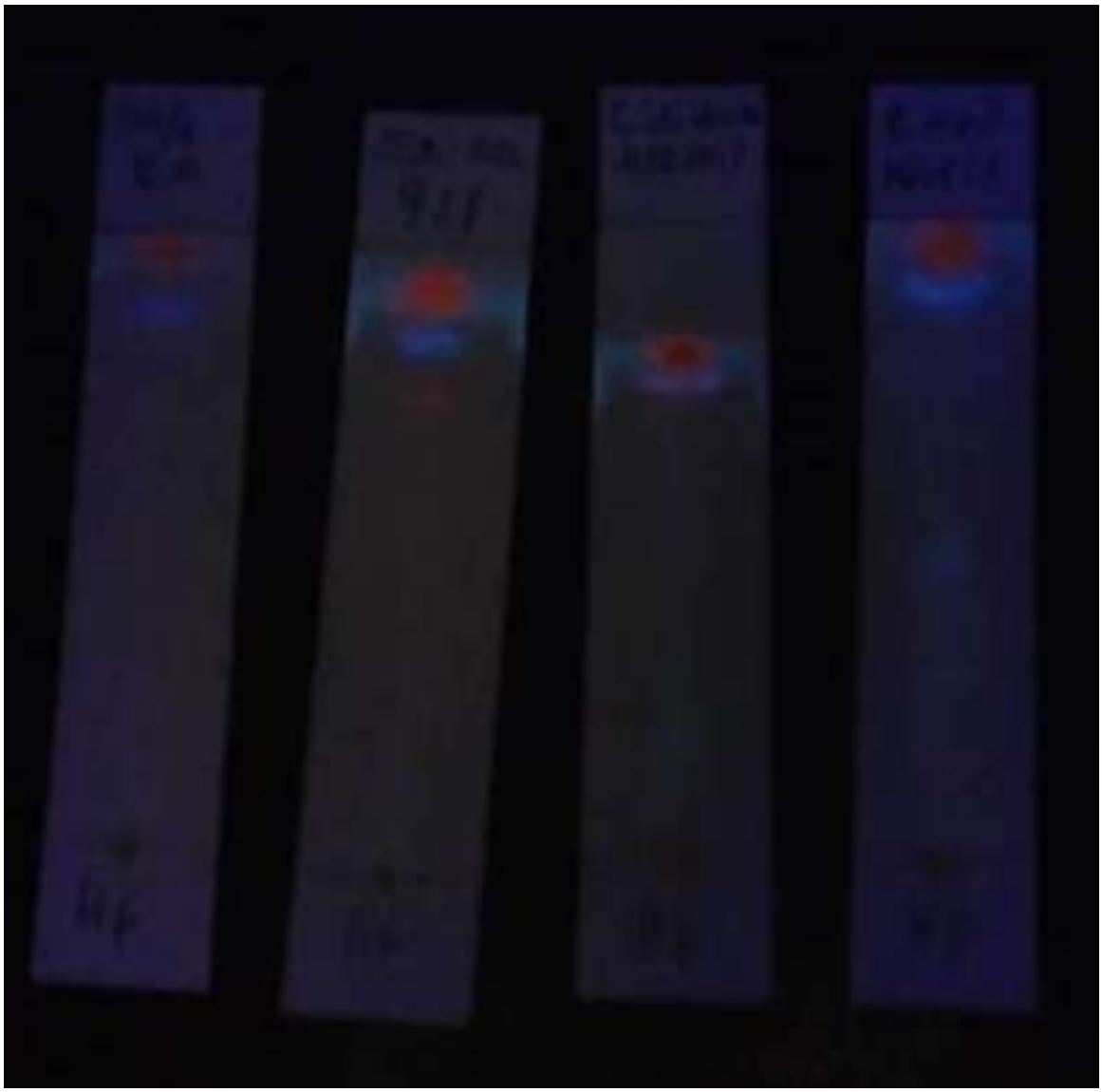

Figure 4.1: TLC plate with: Solvent-I: Ethyl acetate (EA-100%), Solvent system-II: Ethyl acetate + Methanol (EA: Me 9: 1), Solvent-III:chlorophyl +Ethyl acetate + Methanol+ water (CEMW 4:8:4:1) and Solvent-IV: n-butanol + acetic acid + water (BAW 10:1:1).

Figure 4.2: TLC Chromatogram of theextract sprayed with a general spraying reagent (P- anisaldehyde/H_2SO_4)

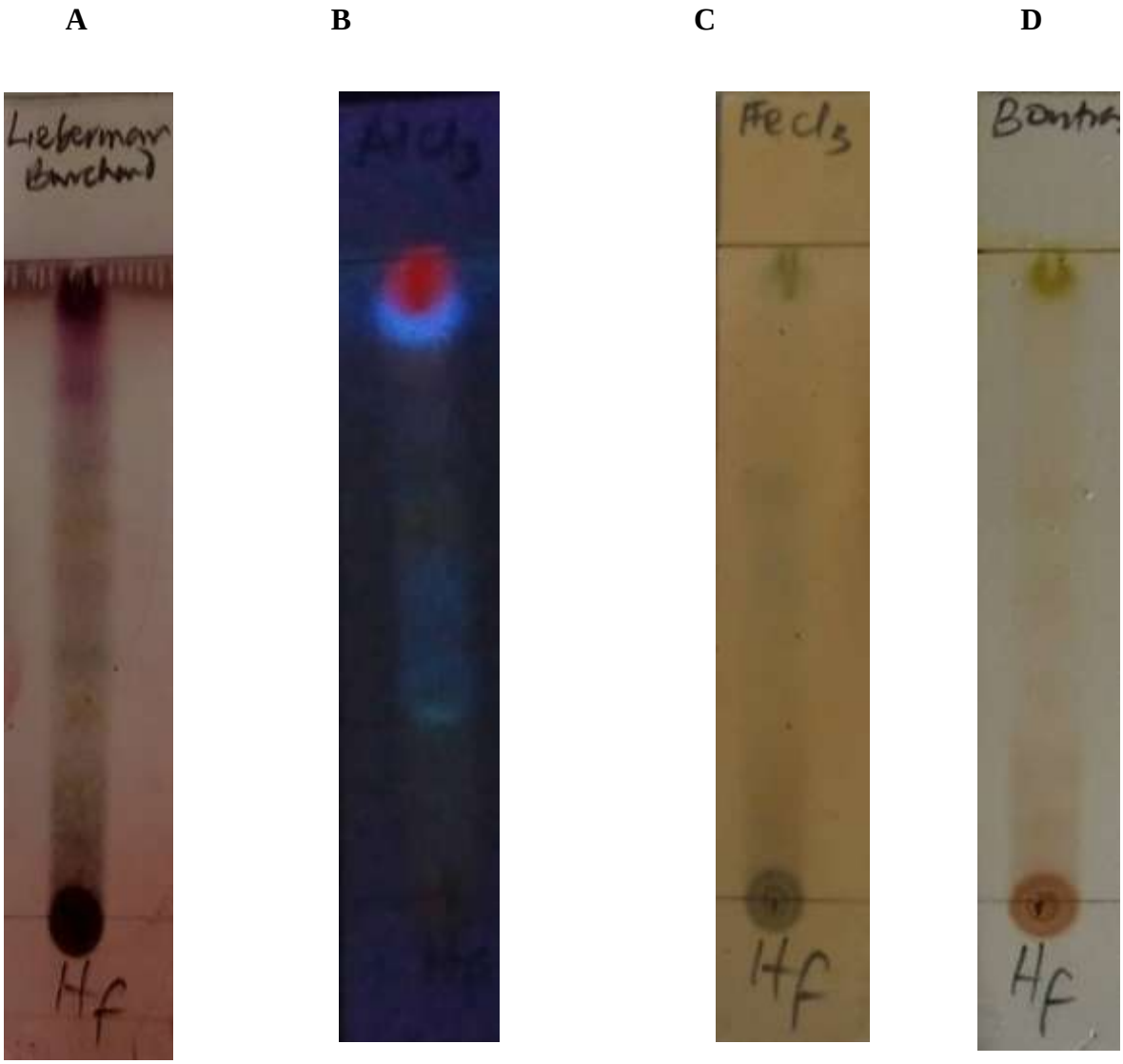

Figure 4.3: TLC Chromatogram of theextract sprayed with Liebermann Bucchard (LB) Reagent (A), Aluminium Chloride ($AlCl_3$) (B), Ferric Chloride ($FeCl_3$) (C), and Bontrager's reagent (D)

4.3 Median Lethal Dose (LD_{50}) of Ethanol Leaf Extract of *Hymenodictyonfloribundum*

The LD_{50} of the ethanol leaf extract of *H. floribundum* was estimated to be greater than 5000mg/kg body weight.

4.4 Effect of Ethanol Leaf Extract of *H. floribundum* on Acetic-acid induced Writhes in Mice

The extract was able to alleviate pain by reducing the number of writhes observed in the animals. The decrease was statistically significant ($p < 0.05$) compared to distilled water and was dose-dependent with mean number writhes of 14.8 ± 1.40, 12.5 ± 1.48 and 7.7 ± 0.49 for 375, 750 and 1500 mg/kg dose of the extract respectively. This low number writhing was more significant ($p<0.01$) in the highest dose of 1500mg/kg than with the other two doses ($p<0.05$) compared to distill water treated group. As expected, morphine produced the most analgesia with a mean writhe number of 0.5 ± 0.5 as shown in figure 4.4.

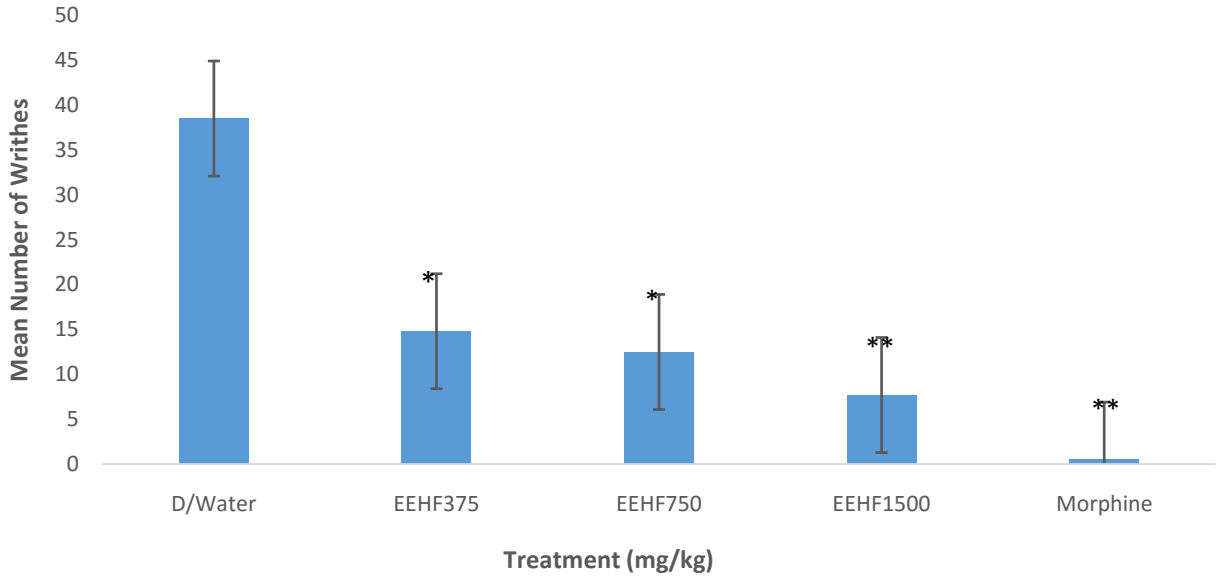

Figure 4.4: Effect of Ethanol leaf Extract of *H. floribundum* on Acetic-acid induced Writhing in Mice

N = 6. Data was analyzed using one-way analysis of variance (ANOVA) followed by Dunnett Post-hoc Test.* $p < 0.05$ compared to Distilled water, ** $p < 0.01$ compared to Distilled water. EEHF = Ethanol Extract of *Hymenodictyonfloribundum;* S.E.M. = Standard Error of Mean.

4.5 Effect of Ethanol Leaf Extract of *H. floribundum* on Reaction time in Hot-plate induced Pain in Mice

Compared to the baseline pain threshold, the extract at all doses (375, 750, 1500mg/kg) was able to increased pain threshold over time and displayed a dose-dependent increased in latency to reaction time in the positive direction. At T2 and T3, only morphine and extract at doses of 750 and 1500m/kg produced a significantly ($p<0.05$) faster reaction time compared to their baseline values. At all-time points, only 1500mg/kg of the extract significantly ($p<0.05$) increased pain threshold when compared to distilled water and the increase in latency to pain at the dose was comparable to that of morphine. (Figure 4.5).

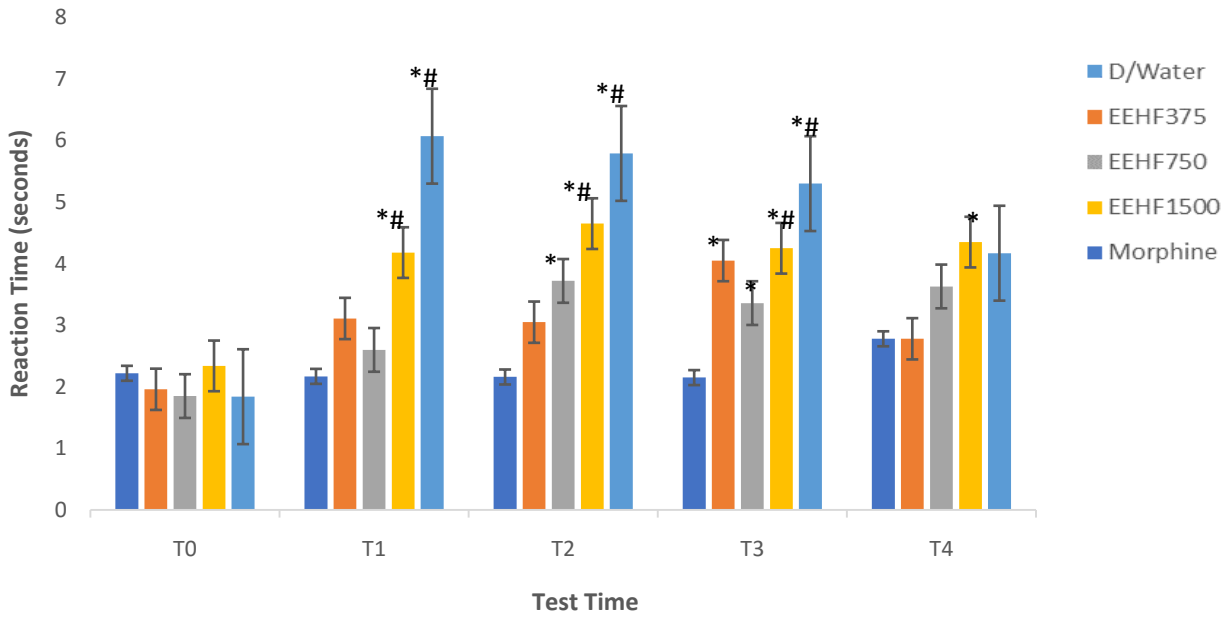

Figure 4.5: Effect of Ethanol Leaf Extract of *H. floribundum* on Reaction time in Hotplate-induced Pain in Mice

N = 6. Data was analyzed using Mixed-Design analysis of variance (ANOVA) followed by Bonferroni Post-hoc Test.* p< 0.05 compared to T0, # p< 0.05 compared to Distilled water. EEHF = Ethanol Extract of *Hymenodictyonfloribundum;* S.E.M. = Standard Error of Mean. T= Test time where T0 is baseline, T1, T2, T3 and T4 represent 60 min, 90 min, 120 min and 150 min after treatment administration.

4.6 Effect of *H. floribundum* on Carrageenan-Induced Paw Oedema in Rats

Data analysis reveals that rats in all groups had comparable paw-size at base line (T0) since there was no statistically significant difference between the paw-sizes. However, as time progressed, there was gradual increased in paw oedema in all groups which started declining after the third hour (T4 and T5). At first hour (T1), only piroxicam-treated rats had significantly ($p<0.05$) smaller paw oedema (2.38 ± 0.06) than distilled water-treated group (2.67 ± 0.06),but at 2^{nd}, 3^{rd}, 4^{th} and 5^{th} hours (T2, T3, T4, T5), piroxicam-treated and extract-treated groups had significantly ($p<0.05$) lower paw oedema sizes (2.40 ± 0.06, 3.23 ± 0.13, 2.84 ± 0.08, 2.55 ± 0.07 for extract at 375mg/kg; 2.36 ± 0.06, 2.69 ± 0.13, 2.68 ± 0.08, 2.36 ± 0.07 for extract at 750mg/kg; 2.35 ± 0.06, 2.76 ± 0.13, 2.72 ± 0.08, 2.51 ± 0.07 for extract at 1500 mg/kg and 2.27 ± 0.06, 2.65 ± 0.13, 2.39 ± 0.08, 2.25 ± 0.07 for piroxicam) than distilled water (2.76 ± 0.06, 3.64 ± 0.13, 3.37 ± 0.08, 3.00 ± 0.07). At the 3^{rd} hour (T3), only the higher doses (EEHF750 and EEHF1500) of extract-treated groups had significantly (*$p<0.05$*) less paw oedema sizes than the distilled water group whereas at T5 only EEHF1500 had significantly lower paw oedema size than distilled water group. At all the time points, none of the treatment was able to significantly reduce the paw oedema size to the baseline size except piroxicam at the 5^{th} hour. (Figure 4.6).

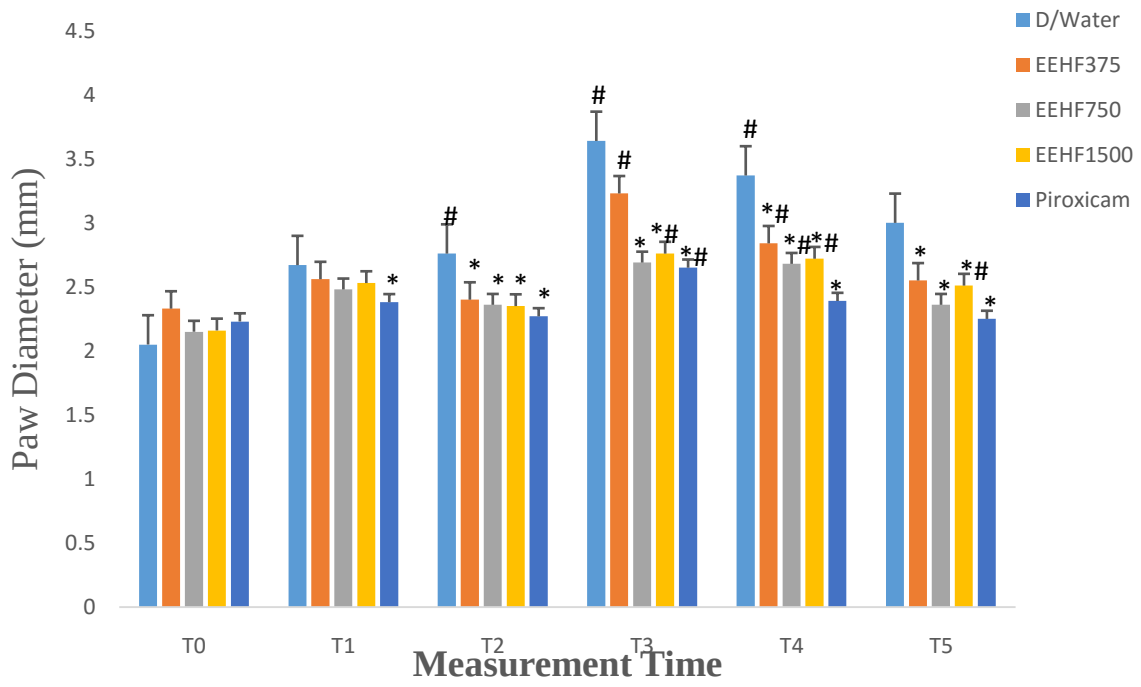

Figure 4.6: Effect of *Hymenodictyonfloribundum* on Paw Size in Carrageenan-Induced Paw oedema in Rat

N = 6.Data was analysed using Mixed-Design ANOVA. * $P< 0.05$ compared to Distilled water, # $p<0.05$ compared to TI. EEHF = Ethanol Extract of *Hymenodictyonfloribundum*, ANOVA = Analysis of Variance, Distilled water= Distilled water.T= Test time where T0 is baseline, T1, T2, T3, T4 and T5 represent 1hr, 2hrs, 3hrs, 4hrs and 5hrs after treatment administration.

4.7 Result of Mechanistic Study

4.7.1 Interaction between *H. floribundum* and some receptors

4.7.1.1Interaction between H. floribundum and potassium-channel pathway

The mean number of writhes produced by mice treated with glibenclamide was significantly ($p > 0.05$) higher than that of the extract at 1500mg/kg ($p< 0.05$) but was comparable to that produced by distilled water treated group ($p > 0.05$). However, the analgesic activity of the extract as well as that of morphine were unaffected in the presence glibenclamide a K^+Channel blocker.(Figure 4.7)

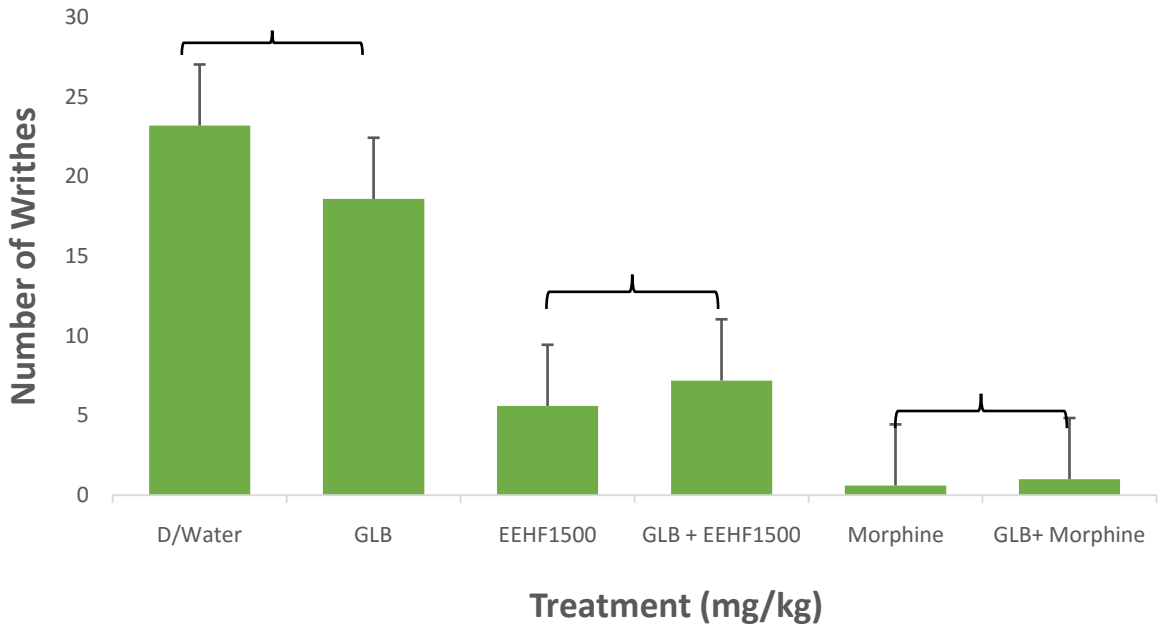

Figure 4.7: Effect of Ethanol Extract of *H. floribundum* on K^+-Channel Pathway

N= 6. Each treatment pair was analyzed using student independent t-test. * is p< 0.01. D/Water = Distilled Water; EEHF = ethanol Extract of *Hymenodictyon floribundum*; GLB = Glibenclamide; N= Number of mice in each treatment group.

4.7.1.2. Effect of H. floribundum on α_2-adrenergic receptor

The effect of yohimbine was comparable to that of distilled water ($p>0.05$) but was significantly ($p<0.01$) different from the extract. The analgesic activity of the extract in the presence of yohimbine was significantly ($p<0.01$) reduced compared to when the extract was administered alone.

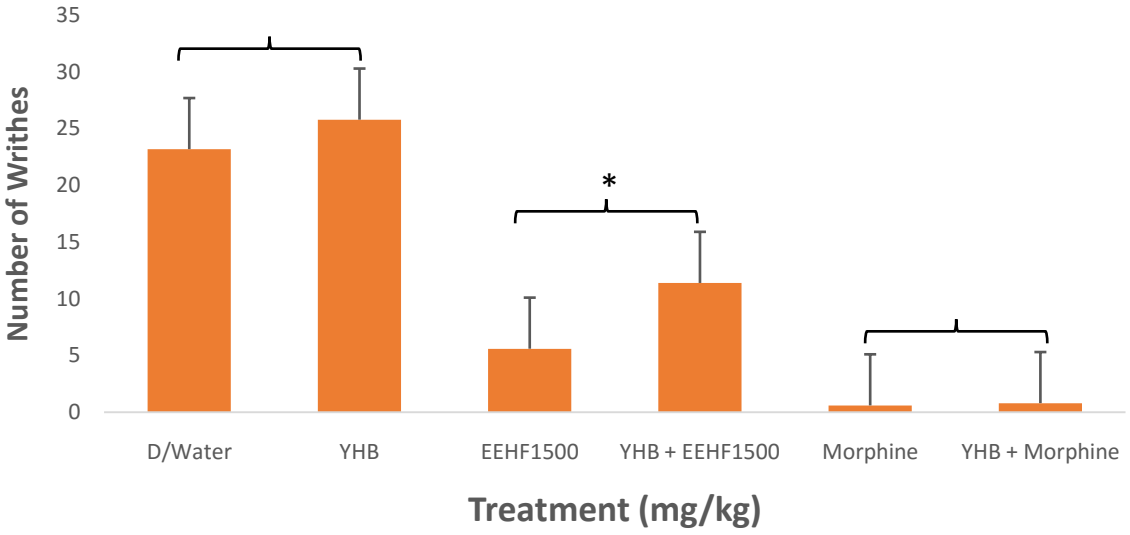

Figure 4.8: Effect of Ethanol Leaf Extract of *H. floribundum* on α_2-receptor

N= 6. Each treatment pair was analyzed using student independent t-test. * is $p<0.01$. D/Water = Distilled Water; EEHF = ethanol Extract of *Hymenodictyon floribundum*; YHB = Yohimbine; N= Number of mice in each treatment group.

4.7.1.3 Effect of H. floribundum on $\alpha_1$adrenergic receptor

The effect of prazosin was comparable *(*$p>0.05$) to that of distilled water. The analgesic activity of the extract and morphine were unaltered ($p>0.05$) in the presence of prazosin. (Figure 4.9)

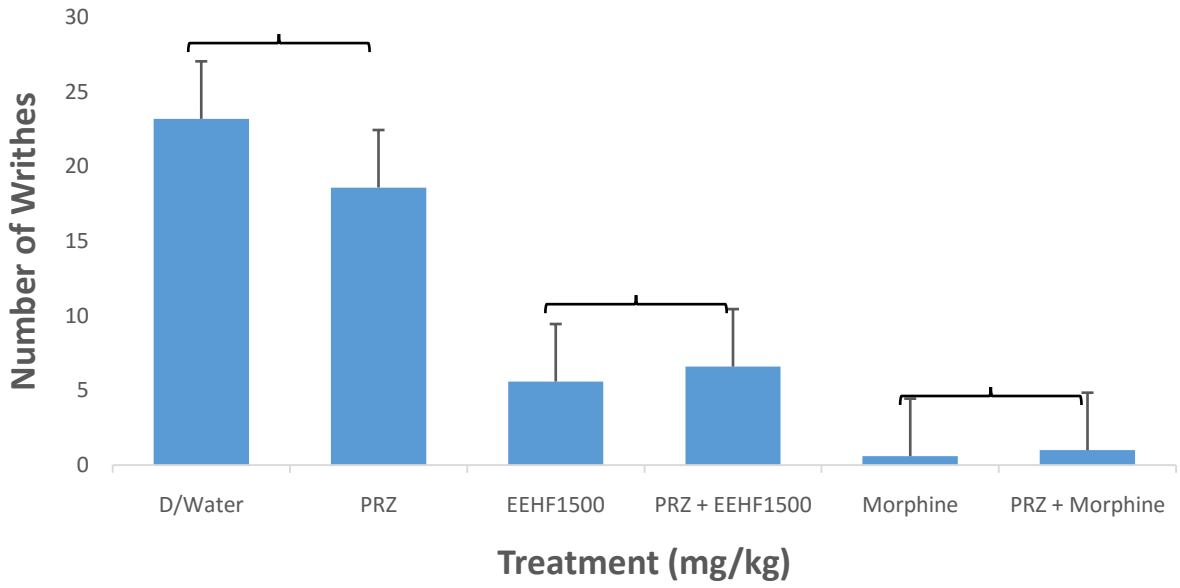

Figure 4.9: Effect of Ethanol Extract of *H. floribundum* on α_1-receptor

N= 6. Each treatment pair was analyzed using student independent t-test. * is $p<0.01$. D/Water = Distilled Water; EEHF = ethanol Extract of *Hymenodictyon floribundum*; PRZ = Prazosin; N= Number of mice in each treatment group.

4.7.1.4 Effect of H. floribundum on β- adrenergic pathway

Propranolol, given alone, produced activity that was similar to that produced by distilled water ($p>0.05$) but significantly ($p<0.01$) reduced the analgesic activity of the extract. The analgesic activity of was unaltered in the presence of propranolol.

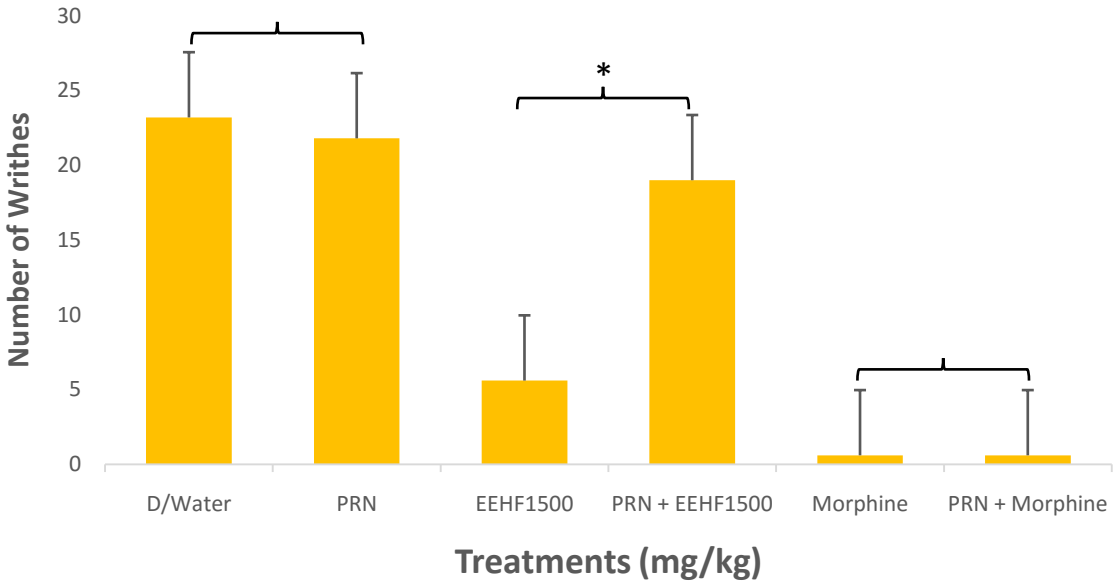

Figure 4.10: Effect of Ethanol Extract of *H. floribundum* on β-receptorPathway

N= 6. Each treatment pair was analyzed using student independent t-test. * is p< 0.01. D/Water = Distilled Water; EEHF = ethanol Extract of *Hymenodictyon floribundum*; PRN = Propranolol; N= Number of mice in each treatment group.

4.7.1.5 Effect of H. floribundum on opioid pathway

Naloxone significantly increased the number of writhes when given alone and when administered before the extract. The analgesic activity of the extract and morphine were significantly reduced ($p > 0.05$) in the presence of naloxone.(Figure 4.11).

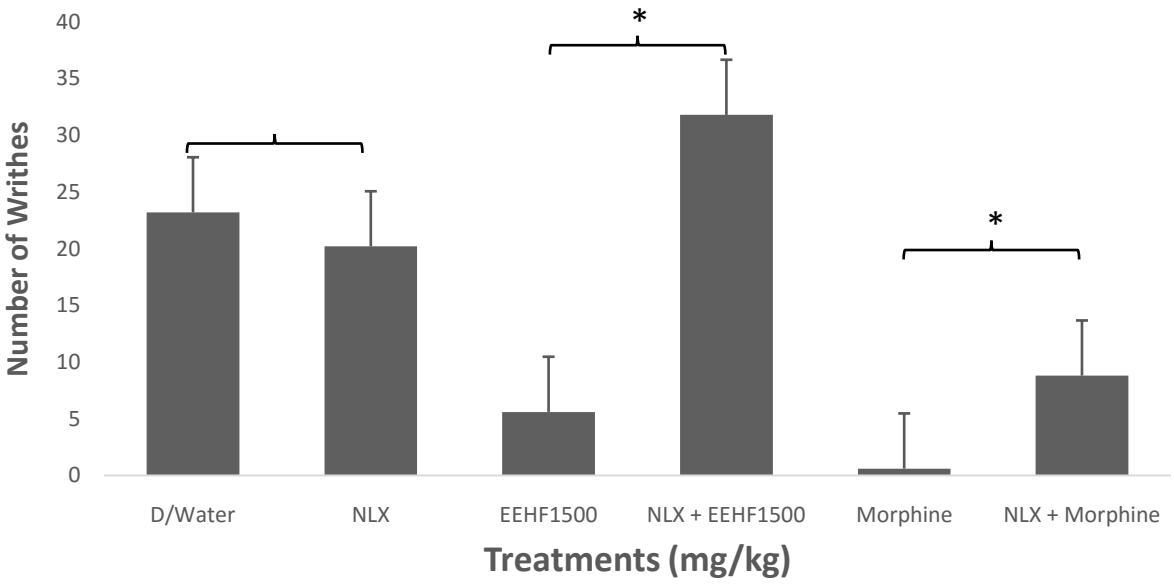

Figure 4.11: Effect of Ethanol Extract of *H. floribundum* on Opioid Receptor pathway

N= 6. Each treatment pair was analyzed using student independent t-test. * is p< 0.001. D/Water = Distilled Water; EEHF = ethanol Extract of *Hymenodictyon floribundum*; NLX = Naloxone; N= Number of mice in each treatment group.

4.7.2 Effect of *H. floribundum* on inflammatory cytokines

There was comparable level of inflammatory markers (IL-6, IL-β, TNF-α, PgE) in both the extract-treated group (EEHF750 and EEHF1500) and distilled water group. However, morphine significantly reduced the levels of PGE while ethanol extract of *H. floribundum*at lower dose (EEHF375) caused a significant rise in the level of IL-6.(Figure 4.12)

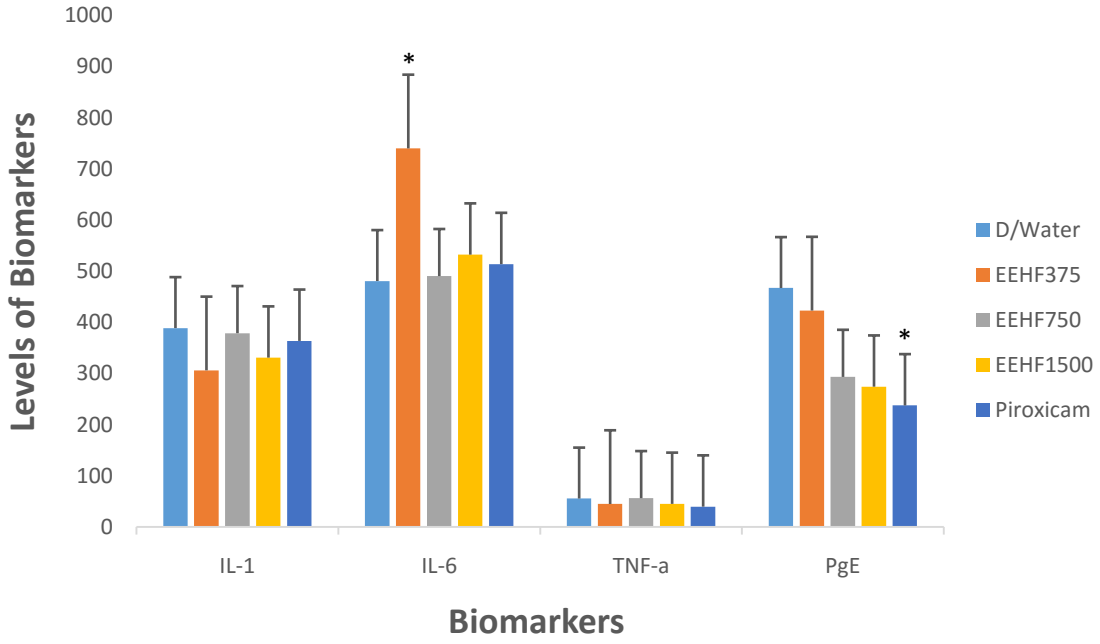

Figure 4.12: Effect of Ethanol Extract of H. floribundum on Inflammatory Cytokines

N = 5.Data were analyzed using one-way Analysis of Variance (ANOVA) followed by Bonferroni Post-hoc test. * is p< 0.05 compared to N/Saline. Distilled water= Distilled water; EEHF = ethanol Extract of *Hymenodictyonfloribundum*; IL= Interleukin, PG= Prostaglandin.

CHAPTER FIVE

5.0 DISCUSSION

The analgesic and anti-inflammatory activity of *Hymenodictyonfloribundum* was successfully investigated. Oral LD_{50} of the extract was evaluated to be greater than 5000 mg/kg in both mice and rats. The Organization for Economic Cooperation and Development (OECD, Paris, France) Walum (1998) categorized acute toxicity established on oral LD50 values as: very toxic, < 5 mg/kg; toxic, > 5 < 50 mg/kg; harmful, > 50 < 500 mg/kg; and not harmful, > 500 < 2,000 mg/kg. Based on this, the oral LD_{50} up to 5,000 mg/kg established for both mice and rats is indicative of relative oral safety. Lack of overt toxicity signs in these experimental animals also pointed to that fact.

Acetic-acid causes pain through peripheral pain route (Ma and Zhang, 2011). This method is highly responsive and quick in blocking the detection of painful effects of compound(s) at dose level that may appear inactive in other procedures like tail-flick test (Collier *et al.*, 1968; Bentley *et al.*, 1981). The irritant indirectly activates the release of nociceptive endogenous mediators (bradykinin, serotonin, and prostaglandin) and pro-inflammatory cytokines (TNF-α and IL-1β) to bring about painful sensation (Kakoti *et al*., 2013). The abdominal constriction produced by acetic acid can be used to evaluate peripherally acting analgesics like non-steroidal anti-inflammatory drugs (NSAIDs) (Aiyelero *et al*., 2009; Mishra *et al*., 2011; Kakoti *et al*., 2013) and centrally acting analgesics like morphine (Donkor *et al*., 2013; Kakoti *et al*., 2013). Acetyl salicylic acid (ASA) and NSAIDs decrease writhes caused by acetic acid by interfering with cyclo-oxygenase (COX) in peripheral tissuesby inhibiting the release and/or synthesis of inflammatory

mediators (Donkor *et al*., 2013).The ability of the extract to dose-dependently reduce pain caused by acetic acid is an indication of its peripheral-acting analgesic potential.

In the hotplate test, a well-established centrally-mediated pain pathway, the extract was also significantly able to increase pain threshold although the analgesic effect was more pronounced at the highest dose of the extract after 2 hours of administration (Bhalke and Pal, 2012). This time-dependent effect is an indication of slow and possibly a complete absorption across the blood brain barrier since the analgesic effect started few hours after drug administration and lasted several hours before wearing off (Nick, 2018). The fact that the analgesia was more pronounced at the highest dose is an indication of several factors; the extract is greatly metabolized before it gets to the central compartment leading to insufficient amount in the CNS with lower doses; and secondly, there is a high possibility that receptor occupancy plays a key role in the analgesic activity of the extract (Nick, 2018).

Majority of analgesic agents are known to block several nociceptive pathways in addition to the inhibition of prostaglandin synthesis. These pathways include; the K^+-Channel (Tsantoulas and McMahon, 2014), adrenergic α_1, α_2 (Daniel *et al.,* 2009) and β receptors (Elif *et al.,* 2010) as well as opioidergic pathway (Machelska and Melih, 2018). All these pathways have known antagonists which have been employed in this study to determine the possible mechanism of action of the extract. The K^+- channel is blocked by sulphonylurea such as glibenclamide (Kenia *et al.,* 2006); α_1 and α_2 adrenoceptors are blocked by prazosin (Mycek, 1997) and yohimbine (Katzung and Masters, 2013) respectively; propranolol is a non-selective β- adrenoceptor blocker (Perez, 2006) while

the opioid receptors situated at the spinal and supraspinal area of central nervous system are blocked by naloxone (Mary-Jeanne *et al.,* 1983).

This study revealed that glibenclamide, yohimbine, prazosin, propranolol and naloxone are all devoid of analgesic activity since the mean number of writhes produced by mice treated with these antagonists/blockers were significantly higher than that produced at 1500mg/kg of the extract but were comparable to that produced by distilled water treated group.

The extract most probably does not act via ATP gated K^+- Channel pathway since blockade of this channel did not negatively impair its analgesic activity. Yohimbine, an α_2-adrenoceptor antagonist was able to impair the analgesic activity of the extract by significantly increasing the average writhes number in mice treated with the extract following pretreatment of the antagonist- this is an indication that the extract most likely acts via the activation of α_2- receptor in the sympathetic pathway. It is unlikely that the extract acts via α1- adrenoceptor pathway since even in the presence of a known α1-adrenoceptor blocker, prazosin, the analgesic activity of the extract remained unaltered. Propranolol is a non-selective β-adrenoceptor antagonist. In the presence of this blocker, the analgesic activity of the extract at the highest dose was significantly altered, an indication that the extract owes it analgesic activity partly to the activation of β-receptor.

The analgesic activity of the extract might also be attributed to the activation of some or all of the opioid receptors since its analgesic activity was greatly impaired by the administration of an opioid antagonist prior to its own administration.

The extract may likely owe its analgesic activity to the activation of α_2, β adrenoceptor and opioidergic pathways. The results obtained in the present work suggest the involvement of

three main systems in the analgesic activity of the extract in the acetic acid induced writhing test in mice. The analgesic activity of the extract was found to be related to opioidergic, α_2 and β-adrenergic receptors.

In this study, pretreatment of mice with prazosin and glibenclamide both failed to reverse the analgesic activity of the extract. These suggest the non-involvement of the α_1 adrenoceptor antagonist and potassium channel blocker pathways in the analgesic activity of the extract. But pretreatment of animals with naloxone, yohimbine and propranolol significantly reversed the analgesic action of the extract. These suggest the involvement of the opioidergic, α_2 and β-adrenergic pathways in the analgesic activity of the extract.

The ability of prazosin to potentiate morphine analgesic has been reported (Ozdogan *et al.*, 2003; Ozdogan *et al.*, 2004). The analgesic potential of propranolol has been reported (Sadrabadi *et al.*, 2011). The therapeutic uses of propranolol include; the management of migraine headache and manyexaminations with propranolol reduces the prevalence and recurrence of migraine of about 80% of patients (Caruso *et al.*, 2000; Sadrabadi *et al*., 2011).

Carrageenan induced paw oedema is the most common test for screening anti-inflammatory agents that exhibits a high degree of reproducibility (Winter *et al.*, 1962). Carrageenan is phlogistic agent that is known to be non antigenic and does not have any systemic consequence (Chakraborty *et al.*, 2006). Swelling or edema, which is a sign of acute inflammation, is an important factor to consider when testing compounds with a probable anti-inflammatory activity (Morris, 2003). The oedema caused by carrageenan is simple, rapid and gives reasonable results that you can rely upon (Kaneira *et al.*, 2007).

After the carrageenan injection, oedema develops mainly in two phases: the first 30 minutes after the injection, the second beginning at the end of the first hour and lasting until the third hour after injection (Ma and Zhang, 2011). The first phase has been attributed to the release of histamine, serotonin and bradykinin on vascular permeability and the later phase has been due to over production of prostaglandin in tissues (Bhalke and Pal, 2012). The anti-inflammatory activities observed in EEHF may be via the inhibition of inflammatory mediators such as histamine, serotonin and prostaglandins. The results of this study indicate that the ethanolic leaf extract of *Hymenodictyonfloribundum* significantly reduced carrageenan induced paw oedema in rats. Therefore, the mechanism of action may be by inhibition of histamine, serotonin or prostaglandin synthesis. Usually most anti-inflammatory and analgesic drugs possess antipyretic activity. In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus. (Hayare *et al.,* 2000). Inflammatory cytokines are known to be upregulated during pain and inflammation (Zhang *et al.,*2007). However, there was no significant reduction in the level of these cytokines in the extract-treated groups, which is an indication that the anti-inflammatory activity of the extract is not via interaction with inflammatory cytokines.

Preliminary phytochemical screening gives an idea of the subjectiveidentity of the active phytochemical constituents present in the ethanol leaf extracts of *Hymenodictyonfloribundum* that are responsible for several pharmacological activities which will help to advance studies or separate the active constituents (Mishra *et al.,* 2011). Several phytochemicals such as saponins, tannins, and cardiac glycosides which are present in the ethanol leaf extract of *Hymenodictyonfloribundum* are known to have

analgesic activity and inflammatory activities (Calixto *et al.,* 2009; Anikumar, 2010). Saponins have been reported to inhibit inflammatory mediators (Bellik *et al.,* 2013). Flavonoids are known to be essential in acute inflammation (Rajnarayana *et al.,* 2001). There are also reports on the analgesic effects of alkaloids, and saponins (Choi *et al*., 2005; Reanmongkol *et al*., 2005). The analgesic and anti-inflammatory effect of the extract may therefore, be due to the presence of flavonoids, tannins, alkaloid or saponins acting in synergy or otherwise.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The findings of this study are summarized below;

1. Ethanol leaf extract of *Hymenodictyonfloribundum* contains carbohydrates, steroids, terpenoids, saponin, tannins, alkaloids, anthraquinones, flavonoids and cardiac glycosides
2. Ethanol leaf extract of *H. floribundum* has analgesic and anti-inflammatory activity
3. The analgesic effect of the extract is likely due to interaction with opioidergic, α_2 and β-adrenergic receptor
4. The extract did not influence the levels of inflammatory biomarkers (1L-β, IL-6, TNF-α, and PgE).

6.2 Conclusion

These findings suggest that the ethanol leaf extract of *Hymenodictyonfloribundum* contain bioactive constituents that has analgesic and anti-inflammatory potentials and its most likely produce analgesia via α2, β and Opioid receptors, which further support the ethnomedical claim of the use of the plant in the management of pain and inflammation.

6.3 Recommendations

1. Isolation of the active constituents responsible for the analgesic and anti-inflammatory action of the extract should be done

2. Chronic and sub-acute toxic effect of the extract should be conducted

3. Effect of the extract on other markers of pain and inflammation should be conduct

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APPENDICES

Appendix 1.0: Effect of Ethanol Leaf Extract of *H. Floribundum* on Acetic Acid-Induced Writhes in Mice

Treatment	**Number of Writhes**
D/ Water (10mls/kg)	38.5 ± 1.34
EEHF375	14.8 ± 1.40*
EEHF750	12.5 ± 1.48*
EEHF1500	7.7 ± 0.49**
Morphine	0.5 ± 0.50**

N = 6. Data was analyzed using one-way analysis of variance (ANOVA) followed by Dunnett Post-hoc Test.* p< 0.05 compared to distilled water, **p< 0.01 compared to distilled water. EEHF = Ethanol Extract of *Hymenodictyonfloribundum;* S.E.M. = Standard Error of Mean.

Appendix 2.0: Effect of Ethanol Leaf Extract of *H. floribundum* on Reaction Time in Hotplate Test in mice

Treatment	**Reaction Time (Seconds)**				
	T0	T1	T2	T3	T4
D/Water (10mls/kg)	2.22 ± 0.28	2.17 ± 0.45	2.16 ± 0.52	2.15 ± 0.45	2.78 ± 0.56
EEHF375	1.96 ± 0.28	3.11 ± 0.45	3.05 ± 0.52$^{\#}$	4.05 ± 0.45*$^{\#}$	2.78 ± 0.56$^{\#}$
EEHF750	1.85 ± 0.28	2.60 ± 0.45	3.72 ± 0.52*	3.36 ± 0.45*	3.63 ± 0.56
EEHF1500	2.34 ± 0.28	4.18 ± 0.45*$^{\#}$	4.65 ± 0.52*$^{\#}$	4.25 ± 0.45*$^{\#}$	4.35 ± 0.56*
Morphine	1.84 ± 0.28	6.07 ± 0.45*$^{\#}$	5.79 ± 0.52*$^{\#}$	5.30 ± 0.45*$^{\#}$	4.17 ± 0.56

N = 6. Data was analyzed using Mixed-Design analysis of variance (ANOVA) followed by Dunnett Post-hoc Test.* $p < 0.05$ compared to T0, # $p < 0.05$ compared to distilled water. HF = Ethanol Extract of *Hymenodictyonfloribundum;* S.E.M. = Standard Error of Mean. T= Test time.

Appendix 3.0: Effect of Ethanol Leaf Extract of *H. floribundum* on Carrageenan-induced Paw Oedema in Rats

Treatment	Paw Diameter (mm)					
	T0	**T1**	**T2**	**T3**	**T4**	**T5**
D/Water (10mls/kg)	2.05 ± 0.08	2.67 ± 0.06*	2.76 ± 0.06#	3.64 ± 0.13#	3.37 ± 0.08#	3.00 ± 0.07#
EEHF375	2.33 ± 0.08	2.56 ± 0.06	2.40 ± 0.06*	3.23 ± 0.13#	2.84 ± 0.08*#	2.55 ± 0.07*
EEHF750	2.15 ± 0.08	2.48 ± 0.06	2.36 ± 0.06*	2.69 ± 0.13*	2.68 ± 0.08*#	2.36 ± 0.07*
EEHF1500	2.16 ± 0.08	2.53 ± 0.06	2.35 ± 0.06*	2.76 ± 0.13*#	2.72 ± 0.08*#	2.51 ± 0.07*#
Piroxicam	2.23 ± 0.08	2.38 ± 0.06	2.27 ± 0.06*	2.65 ± 0.13*	2.39 ± 0.08*	2.25 ± 0.07*

N = 6. Data was analysed using Mixed-Design ANOVA. * P< 0.05 compared to D/SWater, # p<0.05 compared to TI. EEHF = Ethanol Extract of *Hymenodictyonfloribundum*, ANOVA = Analysis of Variance, Distilled water= Distilled water.

Appendix 4.0: Effect of *H. floribundum* on Various Receptors

Treatment	Receptors				
	K^+-Channel	**Opioid**	**Alpha1**	**Alpha2**	**B-Receptor**
	B = Glibenclamide	**B = Naloxone**	**B = Prazosin**	**B = Yohimbine**	**B = Propranolol**
D/Water (10mls/kg)	23.2 ± 0.05*	23.2 ± 0.05*#	23.2 ± 0.05*	23.2 ± 0.05*	23.2 ± 0.05*
EEHF	5.6 ± 0.46	5.6 ± 0.46	5.6 ± 0.46	5.6 ± 0.46	5.6 ± 0.46
EEHF + B	0.6 ± 0.05	31.8 ± 1.32*	6.6 ± 0.98	11.4 ± 0.98*	19 ± 1.11*
B Alone	15.6 ± 1.12	29.2 ± 1.08*	16.6 ± 0.05*	25.8 ± 1.02*	21.8 ± 0.82*
Morphine	0.6 ± 0.05	0.6 ± 0.05	0.6 ± 0.05	0.6 ± 0.05	0.6 ± 0.05
Morphine + B	1± 0.07	8.8 ± 0.05#	1± 0.07	0.8 ± 0.03	0.6 ± 0.05

N = 6. Data was analysed using one-way ANOVA for each receptor. * P< 0.05 compared to EEHF, # p<0.05 compared to Morphine. EEHF = Ethanol Leaf Extract of *Hymenodictyonfloribundum,* ANOVA = Analysis of Variance, D/Water= Distilled water, B = Blocker of each receptor.

Appendix 5.0: Effect of *H. floribundum* on Inflammatory Markers

Treatment	**Inflammatory Biomarkers**			
	IL-1β	**1L-6**	**TNF-α**	**PgE**
D/Water (10mls/kg)	388.9 ± 31.92	480.9 ± 36.60	55.60 ± 6.90	467.2 ± 42.10
EEHF375	306.3 ± 11.90	740.2 ± 104.4*	44.95 ± 7.70	423.2 ± 81.20
EEHF750	378.9 ± 43.00	490.5 ± 20.60	56.30 ± 5.00	293.4 ± 26.50
EEHF1500	331.1 ± 14.80	532.5 ± 35.40	45.20 ± 2.50	274.3 ± 46.80
Morphine	363.8 ± 39.90	513.9 ± 08.50	39.70 ± 5.20	237.6 ± 48.80*

N = 6. Data was analyzed using One-way ANOVA, Followed by Bonferroni Post-hoc test. * P< 0.05 compared to N/Saline. EEHF = Ethanol Extract of *Hymenodictyonfloribundum*, ANOVA = Analysis of Variance, Distilled water= Distilled water.