ANALGESIC, ANTI-INFLAMMATORY AND ANTI-CONVULSANT ACTIVITIES OF STEM BARKEXTRACT OF *Erythrophleumivorense*(A Chev) IN RATS AND MICE

BY

Olayemi Kamoru WAKEEL

BSc, MSc (Lagos)

A THESIS SUBMITTED TO THE

DEPARTMENT OF

PHARMACOLOGY AND THERAPEUTICS,

UNIVERSITY OF IBADAN

COLLEGE OF MEDICINE

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE OF MASTER OF PHILOSOPHY

(PHARMACOLOGY) OF THE UNIVERSITY OF

IBADAN, NIGERIA

CERTIFICATION

I certify that Mr Olayemi Kamoru WAKEEL carried out this work titled '**ANALGESIC, ANTI-INFLAMMATORY AND ANTI-CONVULSANT ACTIVITIES OF STEM BARK EXTRACT OF *Erythrophleumivorense* (A Chev) IN RATS AND MICE u**nder my supervision in the Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Ibadan, Nigeria.

SUPERVISOR

Pro*fessor* O. G ADEMOWO **Date**

Ph.D (Ibadan, AIBMLS (London)

Department of Pharmacology and Therapeutics,

University of Ibadan, Nigeria

.

DEDICATION

This work is dedicated

To

The Almighty GOD

And

The memory of my Late Father

MrWakeel Akanmu

And my Late Mother

Alahja Robiat Wakeel

ACKNOWLEDGEMENT

Glory, honour and power to God our father in Heaven, for His grace, strength and mercy that saw me through this study, in spite of so many odds. I am highly indebted to the exceptional, brotherly advice that I received from my supervisor, Professor O.G.Ademowo, it is through his continued encouragement and meticulous supervision that I managed to accomplish what I have achieved in this study. Also appreciates my dearest friends DrFehintolaFatai and Professor Farombi E.O (Dean, Faculty of basic Medical Sciences, University of Ibadan) for their unrelented encouragement. I appreciate Dr. A.O.Aderibigbe for his advice, support and his willingness to assist in the study. I am grateful to Dr. S.Ummukoro for his encouragement at all time and his willingness to look through my bench work.

I also appreciate the support of the Mother of all, Professor (Mrs).C.O.Falade (Head of Pharmacology and Therapeutics, College of Medicine University of Ibadan) for hasting the administrative aspect of the study.

My appreciation goes to Dr. E.O. Awe (Acting Head of Department, Pharmacology and Therapeutics, LAUTECH, Osogbo) for his brotherly advice at all time and my colleagues- Dr (Mrs). O.O.Abiodun, Mr. O.T.Kolawole, Mr. S.Bakare, Dr. A.Ayankunle.

Special thanks to my wife, Mrs.OluremiYetundeWakeel for her prayers, love, encouragement and support. I also appreciate my children;Damola, Sewa, Segun and Bukola for their endurance.

Finally, I give all glory and praises to my Rock, Lord and true foundation- Jesus Christ; He is the reason for it all.

Thank you all

Olayemi Kamoru WAKEEL

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

OC R-	-Opiate-like Receptor
OGFR	-Opioid Growth Factor Receptor
GAD	-Glutamic Acid Decarboxylase
ImSAID	-Immune Selective Anti-Inflammtory Derivative
DNA	-Deoxyribonucleic Acid
GABA	- γ–Amino Butyric Acid
AMPA	-α-amino-3-hydroxy-5-methylisoxazole-4 propionic acid
NMDA	-N-Methyl-d-Aspartate
AED	-Anti Epileptic Drug
EEG	-Eletroencephalography
KOR	- Kappa-Opioid Receptor
DMSO	-Dimethyl SufOxide
COX	- Cyclooxygenase
NSAID	-Non-Steroidal Anti-inflammatory Drug

ABSTRACT

Erythrophleumivorense isused in traditional medicine for the treatment of convulsion, swellings, body pain and as emetic agents. There is a dearth of scientific information in support of the traditional claims. This study was therefore designed to investigate the analgesic, anti-inflammatory and anticonvulsant properties of the methanol extract and fractions of*Erythrophleumivorense.*

Fresh Stem bark of *E. ivorense* was air dried,ground to powdery form and was extracted using 75% methanol. Crude Methanol Extract (CME, 50g) was fractionated, using ethyl acetate, dichloromethane and n-hexane to yield Ethyl Acetate Fraction (EAF), Dichloromethane Fraction (DCF), and n-Hexane Fraction (HF). Four hundred and eighty male Swiss albino mice (20-25 g) were divided into six experimental groups of twenty animals each for CME and the three fractions. Animals in each group were randomly divided into four treatment groups. Group 1 received 2% DMSO (10mL/kg) (control) while CME or fractions (5, 10, 20 mg/kg) was given to groups 2-4intraperitoneally. Animals were pretreated thirty minutes before injection of acetic acid, formalin or placed in hot plate and picrotoxin, leptazol, strychnine for evaluation of analgesic and anticonvulsant activities respectively. Twenty male Wistar rats (180-200 g) were used for anti-inflammatory study and were randomly divided into four treatment groups. Group 1 received 2% DMSO (10mL/kg) while CME or fraction (5, 10, 20 mg/kg) was given to groups 2-4. All animals were pretreated intraperitoneally thirty minutes before induction of acute inflammation with subplantal injection of carrageenan (0.1ml).Thereafter, the anti-inflammatory activity was evaluated by measuring the oedema size of the right hind paw using cotton thread method.Data were analyzed using descriptive statistic and ANOVA at p=0.05.

The CME (20mg/kg), EAF (20mg/kg)and DCF (20mg/kg)caused significant reductionin the number of writhes of acetic acid-induced writhing (3.8±0.4, 3.6±0.2 and 11.9±0.4 respectively) compared with control (37.7±2.6). In hot plate test, CME (7.0±0.5) and EAF (6.6±0.4) significantly prolonged the reaction time (seconds) to noxious heat, while DCF (2.8±0.4) and HF (2.6±0.5) did not significantly change the responses compared with control (2.2 ±0.2). The CME (35.6±0.4, 67.0±0.8) and EAF (35.2±0.4, 74.2±0.7), but not HF (55.5±0.4, 146.0±0.9) significantlyreduced duration of paw licks (seconds) in boththe neurogenic and inflammatory phases of formalin-induced paw licks compared with control (56.0±0.5, 148.0 ± 0.3), while DCF

produced significant reduction in duration of paw licks in inflammatory (53.0±0.3) but not the neurogenic (55.5±0.4) pain induced by formalin when compared with the control. The CME and EAF significantly ($p<0.05$) delayed the onset, shortened duration of action and offered protection against picrotoxin and leptazol-induced convulsions. However, CME and its fractions did not protect strychnine-induced convulsions. The CME, EAF and DCFat 5, 10, 20mg/kg significantly reduced paw oedema size with percentage inhibitions of(25.0, 41.7, 58.3), (46.7, 46.7, 60.0) and (0.0, 9.1, 54.5) respectively compared with control.

The crude methanol extract, ethyl acetate and dichloromethane fractions exhibited analgesic, anti-inflammatory and anticonvulsant activities. These support ethnomedicinal uses of the plant in the management of pain and convulsive disorders.

Keywords: *Erythrophleumivorense*, Analgesic, Anti-inflammatory, Anti-convulsant,

Word count: 497

CHAPTER ONE

1.0 INTRODUCTION

1.1 Medicinal plants

Knowledge of herbs has been handed down from generation to generation for thousands of years. The revival of interest in natural medicine started in 2004 mainly because of the wide spread belief that green medicine is healthier than synthetic products. In the recent past, there has been tremendous increase in the use of plant-based health products in developing as well as developed countries resulting in an exponential growth of herbal product globally. According to World Health Organization (WHO), about 80% of the world's populations in developing countries rely on traditional medicines for the treatment of various diseases (Padmaa *et al*., 2010). However, due to over population, urbanization, and continuous exploitation of these herbal reserves, the natural resources along with their related traditional knowledge are depleting day by day (Pande *et al*., 2007).

In the present era of drug discovery and development of newer drug molecules, many plantproducts have been specifically evaluated on the basis of their traditional uses. In many developed countries, traditional medicines is increasingly being used in parallel to allopathic medicines, particularly for treating and managing chronic diseases (Heinrich *et al*., 2005).These herbal preparations are generally considered to be less toxic when compared to synthetic drugs. However, most of the preparations have not been evaluated scientifically for safety and efficacy. It is therefore imperative to investigate scientifically such preparations used in traditional medicine for the treatment of various diseases (Acharya *et al.,* 2008). Scientific investigation may provide the necessary information required to promote the rational use of medicinal plants or herbal medicines.

In addition, the history of drug discovery showed that plants are richin active compounds and they have become challenged to modern pharmaceutical industry. Many synthetic drugs owe their origin to plant-based complementary medicine (Howes *et al.*, 2003; Orhan *et al*., 2004)

1.2 African Traditional Medicine

African traditional medicine is the oldest, and perhaps the most assorted, of all therapeutic systems. Africa is considered to be the cradle of mankind with rich biological and cultural differences in healing practices (Chintamunee and Mahomoodally, 2012). African traditional medicine in its varied forms is holistic involving both the body and mind. The traditional healer typically diagnoses and treats the psychological basis of an illness before prescribing medicines, particularly medicinal plants to treat the symptoms (Gurib-Fakim, 2004).The sustained interest in traditional medicine in africa health care system can be justified by two major reasons. The first one is inadequate access to allopathic medicines and western forms of treatments, whereby the majority of people in Africa cannot afford access to modern medical care either because it is too costly or because there are no medical service providers. Secondly, there is a lack of effective modern medical treatment for some ailments such as malaria, HIV/AIDSand or Ebola, which, although global in distribution, disproportionately affect Africa more than other areas in the world. Indeed, Africa is blessed with enormous biodiversity recourses and it is estimated to contain between 40 and 45,000 species of plant with a potential for development and out of which 5,000 species are used medicinally. This is not surprising since Africa is located within the topical and subtropical climate and it is a known fact that plants accumulate important secondary metabolites through evolution as a natural means of surviving in a hostile environment (Manach *et al.*, 2004).The documentation of medicinal uses of African plants and traditional systems is becoming a pressing need because of the rapid loss of the natural habitats of some of these plants due to anthropogenic activities and also due to an erosion of valuable traditional knowledge. It has been reported that Africa has some 216 million hectares of forest, but the African continent is also notorious to have one of the highest rates of deforestation in the world, with calculated loss of 1% per annum (Gurib-Fakim and Mahomoodally, 2013). Interestingly, the continent also has the highest rate of endemism, with the Republic of Madagascar topping the list by 82%, and it is worth to emphasize that Africa already contributes nearly 25% of the world trade in biodiversity. Nonetheless, the paradox is that in spite of this huge potential and diversity, the African continent has only few drugs commercialized globally (Gurib-Fakim, 2006; Atawodi, 2005). The scientific literature has witnessed a growing number of publication geared towards evaluating the efficacy of medicinal plants from Africa which believed to have an important contribution in the maintenance of health and in the introduction of new treatments. Nonetheless, there is still a dearth of promising medicinal plants from Africa continent.

1.3 History of medicinal uses of plants

The use of plants as medicine predates written human history. Many of herbs and spices used by humans to season food also yield useful medicinal compounds (Lai*et al*., 2004 : Tapsell *et al*., 2006). The use of herbs and spices in cuisine developed in part as a response to the threat of food-borne pathogens. Studies show that in tropical climates where pathogens are the most abundant, recipes are the most highly spiced. Further, the spices with the most potent antimicrobial activity tend to be selected (Billing *et al*., 1998). Many of the common weeds that populate human settlements, such as nettle, dandelion and chickweed, also have medicinal properties (Stepp *et al*., 2001).

A large amount of archeological evidence exist which indicates that human were using medicinal plants during the Paleolithic, approximately 60,000 years ago. Furthermore, other non-human primates are also known to ingest medicinal plants to treat illness (Sumner *et al*., 2000). In the written record, the study of herbs dates back over 5000 years to the Sumerians, who created day tablets with list of hundreds of medicinal plants (such as myrrh and opium) (Sumner *et al*., 2000). In 1500 B.C., the ancient Egyptians wrote the Ebers Papyrus, which contains information on over 850 plants medicines, including garlic, juniper, cannabis, castor bean, aloe, and mandrake (Sumner *et al* ., 2000).

In India, Ayurveda medicine had used many herbs such as turmeric, possibly as early as 1900 B.C. (Aggrawal *et al*., 2007). Sankrit writings from around 1500 B.C., such as the Rig Veda, are some of the earliest available documents detailing the knowledge that form the basis of the Ayurveda system. Many other herbs and minerals used in Ayurveda were later described by acient Indian herbalist such as Charaka and Sushruta during the 1st millennium B.C.

The Chinese emperor Shen Nung is said to have written the first Chinese herbal; the Pen Tsao. The Pen Tsao lists 365 medicinal plants and their uses, including Ephedra (the shrub that introduced the drug Ephedrine to mothern medicine), hemp, and chaulmoogra (one of the first effective treatments for leprosy) (Sumner *et al*., 2000).

The earliest known Greek herbals were those of Diocles of Carytus, written during the third century B.C and one by Krateuas from the 1st century B.C. only a few fragments of these works have survived intact, but from what remains scholars have noted that there is a large

amount of overlap with the Egyptian herbals (Robson *et al*., 2009). Greek and Roman medicinal practices, as preserved in the writings of Hippocrates (e.g De herbis et curis) and especially –Galen (e.g Therapeutics), provided the pattern for later western medicine (Loudon *et al*., 2002). Sometime between 50 and 68 A.D., a Greek physician known as Pedanius Dioscorides wrote what is called De Materia Medical, a compendium of more than 600 plants, 35 animal products and 90minerals. De Materia Medical remained the authoritative reference of herbalism into the 17th century (Collins *et al.,* 2000). Similarly important for herbalists and botanists of later centuries was Theophrastus' Historia Plantarum, written in the 4th century B.C which was the first systematization of the botanical world (Gene *et al.,* 2004).

Benedictine monasteries were the primary source of medical knowledge in Europe and England during the early middle ages. However, most of these monastic scholars' efforts were focused on translating and copying ancient Greco-Roman and Arabic works, rather than creating substantial new information and practices. Nabati introduced empirical techniques in the testing, description and identification of numerous material medica, and he separated unverified reports from those supported by actual test and observations. This allowed the study of material medical to evolve into the science of pharmacology (Huff *et al.*, 2003).

Baghdad was an important center for Arab herbalism, as was Al-Andalus between 800 and 1400. Abulcasis (936-1013) of Cordoba authored the book of simples, an important source for later European herbals, while ibn al-Baitar (1197-1248) of Malaga authored the Corpus of simples, the most complete Arab herbal which introduced 200 new healing herbs, including tamarind, Aconitum and nux formica (Castleman *et al*., 2001).

The 15th, 16th, 17th centuries were the great age of herbals, many of them available for the first time in English and other languages rather than latin or Greek. The first herbal to be published in English was the anonymous Grete Herball of 1526. The two best-known herbals in English were the Herball or general history of plant (1597) by John Gerard.

The use of herbs to treat diseases is almost universal among non-industrialized societies (Edgar *et al*., 2002).

1.4Uses of medicinal plants

Medicinal plants are various plants used in herbalism and thought by some to have medicinal properties, few plants or their phytochemical constituents have been proven to have medicinal effects by rigorous science or have been approved by regulatory agencies such as the United States Food and Drug Administration and National Agency for food and Drug Administration and Control

Rauwolfia serpentine: contains a number of bioactive chemicals, including yohimbine, reserpine, ajmaline, deserpidine, rescinnamine and serpentinine. The extract has been used for millennia in India-Alexander the great administered this plant to cure his general Ptolemy of a poisoned arrow. It was reported that Mahatma Gandhi took it as a tranquilizer during his lifetime. A compound which it contains called reserpine, is used to treat high blood pressure and mental disorders including schizophrenia and was particular for that purpose in the west from 1954 to 1957 (Sumit *et al.*, 2006). It has been used for millennia to treat insect stings and the bites of venomous reptiles.

Nicotiana glauca: is a species of wild tobacco known by the common name tree tobacco native of South America used for a variety of medicinal purposes and smoked by Native American groups. The Cahuilla Indians used leaves interchangeably with other tobacco species in hunting rituals and as a poultice to treat swellings, bruises, cuts, boils, inflamed throat and swollen glands. The plant contains the toxic alkaloid nicotine. Ingestion of the leave can be fatal (Foster *et al.*, 2002). It is being investigated for use as a biofuel (Gibson, 2009).

*Rubus occidentalis:*This is a species of North America. It contains anthocyanins and ellagic acid. This has led to their being very useful as natural dyes. Anthocyanins are also antioxidants (Krestye*t al.*, 2006) *Bridelia ferrugineas* is commonly used in traditional Africa medicine fortreating various inflammatory conditions. It was reported to have inhibited the production of PGE2, nitrate, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) as well as COX-2 and iNOSs protein expression in LPS-activated microglia cells (Olajide *et al.*, 2012)

Ricinus communis: also been documented to have being used in medicine and other applications. Alcoholic extract of the leaf was hepatoprotective in rat (Sabina *et al.*, 2009). Methanolic extract of the leaves was shown to have antimicrobial properties. The pericap of castor bean showed central nervous system effects in mice at low doses. Water extract of the root bark showed analgesic activity in rats (Oyewole *et al.*, 2008). Antihistamine and antiiflammatory properties were found in ethanolic extract of Ricinus communis root bark

(Lomaash *et al.*, 2010).

Phyllanthus niruri is an important plant of Indian Ayurvedic system of medicine which is used for the problem of stomach, genitourinary system, liver, kidney and spleen (Patel *et al.*, 2011). A clinical study with Phyllanthus niruri, indicated that it may reduced the level of urinary calcium (Nishiural *et al.*, 2004). Phyllanthus niruri has been shown to interfere with many stages of stone formation, reducing their structure and composition as well as altering the interaction of crystals with tubular cells leading to reduced subsequent endocytosis (Bolm *et al*., 2010)

Drimia maritime have been reportedly used for medicinal purposes. The bulbs contain cardiac glycosides which stimulate the heart and act as diuretics in moderate doses and are emetic and poisonous in larger doses.

Ephedra sinica and others have traditionally been used by indigenous people for a variety of medicinal purposes, including treatment of asthma, hay fever and the common cold (Abourashed *et al.*, 2003). The alkaloids ephedrine and pseudoephedrine are active constituents of *E. sinica* and others member of the genus. These compounds are sympathomimetics with stimulant and decongestant qualities and are related chemically to the amphetamines.

The leaves of *Ginkgo biloba*contain flavonoid, glycosides (myricetin and quercetin) and terpenoids and have been used pharmaceutically. These extracts are shown to exhibit reversible, nonselective monoamine oxidase inhibition, as well as inhibition of reuptake at the serotonin, dopamine and norepinephrine transporters, with all but norepinephrine reuptake inhibition fading in chronic exposure (Fhske *et al.*, 2009). Recently, a meta-analysis patients (Weinmann*et al*., 2010) but not preventing the onset of Alzheimer's disease in normal people (Dekosky *et al*.,2008).

*Bitter melon*also reportedly used in various Asian and African herbal medicine systems for a long time. (Grover *et al.*, 2004; Beloin *et al.*, 2005). The plant contains several biologically active compounds, chiefly momordicin I and momordicin II, and cucurbitacin B (Ftope *et al.*, 1990). The plant also contains several bioactive glycosides and other terpenoid compounds (Kimura *et al.*, 2005). It also contains cytotoxic proteins such as momordin and momorcharin (Arnason, 2005). Two compounds extracted from Bitter melon, α-eleostearic acid and 15,16-dihdroxy-α-eleosterearic acid have been found to induce apoptosis of leukemia cells in vitro

(Koboro *et al.*, 2008). Diets containing 0.01% bitter melon oil were found to prevent azoxymethane-induced colon carcinogenesis in rats (Kohno *et al.*, 2004)

Kaempferia rotunda is a spicy plant and is having many medicinal uses in Ayurvedic and allopathic medicinal systems. The flower contains the toxin benzyl benzoate that is used to make ointments to treat scabies (Nugroho *et al.*, 1996).

Mammea Americana reportedly used traditionally in Central and South America against parasitic skin; ground seeds are stirred into hot water to obtain an anthelmintic infusion. In Trinidad and Tobago, the grated seeds are mixed with rum or coconut oil to treat head lice and chiggers (Mendes *et al.*, 1986). Unripe fruits are riched in pectin and the tree bark is high in tannin.

*Lobelia cardinalis*a native of North America and indigenous people used root tea for a number of intestinal ailment and syphilis. Leaf teas were used for bronchial problem. The Meskwaki people used it as part of an inhalant against catarrh.

Hypoxis hemerocallidea is amedicinal plant native to South Africa in the Hypoxidaceae family (Gillmer *et al.*, 1999). Used in the treatment of benign prostatic hyperplasia, (Wilt *et al*., 2000). However hypoxis alters the activity of cytochrome p450, suggesting that it may interfere with the effectiveness of other drugs or supplements, such as antiretrovirals (Mills *et al.*, 2005).

Rhigiocarya racemifera and Kolobo petalumwere reported to have analgesic effects attributed to O-methyl flavinanthine which has a structure similar to that of morphine (Oliver-Bever, 1986.) *Epidendrum mosenii* Contain titerpenes, pholidotin and 24-methylene cycloartanol and reported to have analgesic activity (Floriani *et al*., 1998) Fresh leaves of *Clerodendron infortunatum*are given for the treatment of diarrhea, liver disorders, and headache, leaves and root are also applied externally over the skin diseases especially fungi infection and alopecia (Khare, 2008)

Abies pindow contains phyto-constituents such as flavonoids and triterpenes (Manju *et al.*, 2000) and reportedly used to treat pain

Ceropegia juncea :Was reported to have central analgesic effect due to the presence of Cerpegin, a novel furopyridine (Adibatti *et al*., 1995)

Hibiscus vitifolius: has been shown to have antinociceptive activity attributed to the presence of gossypin, a biof lavonoid (Vas, 1998)

1.5 Ethnobotany

Ethnobotany (from "ethnology"-study of culture and "botany" study of plants) is the scientific study of the relationships that exist between people and plants. Ethnobotanist aims todescribe,explainanddocumentcomplex relationships between cultures and the uses of plants, focusing primarily on how plants are used, managed and perceived across human societies. This includes use for food, clothing, currency, rituals, medicine, dye, cosmetics and more (Acharya *et al.*, 2008).The field of ethnobotany experienced a shift from the raw compilation of data to a greater methodological and conceptual reorientation. This is also the beginning of academic ethnobotany. The so-called father of this discipline is Richard Evans Schultes even though he did not actually coin the term "Ethnobotany". Today the field of ethnobotany requires a variety of skills: botanical training for the identification and preservation of plants specimens; anthropological training to understand the cultural concepts around the perception of plants; linguistic training, at least enough to transcribe local terms and understand native methodology, syntax and semantics.(Sood *et al*., 2001).The native healers are often reluctant to accurately share their knowledge with outsiders.

1.6 Rationaleforthe study

The traditional usefulness of *Erythrophleum ivorense* have been highly emphasized in literature (Burkil, 1995). However, this plant has not been subjected to pharmacologicalwork on the alkaloid. Hence, it was considered that investigations for these medicinal properties may give scientific authentication to the traditional claims.

1.7 Aim of the present study

To carry out the analgesic, anti-inflammatory and anti-convulsant activities of stem bark extracts of*Erythrophleum ivorense*

1.8 Specific objective are:

(1) To establish analgesic, anti-inflammatory and anti-convulsant activities of methanol extractof*Erythrophleum ivorense* stem bark(2) To cCarry out an activity-directed fractionation of crude methanol extract of the plant in the bid to obtain analgesic, anti-inflammatory and anti-convulsant activities.

CHAPTER TWO

2.0 LITERATURE REVIEW

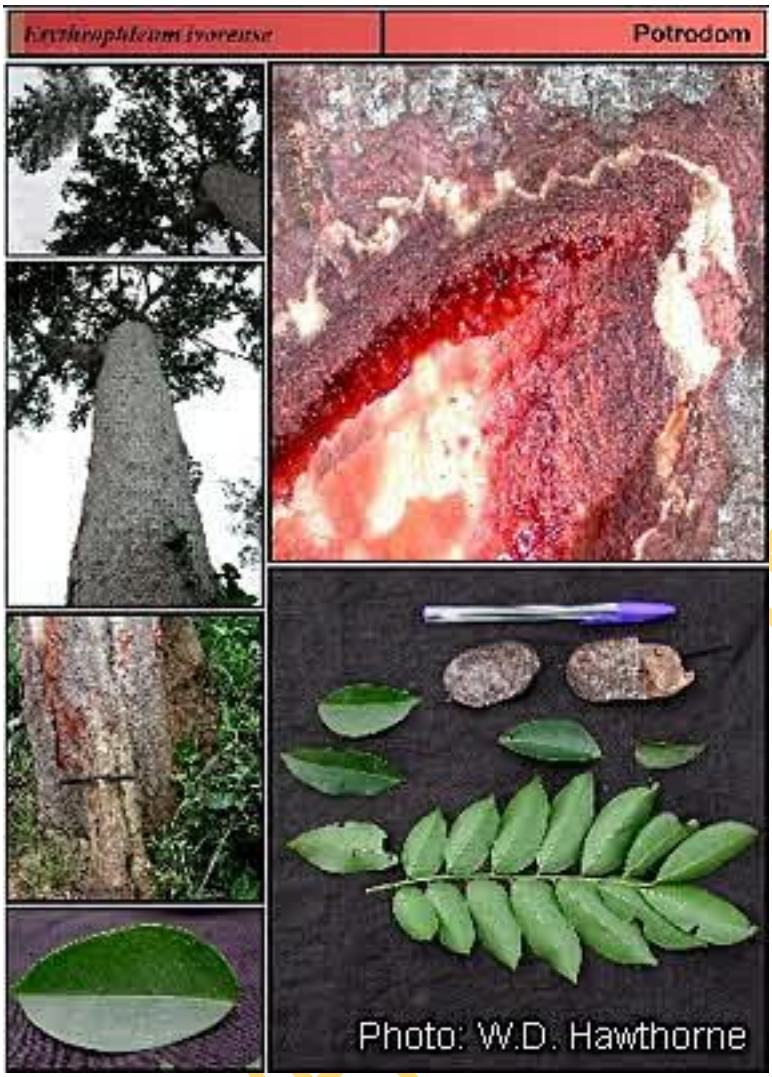
2.1 The plant under study

The plant *Erythrophleum ivorense* belongs to the family Fabaceae. The stem bark is called "epo-obo" among Yoruba people of South Western, Nigeria. It is also called several other vernacular names in West Africa such as forest ordeal tree, red water tree, sasswood tree, Bois rouge tali and mancone.

2.1.1Description of the plant

Erythrophleum ivorense is a medium-tree up to 25-30m tall, often branching low; bark finely fissured, scaly, grey; twigs glabrous. Leaves alternate, bipinnately compound with 2-4 pairs of pinnae; stipules minute, soon falling; petiole and rachis up to 35cm long, petiole thickened at base; leaflets alternate, 7-14 per pinnae, ovate-ovate elliptical, up to 9cm x 5.5cm long, base asymmetrical, apex obtusely acuminate. Inflorescence an axillary panicle, consisting of spike-like racemes up to 12cm long, shortly yellowish hairy. Flowers bisexual, regular, 5-merous, yellowish white to greenish yellow; pedicel 1.5mm long, reddish hairy; calyx lobes 1-1.5mm long; petals 2-3mm x 0.5mm, short hairy at margins; stamens 10, ovary superior, rusty hairy, 1-celled, stigma cup shaped. Fruit a flat, slightly curved, dehiscent pod 5-17 cm x 3-5cm, stipe often lateral, broadly rounded at apex, pendulous, 6-11-seeded. Seeds oblong-ellipsoid, 15mm x 11mm x 5mm.

Fig 1 Photograph of *Erythrophleumivorense*



2.1.2 Habitat and Distribution

Erythrophleum ivorense is found by stream sides and in forest at low altitudes distributed from Senegal in West Africa to Sudan and Kenya in the east and from there south to Zimbabwe and Mozambique. It has been introduced as an ornamental in tropical Asia (Arbonnier, M., 2004)

2.1.3 Medicinal uses of the plant

The stem bark of *Erythrophleum ivorense* is traditionally used in the treatment of convulsive disorderscalled 'Giri' among the Yoruba in southwest of Nigeria. The bark extract is taken orally in Sierra Leone as an emetic and laxative and is applied externally to relieve pain. In Cote d'lvoire , the bark of young branches of *Erythrophleum ivorense* is crushed in water and rubbed on the skin to treat smallpox. (Oliver-Bover,1986). The crushed bark is applied to swellings caused by filarial worms. In Congo the dried powdered bark is taken as a snuff to cure headache. In Kenya a diluted decoction of the roots is used as an anthelmintic, especially against tapeworm. In Malawi a decoction of the root and bark is applied to soothe general body pain. Pieces of root or bark are used as a protective and as love charm (Burkill, 1995).

2.1.4 Phytochemistry of the plant

The bark and seeds of the plant was reported to contain erythrophleine (Paris *et al*.,1941), and also cassaine, cassaidine, nicassaidine, coumingine, erythropguine (Lindwall *et al*.,1965). Catechuic tannin, saponin and fluvonoside have been reportedly isolated from the bark, as well as wax with high proportion of hexacosanol. (Cronlund, 1976)

2.1.5 Pharmacology of the plant

The alkaloid (Erythrophleine) present in the plant has stimulant effects on the heart similar to that of the cardenolides digitoxin (from digitalis) and Oabain (from *Strophantus gratus*), but the effect is very short-lasting as the alkaloids are quickly metabolized in the organism(Cotten *et al.*, 1952). Cassaine and cassaidine have strong anesthetic and diuretic effects and increase contraction of the intestine and uterus. Apart from an increase of heart contraction in systole, the alkaloid also demonstrated an increase in diastole. In addition, cassaidine caused depressive effects, while cassaine caused a violent state of excitation (Bosch, 2006).

2.2Neurotransmitter

Neurotransmitters are endogenous chemicals that transmit signals from a neuron to a target cell across a neuronal synapse. Neurotransmitters are stored in synaptic vesicles clustered beneath the membrane in the axon terminal, on the presynaptic side of a synapse. They are released and diffuse across the synaptic cleft, where they bind to specific receptors in the membrane on the postsynaptic side of the synapse (Elias *et al*., 2005). Release of neurotransmitters usually follows arrival of an action potential at the synapse, but may also

follow graded electrical potentials. Low level baseline release also occurs without electrical stimulation. Neurotransmitters are synthesized from plentiful and simple precursors, such as amino acids, which are readily available from the diet and which require only a small number of biosynthetic steps to convert (Robert *et al.*, 2005).

Until the early 20th century, scientists assumed that the majority of synaptic communication in the brain was electrical. However, through careful histological examination by Ramon Cajal (1852-1934) 20 to 40 nm gaps between neurons, known today as the synaptic cleft, was discovered. The presence of such gap suggested communication via chemical messengers traversing the synaptic cleft and in 1921 German pharmacologist Otto Loewi (1873-1961) confirmed that neurons can communicate by releasing chemicals (Mayers *et al.*, 2000). Through a series of experiments involving the vagus nerves of frogs, Loewi was able to manually slow the heart rate of frogs by controlling the amount of saline solution present around the vagus nerve. Upon completion of this experiment, Loewi asserted that sympathetic regulation of cardiac function can be mediated through changes in chemical concentrations. Furthermore, Otto Loewi is accredited with discovering acetylcholine (ACh) - the first known neurotransmitter (Saladin *et al.*, 2009). Some neurons do, however, communicate via electrical synapse through the use of gap junctions, which allow specific ions to pass directly from one cell to another. There are many different ways to classify neurotransmitters. Dividing them into amino acids, peptides and monoamines is sufficient for some classification purpose

2.2.1 Major neurotransmitters:

Amino acids: glutamate, aspartate, D-serine, γ-aminobutyric acid (GABA), glycine.

Monoamines and other biogenic amines: dopamine, norepineprhine, epinephrine, histamine, serotonin.

Peptides: somatostatin, substance P, opioid peptides.

Others: acetylcholine, adenosine, nitric oxide etc.

β-endorphin is a relatively well known example of a peptide neurotransmitter; it engages in highly specific interactions with opioid receptors in the central nervous system.

Single ions, such as synaptically released zinc, are also considered neurotransmitters by some, as are some gaseous molecules such as nitric oxide, hydrogen sulphide and carbon monoxide (Kodirov *et al*.,2006).

Glutamate is abundant in the human body, particularly in the nervous system and especially prominent in the human brain where it is the body's most prominent excitatory neurotransmitter.(Robert, 2005). The next most prevalent is GABA, which is inhibitory at more than 90% of the synapses. Even though other transmitters are used in far fewer synapses, they may be very important functionally. The great majority of psychoactive drugs exert their effects by altering the action of some neurotransmitter systems, often acting through transmitters other than glutamate or GABA. Addictive drugs such as cocaine and amphetamine exert their effects primarily on the dopamine system. The addictive opiate drugs exert their effects primarily as functional analogs of opioid peptides, which in turn, regulate dopamine levels.

Some neurotransmitters are commonly described as excitatory or inhibitory. The direct effects of a neurotransmitter are to activate one or more types of receptors. The effect on the postsynaptic cell depends, therefore, entirely on the properties of those receptors. Some neurotransmitters (for example, glutamate), the most important receptors all have excitatory effects: that is, they increase the probability that the target cell will fire action potential. For other neurotransmitters, such as GABA, the most important receptors all have inhibitory effects. There are, however, other neurotransmitters, such as acetylcholine, for which both excitatory and inhibitory receptors exist; and there are some types of receptors that activate complex metabolic pathways in the postsynaptic cell to produce effects that cannot appropriately be called either excitatory or inhibitory. Thus, it is an over-simplication to call a neurotransmitter excitatory or inhibitory- nevertheless it is convenient to call glutamate excitatory and GABA inhibitory so this usage is seen frequently.

Glutamate:Glutamate used at great majority of fast excitatory synapse in the brain and spinal cord. It is also used at most synapse that is modifiable, i.e capable of increasing or decreasing in strength. Excess glutamate can over stimulate the brain and causes seizure. Modifiable synapses are thought to be the memory-storage elements in the brain. Excess glutamate release can lead to excitotoxicity causing cell death (Jawal *et al.,* 2009).

GABA:Gamma aminobutyric acid used at the great majority of fast inhibitory synapses in virtually every part of the brain. Many sedative/tranquilizing drugs act by enhancing the effects of GABA. Correspondingly glycine is the inhibitory transmitter in the spinal cord.

Acetylcholine is an organic, polyatomic cation that acts as a neurotransmitter in both the peripheral nervous system and central nervous system in many organisms including humans. It is an ester of acetic acid and cholin, with systematic name 2-acetoxy-N,N,N-trimethylethanamimium. Acetylcholine is one of many neurotransmitters in the autonomic nervous system and is the only neurotransmitter used in motor division of the somatic nervous system. Acethylcholine is also the principal neurotransmitter in all autonomic ganglia.

In cardiac tissues, acetylcholine neurotransmission has an inhibitory effect, which lowers heart rate. However, acetylcholine also behaves as an excitatory neurotransmitter at neuromuscular junction in skeletal muscle (Campbell *et al*., 2002)

Dopamine: Has a number of important functions in the brain; this includes regulation of motor behavior, pleasure related to motivation and emotional arousal. It plays critical role in the reward system; people with Parkinson's disease have been linked to low levels of dopamine (Schacter *et al.*, 2009).

Serotonin:Serotoninor 5-hydroxytryptamine (5-HT) **is** a monoamine neurotransmitter. Biochemically derived from tryptophan, serotonin is primarily found in the gastrointestinal tract, platelets and in the central nervous system of animal including humans. It is popularly thought to be a contributor to feelings of well-being and happiness (Young, 2007). Approximately 90% of human body,s total serotonin is located in the enterochromoffin cells in the alimentary canal where it is used to regulate intestinal movements (Berger *et al.*,2009). The remainder is synthesized in serotonergic neurons of the central nervous system, where it has various functions. These include the regulation of mood, appetite and sleep. Serotonin also has some cognitive functions, including memory and learning. Modulation of serotonin at synapses is thought to be a major action of several classes of pharmacological antidepressants.

Serotonin secreted from the enterochromaffin cells eventually finds its way out of tissues into the blood. There, it is actively taken by the blood platelets, which store it. When the platelets bind to a clot, they disgorge serotonin, where it serves as a vasoconstrictor and helps to regulate hemostasis and blood clotting. Serotonin also is a growth factor for some types of cells, which may give it a role in wound healing. In addition to animals, serotonin is found in fungi and plants (Kang *et al*., 2009). Serotonin presence in insect venoms and plant spines serve to cause pain, which is the side effect of serotonin injection.

Substance P:In the field of neuroscience, substance P is a neuropeptide an undecapeptide that function as a neurotransmitter and as a neuromodulator (Harrison *et al*.,2001; Datar *et al*.,2004). It belongs to the tachykinin neuropeptide family. Substance P and related neuropeptide neurokinin A are produced from a polyprotein precursor after differential splicing of the prepprotachykinin A gene. The deduced amino acid sequence of substance P is as follows (Campbell *etal*.,2001) Arg Pro Lys Pro Gli Phe Gly Leu Met with an amidation at the C-terminus (Wong *et al*., 1994). Substance P is released from the terminals of specific sensory nerves; it is found in the brain and spinal cord and associated with inflammatory proceses and pain. Substance P is involved in nociception, transmitting information about tissue damage from peripheral receptors to the central nervous system to be converted to sensation of pain. It has been theorized that it play a part in fibromyalgia. Capsaicin has been shown to reduce the level of substance P

Opioid peptides: They are neurotransmitters that act within pain pathways and emotional center of the brain; some of them are analgesic and elicit pleasure or euphoria (Schacter *et al*., 2009)

Norepinephrine: A catecholamine with multiple roles including as a hormone and a neurotransmitter. Areas of the body that produce or are affected by norepinephrine are described as noradrenagic. One of the most important functions of norepinephrine is its role as the neurotransmitter released from sympathetic neurons affecting the heart. An increase in norepinephrine from the sympathetic nervous system increases the rate of contractions (Guyton *et al*., 2006).

As a stress hormone, norepinephrine affects part of the brain, such as the amygdale, where attention and responses are controlled (Tanaka. 2000). Along with epinephrine, norepinephrine also underlies the fight-or-flight response, directly increasing the heart rate, triggering the release of glucose from every store, and increasing blood flow to skeletal muscle. It increases the brain's oxygen supply; norepinephrine can also suppress neuroinflammation when released diffusely in the brain from the locus coeruleus (Heneka *et al.,* 2010).

Norepinephrine is synthesized from dopamine by dopa β-hydroxylation in the secretory granules of the medullary chromaffin cells. It is released from the adrenal medulla into the

blood as a hormone and also as a neurotransmitter in the central nervous system and sympathetic nervous system where it is released from the adrenergic neurons in the locus coeruleus. The actions of norepinephrine are carried out via the binding to adrenergic receptors

Histamine:Histamine **i**s an organic nitrogen compound involved in local immune responses as well as regulating physiological function in the gut and acting as a neurotransmitter (Marieb, 2001). Histamine triggers the inflammatory response. As part of an immune response to foreign pathogens, histamine is produced by basophils and by mast cells found in nearby tissues. Histamine increases the permeability of the capillaries to white blood cells and some proteins, in the infected tissues (Di Gluseppe*et al;* 2003).

2.3 Concept of Pain

2.3.1 Pain

The traditional medicine has used medicinal plants to relieve pain without noticeable side effect. Therefore, it can introduce novel drugs with less complications and cost. Pain is described as a discomforting feeling in a certain part of the body. The obligation of medical science is to maintain human health and ease pain. Understanding the concept of pain, therefore, is one of the most essential tools in realizing these goals. Since pain is considered an indicator of disease, all around the world and in all cultures, it is the most common symptom that compels patient to visit doctors (Kruger, 2001; Fatemeh *et al*., 2014). Different classes of drugs have been considered and used for pain relief. Opioid and non-steroidal anti-inflammatory drugs were included. Currently, the side effects of these drugs are causing problem in treatment (Katzung *et al*., 2007). Analgesics, particularly medicinal plants, which have less side effects and additive characteristics, can gain importance.
The International Association for the study of pain defined pain as an unpleasant, sensory and emotional experience associated with actual or potential tissue damage. Pain motivates the individual to withdraw from damaging situations, to protect a damaged body part while it heals, and to avoid similar experiences in the future. Most pain resolves promptly once the painful stimulus is removed and the body has healed, but sometimes pain persists despite removal of the stimulus and apparent healing of the body; and sometimes pain arises in the absence of detectable stimulus, damage or disease.

Pain is the most common reason for physician consultation in the United State.(Turk *et al*., 2004) it is a major symptom in many medical conditions and can significantly interfere with a person's quality of life and general functioning (Breivik *et al*., 2008) Psychological factors such as social support, hypnotic suggestion, excitement, or distraction can significantly modulate pain's intensity or unpleasantness.

2.3.2Classification of pain

Pain is usually transitory, lasting only until the stimulus is removed or the underlying damage or pathology has healed, but some painful conditions, such as rheumatoid arthritis, peripheral neuropathy, cancer and idiopathic pain, may persist for years. Pain that lasts a long times is called chronic and pain that resolves quickly is called acute.

Traditionally , the distinction between acute and chronic pain has relied upon an arbitrary interval of time from onset ; the two most commonly used markers being three and six months since the onset of pain,(Turk *et al*., 2004) though some theorists and researchers have placed the transition from acute to chronic pain at twelfth months(Spanswick, 2000). Others apply acute to pain that last less than 30 days, chronic to pain of more than six months duration and sub-acute to pain that lasts from one to six month (Thienaus *et al*., 2000). A popular alternative definition of chronic pain, involving no arbitrarily fixed durations is "pain that extends beyond the expected period of healing" (Turk *et al*., 2004). Chronic pain may be classified as cancer pain or benign (Thienaus *et al*., 2000). In 1994, responding to the need for a more useful system for describing chronic pain, the International Association for Study Pain (IASP) classified pain according to specific characteristics: (a) region of the body involved (e.g, abdomen, lower limbs), (b) system whose dysfunction may be causing the pain (e.g nervous, gastrointestinal), (c) duration and pattern of occurrence, (d) intensity and time of onset and (e) etiology (Mersky *et al.,* 1994). This system has been criticized by Clifford J. Woolf and others as inadequate for guiding research and treatment (Woolf *et al.,* 1998). According to Woolf *et al*; (1998) there are three classes of pain: nociceptive pain, inflammatory pain and pathological pain which is a disease state caused by damage to the nervous system (neuropathic pain) which is associated with tissue damage and infiltration of immune cells its abnormal function (dysfunctional pain, like in fibromyaigia, irritable bowel syndrome, tension type headache, etc.)(Woolf, 2010).

Nociceptive pain : Nociceptive pain is caused by stimulation of peripheral nerve fibers that respond only to stimuli approaching or exceeding harmful intensity (nociceptors), and may be classified according to the mode of noxious stimulation; The most common categories being "thermal" (heat or cold), "mechanical (crushing, tearing etc)" and "chemical" (iodine in cut, chili powder in eyes). Nociceptive pain can also be divided into visceral, deep somatic and superficial somatic pain. Visceral structure are highly sensitive to stretch, ischemia and inflammation, but relatively insensitive to other stimuli that normally evoke pain in other structures, such as burning and cutting. Visceral pain is diffused, difficult to locate and often refer to a distant, usually superficial, structure. It may be accompany by nausea and vomiting and may be described as sickening, deep, squeezing, and dull. Deep somatic pain is initiated by stimulation of nociceptors in ligaments, tendons, bones, blood vessels and muscles, dull, aching and poorly localized pain. Example includes broken bones. Superficial pain is initiated by activation of norciceptors in the skin or other superficial tissues and is sharp, well-defined and clearly located. Example of injuries that produced superficial somatic pain include minor wounds and minor burns (Spanswick, 2000).

Neuropathic pain: Neuropathic pain is caused by damage or diseased affecting any part of the nervous system involved in bodily feeling (Treede *et al*., 2008). Peripheral neuropathic pain is often described as burning tingling, electrical, stabbing or pins and needles (Paice, 2003).

Psychogenic pain:Psychogenic pain is a pain disorder associated with psychological factors. Some types of mental or emotional problems can cause, increased, or prolonged pain. A person with a psychogenic pain disorder may complain of pain that does not match his or her symptoms (Stephen, 2006) Headache, back pain and stomach pain are some of the most common types of psychogenic pain. It may occur, rarely, in persons with mental disorder, but more commonly it accompanies or is induced by social rejection, broken heart, grief, love sickness, or other such emotional events.

2.3.3 Endogenous opioids (Opioid peptides) involved in pain modulation

Opioid peptides are short sequences of amino acids that bind to opioid receptors in the brain; Opiates and opioids mimic the effects of these peptides. Opioid peptides may be produced by the body itself, for example endorphins. The effects of these peptides vary, but all resemble opiates. Brain opioid peptides are known to play important role in motivation, emotion,

attachment behavior, response to stress and pain, and the control for food intake (Mollereau *et al*., 1996). Opioid peptides that are produced in the body include: Endorphin, Enkerphalins, Dynorphins, Endomorphins and Nociceptins

Endorphins: They are endogenous opioid peptides that function as neurotransmitters (Oswald, 2000). They are produced by the pituitary gland and the hypothalamus in vertebrates during exercise, excitement, pain, consumption of spicy food, love and orgasm and they resemble the opiates in their abilities to produce analgesia and a feeling of well-being. It consists of two parts: endo- and –orphin; these are short form of words endogenous and morphine, intended to mean "a morphine-like substance originating from within the body (Goldstein, 1975). The term "endorphin rush" has been adopted in popular speech to refer to feelings of exhilaration brought on by pain, danger, or other forms of stress, supposedly due to the influence of endorphins. When a nerve impulse reaches the spinal cord, endorphins that prevent nerve cells from releasing more pain signals are released.

Enkephalins:Enkephalin is a pentapeptide involved in regulating nociception in the body. The enkephalins are termed endogenous ligands, as they are internally derived and bind to the body's opioid receptors. onecontained leucine and the other containing methionine. Both are products of the proenkephalin gene (Noda *et al*., 1982). Met-enkephalin and Leu-enkephalin.

Dynorphins:They are class of opioid peptides that arise from the precursor protein prodynorphin. When prodynorphin is cleaved during processing by proprotein convertase,two multiple active peptides are released: dynorphin A, dynorphin B and α/β-neo-endophin (Day *et al*., 1998). Depolarization of a neuron containing prodynorphin stimulate proprotein convertase 2 processing, which occurs within synaptic vesicles in the presynaptic terminal (Yakovleva *et al*.,2006). Occasionally, prodynorphin is not fully processed, leading to the release of "big dynorphin". These 32-amino acid molecules consist of both dynorphin A and dynorphin B (Nyberg *et al*., 2007).

Dynorphin A, dynorphin B and Big dynorphin all contain a high proportion of basic amino acid residues, in particular lysine and arginine (Marinova *et al*.,2005). Although dynorphins are found widely distributed in the CNS, they are the highest concentrations in the hypothalamus, medulla, pons, midbrain and spinal cord (Goldstein *et al*., 1980). Dynorphin are stored in large (80-120nm diameter) dense-core vesicles that are considerably larger than

vesicle storing neurotransmitter. Dynorphin exert their effect primarily through the κ-opioid receptor (KOR), a G- protein-couple of receptor. Two subtypes of KORs have been identified: K1 and K2 (Nyberg, 2007). Although KOR is the primary receptor for all dynorphin, the peptide does have some affinity for the μ-opioid receptor, δ-opioid receptor, N-methyl-D-aspartic acid-type glutamate receptor (Drake *et al*., 2007). Both big dynorphin and dynorphin A are more potent and more selective thandynorphin B (Merg *et al.*, 2006). Dynorphin has been shown to be a modulator of pain response. Han and Xie found that injecting dynorphin into the subarachnoid space of the rat spinal cord produced dose-dependent analgesia that was measured by tail-flick latency (Han *et al.*, 1984). Analgesia was partially eliminated by opioid antagonist naloxone (Han *et al*., 1984).

Endomorphins **:**Are two endogenous opioid peptides. Endomorphin-1 and endomorphin-2 are tetrapeptides with the highest known affinity and specificity for the μ opioid receptor. Endomorphin-1 is located in the nucleus of the solitary tract, the periventricular hypothalamus and dorsalmedial hypothalamus, where it is found within histaminic neurons and may regulate sedative and arousal behaviours (Greco *et al*., 2008). It is assumed that endomorphins are the cleavage products of a larger precursor, but this polypeptide or protein has not yet been identified.

Nociceptin:Nociceptin is a17 amino acid neuropeptide, is the endogenous ligand for the nociceptin receptor (NOP, ORL-1). It is derived from the prepronorciceptin protein, as are a further 2 peptides, nocistatin and Nocil (Okuda-Ashitaka *et al.*, 1998). The gene coding for prepronorciceptin is located onCh8p21 in humans (Mollereau *et al.*, 1996).

Nociceptin is an opioid-related peptide, but it does not act at the classic opioid receptors (mu, kappa and delta opioid receptors) and its actions are not antagonized by the opioid antagonist naloxone but nociceptin is a potent anti-analgesic. Nociceptin is widely distributed in the CNS; it is found in many regions of the hypothalamus, brainstem and forebrain, as well as in the ventral horn and dorsal horn of spinal cord. Nociceptin acts at the nociceptin receptor. The receptor is also widely distributed in the brain, including the cortex, anterior olfactory nucleus. There is some evidence that nociceptin may be involved in the phenomenon of opioid-induced hyperalgelsia

2.3.4 Opiate receptors

Opioid receptors are a group of G protein- coupled receptors with opioids as the ligands.(Dhawan *et al.*, 1996 ;Janecka *et al.*, 2004). The endogenous opioids are dynorphins, enkephalins endorphin, endomorphins and norciceptin. The opioid receptors are 40% identical to somatostatin receptors. Opioid receptors are widely distributed in the brain and are found in the spinal cord and digestive tract. By the mid-1960s, it had become apparent from pharmacologic studies that opiates drugs were likely to exert their actions at specific receptors sites and that there were likely to be multiple of such sites (Ingoglia and Dole 1970). Early studies indicated that opiates appeared to accumulate in the brain (Martins, 1967). The receptor were first identified as specific molecules through the use of binding studies, in which opiates that had been labeled with radioisotopes were found to bind to brain membrabne homogesates. The first such study was published in 1971, using 3H-levorphanol (Goldstein *et al*.**,** 1971). In 1973, Candace Pert and Solomon Snyder published the first detailed binding study of what would turn out to be the μ opioid receptor, using 3H-naloxone (Pert and Synder, 1973). The study has been widely credited as the first definitive finding of an opioid receptor (Terenius, 1973). Purification of the receptor further verified its existence. The first attempt to purify the receptor involved the use of a novel opiod antagonist called chlornaltrexamine that was demonstrated to bind to the opioid receptor (Caruso, *et al.*, 1979). Caruso *et al*., 1979 purified the detergent-extracted component of rat brain membrane that eluted with the specifically bound 3H-chlornaltrexamine. There are four major types of opioid receptors; (Corbett *et al*., 2006) delta (ð), kappa (k), mu (μ) and nociceptin receptors.

Table 1: Types of Opioid Receptor (Waldhoer *et al*., 2004)

Receptor	**Subtypes**	**Locations**	**Functions**
Delta (δ)	δ1 δ2	• Brain Pontine Amygdale	• Analgesia • antidepressant effects • convulsant effects

		Olfactory bulbs Deep cortex • Peripheral sensory neuron	• physical dependence
Kappa (κ)	κ1 κ2 κ3	• Brain Hypothalamus Periaqueductal Claustrum • Spinal cord Sudstantia gelatinosa • Peripheral sensory neurons	• analgesia • sedation • miosis • inhibition of ADH release • dysphoria
	μ1 μ2 μ3	• brain cortex thalamus striosomes periaqueductal • spinal cord substantial gelatinosa • peripheral sensory neurons	μ1: analgesia physical dependence μ2: respiratory depression miosis euphoria reduced GI motility physical dependence μ3: possible vasodilation
Nociceptin	ORL1	• brain • spinal cord	Anxiety Depression Appetite / satiety Development of tolerance

The receptors were named in association with the first letter of the ligand that was found to bind to them. Morphin was the first chemical shown to bind to mu receptors. The first letter of the drug morphin is m, but in biochemistry there is a tendency to use Greek letters, thus turning the m to μ. In similar manner, a drug known as ketocyclazocine was first shown to attach itself to k receptors,(Anil Aggrawal, 1995) while the ð receptors was named after the

mouse vas deferens tissues in which the receptor was first characterized(Lord *et al.,* 1977). Another opioid receptor was later identified and cloned base on homology with the cDNA. This receptor is known as the nociceptin receptor or OLR (opiate-like receptor 1). ∂ Receptor was once considered to be opioid receptors due to the antitussive action of many opioid drugs being mediated via ∂ receptors and the first selective a receptor agonist being derivative of opioid drugs (e.g, allylnormetazocine). However, a receptor were found not to be activated by endogenous opioid peptides, and are quiet different from the other opioid receptors in both function and gene sequence, so they are not usually classified with the opioid receptors. In a similar manner, zeta opioid receptor was also identified and has been shown to be a cellular growth factor modulator with met-enkephalin being the endogenous ligand. This receptor is now most commonly referred to as the opioid growth factor receptor (OGFr) (Zagon *et al*., 2002). Another postulated opioid receptor is the € (epsilon) receptors. The existence of this receptor was suspected after the endogenous opioid peptides beta-endophin was shown to produce additional actions that did not seem to be mediated through any of the known opioid receptors (Wuster *et al*., 1979). Activation of this receptor produces strong analgesia and release of met-enkephalin, and a number of widely used opioid agonist such as the μ agonist etorphine and the k agonist bremazocine have been shown to act as agonist for this effect (Narita *et al*., 1998), while buprenorphine has been shown to act as an epsilon antagonist. Several selective agonist and antagonist are now available for the putative epsilon receptor (Fujil *et al*., 2006) however efforts to locate a gene for this receptor have been unsuccessful and epsilon-mediated effects were absent in μ/k/ð triple knockout mice, (Contet *et al.,* 2004) suggesting the epsilon receptor is likely to be either a spice variant derived from alternative post-translational modification, or a heteromer derived from hybridization of two or more of the known receptors

2.3.5 Mechanism of pain

The perception of pain involves two processes; the detection of pain, or nociception; the second is the conscious experience associated with perception of pain. Special sensory nerves or nociceptive nerve endings are stimulated as a result of pain stimuli. These nerve afferents

are unmyelinated or small myelinated fibres, which run into spinal cord. They then synapse in the dorsal horn of the spinal cord on to a neuron, which projects in the contralateral side in the lateral spinothalamic tract. Pain elicited by stimulation of nociceptive afferent nerve fibres is known as neurogenic pain (Goodman *et al*., 1996).
The sensory detection of nociception may involve stimuli of diverse kinds, including mechanical, thermal, and chemical. When these receptors are activated, the impulses generated are transmitted along peripheral nerve fibres to the central nervous system. Some of the chemicals and natural substances known to be involved in pain are: acetylcholine, potassium chloride, serotonin, histamine, bradykinin, prostaglandins, substances P, somatostatin, vasoactive intestinal polypeptide and noradrenalin. Reflex sympathetic dystrophies, for example can be very painful and may be accompanied by inappropriate sweating and changes in the caliber of blood vessels (Melzack *et al*., 1965).

2.3.6 Pain management (Analgesia)

An analgesic is any member of the group of drugs used to achieve analgesia. The word analgesic derives from Greek av- ("without") and άλγος- ("pain"). (Harper, 2001) Analgesic drugs act in various ways on the peripheral and central nervous systems. They are distinct from anaesthetics, which reversibly eliminate sensation.Analgesics include paracetamol, the non steroidal anti-inflammatory drugs (NSAIDs) and the opioid drugs such as morphine and opium. In choosing analgesics, the severity and response to other medication determines the choice of agent; the World Health Organization (WHO) pain ladder specifies mild analgesics as its first step. Analgesics choice is also determined by the type of pain: for neuropathic pain, traditional analgesics are less effective, and there is often benefit from classes of drugs that are not normally considered analgesics, such as tricyclic antidepressant and anticonvulsants (Dworkin *et al.*, 2003). Analgesic is classified into narcotic and non-narcotics. Example of narcotic is opioids and non-narcotics are non-steroidal anti-inflammatory drugs (NSAIDs).

Paracetamol and NSAIDs:**T**he exact mechanism of action of acetaminophen is uncertain but appears to act centrally in the brain rather than peripherally in nerve endings. Aspirin and the other NSAIDs inhibit cyclooxygenases, leading to a decrease in prostaglandin production. In contrast to acetaminophen and the opioids, this is not only reduces pain but inflammation as well.Acetaminophen has few side effects and is regarded as generally safe, although excess or

sustained use can lead to potentially life-threatening liver damage and occasionally kidney damage (Buck, 2011). While acetaminophen is usually taken orally or rectally, an intravenous preparation introduced in 2002 has been shown to improve pain relief and reduces opioid consumption in perioperative setting.

NSAIDs predispose patients to peptic ulcer, renal failure, allergic reactions and occasionally hearing loss and they can increase the risk of hemorrhage by affecting platelet function

COX-2 inhibitor**:** Cyclooxygenase enzyme inhibited by NSAIDs was discovered to have at least two different types; COX-1 and COX-2. Research suggested that most of the adverse effects of NSAIDs were mediated by blocking the COX-1 (constitutive) enzyme, with analgesic effects being mediated by COX-2 (inducible) enzyme. The COX-2 inhibitors were thus developed to inhibit only the COX-2 enzyme. These drugs (such as rofecoxib, celecoxib, and etoricoxib) are equally effective analgesics compared with NSAIDs, but cause less gastrointestinal haemorrhage in particular (Conaghan 2012). After widespread adoption of the COX-2 inhibitors, it was discovered that most of the drugs in this class increased the risk of cardiovascular events by 40% on average. This led to the withdrawal of rofecoxib and valdecoxib and warnings on others. Etoricoxib seems relative safe, with the risk of thrombotic events similar to that of non-coxib NSAID diclofenac (Conaghan 2012)

Opiates and morphinomimetics:Morphine and various other substances (e.g. codeine, oxycodone, hydrocodone, dihydromorphine, pethidine) all exert a similar influence on the cerebral opioid receptor system. Buprenorphine is thought to be a partial agonist of the opioid receptor and tramadol is an opiate agonist which is structurally closer to venlafaxine than to codeine and delivers analgesia by not only delivering opiate-like effects(through mild agonism of the mu receptor) but also by acting as a weak but fast-acting serotonin-releasing agent and norepineprine reuptake inhibitors(Driessen *et al.,* 1992; Bamigbade, *et al*., 1997). Dosing of all opioids may be limited by opioid toxicity (confusion, respiratory depression, myoclonic jerks and pinpoint pupils), seizure (tramadol), but there is no dose ceiling in patients who accumulate tolerance.

Opioid, which is very effective analgesics, may have some unpleasant side-effects. Patients starting with morphine may experience nausea and vomiting (generally relief by a short course of antiemetics such as phernegan). Itching may require switching to different opioids.

Constipation occurs in almost all patients on opioids, and laxative is typically co-prescribed (Gobbi *et al.,* 2002).

When used appropriately, opiods and similar narcotic analgesics are otherwise safe and effective; however risks such as addiction and body becoming used to the drug (tolerance) can occur. The effect of tolerance means that frequent use of the drug may result in its diminished effect so, when safe to do so, the dosage may need to be increased to maintain effectiveness. This may be of particular concern in patients suffering with chronic pain.

2.4 Concept of Inflammation

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants (Ferrero-Miliani *et al.*, 2007). Inflammation is a protective attempt by the organism to remove the injurious stimuli and initiate the healing process. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection. Although infection is caused by a microorganism, inflammation is one of the responses of organism to damage caused by pathogen. However, inflammation is a stereotype response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen (Abbas *et al*., 2009).

Without inflammation, wounds and infections would never heal. Similarly, progressive destruction of the tissues would compromise the survival of organism. However chronic inflammation can also lead to host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis and even cancer.

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leucocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system and various cells within the injured tissues. Prolonged inflammation known as chronic inflammation, can leads to a progressive shift in type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of tissue from the inflammatory process.Acute inflammation is a short-term process, usually appearing within a

few minutes or hours and ceasing upon the removal of the injurious stimulus (Cotran *et al*., 1998). It is characterized by five cardinal signs: (Parakrama *et al*., 2005). The traditional names for signs of inflammation come from Latin: Dolor (pain), Calor (heat), Rubor (redness), Tumor (swelling) and Functio laesa (loss of function) (Walters, 2009). The first four were described by Celsus (Vogel *etal*., 2009) while loss of function was added later by Galen(Porth, 2007) even though the attribution is disputed and the origination of the fifth sign has been ascribed to Thomas Sydenham(Dormandy, 2006) and Virchow(Cotran *et al*., 1998). Redness and heat are due to increased blood flow at the body core temperature to the inflamed site; swelling is caused by accumulation of fluid; pain is due to release of chemicals that stimulate nerve endings. Loss of function has multiple causes (Parakrama *et al.*, 2005). Inflammation can also be defined as a disorder involving localized increase in number of leukocytes and a variety of complex mediator molecules (Mantri and Witiak, 1994). Prostaglandins are ubiquitous substances that indicate and modulate cell and tissues response involve in inflammation. Their biosynthesis has been implicated in the pathophysiology of cardiovascular diseases, cancer and colonic adenoma (Smith and De Witt, 1995).

2.4.1 Inflammatory mediators

Biochemical mediators' released during inflammation intensifies and propagates the inflammatory response. These inflammatory mediators are soluble, diffusiblemolecules that can act locally and systemically. Mediators derivedfrom plasma include complement and complement derived peptides and kinins. These are release via classic or alternative pathway of the complement cascade.Complement derived peptides (C3a, C3b and C 5a) increase vascular permeability, causes smooth muscle contraction, activate leukocytes and induced mast cell degranulation. C5a is a potentchemotactic factor for neutrophils and mononuclear phagocytes. The kinins are also important inflammatory mediators. They are derived from α2 globulin (high and low molecular weight kininogen) through proteolytic cleavage by a variety of enzymes, the most important of which are plasma and tissue kallikreins (Proud and Kaplan, 1988). Three distinct kinins have been identified in human: kallidin, bradykinin and met-lys-bradykinin (Bhoola *et al.*, 1962). They increases vascular permeability and vascular vasodilation and importantly, activate phospholipaseA2 (PLA2) to liberate arachidonic acid (Keele, 1969). They are also a major mediators involved in pain response.Other mediators derived from injured tissues cell or leukocytes recruited to the site of inflammation. Mast cell,

platelets and basophils produce the vasoactive amine serotonin and histamine. Histamine causes arteriolal dilation, increase in capillary permeability, contraction of nonvascular smooth muscle and eosinophils chemotaxis and can stimulate nociceptors responsible for the pain response (Metcalfe et al., 1981). Its released is stimulated by the complement components C3a and C5a(Carswell et al., 1985). It exerts its effect on variety of cells type including smooth muscle cells and cells of immune system (Pearce, 1991). In addition to its role immediate hypersensitivity reactions, histamine canexert H2-receptor-mediated antiinflammatoery activity including inhibition of human neutrophil lysosomal enzyme release, inhibition of IgE-mediated histamine release from the periphera leucocytes and activation of suppressor T- lymphocytes (Metcalfe et al).Cytokines, including interleukins 1-10, tumor necrosis factor (TNF-α) and Interferon γ (INF-γ) are produced predominantly by macrophages and lymphocytes but can be synthesized by other cell types as well. Their role in inflammation is complex. These polypeptides modulate the activity and function of other cells to coordinate and control inflammatory response. Two of the more important cytokines are Interleukin-1 (IL-1) and (TNF-α), mobilized and activate leukocytes, enhance proliferation of B and T cells and are involved in the biologic response to endotoxins. Leukotriene are derived from arachidonic acid which is made available from cell membrane phospholipids by the actions of phospholiphase A2 (Ho and Orange, 1978).They are also generated by most cells types that participate in inflammatory reactions including mast cell basophils, eosinophil, neutrophil and monocytes (Naclero *et al*., 1991).Among many mediators of inflammation, prostaglandings are one of the most important. The key enzyme in their synthesis is prostaglandin endoperoxide synthase or cyclooxygenase.Cyclooxygenase exist in two isoforms COX-1 and COX-2 (Vane and Botting, 1998). COX-2 is induced by inflammatory stimuli and by cytokines in migratory and other cells, suggesting that the anti-inflammatory action of of NSAIDS are due to inhibition of COX-2, whereas the unwanted side effects such as irritation of the stomach lining and toxic effect on the kidney are due to inhibition of the constitutive enzyme, COX-1 (Vane and Botting, 1998).

2.4.2 Role of nitric oxide in inflammation

A number of laboratories have sought to elucidate the roleof nitric oxide in both the acute and chronic inflammatory diseases. It is now well appreciated that nitric oxide can influence many aspects of the inflammatory cascade ranging from its own expression to recruitment of

leukocytes to the affected tissues. Infiltration of leukocytes to the site of injury or infection is a hallmark feature of inflammation and one that can be profoundly influenced by nitric oxide. Nitric oxide has been shown to inhibit the expression of the selectin adhesion molecules on neutrophils (Banich *et al*., 1997). Inhibition of nitric oxide synthesis result in a marked increase in leukocytes adherence to the endothelium (Kubes *et al*., 1991), while adherence of leukocytes to the vascular endothelium in response to stimulation with a chemotactic factor can be markedly suppressed by nitric oxide donors (Wallace *et al*., 2002). The rate of release of nitric oxide from the mastcells can be rapidly up-regulated by stimulation with interleukin-1b (Hogaboam *et al.,* 1993). Interestingly, nitric oxide produced by mast cells appears to down-regulate the release of a number of other inflammatory mediators from these cells, including histamine, plasma activating factor, and tissues necrosis factors (Salvemini *et al*., 1990). Platelet plays a crucial role not only in blood clotting and thrombosis, but also in inflammatory processes. Platelets can release numerous pro-inflammatory mediators including serotonin, thromboxane and lipoxins. Platelets also contain a number of factors capable of regulating the process of angiogenesis, including vascular endodothelial growth factor and endostatin. The ability of platelets to adhere to the vascular endothelium and to aggregate is under the control of many soluble mediators, including nitric oxide thus, nitric oxide acts to down-regulate platelet aggregation and adherence and therefore plays an important role in down-regulating inflammatory processes. Nitric oxide also mediates at least in some of the pro-angiogenic effects of vascular endothelial growth and thereby affects healing processes and tumor growth (Ziche *et al*.., 1997. Nitric oxide has the capacity to interact with a variety of enzymes, thereby altering their function and influencing inflammatory reactions. For example, nitric oxide can inhibit many iron-containing enzyme functions, including mitochondria electron transfer (Beckman and Koppenol, 1996), which may contribute to tumoricidal activity of macrophages. Nitric oxide has also been shown to interact with cyclooxygenase, another heme-containing enzyme, resulting in an increase in activity (Salvemini *et al* 1993). Nitric oxide can inhibit transcriptional events by inhibiting the transcription factor NF-kB (Katsuyama *et al*.,1998). This has suggested being an important mechanism underlying the anti-inflammatory actions of some nitric oxide releasing drugs (Florucci et al., 2002). Likewise, interactions of nitric oxide with the glucocorticoid receptor appear to contribute to enhanced anti-inflammatory effects of some nitric oxide donating drugs (Paul-Clack *et al*., 2003).

2.4.3Free radicals and inflammatory processes

Most oxidants generated during the inflammatory response derived from pathogenic cells (neutrophils, macrophages and monocytes) and are released into the extracellular environment, in part because one of the oxidants (nicotinamide adenine dinucleotide phosphate oxidase) or NADPH oxidase is assembled in an enzymatically active form on the surface of phagocytic cells (Ward *et al*., 1999). The principal oxidant generating pathway include NADPH oxidase and inducible nitric oxide synthase (Royall *et al.,* 1995). NADPH exist as inactive subunits that are located both on the cell membrane and in the cytosol. Cell activation causes translocation of cytosolic sub-units to the cell membrane, resulting in a multimeric complex that exhibits oxidase activity. The pathway of oxidant generation by NADPH oxidase is characterized by a series of single additions of electrons. In the presence of the oxidase NADPH undergoes oxidation. The released electrons interact with molecular oxygen to cause its reduction, to form the superoxide anion. One of the functions of oxygen is to reduce intracellular iron by converting ferric iron to ferrous iron. A further electron addition to oxygen converts it to hydrogen peroxide which can be further reduced to the most active of all oxygen-centred radicals, the hydroxyl (HO)radicals. Generation of hydroxyl radical requires a heavy metal such as iron in its transition state (Ward et al., 1999). Hydroxyl radical is highly reactive and damaging radical. If it is further reduced, the product is water. In the context of phagocytic cells, such as neutrophils release of myeloperoxidase in the presence of a halide, such as chloride, will enzymatically convert hydrogen peroxide to hypochlorous acid, another potent oxidant (Ward *et al*., 1999). The second major oxidant generating pathway in phagocytic cells involves inducible nitric oxide sythase, which is typically not expressed in resting cells, especially macrophages. On cell activation, however, inducible nitric oxide synthase is transcriptionally up regulated, reacting with L-arginine to generate nitric oxide, which relaxes smooth muscle cells. Nitric oxide is converted to peroxynitrile anion, which is highly reactive with thio groups. Finally peroxynitrite anion is broken down into nitrite and nitrate radicals, which serves as convenient quatitative makers of nitric oxide (Ward *et al*., 1999). These two oxidant generating processes in phygocytic cells account for many tissue-damaging outcomes of inflammatory responses and may well impair physiological responses to injury. Oxidants may perturb phagocytic cells to inappropriately generate mediators, such as cytokins and chemokins. While, chemically synthesized version of L-arginine effectively

antagonized the ability of inducible nitric oxide synthase to react with its natural substrate, the in-vivo use of such compounds lead to problems, because they also antagonized constitutive nitic oxide synthase of endothelia cells, leading to a loss in the regulation of vascular smooth muscle tone, resulting in systemic hypertension.

2.4.4 Anti-inflammatory agents

Anti-inflammatory agent refers to property of a substance or treatment that reduces inflammation.Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system

2.4.4.1Types ofAnti-inflammatory agents

Anti-inflammatory refers to the property of a substances or treatment that reduces inflammation. Antiinflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system.

Steroidal anti-inflammatory agents: Many steroids, (glucocorticoids,) reduce inflammation or swelling by binding to glucocorticoid receptors. These drugs are often referred to as corticosteroids

Non-steroidal anti-inflammatory agents: Non-steroidal antiinflammatory agents (NSAIDs), alleviate pain by counteracting the cyclooxygenase (COX) enzyme. On its own, COX enzyme synthesizes prostaglandins, creating inflammation. The NSAIDs inhibit prostaglandin synthesises, reducing or eliminating the pain.

Some common examples of NSAIDs are: aspirin, ibuprofen, and naproxen. The newer specific COX-inhibitors although, it is presumed, sharing a similar mode of action are not classified together with the traditional NSAIDs. Long term use of NSAIDs can cause gastric erosion, which can become stomach ulcers and in extreme cases can cause severe haemorrhage, resulting in death. The risk of death as a result of the use of NSAIDs is 1 in 12000 for adults aged 16-45. The risk increases almost twentyfold for those over 75 (Trelle *et al*., 2011). Apart from aspirin, prescription and over-the-counter NSAIDs also increase the risk of myocardial infarction and stroke (Trelle *et al*., 2011).

Immune selective anti-inflammatory derivative (ImSAIDs):ImSAIDs are class of peptides being developed by IMULAN Bio Therapeutics, LLC, which were discovered to have diverse biological properties, including anti-inflammatory properties. ImSAIDs work by altering the activation and migration of imflammatory cells, which are immune cells responsible for amplifying the inflammatory response (Bao *et al.,* 2006). The ImSAIDs represent a new category of anti-inflammatory and unrelated to steroid hormones or non steroidal anti-inflammatory agents.

The ImSAIDs were discovered by scientists evaluating biological properties of the submandibular gland and saliva. Early work in this area demonstrated that the submandibular gland release a host of factors that regulate systemic inflammatory responses and modulate systemic immune and inflammatory reactions. It is now well accepted that the immune, nervous and endocrine system communicate and interact to control and modulate inflammation and tissue repair (Mathison *et al.,* 1994)

2.4.4.2 Mechanism of action of anti-inflammatory drugs

Most NSAIDs act as nonselective inhibitors of the enzyme cyclooxygenase (COX), inhibiting both cyclooxygenase-1(COX-1) and cyclooxygenase-2 (COX-2) isoenzymes. COX catalyses the formation of prostaglandins and thromboxane from arachidonic acid (itself derived from the cellular phospholipid bilayer by phospholipase A2). Prostaglandins act as messenger molecules in the process of inflammation (John, 2004). COX-1 is a constutively expressed enzyme with house-keeping role in regulating many normal physiological processes. One of these is in the stomach lining, where prostaglandins serve a protective role, preventing the stomach mucosa from being eroded by its own acid. COX-2 was discovered in 1991 by Daniel Simmons at Brigham Young University. COX-2 is an enzyme facultatively expressed in inflammation and it is inhibition of COX-2 that produces the desirable effects of NSAIDs

When nonselective COX-1 and COX-2 inhibitors (such as aspirin, ibuprofen and naproxen) lower stomach prostaglandin levels, ulcer of the stomach or duodenum internal bleeding can result. Discovery of COX-2 led to research to development of selective COX-2 inhibiting drugs that do not cause gastric problems characteristic of older NSAIDs.

Acetaminophen is not considered an NSAID because it has little anti-inflammatory activity. It treats pain mainly by blocking COX-2 mostly in the central nervous system, but not much in the rest of the body (Hinz *et al*., 2008). NSAIDs are also used in the acute pain caused by gout because they inhibit urate crystal phygocytosis besides inhibition of prostaglandin synthase (Koeberli *et al*., 2009). NSADs have antipyretic activity and can be used to treat fever (Aronoff *et al*., 2001). Fever is caused by elevated levels of prostaglandin E2, which alters the firing rate of neurons within the hypothalamus that control thermoregulation (Nabulsi, 2009). Antipyretics work by inhibiting the enzyme COX, which causes the general inhibition of prostanoid biosynthesis (PGE2) within the hypothalamus. PGE2 signals to the hypothalamus to increase the body's thermal set point (Coceani *et al*., 1986). Ibuprofen has been shown more effective as an antipyretic than acetaminophen (Rainsford, 2009).

2.5 Concept of convulsion

Neurological, mental, and behavioural disorders represent a huge health burden to society, affecting more than 450 million people globally (Mathers *et al.*, 2001) Research data suggest that psychiatric neurological disorders are a growing and important cause of morbidity (Sperling *et al.*, 2005) . Among the most common neurological disorder are depression, schizophrenia, anxiety disorder and epilepsy.

Epilepsy is the second most common neurological disease in the world, affecting approximately 1% of the population (Blum, 1998). It is defined as a chronic disorder of the brain characterized by recurrent and spontaneous unpredictable seizure activity, which is triggered by an abnormal discharge of neurons (Tunnicliff*et al*., 1996) and it has psychological, cognitive, neurological, and social consequences for the patients (Guerrini*et al*.,2006).Convulsion is often a symptom of an epileptic seizure, the term convulsion is sometimes used as a synonym for seizure. However, not all epileptic seizure leads to convulsions and not all convulsions are caused by epileptic seizures. Convulsions are also consistent with an electric shock. The word "fit" is sometimes used to mean a convulsion or epileptic seizure. However, over 30% of people with epilepsy do not have seizures control even with the best available medication (Yemitan and Adeyemi, 2013). Currently available antiepileptic drugs are associated with serious side effects, including teratogenecity, chronic toxicity and adverse effects on cognition and behavior (Johannesen and Patsalos, 2010).

Almost, all the currently available antiepileptic drugs are associated with drug interaction making it difficult to attain easy seizure control (Hela. *et al.,* 2013). There is an urgent need for the development of newer antiepileptic agents with better safety and efficacy profile. There is a reawakeninginterest in traditional medicine in the management of epilepsy, especially in developing countries (Magaji *et al.,* 2012). Researches are needed to validate the folkloric use of these medicinal plants in order to provide evidence of their safety and efficacy (Maiha *et al.,* 2009). One of such medicinal plant used in the traditional management of epilepsy but with paucity of scientific verification literature is *Erythrophleum ivorense*

2.5.1 Types of seizure

Many different types of seizures can be identified on the basis of their clinical phenomena (Loscher, 1998). Seizures are fundamentally divided into two major groups: partial and generalized. Partial (focal, local) seizure are those in which clinical or electrographic evidence exists to suggest that the attacks have a localized onset in the brain, usually in a portion of one hemisphere, while generalized seizure are those in which evidence for a localized onset is lacking. Partial seizure are further subdivided into simple partial, complex partial and partial seizure evolved to secondarily generalized seizure, while generalized seizure are categoralized into absence (nonconvulsive), myoclonic, clonic, tonic, tonic-clonic and atonic seizures. In addition to classifying the seizure that occur in patients with epilepsy, patients are classified into appropriate types of epilepsy or epileptic syndromes characterized by different seizure types, etiologies, ages of onset and electroencephalographic (EEG) features (Commission, 2003)

2.5.2 Anticonvulsant drugs

Anticonvulsants, (commonly known as anti-epileptic drugs) are a diverse group of pharmaceuticals used in the treatment of epileptic seizures. The 20th century has witnessed considerable progress in anticonvulsant drug development (Loscher *et al.,* 1994). The major drugs in clinical use, i.e. phenytoin, carbamazepine, valproate, benzodiazepines, ethosuximide, phenobarbital and primidone, were developed and introduced between 1910 and 1970 and are referred to as old drugs or first generation drugs. After a hiatus of over 20 years, several new anticonvulsant drugs, i.e., vigabatrin, gabapentin, felbamate, lamotrigine, oxcarbazepine, tiagabine and topiramate, have been introduced into clinical practice and were

referred to as new drugs or second generation drugs. The goal of an anticonvulsant is to suppress the rapid and excessive firing of neurons that start a seizure. An effective anticonvulsant would prevent the spread of the seizure within the brain and offer protection against possible excitotoxic effects that may result in brain damage. Some studies have reported that anticonvulsants themselves are linked to lowered intelligent quotient in children (Loring *et al.*, 2005). However these adverse effects must be balanced against the significant risk epileptoform seizures posed to children and the distinct possibility of death and devastating neurological sequel secondary to seizures. Anticonvulsants are more accurately called antiepileptic drugs ("AEDs") and sometimes referred to as anti-seizure drugs. While the term anticonvulsant is a fair description of AEDs, the use of this term tends to lead to confusion between epilepsy and non-epileptic convulsions. Convulsive seizures non-epileptic seizures are quiet common, and these types of seizures do not respond to antiepileptic drugs. In epilepsy, an area of the cortex is typically hyper-irritable. This condition can often be confirmed by completing a diagnostic EEG. Antiepileptic drugs function to help reduce this area of irritability and thus prevent epileptiform seizure.

The major molecular targets of marketed anticonvulsant drugs are voltage-gated sodium channels and components of the GABA system, including GABA-A receptors, GAT-T GABA transporter, and GABA-T (Rogawski *et al*., 2004). Additional targets include voltage-gated calcium channels, SV2A and α2δ (Rogawski *et al*., 2008). Some anticonvulsants have shown antiepileptogenic effects in animal models of epilepsy. That is, they either prevent the expected development of epilepsy or can halt or reverse the progression of epilepsy. However, no drug has been shown to prevent epileptogenesis (the development of epilepsy after an injury) in human trials. (Abou-Khalil, 2007). Approximately 70% of patients with epilepsy are well controlled by monotherapy with currently available antiepileptic drugs. Another 5-10% of patients are stabilized by the addition of another antiepileptic drugs but there remains over 20% of patients whose seizures are not controlled (Richens *et al*., 1993). Therefore, phytomedicines can potentially play an important role in the development of new antiepileptic drugs for pharmacoresistant patients (Nsour *et al*., 2000).

2.5.3 Animals models for testing anticonvulsant drugs

The models employed in the early phase of AED discovery are highly predictive of subsequent efficacy in easy-to-manage generalized and partial epilepsy (Smith *et al.,* 2007). Thus, animal models more employed were leptazol-induced seizure, maximal electroshock seizure, metrazole-induced seizure, picrotoxin-induced convulsion, pilocarpine and strychnine-induced seizures. However, maximal electroshock seizure, picrotoxin-induced seizure and pentylenetetrazole-induced seizure models continue to represent the three most widely used animal seizure models employed in the search for new AEDs (While *et al.,* 2002).

2.5.4 Mechanism of action of anticonvulsant drugs

It is important to understand the mechanism of action and the pharmacokinetic of anticonvulsants so that these can be used effectively in clinical practice, especially in multi-drug regimen. Many structures and processes are involved in the development of a seizure, including neurons, ion channels, glia, and inhibitory and excitatory synapses. The AEDs are designed to modify these processes so as to favour inhibition over excitation and thereby stop or prevent seizure activity. AEDs can be grouped according to their main mechanism of action, although many of them have several actions and others have unknown mechanism of action. The main group includes Sodium channel blockers, calcium current inhibitors, gamma-aminobutyric acid enhancers, glutamate blockers,

Sodium channel blockers: The firing of an action potential by an axon is accomplished through sodium channels. Each sodium channel dynamically exists in the following 3 states: A resting state, during which the channel allows passage of sodium into the cell. An active state, in which the channel allows, increased influx of sodium into the cell. An inactive state, in which the channel does not, allows passage of sodium into the cell. During an action potential, these channels exist in the active state and allow influx of sodium ions. Once the activation or stimulus is terminated, a percentage of these sodium channels become inactive for a period known as the refractory period. With constant stimulus or rapid firing, many of these channels exist in the inactive state, rendering the axon incapable of propagating the action potential. AEDs that target the sodium channels prevent the return of these channels to the active state by stabilizing them in the inactive state. In doing so, they prevent repetitive firing of the axon.

Calcium channel blockers:Calcium channels exist in three known forms in the human brain: L, N and T. These channels are small and are inactivated quickly. The influx of calcium currents in the resting state produces a partial depolarization of the membrane, facilitating the development of action potential after rapid depolarization of the cell. Calcium functions as the pacemakers of normal rhythmic brain activity. This is particularly true of the thalamus. T-calcium channels have been known to play a role in the three per second spike-and-wave discharges of absence seizure. AEDs that inhibit these T-calcium channels are particularly useful for controlling absence seizure (Nelson, 2010).

GABA enhancers:Gamma-aminobutyric acid (GABA) has 2 types of receptors, A and B. when GABA binds to a GABA-A receptor, the passage of chloride, a negatively charged ion, into the cell is facilitated via chloride channels. This influx of chloride increases the negativity of the cell. These cause the cell to have greater difficulty reaching the action potential. The GABA-B receptor is linked to a potassium channel. The GABA system can be enhanced by binding directly to GABA-A receptors, by blocking presynaptic GABA uptake, by inhibiting the metabolism of GABA by GABA transaminase, and by increasing the synthesis of GABA. GABA is produced by decarboxylation of glutamate mediated by the enzyme glutamic acid decarboxylase (GAD). Some AEDs may act as modulators of this enzyme, enhancing the production of GABA and down-regulating glutamate. Some AEDs function as an agonist to chloride conductance, either by blocking the reuptake of GABA (e.g tiagabine) or by inhibiting its metabolism as mediated by GABA transaminase (e.g vigabatrine), resulting in increased accumulation of GABA at the postsynaptic receptors.

Glutamate blockers:Glutamate receptors bind glutamate, an excitatory amino acid neurotransmitter. Upon binding glutamate, the receptors facilitate the flow of both sodium and calcium ions into the cells, while potassium ions flow out of the cell, resulting in excitation. The glutamate receptors have 5 potential binding sites as follows (Kim *et al*., 2002): Alpha-amino-3-hydroxy-5-methylisoxazole-4 propionic(AMPA) site, Kainite site, N-methyl-D-aspartate (NMDA) site, glycine site and the metabotropic site, which as 7 subunits (GluR1-7).

AEDs that modify these receptors are antagonistic to glutamate. Responses to glutamate antagonists differ, depending on the site being affected.

SV2A-binding agents:Synaptic vesicle protein 2A (SV2A) is ubiquitously expressed in the brain, but its function has not been clearly defined. SV2A appears to be important for the anailability of calcium-dependent neurotransmitter vesicles ready to release their content (Xu and Bajjalieh, 2001). The lack of SV2A results in decreased action potential-dependent neurotransmission, while action potential-independent neurotransmission remain normal. The role of SV2A in epilepsy is confirmed by the finding that SV2A knockout mice develop a strong seizure phenotype a few weeks after birth (Janz *et al.,* 1999). The anticonvulsant potency of SV2A ligands is correlated with their binding ability in the audiogenic seizure-prone mice. Levetiracetam binds the SV2A (Lynch *et al.*, 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plants Materials

Fresh stem bark of *Erythrophleum ivorense* was collected from Iwo in Iwo Local Government area of Osun State, South West, Nigeria. It was authenticated in the herbarium, Department of Botany, Obafemi Awolowo University Ile-Ife, where voucher specimen was deposited with voucher number given as 16878

3.1.1 Preparation of plants material

The stem bark of *Erythrophleum ivorense* was dried and reduced to coarse powdery form using electric blending machine.Air-dried powder of *Erythrophleum ivorense* was extracted in 75% methanol. Crude methanol extract (CME) was fractionated using solvents of different polarity including ethyl acetate (EA), dichloromethane (DCM) and n-hexane (NH) according to standard protocol (Uddin *et al*.,2012). A total of 50g of CME of ***Erythrophleum ivorense*** was suspended in water and extracted successively with ethyl acetate, dichloromethane and n-hexane in a separating funnel. At each stage of partitioning the organic fractions was pooled and the solvent removed.CME and fractions were concentrated to yield ethyl acetate fraction (EAF), dichloromethane fraction (DCMF), n-hexane fraction (N-HF) (Table 2). The crude methanol extract and fractions were prepared by dissolving in 2% dimethylsuphoroxide (DMSO). The extract and fractions were administered intraperitoneally at doses of 5 to 20 mg/kg body weight to experimental animals.

Table 2: Yields of fractions obtained from the solvent-solvent fractionation of CME of *Erythrophleum ivorense*

Fraction	Weight of yield	% yield
Ethylacetate Dichloromethane	27.6	55.2
N-hexane	15.7	31.4
	6.7	13.4

The starting CME was 50.0g.

3.2 Animal materials

The animals used in the study were mice (Swiss strain, male, 20-30g). They were obtained and housed in preclinical Animal House, College of Medicine, University of Ibadan. They were kept in standard cages with a maximum of six animals in a cage. The animals were housed under standard environmental conditions in the Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan Animal house. Animals were fed with standard diet (Ladokun feeds Ltd, Ibadan) for two weeks prior to experimentation and allowed free access to clean drinking water delivered water dispensing bottles.

3.3 Acute toxicity of CME

Toxicity of CME was assessed by determination of lethal dose. Male Swiss mice were intraperitoneally (i.p) injected with CME (5, 10, 20, 40, 80, 160mg/kg). Each dose was tested in five animals. Animals were observed for symptoms of toxicity and the number of death was registered within 24 hours of treatment (Miller *and Tainter*, 1944; Lorked, 1983)

3.4 Phytochemical screening

The preliminary phytochemical screening of the extracts (CME, EAF, DCMF and HF) of *Erythrophleum ivorense* stem bark was carried out using standard procedures (Treese and Evan 1989; Harborne, 1998)

Flavonoids- Some magnesium ribbons and 5ml of concentrated HCl were added to 0.5g of extract and fractions. Red colouration indicates the presence of flavonoid.

Tannins- Each of the extract and fractions **(**0.5g) were boiledin 10ml of water for 15 minutes, filtered and made up to 10ml. To 2ml of the filtrate was added 10ml of water and 1 drop of ferric chloride. Blue or green colouration indicates the presence of tannins.

Phlobatannins- To 5ml of the filtrate from the test on tannins was added 3 drops of 40% formaldehyde and 6 drops of dilute HCl. The temperature of the mixture was raised to boiling point, cooled and the bulk precipitate formed was washed with hot water, alcohol and warm 5% potassium hydroxide. The presence of coloured residue indicates the presence of phlobatannins.

Cardiac Glycosides- Each of the extract and fractions **(**0.5g) was dissolved in 2ml chloroform and sulphuric acid was carefully added to form a layer. Reddish-brown colouration at interfase indicates the presence of steroidal cardiac glycoside.

Alkaloids – About 250mg of extract was dissolved in water and filtered. The filtrate was acidified with 1ml HCl. One milliliter of the filtrate was treated with 2 drops of Mayers reagent. A precipitate was taken as an evidence of the presence of alkaloids. Another 1ml of the filtrate was treated with Dragendorff's reagent. Presence of turbidity or a precipitate further indicates the presence of alkaloids.

Saponins -Each of the extract and fractions **(**0.5g) was shaken with hot water in a test tube, persistent frothing indicate the presence of saponins

3.5 Analgesicstudies

3.5.1 Acetic acid-induced writhing test

Writhing test is a chemical method used to induce pain of peripheral origin by injection of irritant principles like phenylquinone and acetic acid in mice. Analgesic activity of the test compound is inferred from the decrease in the frequency of writhing. The manifestations of abdominal writhing in mice were first described by Stevens et al., 1971 as an arching of back, extension of hind limbs and contraction of abdominal musculature. The writhing response is considered as a reflective test and is without clinical counterparts as it cannot be performed in human. Writhing generated by parenteral administration of acetic acid in mice are due to prolonged pain of endogenous nature which recur for a prolonged period of time. Due to

irritant nature, these principles are also prone to induce lesions. Writhing is an overt response to intense pain induced by irritant principles via nociceptors characterize by episodes of retraction of abdomen and stretching of hind limbs. The signals transmitted to central nervous system in response to pain due to irritation, caused release of mediators such as prostaglandin, which contributes to increased sensitivity to nociceptors. In this experiment Writhing in mice was induced according to the method described by Koster *et al*., 1959.

The mice were randomly divided into five groups, group 1 received normal saline (10ml/kg, i.p) while CME (5-20mg/kg, i.p) was given to groups (2-4). Each mouse was given 0.6% aqueous solution of acetic acid and then placed in an observer box. The animals were pretreated for 30minutes before acetic acid administration. Nociception was evaluated by counting the number of abdominal constriction for 20minutes after administration of acetic acid. Percentage protection against abdominal constriction was taken as an index of analgesia .The processes was repeated using EAF (5-20mg/kg,i.p), DCMF (5-20mg/kg, i.p), n-HF (5-20mg/kg, i.p).Acetylsalicylate (ASA, 150mg/kg, i.p), served as reference drug

3.5.2 Hot plate test

The hot plate test is a test of the pain response in animals, similar to the tail flick test. It is used in basic pain research and in testing the effectiveness of analgesics by observing the reaction to pain caused by heat. It was proposed by Eddy and Leimbach in 1953. They used a behavioural model of nociception where behaviours such as jumping and hind-licking are elicited following a noxious thermal stimulus. Licking is a rapid response to painful thermal stimuli that is a direct indicator of nociceptive threshold. Jumping represent a more elaborated response, with latency and encompasses an emotional component of escaping (Espejo and Mir, 1993). In this experiment the pain episode was induced by thermal stimulus as described by Hunskaar *et al.*, 1986. The mice were randomly divided into five groups, group 1 received normal saline (10ml/kg, i.p) while CME (5-20mg/kg, i.p) was given to groups (2-4). The animals were pretreated for 30minutes; each mouse was placed in a hot plate maintained at 55±0.5º Nociception was evaluated when the animal began to lick its hind paw or attempt to jump out of the hot plate. The time taken to lick the hind paw was taken as reaction time Anti-nociceptory activity was expressed as the increased in reaction time.The processes was repeated using EAF (5-20mg/kg,i.p), DCMF (5-

20mg/kg, i.p), n-HF (5-20mg/kg, i.p).Morphine sulphate (5mg/kg, i.p) served as reference drug.

3.5.3 Formalin-induced paw lick test

The formalin assay is the most popular chemical assay of nociception. It entails the injection of a dilute solution of formalin into the surface of the rodent's hind paw, followed by scoring of stereotypical behaviours such as flinching; licking and biting of the affected hind paw (Carter *et al.,* 2010). The behavior last for approximately one hour, with the early or acute phase (directly after injection) reflecting direct activation of nociceptors and late phase (15 or 20 minutes after injection) reflecting inflammation (Carter *et al.*, 2010). In this experiment paw lick in rats was induced by formalin according to the method described by Hunskaar and Hole 1987.The mice were randomly divided into five groups, group 1 received normal saline (10ml/kg, i.p) while CME (5-20mg/kg, i.p) was given to groups (2-4). Each mouse was injected at right hind paw with formalin (1%, 2ul). The animals were pretreated for 30minutes before injection of formalin. Norciception was evaluated when the animal began to lick its paw at 0-5 minutes (early phase) and 20-30 minutes (late phase). Antinorciceptory activity was expressed as the reduction in duration of paw lick.The processes was repeated using EAF (5-20mg/kg,i.p), DCMF (5-20mg/kg, i.p), n-HF (5-20mg/kg, i.p).Morphine sulphate (5mg/kg, i.p) served as reference drug.

3.6 Antiinflammatory Activity of extract andfractions of *Erythrophleum ivorense*

3.6.1Carrageenin-induced paw lick

Carrageenin-induced rat paw oedema test is useful in detecting orally active anti-imflammatory agents (Dirosa, Giroud and Willoughby., 1971). Carrageenin is a mucopolysaccharide from the Irish moss *Chondrus crispus*(Dirosa, 1972). It produces inflammatory oedema by its ability to stimulate the release of inflammatory mediators such as prostaglandin, kinin, histamine and serotonin; leading to fluid exudation from within blood capillaries into the extravascular spaces. In this experiment pedal inflammation in rats was induced according to the method described by Winter *et al*., 1962.

The Rats were randomly divided into five groups, group 1 received normal saline (10ml/kg, i.p) while CME (5-20mg/kg, i.p) was given to groups (2-4).Edema was induced by sub-plantar

injection of 0.1ml of freshly prepared 1% carrageen an into the right hind paw of each rat. The animals were pretreated for thirty minutes before sub-plantar injection (0 hour). Inflammation was evaluated by increased in paw volume, paw volume was measure at 0 and 3 hours after carragenin injection using cotton thread. Anti-inflammation was expressed as reduction or increased in percentage inhibition of paw volume. The processes was repeated using EAF (5-20mg/kg,i.p), DCMF (5-20mg/kg, i.p), N-HF (5-20mg/kg, i.p). Indomethacin (5mg/kg, i.p) served as reference drug

3.7 Anticonvulsant activities of extract andfraction of*Erythrophleum ivorense*

3.7.1 Effect of *Erythrophleum ivorense* on Picrotoxin-induced convulsion

Themethod as described by Elisah *et al*., 1988 was used.
The mice were randomly divided into four groups. Group 1 received normal saline (10ml/kg, i.p) while CME (5-20mg/kg, i.p) was given to groups (2-4).The animals were pretreated for thirty minutes before intra-peritoneal administration of picrotoxin. Each mouse was given picrotoxin (10mg/kg i.p) and then placed in an observer box.. Convulsion was evaluated when there was clonic contraction. Anti-convulsant activity was measured when onset of convulsion was prolonged and duration of convulsion shortened.The processes was repeated using EAF (5-20mg/kg,i.p), DCMF (5-20mg/kg, i.p), n-HF (5-20mg/kg, i.p).

3.7.2 Effect of *Erythrophleum ivorense* on Leptazol-induced convulsion in mice

The mice were randomly divided into four groups. Group 1 received normal saline (10ml/kg, i.p) while CME (5-20mg/kg, i.p) was given to groups (2-4). The animals were pretreated for thirty minutes before intra-peritoneal administration of leptazol.Each mouse was given leptazol (85mg/kg i.p) and then placed in an observer box.. Convulsion was evaluated when there was tonic/clonic contraction. Anti-convulsant activity was measured when onset of convulsion was prolonged and duration of convulsion shortened.The processes was repeated using EAF (5-20mg/kg,i.p), DCMF (5-20mg/kg, i.p), N-HF (5-20mg/kg, i.p).

3.7.3 Effect of *Erythrophleum ivorense* on Strychnine-induced convulsion

The method as described by Elisah *et al*., 1988 was used.
The mice were randomly divided into four groups. Group 1 received normal saline (10ml/kg,

i.p) while CME (5-20mg/kg, i.p) was given to group (2-4). Each mouse was given strychnine (3mg/kg i.p) and then placed in an observer box. The animals were pretreated for thirty minutes before intra-peritoneal administration of strychnine. Convulsion was evaluated when there was tonic/clonic contraction. Anti-convulsant activity was measured when onset of convulsion was prolonged and duration of convulsion shortened.The processes was repeated using EAF (5-20mg/kg,i.p), DCMF (5-20mg/kg, i.p), n-HF (5-20mg/kg, i.p).

3.8. Effect of*Erythrophleumivorense*onpentobarbitone-induced sleeping time

The sleep evaluation method was based on prolongation of pentobarbital-induced sleeping time (Rakhshandah *et al.*, 2007; Rakhshandah *et al*.,2012). The mice were randomly divided into four groups. Group 1 received normal saline (10ml/kg, i.p) while CME (5-20mg/kg, i.p) was given to groups (2-4)s. Thirty minutes later, each mouse received pentobarbitone sodium (40mg/kg i.p) Sleep onset was calculated as the interval between the loss and recovery of the righting reflex (Vohora *et al*., 2000). The processes was repeated using EAF (5-20mg/kg,i.p), DCMF (5-20mg/kg, i.p), n-HF (5-20mg/kg, i.p).

3.9. Statistical analysis

Data obtained from this study were expressed as mean±SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Newman Keuls multiple comparison test. P-values less than 0.05 were considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Acute Toxicity Test

Acute toxicity study showed that the crude methanol extract is fairly toxic. Some of the signs of toxicity observed were reduced motor activity, ataxia, scratching and increased respiratory rate and death. The crude methanolic extract showed that at a dose of 87mg/kg body weight, 50% of animals died.

4.2 Phytochemical screening of crude methanolic extract and its fractions

The results of Table 3 shows that the N-HF of *E ivorense* although having a lot of non-polar constituents, is devoid of the main compound families indicated in the table, whereas, the DCMF contained alkaloids, saponins, flavonoids and EAF contained alkaloids, saponins, tannins, flavonoids and cardiac glycosides. Likewise CME showed similar constituents to EAF. Since both CME and EAF were rich sources of most of the phytochemical constituents, one of which will be selected for further study.

Table 3: Phytochemical constituents of the extract and fractions of *Erythrophleum ivorense* stem back

Secondary metabolites	Extracts			
	CME	**EAF**	**DCMF**	**HF**
Alkaloids	++	++	++	--
Saponins	++	++	++	--
Tannins	++	++	--	--
Flavonoids	++	++	++	--
Cardiac glycosides	++	++	--	--

Keys: ++ present -- Absent

4.3 Analgesic activities

4.3.1 Effects of CME, EAF, DCMF, N-HF *Erythrophleum ivorense* on Acetic Acid-Induced WrithingFigure (2 -3) showed that CME and EAF produced significant ($p<0.05$) inhibition of acetic acid-induced writhing (25.8, 83.7, 88.7%) and (32.4, 81.4, 89.4%) respectively in a dose-related manner.

DCMF produced significant ($p<0.05$) inhibition of acetic acid (65.0%) at maximum dose (20mg/kg) (Figure 4).

N-HF (5-20mg/kg) did not significantly ($p>0.05$) inhibit acetic acid-induced contraction of the stomach of mice. (Figure 5)

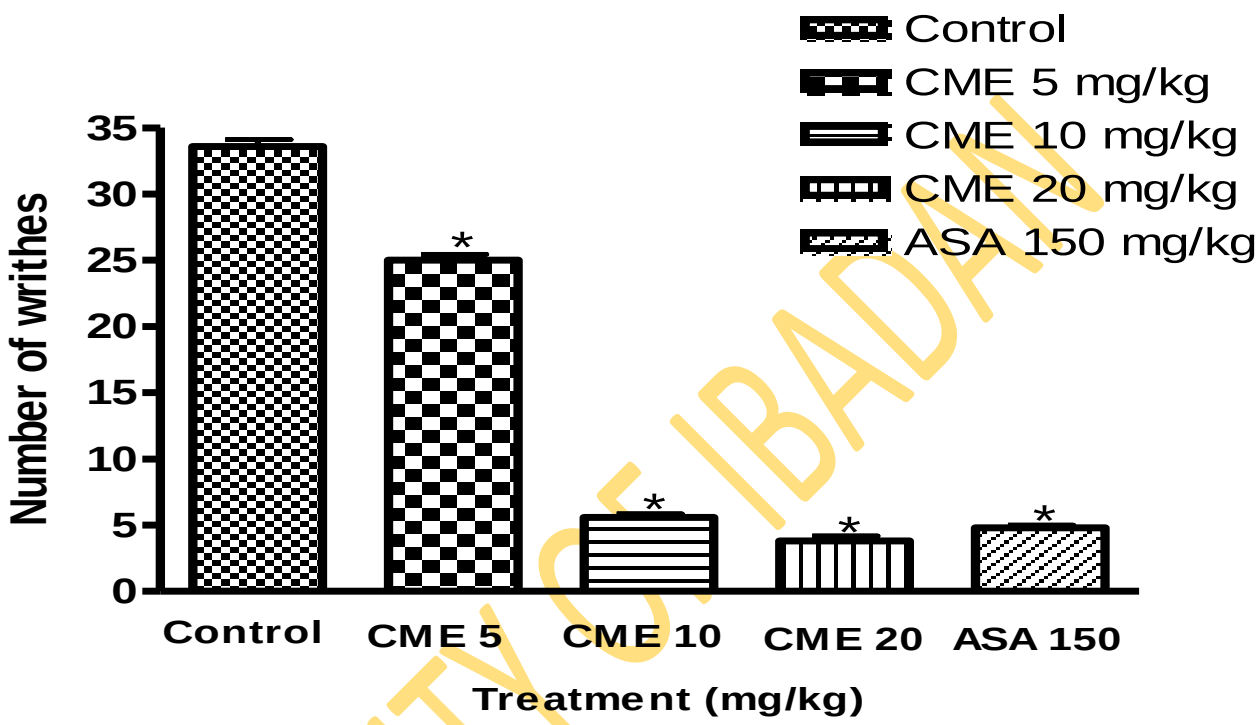


Figure: 2 Effect of Crude methanol extract (CME) of *E.ivorense* on acetic acid induced writhing in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups.

ANOVA followed by Newman-Keuls Multiple Comparison tests.

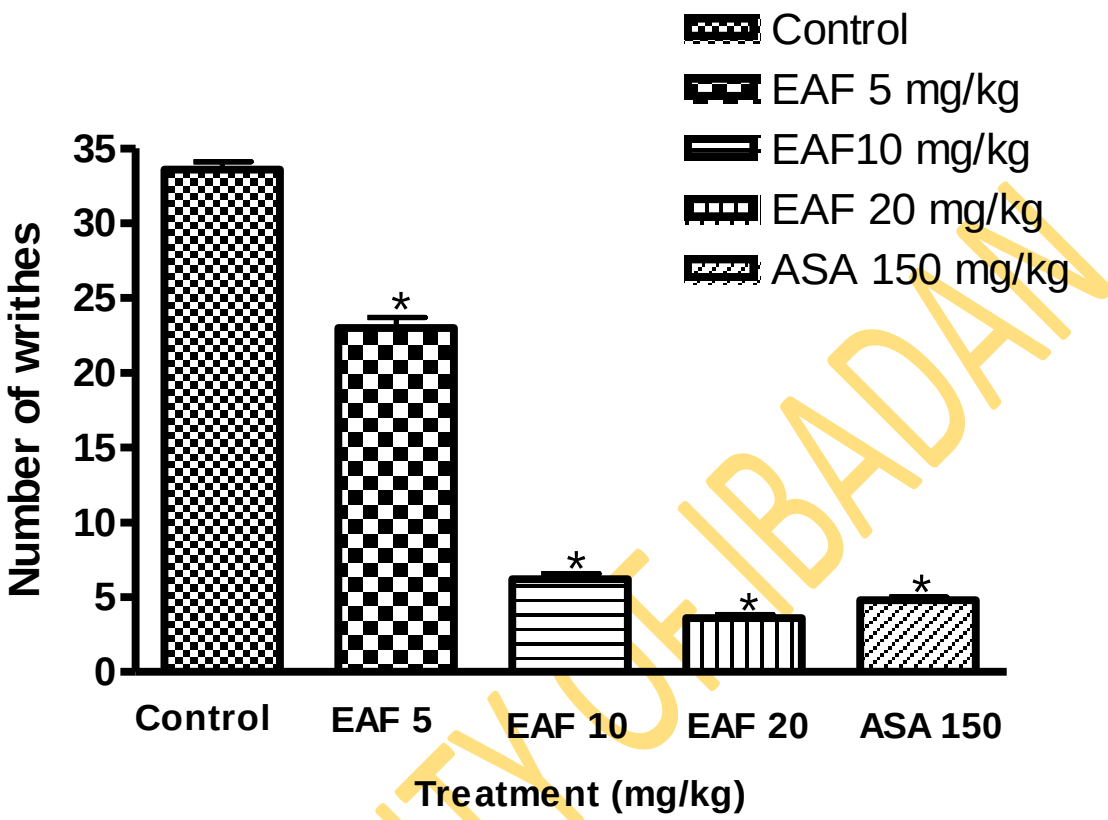


Figure :3 Effect of Ethyl acetate fraction (EAF) of *E.ivorense* on acetic acid induced writhing in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups.

ANOVA followed by Newman-Keuls Multiple Comparison tests.

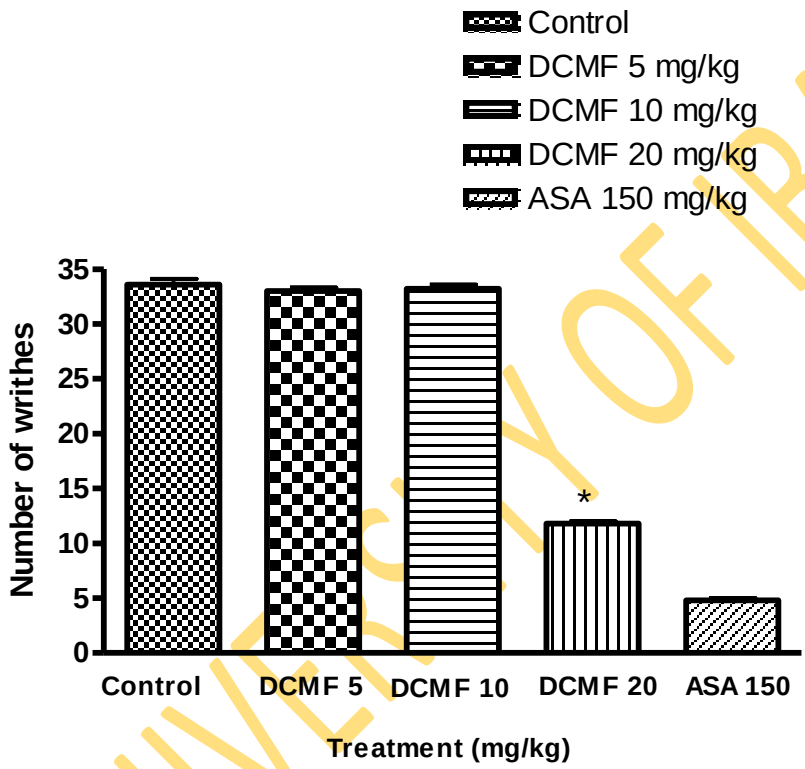


Figure : 4 Effect of Dichloromethane fraction (DCMF) of *E.ivorense* on acetic acid induced writhing in mice.Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison tests.

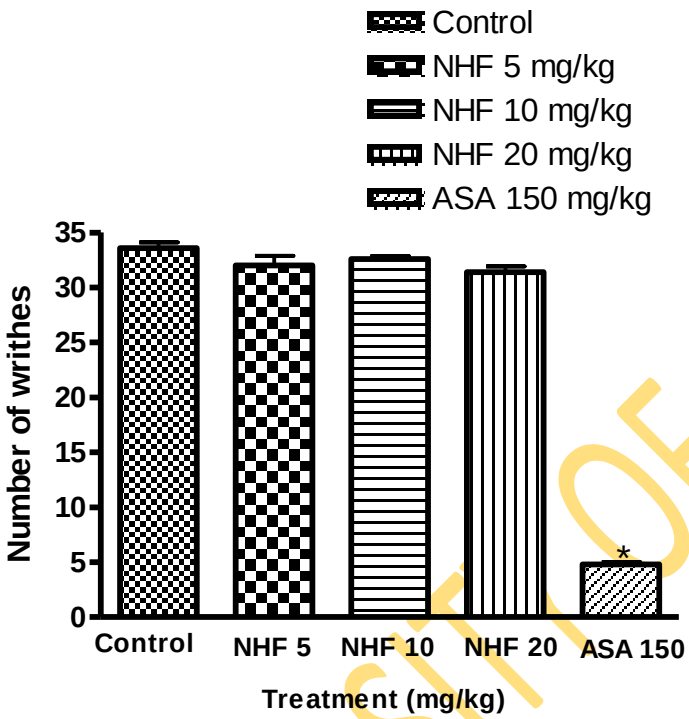


Figure :5 Effect of N-Hexane fraction (NHF) of E.I on acetic acid induced writhing in mice. Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison tests.

4.3.2 Effects of CME, EAF, DCMF, n-HF *Erythrophleum ivorense* on hot plate test in mice

Figure (6 – 7) showed that CME and EAF significantly ($p<0.05$) prolonged the reaction time (seconds) in hot plate test (3.6±0.40, 5.8±0.32, 7.0±0.50) and (3.2±0.30, 4.4±0.25, 6.6±0.45) to noxious heat respectively in a dose related manner, while DCF (2.6±0.24, 2.6±0.24, 2.8±0.40) and HF (2.4±0.35, 2.0±0.20, 2.8±0.5) did not significantly change the responses compared with control (2.2 ±0.20) (Figure 8 - 9).

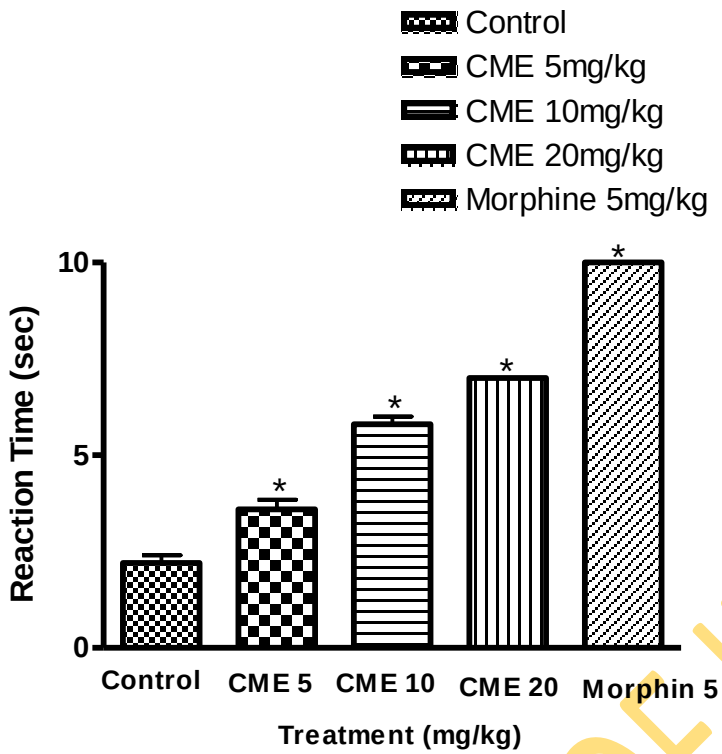


Figure:6 Effect of Crude methanol extract (CME) of *E.ivorense* on Hot plate test in mice. Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

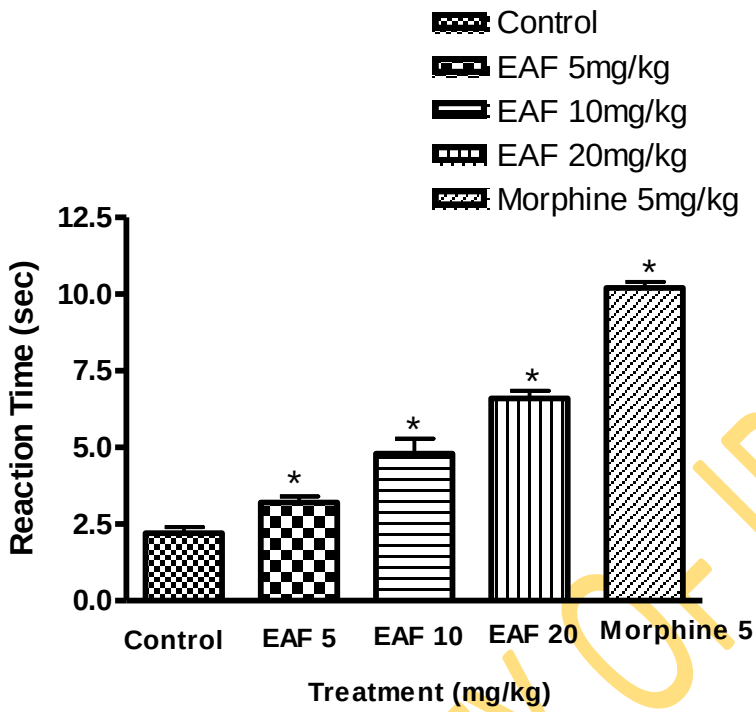


Figure:7 Effect of Ethyl acetate fraction (EAF) of *E.ivorense* on Hot plate test in mice.Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

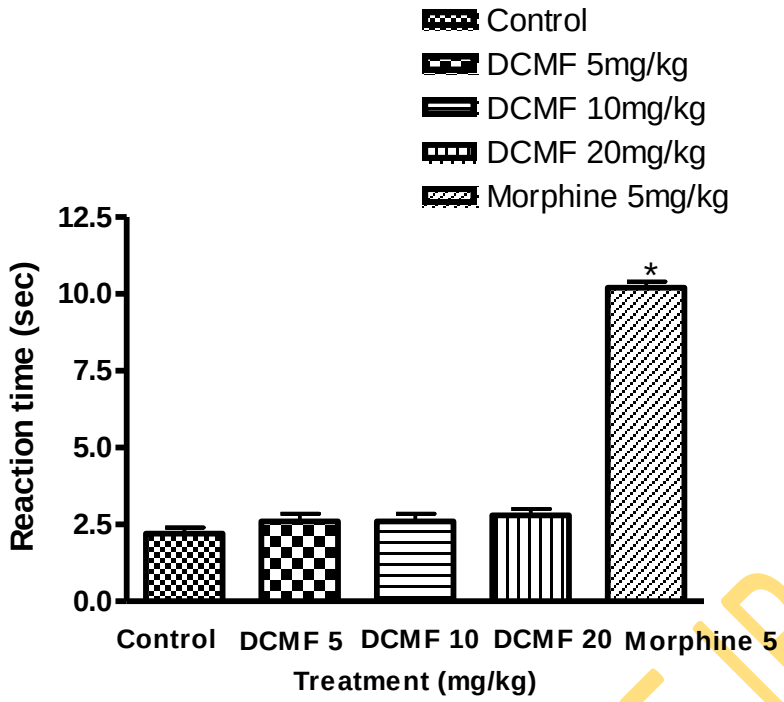


Figure:8 Effect of Dichloromethane fraction (DCMF) of *E.ivorense* on Hot plate test in mice.Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

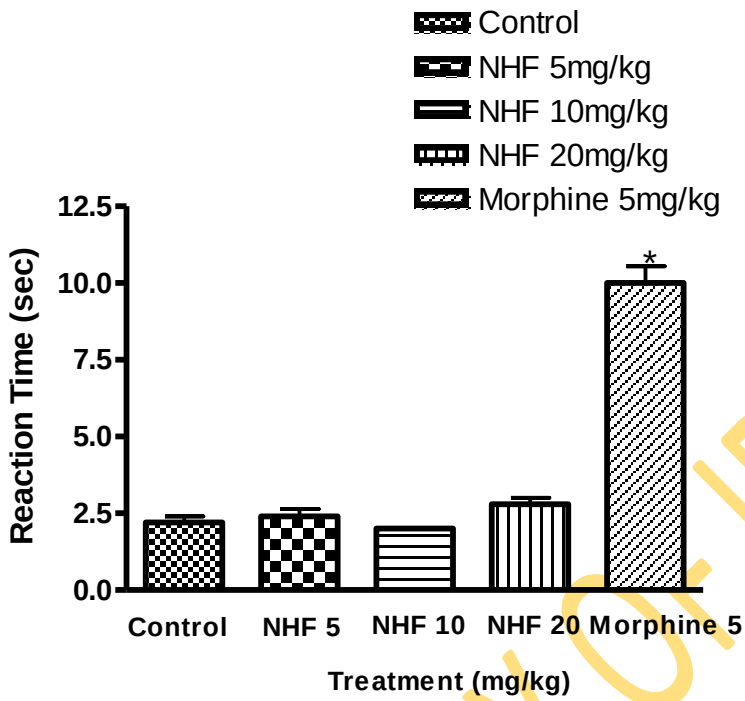


Figure:9 Effect of N-Hexane fraction (NHF) of *E.ivorense* on Hot plate test in mice.Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

4.3.3 Effects of CME, EAF, DCMF, and N-HF *Erythrophleum ivorense* on Formalin-induced paw licks

CME and EAF produced significant ($p<0.05$) inhibition of acute and chronic (inflammatory pain) phases of formalin-induced paw licks in a dose-related manner (38.2±0.45, 116.1±0.5; 35.6±0.40, 67.0±0.85) and (42.5±0.35, 128.3±0.56; 35.2±0.42, 74.2±0.75) respectively compared with control (56.0±0.82, 148.0±1.05) (Figure 10 - 11).

The DCMF (20mg/kg) produced significant ($p<0.05$) inhibition of inflammatory (53.0±0.3) but not the neurogenic (55.5±0.4) pain induced by formalin when compared with the control (Figure12).

However, N-HF did not significantly ($p>0.05$) produce inhibition of either acute phase or chronic phase of formalin-induced paw licking. (Figure 13)

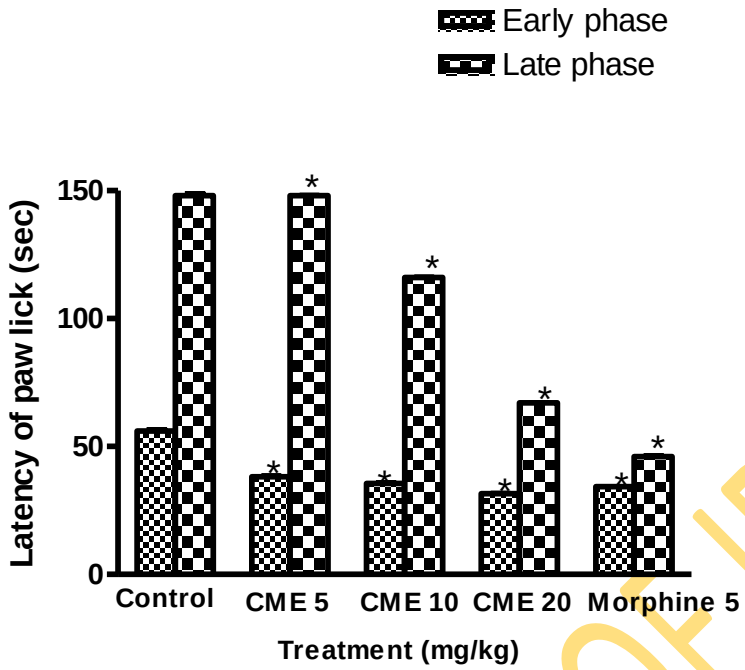


Figure :10 Effect of Crude methanol extract (CME) of *E.ivorense* on formalin induced paw licking in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups

ANOVA followed by Newman-Keuls Multiple Comparison tests.

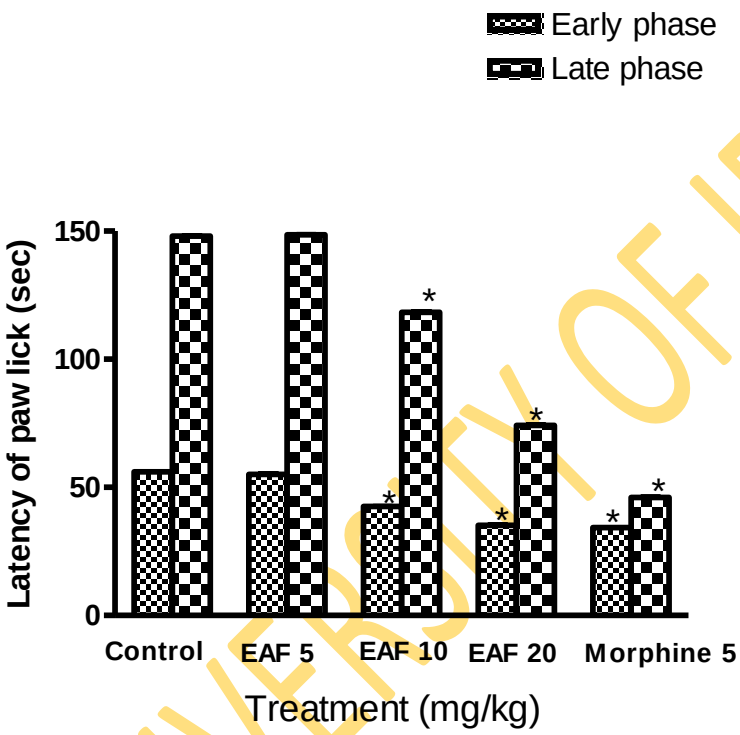


Figure: 11 Effect of Ethyl acetate fraction (EAF) of *E.ivorense* on formalin induced paw licking in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups.

ANOVA followed by Newman-Keuls Multiple Comparison tests.

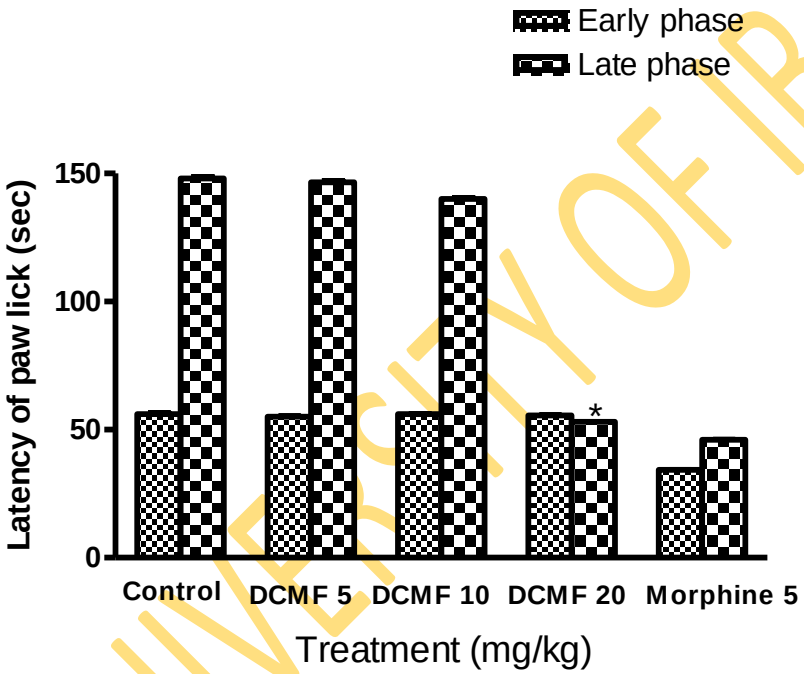


Figure: 12 Effect of Dichloromethane fraction (DCMF) of *E.ivorense* on formalin induced paw licking in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups.

ANOVA followed by Newman-Keuls Multiple Comparison tests.

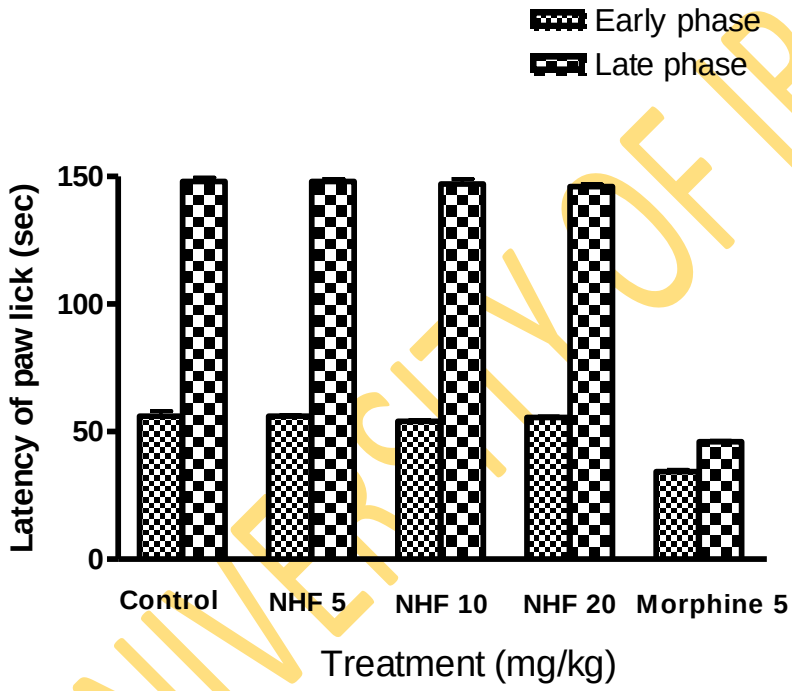


Figure :13 Effect of N-Hexane fraction (NHF) of *E.ivorense* on formalin induced paw licking in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups.

ANOVA followed by Newman-Keuls Multiple Comparison tests

4.4 Antiinflammatory activity

4.4.1 Effect of CME, EAF, DCMF and N-HF on carragenin induced rat paw edema at 3 hours (peak edema period)

Injection of carrageenan into the hind paw induced a progressive edema reaching its maximum at 3hours.

CME and EAF significantly ($p<0.05$) inhibited the paw edema sizes (25.0, 41.7, 58.3% and 46.7, 46.2, 60.0%) respectively in a dose related manner, while DCMF produced a significant ($p<0.05$) inhibition in the paw edema sizes (54.5%) at a maximum dose 20mg/kg

While, N-HF (5-20mg/kg) did not significantly ($p>0.05$) inhibit paw edema sizes. (Table 4)

Table 4: Effects of CME, EAF, DCMF, and N-HF of *E ivorense* on carragenin-induced rat paw edema at 3 hours (peak edema period)

Treatments	Doses (mg/kg)	Paw sizes** (cm)		Percentage inhibition
		Before carragenan	**After carragenan**	
Control(2% DMSO)	0	2.0±0.20	3.2±0.50	0
CME	5	2.0±0.20	2.9±0.40*	25.0
	10	2.0±0.20	2.7±0.32*	41.7
	20	2.0±0.20	2.5±0.41*	58.3
EAF	5	2.0±0.20	2.8±0.25*	46.7
	10	1.9±0.20	2.7±0.23*	46.2
	20	2.0±0.20	2.6±0.29*	60.0
DCMF	5	2.0±0.20	3.1±0.25	0
	10	2.1±0.30	3.1±0.25	9.1
	20	2.0±0.20	2.5±0.05*	54.5
NHF	5	2.0±0.20	3.2±0.50	0
	10	2.1±0.30	3.2±0.50	8.3
	20	2.0±0.20	3.1±0.05	8.3
Indomethacin	5	1.9±0.21	2.3±0.25*	68.7

**Values were recorded as means±SEM of five independent observations. *Values are statistically significant ($p<0.05$) in relation to control. One way ANOVA followed by Dunnett's multiple comparison tests

4.5 Antnticonvulsant activities

4.5.1Effect of CME, EAF, DCMF and N-HF *Erythrophleum ivorense* on picrotoxin-induced convulsion in mice

CME (5-20mg/kg) and EAF (10-20mg/kg) significantly ($p<0.05$) prolonged the onset of seizures and shortened the duration of convulsion and at 5mg/kg EAF exhibited no significant effect on the latency of onset of convulsion (Figure 14 - 15).

DCMF (5-20mg/kg) and N-HF (5-20mg/kg) did not significantly ($p>0.05$) delay onset of convulsion or shortened or offer any protection to animals. (Figure 16-17)

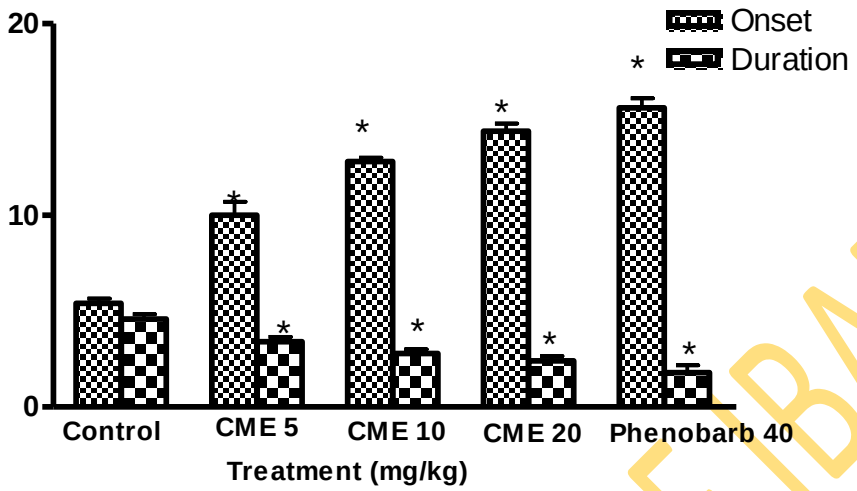


Figure: 14 Effect of Crude methanol extract (CME) of *E.ivorense* on picrotocin-induced convulsion in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison tests

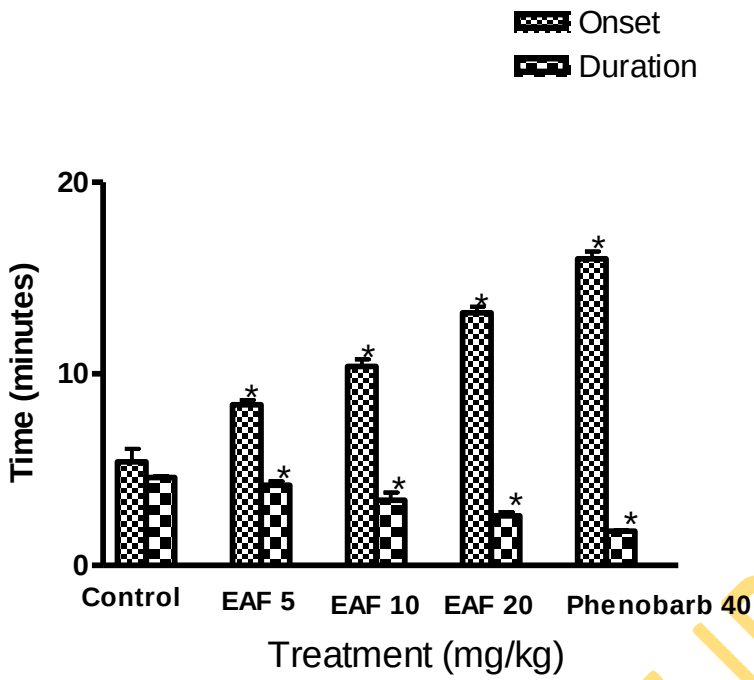


Figure15 Effect of Ethyl acetate fraction (EAF) of *E.ivorense* on picrotocin-induced convulsion in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison tests

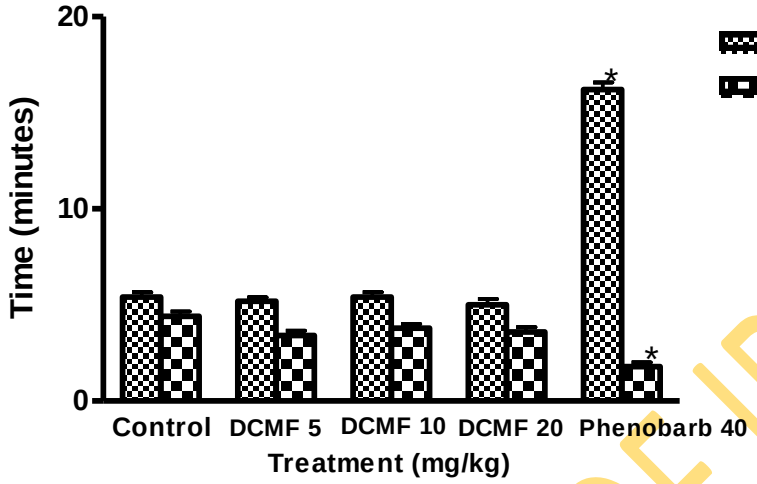


Figure:16 Effect of Dichloromethane fraction (DCMF) of *E.ivorense* on picrotocin-induced convulsion in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison tests

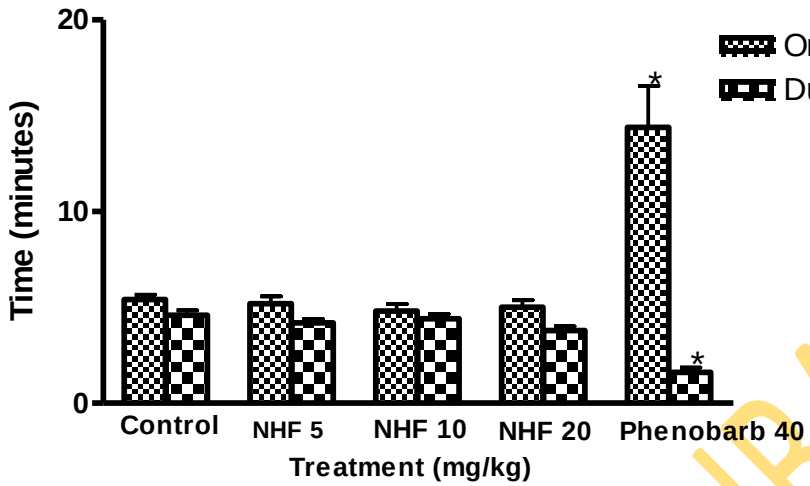


Figure:17 Effect of N-Hexane fraction (NHF) of E.I on picrotocin-induced convulsion in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison tests

4.5.2 Effect of CME, EAF, DCMF and N-HF *Erythrophleum ivorense* on leptazol-induced convulsion in mice

CME and EAF at a maximum dose (20mg/kg) significantly $p<0.05$ prolonged onset of seizure and shortened duration of convulsion (Figure 18-19). However, DCMF and N-HF did not significantly $P>0.05$ prolong the onset of seizures and also failed to shorten the duration of convulsions (Figure 20-21).

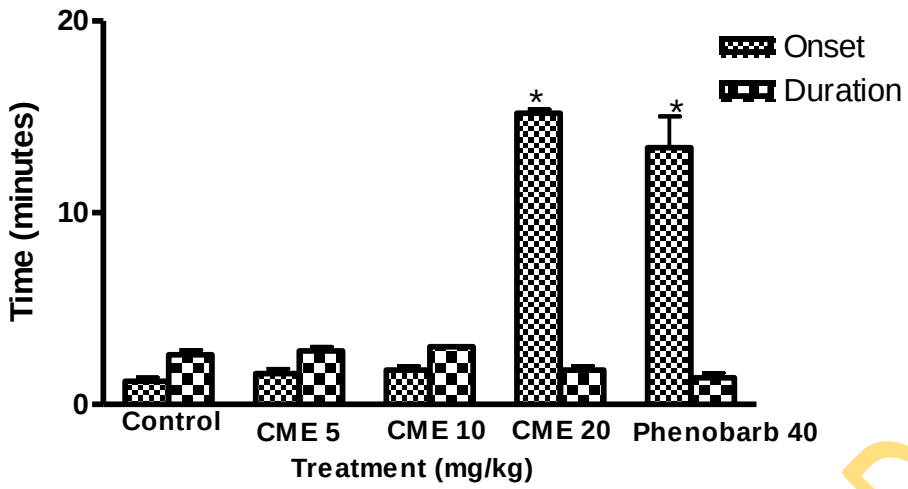


Figure:18 Effect of Crude methanol extract (CME) of *E.ivorense* on Leptazol-induced convulsion in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison tests

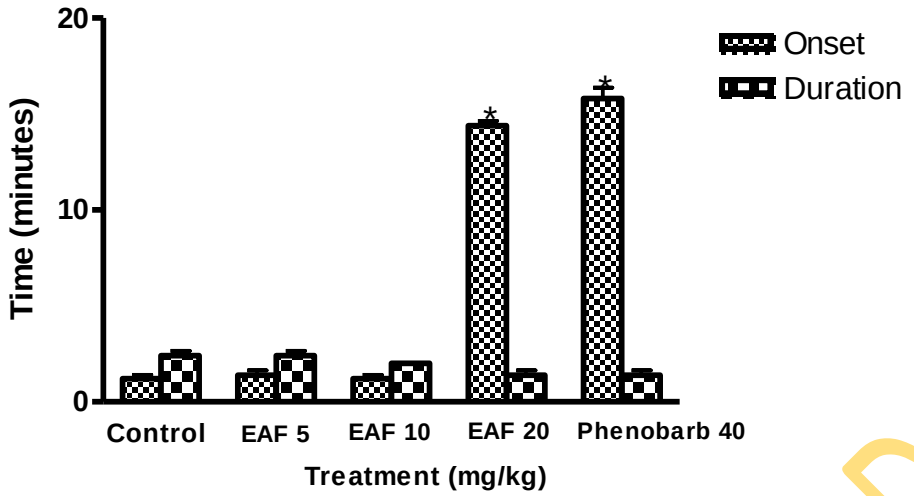


Figure:19 Effect of Ethylacetate fraction (EAF) of *E.ivorense* on Leptazol-induced convulsion in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison tests

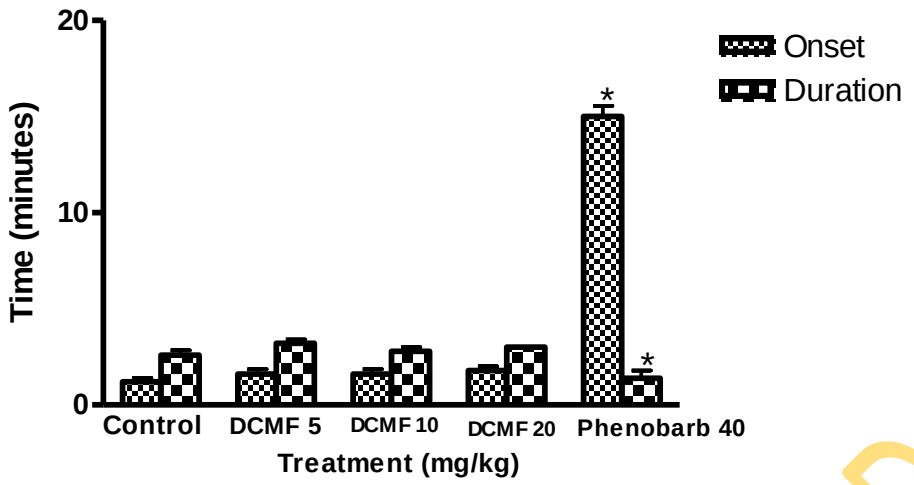


Figure:20 Effect of Dichloromethane fraction (DCMF) of *E.ivorense* on Leptazol-induced convulsion in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison tests

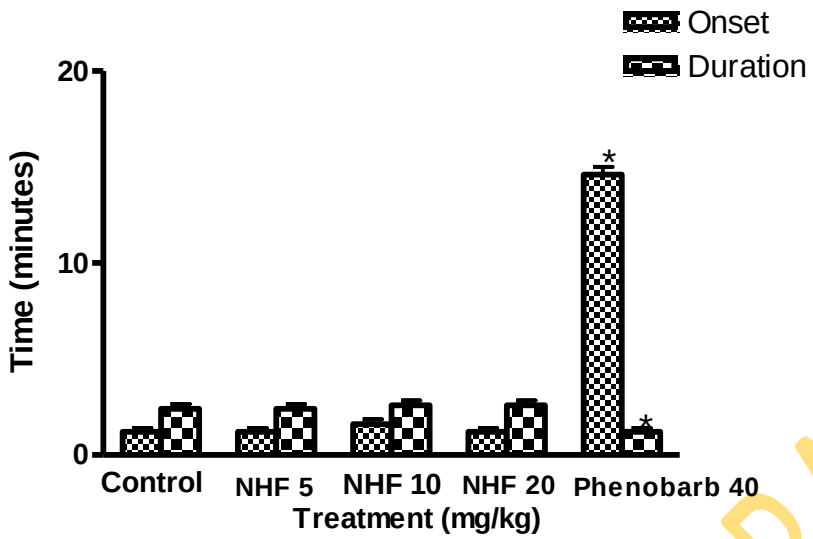


Figure:21 Effect of N-Hexane (NHF) of *E.ivorense* on Leptazol-induced convulsion in mice. Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison tests

4.5.3Effect of CME, EAF, DCMF and N-HF of *Erythrophleum ivorense* on strychnine-induced convulsion

Similarly, the extract and the fractions at the tested doses (5-20mg/kg) did not modify the action of strychnine in mice. They did not significantly ($p>0.05$) prolong the onset of seizures and also failed to shorten the duration of convulsion in strychnine-treated mice. (Figure 22-25)

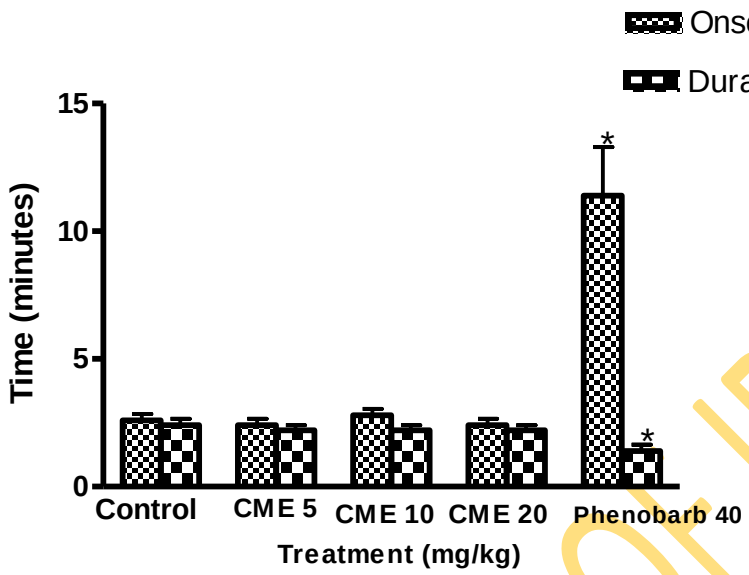


Figure:22 Effect of Crude methanol extract (CME) of *E.ivorense* on Strychnine-induced convulsion in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison tests

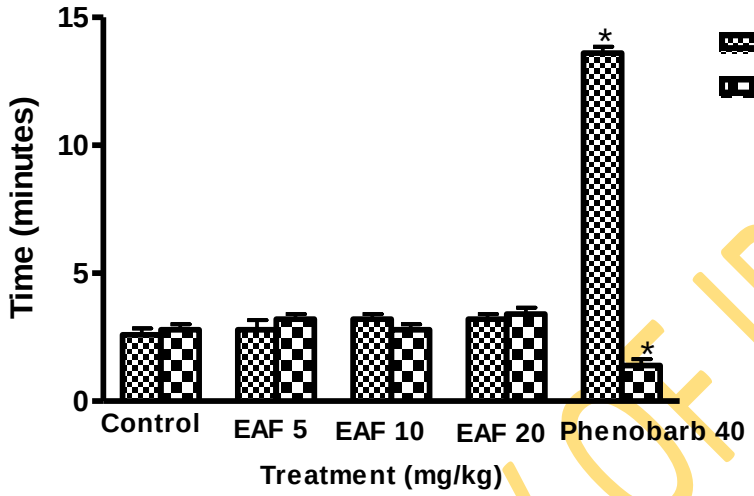


Figure:23 Effect of Ethyl acetate fraction (EAF) of *E.ivorense* on Strychnine-induced convulsion in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison tests

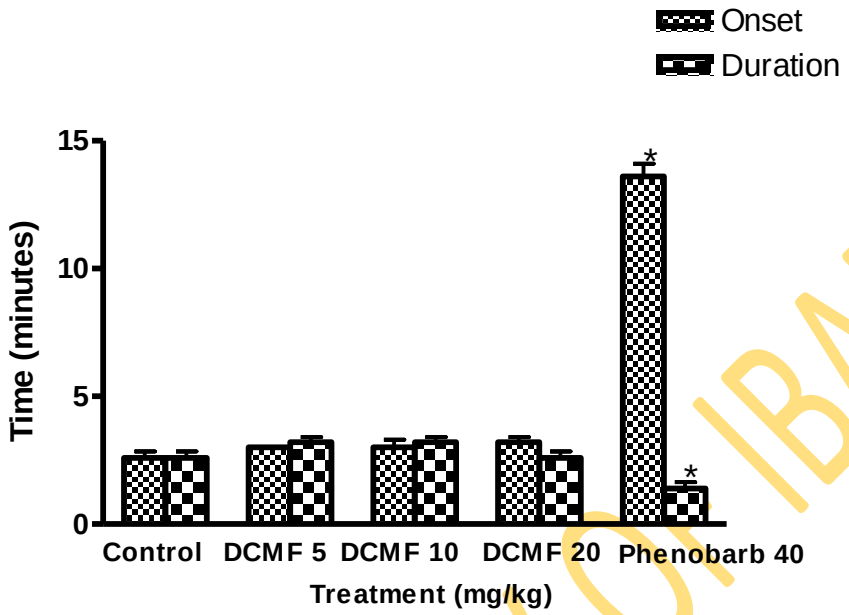


Figure:24 Effect of Dichloromethane fraction (DCMF) of *E.ivorense* on Strychnine-induced convulsion in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison tests

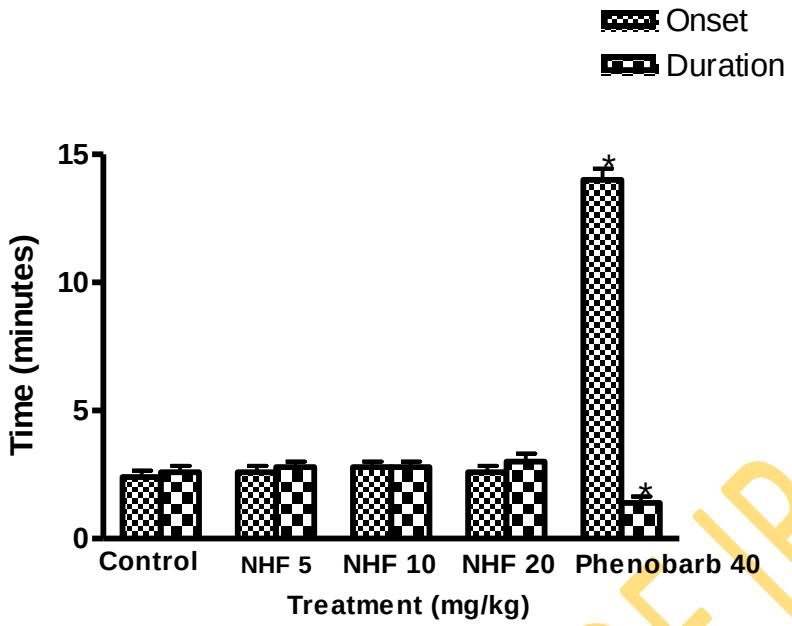


Figure:25 Effect of N-Hexane fraction (NHF) of E.I on Strychnine-induced convulsion in mice.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

4.6 Effect of CME, EAF, DCMF and N-HF of *Erythrophleum ivorense* on Pentobarbitone-induced sleeping time

The extract was found to exhibit sedative property, as shown by its effects on the general behavior of the animals and on pentobarbitone-induced sleeping time in mice. The extract and fractions reduced spontaneous motor activity and response to touch but did not caused loss of righting reflex in mice at the tested doses.CME and EAF significantly ($p<0.05$) prolonged the sleeping time hypnosis of barbiturate-induced sleeping time in a dose related manner (Table 5)

Table 5: Effect of CME, EAF, DCMF and N-HF on Pentobarbital-induced sleeping time in mice

Treatments	Doses mg/kg	Duration of sleep(sec)
Control	0	63.25±2.04
CME	5	281.06±4.02*
	10	295.38±3.96*
	20	325.02±4.21*
EAF	5	293.40±5.05*
	10	307.21±3.01*
	20	341.60±4.45*
DCMF	5	65.60±5.09
	10	58.10±3.31
	20	46.07±2.11
NHF	5	62.21±1.07
	10	63.08±4.14
	20	52.20±4.05
Sodium Pentobarbital	40	275.65±4.12*

Each value represents the mean±SEM of five independent observation *p<0.05 compare with control group. ANOVA followed by Dunnett's multiple comparison tests

CHAPTER FIVE

5.0 DISCUSSIONS

There are several traditional claims regarding the usefulness of *Erythrophleum ivorense* in pain, inflammation and convulsion. However, this plant has not been subjected to any systematic pharmacological screening so far. Hence, it was considered that investigations on these medicinal properties may give scientific authentication to the traditional claims. To assess the acute toxicity of the plant, the crude methanolic extract (CME) was selected as it gives the positive test for most of the constituents during phytochemical screening and because of its high extractive value. The median lethal dose (LD) of the extract was found to be 87mg/kg and this indicates that the extract is fairly toxic compared with toxicity classification (Loomis, 1986). This study was designed to investigate the analgesic, anti-inflammatory and anticonvulsant properties of methanol extract and fractions of *Erythrophleum ivorense.*

Acetic acid mouse writhing is widely used animal model for routing screening of compound with peripheral analgesic activity (Nunez-Guiller *et al*., 1997; Khan *et al*.,2010; Ibrar et al.,2012). The writhing response is considered to be a visceral inflammatory pain (Collier *et al*., 1968; Vanessa *et al.,* 2012) Acetic acid is a chemical irritant that produces tissue necrosis of the peritoneal region accompanied by the release of chemical mediators such as bradykinin, prostaglandin, histamine, substance P, vasoactive polypeptide, which cause pain either by activation or sensitization of nociceptors that encode tissue injury (Gene *et al*., 1998; Ibrar *et al*.,2010; Mazid *et al*.,2010) whilst the hot plate or tail immersion model of pains is generally used to detect centrally acting analgesics (Hunkaars *et al*., 1987). CME and EAF produced dose-dependent and significantly ($p<0.05$) antinociceptive effects in chemically induced nociceptive pain stimuli in mice, whilst DCMF significantly reduced the number of writhes at the maximum dose (20mg/kg). But the inhibitory effect exhibited by CME, EAF and DCMF against nociceptive action of acetic acid in mice may suggest the presence of phytochemically active substances with analgesic property. Indicationssuggesting peripheral action . This suggestion further supported by the finding that CME, EAF and DCMF inhibits the nociceptive (inflammatory pain) behavior produced by formalin in mice. The mechanism of analgesic effect of extract and fractions in acetic acid-induced writhing could be due to the

blockade of the effect or the release of endogenous substances that excites pain nerve endings similar to that of indomethacin and other NSAIDs (Murakami *et al*., 2005)

Formalin is used as chemical noxious stimuli to trigger pain. This test was normally used to study both central as well as peripheral analgesic activity (Tjoisen *et al*., 1992). Injection of formalin is associated with the neurogenic pain during early phase followed by the pain due to inflammation during the late phase (Hunskaar*et al*., 1987; Santos *et al*., 1998). The neurogenic pain is centrally mediated and is attributed to the direct stimulation of nociceptive primary afferents nerve fibers and the release of pain mediators such as kinin, histamine and serotonin. The inflammatory pain is peripherally mediated and it is due to peripheral release of chemical pain mediators that sensitize or activate nociceptors such as prostaglandin (Okuda *et al*., 2001). The peripherally analgesic drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) are only effective against inflammatory pain produced by formalin (Tjoisen *et al*., 1992; Hahn *et al*., 2010). In contrast, the centrally acting analgesic drugs such as morphine inhibit both the neurogenic and inflammatory pains caused by formalin. The inhibitory effect demonstrated by CME and EAF against neurogenic and inflammatory pains may suggest peripheral and central analgesic actions similar to morphine. The inhibitory effect demonstrated by DCMF against inflammatory pain may suggest peripheral analgesic effect similar to NSAIDs. However, further study is needed to identify the active principle(s) and the mechanism underlying the analgesic effects of CME, EAF and DCMF.

Hot plate is a transparent glass cylinder used to keep the animal on the heated surface of the plate (Hunskaar *et al*., 1986). The temperature of hot plate is set using a thermoregulated water-circulated pump. This hot plate test is also considered to be sensitive to drugs acting at the supraspinal modulation level of the pain response (Yaksh, 1977), suggesting atleast a modulatory effect of the investigated extract and fractions. The time of latency or reaction time is defined as the time period between the zero point, when the animal is placed on the hot plate surface, and the time when the animal licks its paw or jumps off to avoid pain (Ripoll *et al*., 2006; Tzschentke, *et al.*, 2007). Hot plate test is normally used to evaluate the centrally acting analgesics (Vogel, 2002). DCMF (5-20mg/kg) and N-HF (5-20mg/kg) did not show any promising analgesic activities, whilst CME (5-20mg/kg) and EAF (5-20mg/kg) did exhibit analgesic activities. Hence, the result of hot plate test supported the result of formalin-induced paw licks and affirmed the presence of centrally acting analgesic activity. However, it is not

known whether the analgesic action is opioid-like in nature and or involves dopaminergic or other mechanism. The use of selective antagonist like Naloxone or metoclopramide might help in understanding the mechanism involved.

Inflammation is typically characterized by increased permeability of endothelial tissues and influxes of blood leuckocytes into the intestitium resulting in oedema. Many different biological mediators' influences each step of inflammation cascade and typically anti-inflammatory agents exhibit therapeutic properties by blocking the actions of synthesis of some of these mediators (Gabor, 1979; Zakaria *et al*., 2012). Carrageenin-induced paw oedema was taken as a prototype of exudative phase of inflammation. This oedema depends on the participation of kinins and polymorphonuclear leucocytes with their proinflammatory factors including prostaglandins (Damas *et al*., 1986). The development of oedema in the rat paw after the injection of carrageenin has been described as a biphasic event (Vinegar *et al*., 1969). The initial phase starts immediately after the injection and reduces within one hour, and is attributed to the release of histamine and serotonin (Crunkhon *et al*., 1971). The second phase of swelling which begin at one hour and remain through three hour is due to the release of prostaglandin-like substances (Borsini *et al*., 1998). It has been reported that the second phase of oedema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents. Generally NSAIDs strongly inhibit the second phase of carrageenin-induced oedema while some inhibit both phases. Indomethacin seems to inhibit both phases (Venigar *et al.,* 1969; Di Rosa *et al*., 1971). Some of the phytochemicals found in certain herbs and plants are reported to demonstrate pain and inflammation-reducing properties (Havsteen, 1983).
The effective anti-inflammatory activity was observed with CME, EAF and DCMF treated animals for three hours measurement. The ability of CME, EAF and DCMF to suppress acetic acid-induced nociceptive and carrageenin-induced inflammation suggests a peripheral analgesic effect similar to NSAIDs. The significant anti-inflammatory effect shown by CME, EAF and DCMF against pain associated with second phase of formalin test and reduced pain episodes elicited by acetic acid may suggest involvement of phytochemically active constituents with prostaglandin synthesis inhibitory properties. Flavonoids have been reported to produce several anti-inflammatory effects (Gaille *et al.,* 1999)

The crude methanolic extract and ethyl acetate fraction of the plant were found to exhibit sedative effects, as shown by their abilities to prolong pentobarbitone-induced sleeping time. It is well known that drugs with sedative properties prolonged the time of sleep produced by barbiturate (Nyeem *et al.,* 2006). Studies have shown that the potentiation of barbiturate hypnosis is an index for central nervous system depression (Dehar *et al.,* 2012). It may therefore be suggested that the ability of the extract and fraction to prolong barbiturate-induced sleeping time indicates that it possesses central nervous system depressant property. Picrotoxin, a GABA-A receptor antagonist, produces seizures by blocking the chloride-ion channels linked to GABA-A receptors, thus preventing the entry of chloride ions into the neurons. This leads to decreased GABA transmission and activity in the brain. Thus, convulsions arising from picrotoxin are due to the decreased GABA-A receptors-mediated inhibition which tips the balance in favour of glutamate-mediated excitatory transmission (Smith *et al.,* 2007). The abilities of CME and EAF to attenuate seizures induced by picrotoxin may possibly be due to an interaction with GABA-A receptors and / or GABAtransmission. Phenobarbitone, a reference anticonvulsant, produced similar effects on picrotoxin-induced seizures. And it is known to enhance GABAergic neurotransmission by increasing chloride ion flux through the chloride channels of GABA-A receptors. Since CME and EAF mimicked, to some extent, the anticonvulsant actions of phenobarbitone, it is possible that CME and EAF antagonizes picrotoxin-induced seizure by opening the chloride channel associated with GABA-A receptors. It is also possible to achieve these effects by suppressing glutamate-mediated excitation. Leptazol-induced convulsion represents a valid model for human generalized and absence seizure (Loscher *et al.,* 1988). Leptazol has been used experimentally to study seizure phenomenon and to identify pharmaceuticals that may control seizure susceptibility. The exact mechanism of epileptogenic action of leptazol at the neuronal level is still unclear, but it has been generally reported to produce seizures by inhibiting gamma-aminobutyric acid (GABA) neurotransmission (De Sarro *et al., 2003*). Enhancement of GABAergic neurotransmission has been shown to inhibit or attenuate seizure, while inhibition of GABAergic neurotransmission or activity is known to promote and facilitate seizure. Anticonvulsant agents such as diazepam and phenobarbitone inhibit leptazol-induced seizure by enhancing the action of GABA-A receptor, thus facilitating the GABA-A receptor mediated opening of chloride-ion channels (Gale, 1992). Thus inhibition of leptazol-induced seizures by CME and EAF suggest that both may produce this effect by

enhancing GABAergic neurotransmission. Also Drugs that promote an increase in onset and shortening of period of convulsion in picrotoxin- and leptazol-induced convulsion are suggesting anticonvulsant activity (Salih *et al.,* 2008). CME and EAF prolonged onset of convulsion, while the average duration of convulsion was markedly reduced. Although the parameters (i.e. onset time of convulsions, decreased in duration of tonic-clonic convulsions) used for evaluation of anticonvulsant activity in the present study are not conclusive. However, it gives a preliminary indication about the anticonvulsant effect of the extract and the fractions.

The extract and fraction were found to exhibits sedative effects, as shown by its ability to prolong pentobarbitone-induced sleeping time. It is well known that drugs with sedative properties, prolonged the time of sleep produced by barbiturate (Occhiuto *et al.,* 1995). Studies have shown that the potentiation of barbiturate hypnosis is an index for CNS depression (Fujimori, 1995). It may be suggested that the ability of the extract to prolong barbiturate-induced sleeping time, indicates that it possesses CNS depressant property. It is therefore, suggestive that the anticonvulsant property of the extract and fractions may be linked; at least in part, to its ability to depress the central nervous system.

The difference in group of chemical constituents of the crude methanol extract and fractions especially alkaloids, flavonoids, saponins, may be responsible for the observed differences in analgesic, anti-inflammatory, anticonvulsantand sedative effects. Flavonoid and saponin are known to inhibit pain perception as well as anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of chemical mediator of inflammation (Sawadogo *et al*., 2006)

In conclusion, crude methanol extract and fractions of *E ivorense* possesses analgesic, anti-inflammatory and anticonvulsant activities. However the result of this experimental animal study lends pharmacological credence to the suggested folkloric, ethnomedicinal uses of the plant in the management and control of painful and inflammatory conditions, as well as in the management of convulsive disorder. There is a need for more precise studies to determine and separate the active compounds and elucidate the mechanism of action responsible for the central nervous system effects of *E ivorense.*

REFERENCES

Abbas A.B and Lichtman A.H (2009).Innate immunity.Basic immunology.Function and disorder of immune system. ISBN 978-14160-4688-2

Abourashed E, Khan I and Walker L (2003).Ephedra in perspective-a current review. Phythotheraphy Research 17 (7): 703-713

Acharya, Deepak and Shrivastava (2008). Indigenous herbal meditions: Tribal formulation and traditional herbal practices. Aavishkar Publishers Distributor, India Pp440

Adibatti N.A and Sukumar E (1995) Apyridine alkaloid from Ceropegea juncea.Phytochemistry 30 (7): 2449-2450

Anil Aggrawal (1995). Opium: the king of narcotic. BLTC Research. Retrieved 2008-03-21

Arggawal B.B, Sundaram C and Malani M (2007). Curcumin: the Indian solid gold. Advances in Experimental. Medicine and Biology 595: 1-7

Arnason J.I (2005) Ethnomedicinal uses of Mormodicacharantia in Togo and relation to its phytochemistry and biological activities. Journal of Ethnopharmacology 96 (2): 49-55

Aronoff D.M and Neilson E.G (2001). Antipyretics: mechanism of action and clinical use in fever suppression. American Journal of Medicine. 111 (4): 304-315.

Atowodi, S.E (2005) "Antioxidant potential of African medicinal plants" African Journal of Biotechnology, 4(2)128-133

Bamigbade T.A Davidson C, and Langford R.M, Stamford JA (1997).Action of tramadol, its enantiometer and principal metabolite.British Journal of Anesthesia. 79 (3): 352-356

Bao,S, Wu Q, Hao Y andMclendon R.E (2006). Stem cell-like glioma cells promote tumor angliogenesis through vascular endothelial growth factor. Cancer Research 66 (16): 7843-8

Beckman J and Koppenol W (1996). Nitric oxide, superoxide and peroxynitrite: the good, the bad and the ugly. American Journal of physiology. 272: C1424-C1437

Barbosa-Filho J.M, MartinsV.K.M, Rabelo L.Aand Maura M.D (2006b)..Natural products inhibitors of angiotensine converting enzyme. RevistaBrasileira de Farmacognsia 16: 421-446

Barbosa-Filho J.M, Medeiros K.C.P, Diniz M.F.F.Mand Batista L.M (2006a).natural product inhibitors of the enzyme acetylcholinesterase. RevistaBrasileira deFarmacognosia 16: 258-285

Beloin N, Gbeasor M, Akpagana K, and Hudson J (2005).Ethnomedicinal uses of Momordical charantia. Jouornal of Ethnopharmacology 96 (1): 49-55

Ben-Ari Y, Galarsa J.Land Tyzio R (2007). GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. Physiological Review 87 (4): 1215-1284

Banik P.D, Chen Q.P and Xu Y.A (1997) Nitric oxide inhibits neutrophils b2 integrin function by inhibiting membrane-associated cyclic GMP synthesis. Journa of cell physiology 172:12-24

Berger M, Gray J.A and Roth B.L (2009).The expanded biology of serotonin. Annual ReviewMedicine 60: 355-366.

Barnerd M (1992) Potentiation of thiopentone sodium induced hypnosis
By Berberis aristata in rodents. Asian Journal of Pharmacy and Clinical Research 5(1): 131-133

Billing, Jennifer and Sherman P.W (1998).Antimicrobial function of spices. Review Biology 73 (1): 3-49

Blum D.E. (1998). New drugs for persons with epilepsy. Advance Neurology 76:57-87

Bhoola, K.D, Collier H.O.J and Schatcher (1962) Action of some peptides on bronchial muscle. British Journal of Pharmacology 9: 190-197

Bosch, C.H., (2006) *Erythrophleum ivorense* A Chev.Protologue Veg. util. African Tropical France 5: 178

Bowery N.G Bettler B and Froestl W (2002) Mammalian GABA-b receptors: structure and function. Pharmacological Review. 54:247-264

Breivik h, Borchgrevink P.C and Allen S.M (2008) assessment of pain British Journal ofAnesthesia 101(1): 17-24.

Brittain, R.T, Lehrer D.N and Spenser, P.S.J (1965) Phynylquinone writhing test: interpretation of data. Nature 200: 895-896

Buck M.L (2011) Intravenous acetaminophen used in infant and children. Pediatric Pharmacology, 17 (4): 21-23

Burkill, H.M., (1995) The useful plants of West Tropical Africa. 2nd Edition.Volume 3, Families J-L. United Kingdom. Pp 857.

Campbell N.A, and Reece J.B (2002).“48” Biology (6thed.) San Francisco CA: Pearson Education, Inc, Pp1037. ISBN 0-8053-6624-5

Carlini E.A, Rodrigues E, Mendes F.R and Tabacch R 2006.Treatment of drug dependence with Brazilian herbal medicines. Revista Brasileira de Famacognosia 16: 690-695.

Carlini E.A (2003). Plants and central nervous system.Pharmacology Biochemical Behaviour 75:501-512.

Caruso T.P, Larson D.L and Portoghese P.S (1979).Chloroxymorphamine an opioid receptor site-directed alkylating agents having narcotic agonist activity. Science 204 (4390): 316-318

Carswell H, Daum P.R and Young J. M (1985).Histamine H1-receptor agonist stimulated breakdown on inositol phospholipids. In: Frontier in histamine Research (edited by Ganellin, C.R and Schwatch, J.C). pp27-38

Castleman, M (2001). The new healing herbs: The classic guide to Nature’s medicine feathuring the top 100 time tested herbs. Radale p. 15

Challem, J (1999). The problem with herbs.Natural Health. Retrieved 2010-12-23

Chauhan A.K, Dobhal M.P and Joshi B 1988.A review of medicinal plants showing anticonvulsant activity. Journal of Ethnopharmacology 22:11-23

Chebb M, Johnston G.A (1999) The ABC of GABA receptors: a brief review. Clinical and Experimental Pharmacology and Physiology 26: 937-940

Chintamunnee V and Mahomoodally M.F (2012) "Herbal medicine commonly used against infectious diseases in tropical island of Mauritus," Journal of Herbal Medicine, 2:113-125

Coceani F, Bishai I, Lees J, Sirko S (1986). Prostaglandin E2 and fever: a continuing debate. Yale Journal of Biology and Medicine. 59 (2): 169-174

Collier H.D.J, Dinnin L.C, Johnson C.A, and Schneider C (1968).The abdominal response and its suppression by analgesic drugs in the mouse. British Journal of Pharmacology and chemotheraphy 32:295-310

CollinsP,andMinta T (2000). Medieval herbals: The illustrative tradition. University of Toronto Press Pp 32

Corbett A.D, Henderson G, McKnight A.T, and Paterson S.J (2006). 75 years of opioid research: the exciting but vain quest for the holy Grail BritishJournal of Pharmacology 147 Suppl 1:S153-62

Cotran T, Collins P,and Kumar C (1998). Robbins pathologic basic of disease. Philadelphia: W.B Saunders Company. ISBN 0-7216-7335-X

Cronlund, A., (1976). The botanical and phytochemical differentiation between Erythrophleum suaveolens and Erythrophleum ivorense. Planta medical 29(2): 123-128

Conaghan P.G (2012). A turbulent decade for NSAIDs: update on current concepts of classification, epidemiology, comparative efficacy and toxicity. Rheumatology International 32 (6): 1491-1450

Damas J,andRemacle-Volon G. (1986). Further study of the mechanism of counter irritation by turpentine.Archives of Pharmacology. 332:196-200

Datar P, Coutinho E and Gevr T (2004). Substance P Structure, Function and Therapeutics Current Topic in Medicinal Chemistry 4 (1): 75-103.

Dehar N, Walia R and Ratol S. (2012). Potentiation of thiopentone sodium induced hypnosis by Berberis aristata in rodents. Asian Journal Pharmacy and Clinical Research. 5(1): 131-133

Dekosky S.T, Williamson J.D, Fitzepatric A.L, Ives D.G and Saxon J.A (2008).Ginko evaluation of memory study, Journal of American Medical Association. 300 (23):2730

Dhawan B.N, Cesselin F, Raghurbir R and Bradley P.B (1996).Classification of opioid receptors. Pharmacology Review. 48 (4): 567-592.

Di Rosa M, Giroud P.J and Willoughby D.A (1971). Study of mediators of acute inflammatory response induced in rats in different sites by carragenan and turpine Journal of Pathology. 101:15-29

Di Giuseppe M and Reece J.B (2003). Nelson Biology 12 Toronto Thomson Canada Pp 473

Dworkin R.H, Backonja M, Rowbothan MC, et al (2003). Advances in neuropathic pain: diagnosis, mechanism and treatment Archives Neurology 60 (11): 1524-1534

Eddy, N.W, and Leimback, D., (1953). Synthetic analgesic. Dithienylbutenyl and dithienylbutylamine Journal of Pharmacology and Experimental Therapeutics 107:385-393

Elias, L.J and Saucier D.M (2005). Neuropsychology: Clinical and experimental foundation, Boston Pearson.

Elisha, E., Maliki, S.J and Abraham B.K (1998).Effect of Jasmium officinale flower on the central nervous system of mouse. International Journal of Crude Drug Research 26: 221-227

Fabricant D.S, and Famswort N.R (2001) The value of plant used in traditional medicine for drug discovery. Enviromental Health Journal 109: 69-75

Fatemeh, Z, Farzaneh E, Malihe A, Amir H.E, Majid V.R, Sadegh S and Kaivan R (2014). Evaluation of topical antinociceptive effect of Artemisia absinthium extract in

mice and possible mechanisms. African Journal of Pharmacy and Pharmacology 8 (19):492-496

Fatope, Majekodunmi, Takeda, Yosio, Yamasitah, Hroyasu (1990). New cucurbitane triterpenoids from Momordical charrantia. Journal of Natural Product 53 (6): 1491-1497

Ferrero-Miliani L,Nelson O.H, Anderson and P.S,Giradin S.E (2007). Chronic inflammation: important of NOD2 and NALP3 in interleukin-1beta generation. Clinical.Experimental.Immunology. 147 (2): 132-142

Florucci S, Antonelli E and Distrutti E (2002). Nitric oxide derivative of prednisolone, enhances regulatory T cells in the lamina propia and protects against 2, 4, 6-trinitrobenzene sulfonic acid-induced colitis in miceProceedings of Natural academia of science. United.State.America. 99: 15770-15775

Funke I,andMelzig M.F (2006). Traditionally used plants in diabetes therapy-phytotherapeutics as inhibitors of amylase activity. Revista Brasileira Farmacognosia 16: 1-5.

Foster H and Steven P (2002).Western Medicinal Plants and Herbs. Boston, NY: Houghton Mifflin Company. Pp 339

Gene R.M, Segura L, Adzet T, Marin E, and Iglesia J (1998). Heterotheca inuloides: anti-inflammatory and analgesic effect. Journal of Ethnopharmacology 60: 157-160

Gillmer, M. and Symmonds, R. (1999) "Seed collection and germination: Hypoxis hemerocallidea". Plantlife 21 (36): 37

Goldman P, (2001). Herbal medicine today and the root of modern pharmacology.Annal of internal medicine. 135 (8): 594-600

Goldstein A, Lowney L.I, and Pal B.K (1971). Stereospecific and nonspecific interactions of the morphine congener levorphanol in subcellular fractions of mouse brain.Proceedings of Natural science. United.State.America 66 (8) 1742-1747

Goldstein A,andGhazarossian V.E (1980). Immunoreactive dynorphin in pituitary and brain.Proceeding Natural. Academic of Science United State of America 77 (10): 6207-6210.

Grene M, (2004). Philosophy of biology Cambridge University press.P. 11. ISBN 978-0-521-64380

Grover J.Kand Yadav S.K (2004).Pharmacological actions and potential uses of Momordical charrantia. Journal of ethnopharmacology 93 (1) 123-132

Guerrini R (2006). Epilepsy in children. Lancet 367:499-524

Gurib-Fakim, A, (2006) "Medicinal plants: traditions of yesterday and drugs of tomorrow ", Molecular Aspects of Medicine 27 (1); 1-93

Gurib-Fakim, A and Mahomoodally, M.F. (2013) "African flora as potential sources of medicinal plants: towards the chemotherapy of major parasitic and other infectious diseases- a review", Jordan Journal of Biological Sciences, 6:77-84

Guyton, Arthur, Hall, John (2006). Rhythmical excitation of the heart. Text book of medical physiology 11th ed. Pp122

Hahn Y.S, and Kim J.G.(2010). Pathogenesis and clinical manifestations of juvenile rheumatoid arthritis.Korea Journal of Pediat.try 53(11): 921-930

Han J.Sand Xie C.W (1984).Dynorphin : Potent analgesic effect in spinal cord of rat. Earth science. 27 (2): 169-177

Hela S.I, Megahed H.S, Salem S.M and Younnes E.R (2013). Monotherapy versus polytherapy in epileptic adolescent. Macedonian Journal of medical Sciences 6(2): 174-177

Heinrict M, Singh H. V.P, and Pandey V.B (2005)."Plant as medicines".In Prance G and Nesbith M. The cultural history of plants pp 205-238 ISBN 0-41592946-3

Harrison S, and Geppeti P (2001). "Substance P". International Journal of Biochemistry and Cell Biology 33 (6): 555-576.

Harborne J.P (1998). Phytochemical methods: A guide to modern technique of plants analysis, 2nd ed. Chapman and Hall, London

Hinz B, Cheremina O, and Brune K (2008). Acetaminophen is a selective cyclooxygenase-2 inhibitor in man. Experimental Biology 22 (2): 383-390.

Hoff T, (2003). The rise of early modern science: Islam,Chinal and the West Cambridge University press Pp 218

Hogaboam C.M, Befus A.D and Wallace J.L (1993). Modulation of rat mast cell reactivity by IL-1b: Divergent effects on nitric oxide and platelet-activating factor release. Journal of immunology 151:3767-3774.

Howes M.J.R,andPerry N.S.L, Hoighton PJ 2003. Plants with traditional uses and activities, relivants to the management of Alzheimer's disease and other cognitive disorders. Phytotherapia Research 17: 1-18

Hunskaar S, and Hole K (1987). The formalin test in mice: Dissociation between inflammatory and non-inflammatory pain. Pain 30: 103-114

Ibrar M, Muhammed N, Barkatullah P, Khan H, Jahan F and Asrha N (2012). Antinociceptive and anticonvulsant activities of essential oils of Zanthoxylum armatum. Phytopharmacology 3(1): 191-198

Ingoglia, N.A and Dole, V.P (1970).Localization of d- and l-methadone after intraventicular injection into rat brain. Journal of Pharmacology and Experimental Therapeutics 175 (1): 84-87

Janecka A, Fichna J, and Janecki T (2004). Opioid receptors and their ligands Current Toppicsin Medicinal Chemistry 4 (1): 1-17

Janz R, Goda Y and,Geppert M (1999). SV2A and SV2B function as redundant Ca2+ regulators in neurotransmitter release. Neuron. 24 (4): 1003-1016.

Jawal M.K, and Zech W.D, Goos M, Lauthbecher C (2009) Impairment of mitpchondrial calcium handling in mtSOD1 cell culture model of motor neuron disease Neuroscience 10: 64. 1471-2202

Johnannessen L.C and Patsalos P.N (2010).Drug interaction involvingthe new second and third generation antiepileptic drugs.Expert Review Neurotherapy. 10(1): 119-140

John R, Webb M, Young A and Stevens P.E (2004). Unreferred chronic kidney disease: a longitudinal study. American Journal ofKidney Diseases 43 (5) : 825-35

Johnston G.A (1996) GABA-C receptors: relatively simple Ingoglia NA, Dole VP (1970).localization of d- and l-methadone after intraventricular injection in rats brains Journal of pharmacology and Experimental Therapeutics 175 (1): 84-87

Kang K, Park S, Kim Y.S, Lee S, and Back K (2009). Biosynthesis and biotechnological production of serotonin derivative.Applied MicrobiologyBiotechnology (1): 27-34.

Katsuki S, Arnold W and Murad F (1977).Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. Journal of cyclic Nucleotide Research 3:23-35

Katzung B.G, Masters S.B and Trevor A.J (2007).Basic and clinical pharmacology.Mc Graw-Hill, New York, pp. 489-511Katsuyama K and Hirata Y (1998). Nitric oxide inhibits cytiokin-induced iNOS expression and NF-kappaB activation by interfering with phosphorylation and degradation of IkB-a . Journal of Cell biology 18: 1796-1802

Keele C.A (1969). Clinical pathological aspect of kinins in man. Proceeding of royal Society of Biology 173: 361-369

Kendall D.A, Fox D.A, and Enna S.J. (1981).Effect of gamma-vinyl GABA on bicuculline-induced seizures.Neuropharmacology 20: 4-10

Khan H, Saeed M, Gilani A.U, Khan M.A, Dar A, and Khan A (2010).The antinociceptive activity of Polygonatum verticillatum rhizomes in pain models. Journal of Ethnopharmacology 127(2): 52-527

Khare C.P (2008). India medicinal plants: An illustrated dictionary. Springer-varlag Pp 160. ISBN 0-387-70637-2

Kimura R, Yumiko Y and Akihisa K (2005).Cucurbitane-type triterpenoid from the fruit of Mmordical charantia, .Journal of Natural Product. 68 (5): 807-809

Koboro, M, Ohnishi M, Akimoto Y (2008). A-Eleosterearic acid and its derivative. Journal of Agric and food Chemistry 56 (22): 10151-10120

Koeberle A and Werz O (2009). Inhibitors of the microsomal prostaglandin E2) synthase-1 as an alternative to anti-inflammatory drugsCurrent Medicinal Chemitry 16 (32): 4274-4296.

Koster R, Anderson M, and Debeer E.J (1959). Acetic acid induced analgesic screening. Federation Proceeding 18: 418-420

Kresty L.A, Frankel W.L, and Hammond C.D . (2006). Transitioning from preclinical to clinical chemopreventive assessment of lyophilized black raspberries. Nutrition Cancer 54 (1): 148-156

Kruger L (2001). Method in pain research. CRC Company, Florida, pp.18-30Lichteman B.L (2004). Aspirin: The story of wonder Drug. British Medical Journal 329 (7479): 1408

Li K,and Xu E (2008). The role and mechanism of gamma aminobutyricacid during central nervous system development. Neuroscience Bulletin 24 (3): 195-200

Lomash V, Pariha S.K, Jain N.K, and Katiyar A.K (2010) Effect of Solanium nigrum and Ricinus communis extracts on histamine and carrageenan-induced inflammation in chicken skin. Cellular and Molecular Biology.56: 1239-1251

Loomis T.A. (1986) Essential of toxicology. Philladelphia: Lea and Febiger; pp 67-78

Lord J.A, Waterfield A.A and Hughes J (1977). Nature, 267: 495-499. Loscher W (1982). Neuropharmacology. 21:803-810

Loudon, Irvine (2002). Western medicine: An illustrated history. Oxford University Press Pp 54

Lynch B.A, Lambeng N, K and Pensel K (2004) The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam Proceeding National Academy Science U.S.A 101 (26): 9861-9866

Macdonald R.L., and Olsen R.W. (1994) GABA-A receptor channels, Annual Review Neuroscience 17:569-602

Magaji M.G Yaro A.H, Musa A.M, Anuka J.A, Abdul-Aguye I and Hussainin I.M (2012). Central depressant activity of butanol fraction of Securinega virosa root bark in mice. Journal of ethnopharmacology. 141: 128-133.

Maiha B.B, Magaji M.G, Yaro A.H, Ahmmed S.T Hamza A.H and Magaji R.A (2009). Anticonvulsant study on Cochlospermum tinctorium and Paullinia pinnata extracts in laboratory animals. Niger Journal of pharmaceutical science. 8 (1): 102-108.

Manach C, Scabert A, Moran C, Rmesy C and Jimenez L (2004) "Polyphenols: food sources and bioavailability," American Journal of Clinical Nutrition, 79 (5):727-747

Manju T,andPieroniA (2000). Chemical constituents of Abies pindow leaves. India Journal of Natural product 16 (2) : 30-31

Marieb E (2001). Human anatomy and physiology. 5th ed, San Francisco Pp414

Martins W.R (1967).Opioid antagonists.Pharmacological Review 19 (4): 463-521

Mathers C.D, Lopez A, Stein C, and Ma-Fat D (2001) "Disease control priorities project" Available from: http://www.dcp2.org/file/33/wp18.pdf

Mathison R.D, Davison J.F and Scott B (1994). Attenuation of intestinal and cardiovascular anaphylaxis by the salivary gland tripeptide FEG and its D-isomeric analogues Peptides 19: 1037-1042

Mazid M.A, Datta B.K, Nahar L, Rashid M.A, Bachar S.C and Bashar S.A (2010). Analgesic and diuretic properties of alpha-santalone from Polygonum flaccidum, Phytotherpy Research 24:1084-1087

McCall M.A.,Gregg R. G., and Peachey N.S (2002) Elimination of the rh1 subunits abolishes GABA-C receptor expression and alters visual processing in the mouse retinal. J Neurosci. 22: 4163-4174. Meldrum BS (1997). "Identification and preclinical testing of novel antiepileptic compounds" Epilepsia 38:7-15

Metcalfe D.D, Kalliner M and Donium M.A (1981). The mast cell. Critical Review of Immunology 3: 23-26

Merg F, Fillio D, Usynin, and Bazol I (2006)."Big dynorphin as a putative endogenous ligand for the kappa-opioid receptor". Journal of Neurochemistry, 97 (1): 292-301

Merskey H and Bagduk N (1994).Classification of chronic pain.2th edition Seatle: international Association for the study of pain Pp3-4

Meyers Gand Stephen T, 2000.The use of neurotransmitter precursors for the treatment of depression. Alternative medicine review 5 (1): 64-71

Miller L C and Tainter M.L (1944).Estimation of LD50 and its error by means of log-probit graph paper.Proceeding of the Society for Experimental Biology and Medicine 57:261-264

Mills E and Noda M. (2005). "Impact of African herbal medicines on antiretroviral metabolism" Acquire ImmuneDeficiency Syndrome 19 (1): 95-97

Mollereau C, Simons M.J, and Soularue P (1996).Structure, tissue distribution and chromosomal localization of the prepronociceptin gene. Precision National AcademicScience 93 (16): 8666-8670.

Nabulsi M (2009). Is combining or alternating antipyretic therapy more beneficial than monotheraphy for febrile children. British Medical Journal 339: b3540

Noda M, Teranishi Y, Takahashi H, Toyosato M, and Notake M (1982). Isolation and structural organization of human preproenkephalin gene Nature 297 (5865): 431-435

Nsour W.N, Lau C.B.S, and Wong I.C.G 2000. Review on phytotherapy in epilepsy. Seizure 9:96-107.

NugrohoHand Bambang W (1996).Insecticidal constituents from rhizomes of Zingiber cassumunar and Kaempfera rotunda. Phytochemistry 41 (1):129-132

Nunez-Guillen M.E, Silva-Emini J.A, Lmdala A.J, and Souccar C (1997).International Journal of Pharmacolognosy. 35:99-104.

Nyberg F, Hallberg M (2007). Neuropeptides in hyperthermia.Prog. Brain Research 162: 277-293

Nyeem M.A.B, Alam M.A, Awal M.A, Mostafa M, Uddin S.J, Islam N, and Rouf R (2006). CNS depressant effect of the crude ethanolic extract of the flowering tops of Rosa damascene. Iranian Journal of pharmacology and therapeutics. 5(2):171-174

Occhiuto F, Limardi F and Circosta C. (1995). International Journal of Pharmacognosy 33, 186

Okuda-Ashitaka E, Minami T, Tachibana SandYoshihara Y (1998).Nocistatin, a peptide that blocks nociceptin action in pain transmission. Nature, 392 (6673): 286-289.

Olajide A. O, Mutalib A.A, Uchechukwu P.O and Bernd L.F (2012).*Bridelia ferrugina* produces antineuroinflammatory activity through inhibition ofnuclear factor-kappa B and p38 MAPK signaling. Http://dx.doi.org/101155/2012/546873

Oliver-Bever, B (1986). Medicinal plants in tropical West Cambridge University Press, New York. Pp 82-83

Orhan I, Sener B, Choudhary M.I, and Khalid A 2004.Acethylcholinesterase and butyrylcholinesterase inhibitory activity of some Turkish medicinal plants. Journal of Ethnopharmacology 91: 57-60.

Oyewole O.I, Owoseni A.A, and Faboro E.O (2004). Studies on medicinal and toxicological properties of Cajanus cajan, Ricinus communis and Thymus vulgaris leaf extract. Journal of Medicinal Plant Research 4:19-21

Padmaa M.J, Schher M.S, Stein A.D, Armtti S, and Wang Z (1999). Phenobarbital compared with phenytoin for the treatment of neonata seizure. New England Journal of Medicine 341:485-489

Painters M.P, Leena J.P, and Angelin S.T. (2010) Genius salacia: A comprehensive review. Journal of Natural Remedies 8:116-131

Pande P.C, Tiwari L, and Pande H.C. (2007) Ethnoveterinary plants of Uttaranchal: A review. Indian Journal ofTraditional Knowledge 6:444-458

Parakrama C, Clive R,andTaylor G (2005). The host response to injury, acute inflammatory response Isolation of selective 3H-chlomalttrxamine-bound complexes, possible opioid receptor components in brains of mice. Life Science 27 (22): 2063-2069.

Pearce F.L (1991). Biological effect of histamine: an overview. Agents Actions 33:4-7

PatelF,andJay R (2011). Ethnomedicinal uses of Phyllanthus niruri, phytochemistry and pharmacology. Journal of Ethnopharmacology 138 (2):286-333

Pert C.B,andSnyder S.H (1973). Opiate receptors: demonstration in nervous tissue. Science 179 (4077): 1011-1014.

PerceT,andAndrea S (1999). American pharmaceutical association pratical guide to natural medicine. New York Pp338-340

Proud D and Kaplan A.P (1998). Kinin formation: mechanism and role in inflammatory disorders. Annual Review of Immunology 6:49-84

Rainsford K.D (2009). Ibuproven: pharmacology, efficacy and safety. Inflammopharmacology 17 (6): 275-342

Rakhshandah H, Shakeri M.T and Ghasemzadeh M.R (2007). Comparative hypnotic effect of Rosa damascene fractions and diazepam in mice. Iranian Journal of Pharmaceutical Research 6:193-197

Sadeghnia H.R and Ghorbani A (2012). Sleep prolonging effect of Corandrum sativum hydro-alcoholic extract in mice. Natural production research Journal. 26:2095-2098

RamsewakD,andRussei S (2003). Invitro antagonistic activity of monoterpenes and mixture against toe nail fungus pathogens. Phytotherapy Research 17 (4): 376-379

Richens A, and Perucca E (1993). Clinical pharmacology and medical treatment. In: a text book of epilepsy (Eds J. Laidlaw, A. Richens and D. Chadwick). Churchill Livingstone, pp. 495-560

Ripoll N, Hascoet M, and Bourin M. (2006). The four-plates test: anxiolytic or analgesic paradigm? Progress in neuro-psychopharmacology and biological Psychiatry, l. 30 (5): 873-880

Robert S (2005). Biology and human behavior: Neurological origin of individuality. 2nd edition. Teaching company Pp13-14

Robson P, Barry J, and Baek O.K (2009). The down of Medicine to Medical and Pharmaceutical informatics ISBN 9780470289534

Rogawski M.A,andWolgang L (2004). The neurobiology of antiepileptic drug. Nature Reviews Neuroscience57 :553-564

Sabina E.P, Rasool M.K, and Mathew L (2009). Study on protective effect of Ricinus comunnis leaves extract on carbon tetrachloride hepatotoxicity in albino rats Pharmacologyonline 2: 905-916

SaladinK,andKennet S (2009). Anatomy and Physiology: The unity of Form and function McGraw Hill ISBN 0-0727620-05

Salih, M.A.M and Mustafa A.A (2008). Substance in broad beans (Vicia faba) is protective against experimentally induced convulsions in mice. Epilepsy Behaviour, 12:25-29

Schacter T, Gilbert Y and Weger (2009).Psychology. United State of America, print

Schmidt R (2001). Attension in P Robinson (Ed) Cognition and second language instruction (pp 3-32).Cambrige University press.

Shen W., Sand laughter M.M. (2001) multireceptor GABAergic regulation of synaptic communication in amphibian retinal Journal physiology 530: 55-67

Smith M, Wilcox K.S and White H.S (2007). Discovery of antiepileptic drugs. Neurotherapeutics 4: 12-17.

Sood, S.K, Nath, R and Kallia D.C (2001).Ethnobotany of cold Desert tribes of Lahoul-Spiti, Deep publication, New Delhi.

Sofowora A 1993. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd p. 150

Spanswick C.C, and Main C.J (2000). Pain management: an interdisciplinary approach. ChurchillLivingstone; ISBN 0-443-05683-8 p-83.

Sperling M.R, Harris A, Nei M, and Liporace J.D (2005) Mortality after epilepsy surgery. Epilepsia 46:49-53

Stepp,R, John R, and Daniel E (2001). Important of weeds in ethnopharmacology. Journal of ethnopharmacology 75 (1): 19-23

Stephen T (2006). Psychogenic pain. British Journal of Psychiatry 88:91-93

Sumit Iand Shubbam G (2006). Rustom Jal Vakil: his contribution to cardiology. Texas Heart Institute Journal 33 (2): 161-170

SumnerT,andJudith I (2000). The natural history of medicinal plants. Science 190 (4217): 880-881

Suzette M, Laroche S.M, and Helmers S.L (2004). The new antiepileptic drugs: clinical applications. Journal of American Medical Association 291:615-620

Sawadogo W.R,BolyandRLompo M (2006). Antiinflammatory and analgesic activities of ethanol extract of aerial parts of Justicia gendarussa. International Journal of Pharmacology, 6 (3): 278-283

Tapsell L.C, Hemphill I, and Cobiac L (2006). Health benefit of herbs and spices: the past present and future. Medical JournalAnesthesia 185

Tanaka (2000). Norepiniphrine in the hypothalamus involved in the provocation of anxiety: basic studies. Dol: 10.1016/50014-2999(00)00569-0

Terenius L (1973). Stereospecific interactions between narcotic analgesic and a synaptic plasma membrane fraction of rat cerebral cortex. .Acta Pharmacology and Toxicology 32 (3): 317-320

Tjolsen A, Berge O, Hunskaar S, Rosland J.H, and Hole K (1992). The formalin test: an evaluation of the method. Pain 51: 5-14

Tunnicliff G (1996). Basis of the antiseizure actionof phenytoin. General Pharmacology 27:1091-1097

Turk D.C,andDworkin R.H. (2004). What should be the core outcomes in chronic pain clinical trials? Therapeutics 6 (4): 151-154

Treede R.D, Jensen T.S, and Campbell J.N (2008). Neuropathic pain: redefinition and a grading system for clinical and research purpose. Neurology. 70 (18): 1630-1635

Treese G.E and Evan W.C (1989). Pharmacognosy Braillier Tridel can, 13th ed. Macmillian Publisher.

Tzschentke,T.M, Christoph, T, and Kogel, B 2007. A novel opioid receptor agonist/norepineprine reuptake inhibitor with broad spectrum analgesic properties. Journal of pharmacology and experimental therapeutics 323 (1): 265-276

Venessa S.T, Jucelia B.S, Maria S.A Antonia R, Celia H.Y, Mariam A.O.P, Glauciermar D and Orlando V.S (2012). Antinociceptive and Antiinflammatory Effects of Ethanol Extract from Vernonia polyanthes Leaves in Rodents. International Journal of Molecular Science. 13: 3887-3899

Uddin G, Rauf A, Arfan M, Waliullah A,Khan I and Ali M (2012). Pistagremic acid a new leishmanicidal triterpene isolated from Pistacid intergetrima. Journalof Enzyme inhibition and Medicinal Chemistry. 27(5):468-47

Verity C.M Hosking G, and Easter D.J (1995).A multicentre comparative trial of sodium valproate and carbamazepine in paediatric epilepsy. Developmental Medicine and Child Neurology 37:97-108

Vickers A,andZollman C (1999) Herbal medicine. British Medical Journal, 319 (7216): 1050-1053

Vogel H.G, (2002). Drug discovery and evaluation, pharmacological assay, 2nded. New York, Springer. Pp670

Vohora, SB (2000). J.Ethnopharmacology, 16:201

While H.S, Woodhead J.H, Wilcox K.S and Wolf H.H (2002). Discovery and preclinical development of antiepileptic drugs. In: Levy RH, Mattson RH, Meldrum BS, Perrucca E, eds. Antiepileptic drugs. 5th ed. Philadelpia: Lippincott Williams and Wilkins. Pp 36-48

Waldhoer M, Batlett S.E and Whistler J.L(2004).Opioid receptors. Annual Review of Biochemistry73:953-990

Wilt T.J, IshaniA, Rutk I, and MacDonald R (2000). " phytotherapy for benign prostatic hyperplasia". Public Health Nutrition3 (4A): 459-472

Winter, C., Risley E and Nuss O (1962). Carrageenan-induced inflammation in the hind limb of rats Federation Proceeding, 46:118-126

Wong M,andJeng A.Y (1994). Posttranslational modification of glycine-extended substance P by an alpha-amidating enzyme in culture sensory neurons of dorsal root ganglia Neuroscience Research 37 (1): 97-102

Woolf C.J (2010). What is this thing called pain. Journal of clinical investigation 120 (11): 3742-3744.

Woolf C.J, Bennett G.J, and Doherty M (1998).Toward a mechanism-base classification of pain? Pain 77 (3): 227-229

Xu T,andBajjalieh S.M (2001). SV2A modulates the size of the readily releasable pool of secretory vesicles. Nat Cell Biol. 3 (8) 691-698

Yadav.V. (2008) Lrp5 control bone formation by inhibiting serotonin synthesis in the duodenum. Cell 135 (5): 825-837

Yemitan O.K,and Adeyemi O.O (2013). Antiepileptogenic and anticonvulsant actions of Dalbergiasaxatilis in subtoxic chemical kindling and toxic covulsant models. Europian Journal of Medicinal plants 3(2): 288-296.

Young S.N (2007). How to increase serotonin in the human brain without drugs. Review of Psychiatry Neuroscience 32 (6): 394-399

Zakaria Z.A, Hussain M.K, Mohamed A.S, and Abdullah F.C (2012).Anti-inflammatory activity of aqueous extract of ficus deltoider. Biological Research for Nursing 14(1): 90-97