# ANTI-INFLAMMATORY, ANALGESIC AND TOXICITY STUDIES ON ETHANOLIC, AQUEOUS AND PETROLEUM ETHER EXTRACTS OF STEM BARK OF *TRICHILIA MONADELPHA* (THONN) JJ DE WILDE

A THESIS SUBMITTED IN

FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE

OF

MASTER OF PHILOSOPHY

In the

Department of Pharmacology,
Faculty of Pharmacy and Pharmaceutical Sciences,
College of Health Sciences
By

GEORGE OWUSU

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

JUNE, 2009

# DECLARATION

I, George Owusu do sincerely declare that, this submission is my own work towards the MPhil and that, to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any other degree of the University, except where due, acknowledgement has been made in the text.



| | |
|---|---|
| …….. | …….. |
| George Owusu | George Kwaw Ainooson |
| (Candidate) | (Supervisor) |
| Date……... | DATE…… |
| …… | …… |
| Dr. Charles Ansah | Dr. Eric Woode |
| (Supervisor) | (Head of Department) |
| Date…… | Date…… |

# ABSTRACT

*Trichilia monadelpha* (Thonn) JJ De Wilde (fam. Meliaceae) is a medium-sized tree that grows in the tropical rain forest. In Ghana, the aqueous extract of the stem bark of the plant is used to treat various diseases. This project investigated the anti-inflammatory (acute and chronic) and analgesic activities of the aqueous (TWE), alcoholic (TAE) and petroleum ether (TPEE) extracts of stem bark of the plant. Chick carrageenan induced foot edema model was used to test the effect of the extracts on acute inflammation and adjuvant induced arthritic model was used to test the effect of the extracts on chronic inflammation. To induce acute inflammation, 0.1 ml of 1% carrageenan was injected into the right footpads of 7-day-old chicks (n = 5). Extracts (10-300 mg/kg, *p.o.*) were given to chicks 1 hour after carrageenan challenge. Diclofenac (10-100 mg/kg *i.p.*) and dexamethasone (0.1-1.0 mg/kg *i.p.*) were used as reference drugs. Chronic inflammation was induced by inoculating rats (120-320 g) with 0.1 ml of 3 mg/ml of complete Freund's adjuvant (CFA). Rats (n = 5) were treated with the extracts and the reference drugs dexamethasone, diclofenac and methotrexate 10 days after CFA inoculation. Analgesic effects of the extracts were evaluated by using the formalin test in mice. In the formalin test, mice were pretreated with the extracts 1 hour or the reference drugs 30 minutes before injection of 10 µl of 4% formalin into the ventral surface of the right hind paw. Morphine and diclofenac were used as reference drugs. Sub acute toxicity test in rats was employed to assess the safety of the aqueous extract. From the results, diclofenac and dexamethasone significantly inhibited carrageenan-induced foot edema in 7-day-old chicks with maximal percentage inhibitions of 71.85±1.53 at 1.0 mg/kg for dexamethasone and 62.92±2.03 at 100 mg/kg for diclofenac. Similarly, TPEE and TWE inhibited the paw edema significantly with maximum percentage inhibitions of 63.83±1.28 at 300 mg/kg for TPEE and 57.79±3.92 at 300 mg/kg for TWE. On the contrary, TAE had no significant effect on the carrageenan-induced foot edema. In the adjuvant-induced arthritis, the extracts and the reference drugs decreased the joint thickness. Maximal percentage inhibitions were 85.75±2.96

at 1.0 mg/kg, 80.28±5.79 at 100 mg/kg, 74.68±3.03 at 1.0 mg/kg, 62.81±2.56 300 mg/kg, 64.41±5.56 at 300 mg/kg and 57.40±8.57 at 300 mg/kg for dexamethasone, diclofenac, methotrexate, TPEE, TWE and TAE respectively. In the formalin test, TWE, TPEE and morphine significantly reduced the pain scores in both phases. Diclofenac was more effective in phase 2 than phase 1. TAE did not show significant effect on phase 2. The sub acute toxicity test did not give any evidence of adverse effect on the blood, kidney, heart, spleen, liver or stomach of the rats. All together, the observations of these studies showed that the stem bark extracts of *Trichilia monadelpha* have anti-inflammatory as well as analgesic effects and oral administration of aqueous extracts for two weeks gave no evidence of toxicity.

# ACKNOWLEDGEMENT

My greatest gratitude goes to the Almighty God for seeing me through this programme.

A very profound gratitude goes to my supervisors Dr. Charles Ansah and Mr. George Kwaw Ainooson of the Department of Pharmacology, KNUST, for their supervision and inspirations that made these objectives achievable.

I am much grateful to Dr. Eric Woode of the same department for the plant collection and useful suggestions that made this work a success.

I am also grateful to all the postgraduate students of the Department for their support and advice, and Mr. Kingsley Amponsah of the Department of Pharmacognosy, KNUST, for his special assistance during the phytochemical screening of the extracts.

I thank Mr. Thomas Ansah and the entire technical staff of the Department for their support throughout the project.

Finally, I sincerely appreciate the prayer support and encouragement of my family and all my friends.

# TABLE OF CONTENTS

## LIST OF TABLES

# LIST OF FIGURES

# ABBREVIATIONS

| | |
|---|---|
| 5-HT | 5-Hydroxytryptamine |
| AA | Arachidonic acid |
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| AUC | Area under the time course curve |
| CFA | Complete Freund's adjuvant |
| CGRP | Calcitonin gene related peptide |
| COX | Cyclooxygenase |
| DH | Dorsal horn |
| DLF | Dorsolateral funiculus |
| DMARDs | Disease modifying anti-inflammatory drugs |
| $ED_{50}$ | Dose responsible for 50% of the maximum response |
| GABA | Gamma aminobutyric acid |
| GAFCO | Ghana Agro Food Company |
| GM-CSF | Granulocyte monocyte colony stimulating factor |

| | |
|---|---|
| HB | Haemoglobin |
| IFA | Incomplete Freund's adjuvant |
| IL | Interleukin |
| INF | Interferon |
| iNOS | Inducible nitric oxide synthase |
| *i.p.* | Intraperitoneal |
| MCV | Mean corpuscular volume |
| MCHC | Mean corpuscular haemoglobin concentration |
| MCH | Mean haemoglobin concentration |
| MGLUR | Metabotropic glutamate receptors |
| NK | Neurokinin |
| NO | Nitric oxide |
| NMDA | N-methyl-D-aspartase |
| NRM | Nucleus raphe magnus |
| NRPG | Nucleus reticularis paragigantocellularis |
| NSAIDs | Non-steroidal anti-inflammatory drugs |
| PAG | Periaqueductal grey |
| PAF | Platelet activating factor |

| | |
|---|---|
| PGs | Prostaglandins |
| PMNLs | Polymorphonuclear leukocytes |
| *p.o.* | *Per os* |
| PLT | Platelet |
| RA | Rheumatoid arthritis |
| SP | Substance P |
| TAE | Alcoholic extract of *Trichilia monadelpha* |
| TPEE | Petroleum ether extract of *Trichilia monadelpha* |
| TWE | Aqueous (water) extract of *Trichilia monadelpha* |

# *Chapter 1*

# INTRODUCTION

## *1.1 GENERAL INTRODUCTION*

Herbal and natural products of traditional medicine have been used by men since the advent of human race. Every culture, including western culture has evolved indigenous system of traditional healing (Atkinson *et al.*, 1969). Traditional healing in different cultures has a long history of ancestors creating primitive medicine during their struggle against natural calamities and diseases. While searching for food, the ancient humans found that some foods have specific properties of relieving or eliminating certain diseases and maintaining good health (Baliga *et al.*, 2004).

Plants are a great source of medicines, especially in traditional medicine, which are useful in the treatment of various diseases (Bako *et al.*, 2005). Traditional medicine has not only played a vital role in providing healing (Principe, 1991) but has also contributed to the discovery of most pharmaceutically active substances in plants which have been used in the commercial production of drugs (Pearce *et al.*, 1992). It has been estimated that many people in the developing countries use medicinal plants to help meet their primary health care needs (WHO, 2002). Apart from their importance in the primary health care system of rural communities, medicinal plants also improve the economic status of people involved in their sales (Robbins, 2000; Ticktin *et al.*, 2002). About 50, 000 of the flowering plants species in the world have been studied for their medicinal purposes (Govaert, 2001). In India for example, more than

43% of the total flowering plants are reported to be of medicinal value (Pushpangadan, 1995). The World Health Organization (WHO) encourages the development and integration of traditional medicine in the primary health care delivery system. The traditional medicine practitioners are considered repository of knowledge on medicine that could contribute immensely to the discovery of new therapeutic compounds (WHO, 2002). This is based on the sound recognition of the role that traditional medicine is already playing in the health care programmes in most developing countries, especially in Africa, Asia and Latin America (Ghana Herbal Pharmacopoeia, 1992). Ghana, like many developing countries faces a serious shortage of health workers, and their frequent strike action adds to the strain on the health care system. It is estimated that in Ghana, the ratio of allopathic or orthodox doctor to patient is extremely lower than the ratio of traditional doctor to patient (Ghana Herbal Pharmacopoeia, 1992). This could be improved by integrating traditional medicine into Ghana's health care delivery system. Currently, the importance of medicinal plants in the management of diseases has attracted a rapid attention worldwide. Herbal medicine remains the traditional form of medicine in many developing countries whilst it is increasingly gaining popularity in industrialized countries, which had before then, relied mostly on allopathic medicine.

*Trichilia monadelpha*, a tropical forest plant is widely used in traditional medicine to treat many diseases. However, the use is based on community knowledge of existence as there are no scientific data available to support this claim. There is therefore a need for scientific evaluation of pharmacological activities of this useful plant.

## *1.2 TRICHILIA MONADELPHA*

Vernacular names: Otannuro, Otanduro, (Twi); Tenuba (Nzema)

The vernacular name, Otanuro or Otanduro literally means medicine for the enemy because of its bitterness (Irvine, 1961). *Trichilia* as a genus is made up of hard woody plants yielding pit-props and those useful for building boats and canoes (Irvine, 1961).



### *1.2.1 Description*

*Trichilia monadelpha* (Thonn) JJ De Wilde is a medium-sized tree that grows 12-20 m high and up to 0.4 m girth in the secondary forest in moist places. It has a large spreading, dark crown and short trunk (Irvine, 1961). The slash is pale, darkening to orange brown on exposure (figure 1.1). The leaves may grow up to 15 cm long and are pinnate. The leaflets may be 4-6 pairs with a terminal. The leaflets are oblong with numerous lateral nerves. The flowers are usually whitish or greenish yellow, usually scented and occur in axillary panicles. The fruits are often found in clusters and globose up to 2 cm in diameter (Irvine, 1961).

### *1.2.2 Ecology and geographical distribution*

*Trichilia monadelpha* is a tropical forest plant that establishes itself well in the lowland high forest and evergreen semi-deciduous secondary jungles, often near river banks (Burkill, 1985). In West Africa, the plant is distributed in deciduous and semi-deciduous secondary forests, often in wet places in Ivory Coast, Sierra Leone, Nigeria, Benin, Congo and Ghana (Irvine, 1961). In Ghana, the plant is found in the secondary rain forest in Western, Eastern, Ashanti

and Brong Ahafo regions. The plant flourishes best on fertile and well drained soils where the annual temperature is between 17-28°C with moderately high humidity (Burkill, 1985).

**Figure 1.1** *Trichilia monadelpha* tree showing the stem and leaves.

### *1.2.3 Traditional Medicinal uses*

*Trichilia monadelpha* is used in traditional medicine for the following conditions:

- Dysentery: the dried stem bark is ground to powder, boiled with water for one hour and then strained. The decoction is drunk as required (Dokosi, 1998).
- Dyspepsia: It is used as a component of traditional formulate for dyspepsia. For example, stem bark of *Spathodea compalunata, Trichilia monadelpha, Capara pocera,* root of *Maytenus senegalensis*, fruit of *Piper guineense* and rhizome of *Zinziber officinale* are boiled together with water. The decoction is boiled for 45 minutes and one table spoonful is taken three times daily (Dokosi, 1998).
- Skin Ulcer: the stem bark of the plant is boiled to make a decoction. The decoction is bathed daily to cure the ulcer (Abbiw, 1990).
- Yaws: The stem bark of *T. monadelpha* is ground or crushed to powder. The powder is applied to the wounds (Abbiw, 1990).
- Cough: stem bark of *T.monadelpha*, rhizome of ginger and *Zylopia* seeds are boiled together with water. The decoction is taken three times daily after meals to treat the cough in adults and a reduced dose for children (Dokosi, 1998).
- Arthritis or Articular Rheumatism: Stem bark of *T. monadelpha, Kigelia Africana, Khaya ivorensis* and *Pilostigima thoningii*, root of *Nauclea latifolia Clausina anisata* and *Stronphantus hispidus*, 0.5g of *Zylopia aethiopia* and 0.5g of *Monodora myristica* seeds. One table spoonful is taken three times daily to treat arthritis (Mshana, 2000).

- Abortion: Stem bark of *Maesobotrya barteri* var. *sparsiflora* (apotrewa, Twi) and *T. monadelpha* stem bark are boiled together. The decoction is taken as abortifacient (Abbiw, 1990) .
- Abdominal pain (Colic): In Ghana, the stem bark of the plant is boiled with water and the decoction is taken to cure abdominal pain (Irvine, 1961).



- Gastro-intestinal disorders: Stem bark of *T. monadelpha* is boiled with water. The decoction is taken daily to cure the illness (Abbiw, 1990).
- Analgesic: Dried bark of the stem is boiled with water and the decoction is taken as Pain-killer (Burkill, 1985).
- Sedation/ drowsiness: Decoction of the dried bark of the stem causes sedation or drowsiness when taken (Burkill, 1985).
- Palpitation, heart troubles and heartburns: The leaves of the plant are boiled with water. The decoction is taken to treat the above diseases (Abbiw, 1990).
- Lumbago: The bark of the stem is boiled with water and the decoction is taken daily to treat the disease (Abbiw, 1990).
- Edema: Decoction of the stem bark of the plant is drunk daily to treat the disease (Abbiw, 1990).
- Ricket: The bark of the stem is collected and dried. The dried bark is ground to powder. The powder is boiled with water and the decoction is given to children (Abbiw, 1990).

- Gonorrhoea: Decoction of the stem bark of the plant is drunk daily to treat the disease (Abbiw, 1990).
- Sores, wounds and cuts: The barks of *T. monadelpha* and *Khaya ivorensis* are boiled together with water. The decoction is applied as lotion to the affected parts (Abbiw, 1990).

### *1.2.4 Other medicinal uses*

Decoction of unspecified parts of the plant is used to treat Malnutrition in children (Burkill, 1985).

Decoction of unspecified part of the plant is used to treat general body weakness (debility) and pulmonary troubles (Burkill, 1985).

### *1.2.5 Non-medicinal uses*

In the traditional Agri-Horticulture, the flowering of the plant (which mainly occurs in August), serves as indicators of weather and season (Burkill, 1985; Irvine, 1961). This is the time farmers make second planting of corn (apese-buro, Twi) prior to the onset of the second rains. The trunk is preferably used as pit-props, fences, drum barrels and for building boats and canoes due to its ability to resist water (Abbiw, 1990). In Sierra Leone and Ghana, the bark is used as reddish-brown dye for clothes and hides (Irvine, 1961).

## *1.3 INFLAMMATION*

Inflammation is a sequence of responses occurring in body tissues initiated usually by noxious or injurious stimuli (Issekutz *et al.*, 1989). It could also be defined as a natural response of the body to a variety of hostile agents including parasites, pathogenic microorganisms, toxic chemical substances and physical damage to tissues (Laupattarakasem *et al.*, 2003). Inflammatory response has received interest in pharmacology because inflammation accompanies many disease processes (Erlinger *et al.*, 2004; Lucas *et al.*, 2006). Inflammation often elicits a generalized sequence of events known as the acute phase response which is characterized by swelling, redness, heat, pain and sometimes loss of function (Erlinger *et al.*, 2004; Lucas *et al.*, 2006; Serhan, 2004). The ultimate goal of inflammation is to rid the organism of both the initial cause of cell injury (e.g. microbes and toxins) and the consequences of such injuries (Schmid *et al.*, 2006; Serhan, 2004). Inflammatory response has three basic functions: (1) to deliver the effector molecules and cells to the site of infection; (2) to form a physical barrier to prevent the spread of the tissue damage or infections and (3) to heal the wounds and repair tissues (Issekutz *et al.*, 1989).

### *1.3.1 Cellular mediators of inflammation*

Some of the cells that contribute to inflammatory processes include mast cells (Prussin *et al.*, 2003), vascular endothelia cells, polymorphonuclear leukocytes, platelets, neurons and lymphocytes (Cassim *et al.*, 2002)

### *1.3.2 Chemical mediators of inflammation*

Chemical mediators of inflammation include components of the complement system (for example C3a, C3b, C5a etc.) (Mooijaart *et al.*, 2007), kinins (Bhoola *et al.*, 1992), histamine (table 1.1 shows histamine receptors and their functions) (Marieb, 2001), 5-hydroxytriptamine (5-HT) (Taniyama *et al.*, 2000), neuropeptides (Maggi *et al.*, 1996), nitric oxide (Moncada *et al.*, 1991; Salvemini *et al.*, 1996), arachidonic acid metabolites (table 1.2) and cytokines such as interleukins (IL), tumor growth factor (TGF) and tumor necrotic factor (TNF). Figure 1.2 shows the cyclooxygenase pathway and eicosanoids derived from it.

**Table 1.1** Location and functions of the main receptors of histamine. (*Source:Histamine-Wikipedia,the free encyclopedia.htm).*

| Receptor | Location | Function(s) |
|---|---|---|
| $H_1$ | Smooth muscle, Endothelium, CNS | Causes vasodilatation, bronchoconstriction and smooth muscle activation. |
| $H_2$ | Parietal cells | Stimulates gastric acid secretion |
| $H_3$ | CNS tissues. | Decrease neurotransmitter release: histamine, acetylecholine, norepinephrine & serotonin. |

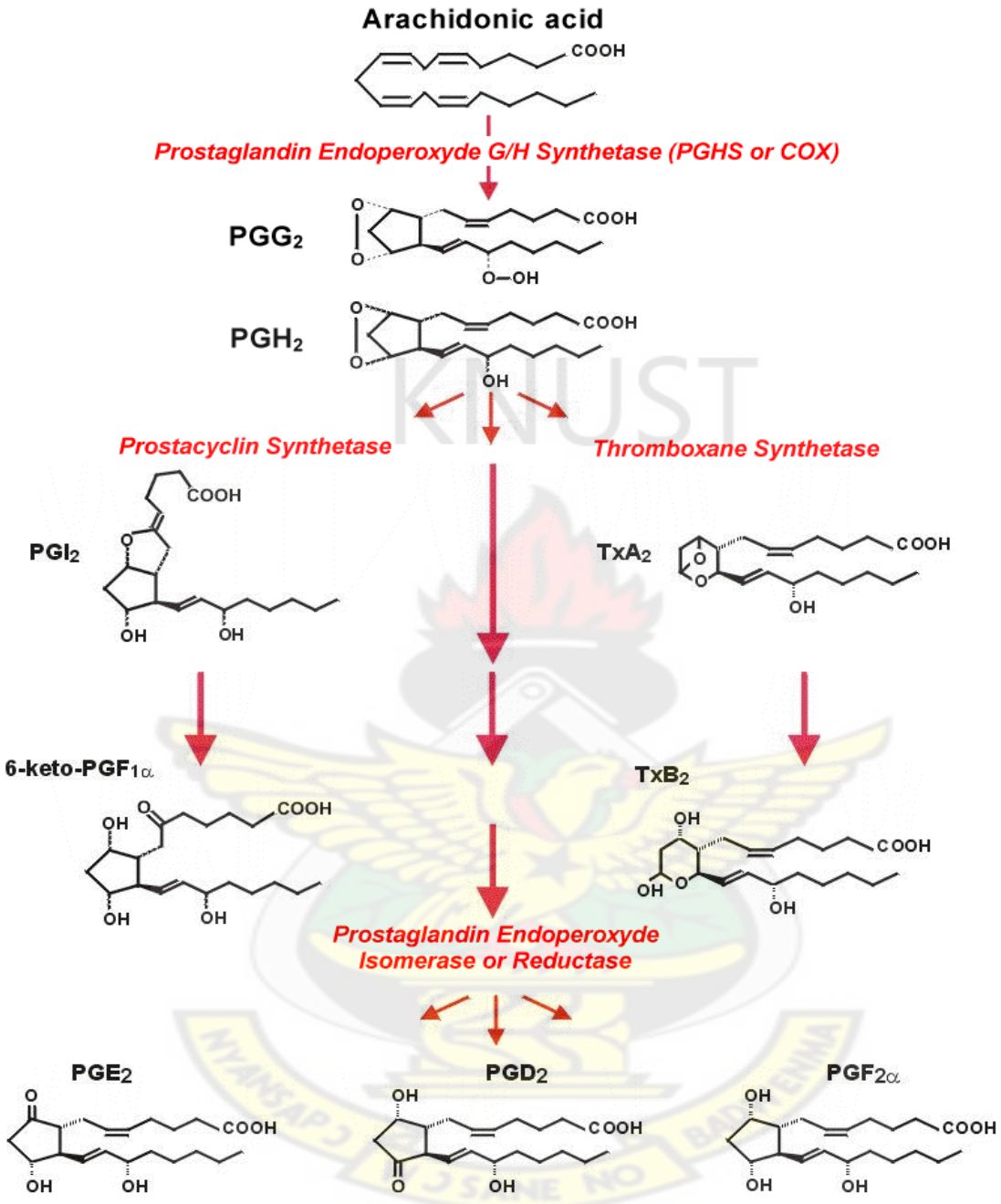



**Figure 1.2** Cyclooxygenase pathway and the main eicosanoids derived from it. (*Source:www.biomol.com)*

**Table 1.2** Receptors of eicosanoids and their functions in inflammation. (*Source: Rang et al: www.studentconsult.com*

| **Eicosanoid** | **Receptor** | **Main function(s)** |
|---|---|---|
| Thromboxane $A_2$ ($TXA_2$) | TP-receptors | Platelet aggregation and vasoconstriction. |
| $PGI_2$ | IP-receptors | Vasodilatation, inhibition of platelet aggregation. |
| $PGE_2$ | $EP_1$-receptors | Contraction of bronchial and GIT smooth muscles |
| | $EP_2$-receptors | Relaxation of bronchial, vascular and GIT smooth muscles. |
| | $EP_3$-receptors | Inhibition of gastric acid secretion, increased gastric mucus secretion, contraction of GIT and pregnant uterus smooth muscles. |
| $PGF_{2\alpha}$ | FP-receptors | Contraction of the uterus. |
| $PGD_2$ | DP-receptors | Vasodilatation, inhibition of platelet aggregation |
| Leucotriene $B_4$ ($LTB_4$) | $LTB_4$-receptors | Chomotactic agent for neutrophils and macrophages. |

### *1.3.3 Models of acute inflammation*

Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increase movement of plasma and leucocytes from the blood into the injured tissue. This is characterized by swelling, redness or erythemia due to an increase in the local blood flow (hyperemia). These early visible responses may be accompanied by platelet and polymorphonuclear leukocytes (PMNL) migration into the tissues (Issekutz *et al.*, 1989). These responses may be induced by injection of inflammatory agents such as killed bacteria (e.g.

*Escherichia coli*), injection of PMNL chemotactic factors, leucotriene B4, vassoactive agents (e.g. PAF and histamine) and Arachidonic acid (Issekutz *et al.*, 1989). Carrageenan, yeast and latex could be injected into various parts of the body to induce inflammation (Meller *et al.*, 1997). Other agents such as mustard oil and zymosan have been used to induce edema (Ma *et al.*, 1996; Neumann *et al.*, 1996). Injection of mustard oil subcutaneously or into the muscle produces edema that lasts up to twenty minutes (Banvolgyi *et al.*, 2004; Yu *et al.*, 1994). Injection of mustard oil into the temporomandibular joint of rats elicits inflammatory response that develops within 30 minutes and reaches maximum in 2 hours (Haas *et al.*, 1992). Intraplantar injection of zymosan produces a persistent dose-and-time-dependent thermal and mechanical hyperalgesia associated with intense inflammation (Meller *et al.*, 1997). Among these models, the Carrageenan induced acute footpad edema in chicks (employed in this project) (Winter *et al.*, 1963) has been widely used by pharmacologists to screen new anti-inflammatory drugs because of its ability to induce an intense and reproducible inflammatory reactions and its sensitivity to inhibition by various anti-inflammatory drugs (Singh *et al.*, 2000).

### *1.3.4 Models of chronic inflammation*

Among the several models of chronic inflammation, the most frequently studied models are the models of arthritis, particularly the polyarthritis induced in rats with *Mycobactreium* (Freund's Adjuvant arthritis) (Whicher *et al.*, 1989). Several species of killed and dried *Mycobacterium* (*M. butyricum, M. tuberculosis and M. plei*) suspended in liquid paraffin could be used to induce arthritis (Liyanage *et al.*, 1975). Alternatively, other bacteria (e.g *Nocardia asteroids*,

*coryne bacterium* etc) (Azuma *et al.*, 1972) or Mycobacterium-derived adjuvant muramyl dipeptide (Kohashi *et al.*, 1980) or a synthetic adjuvant, CP20961 (Chang *et al.*, 1980) could be used to induce polyarthritic syndrome. Acute arthritis could be induced by injection of Carrageenan and kaolin into the cat or monkey knee joint just below the patella (Doughetty *et al.*, 1992; Schaible *et al.*, 1987). Other models of arthritis that have been developed recently include polyarthritis induced by type II collagen in rats and mice (Sluka *et al.*, 1993a), arthritis induced by streptococcal cell walls in rats and implantation of cotton wool pledget subcutaneously. Rat adjuvant arthritis, a model of chronic inflammation, has been extensively employed by industrial pharmacologists principally because of its reproducibility and ability to predict the activity of non-steroidal ant-inflammatory drugs (NSAIDs) (Sluka *et al.*, 1993b). Adjuvant induced arthritis affects not only the joint, since lesions are noted in many of the animal's organs such as the eyes, skin, urinogenital tract, heart etc (Mohr *et al.*, 1976).

### *1.4 PAIN/NOCICEPTION*

Pain is defined by the international association for the study of pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage ((IASP), 1983; Merskey *et al.*, 1979). It could also be defined as a direct response to an untoward event associated with tissue damage such as injury, inflammation or cancer. Pain can be said to be acute or chronic. Acute pain can be defined as pain of recent onset and probable limited duration and usually has an identifiable temporal and causal relationship to injury or disease (Ready, 1992). Acute pain serves as protective purposes of warning the individual of danger

and limits the use of the injured body part (Otsuka *et al.*, 1993). Chronic pain is pain that continues when the causative stimulus is no longer present (Nicoll *et al.*, 1980). Chronic pain persists despite normalization after injury or disease, ultimately interfering with productive activity and reducing quality of life (Nicoll *et al.*, 1980)

### *1.4.1 Basic neuroanatomy and mechanism of pain*

The skin has a number of different types of nerve fibres that transmit information on different stimuli to the spinal cord and brainstem. There are several subtypes of primary afferent neurons, but they can be broadly divided into three major categories. These are; A-β fibres, which convey information on innocuous touch, vibration and pressure; A-δ fibres which are myelinated and conduct impulse more rapidly but respond to intense and potentially dangerous mechanical or mechanicothermal stimuli. A-δ fibres cause a sensation of sharp, well-localized pain and C-fibers which are non-myelinated with low conduction velocities (< 1m/s) and respond to a wide range of thermal, mechanical, and chemical stimuli. Because of its very broad sensitivity, this type of C-fibre is referred to as C-polymodal nociceptor (Masu *et al.*, 1987; Schultzberg *et al.*, 1980).

### *1.4.2 Pain perception*

Perception of pain is best viewed as a three-stage process: (i), Activation of peripheral nociceptors followed by (ii), transmission into the dorsal horn of the spinal cord and (iii), onward passage of pain into the higher centers of the brain.

*Activation of peripheral nociceptors*: Peripheral nociceptive afferents are widely distributed throughout the body (skin, muscle, joints, viscera and meninges) and comprise both medium-diameter lightly myelinated A-δ fibres and small diameter, slow conducting unmyelinated C-fibres. Tissue damage, such as that associated with infection or inflammation produces chemical mediators (e.g. Bradikinin, 5-Hydroxytryptamine, prostaglandins etc.) to activate and / or sensitize nociceptors (Yoshimura *et al.*, 1998).

*Transmission of pain information*: The central terminals of C- and A-δ fibers convey information to nociceptive-specific areas within laminae I and II of the superficial dorsal horn and also to wide dynamic range of neurons in lamina V. Large myelinated A-β fibres transmit light touch or innocuous mechanical stimuli to deep laminae III and IV.

*Pain transmission in the spinal cord*: Primary afferent terminals contain both excitatory amino acid (e.g. Glutamate) and peptide (e.g. Substance P) neurotransmitters. Depolarization of the primary afferent terminals results in Glutamate release, which activates postsynaptic ionotropic α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors to facilitate fast transmission at the first synapse in the dorsal horn (Woolf *et al.*, 2000).

### *1.4.3 Central projection of pain pathways*

The spinothalamic pathway which originates from deeper dorsal horn (lamina V), receives inputs from the superficial dorsal horn (lamina I and II) and distributes nociceptive information to the areas of the thalamus (Simone *et al.*, 1991).

### *1.4.4 Descending pathway and the sites of action of opioids on pain transmission*

The descending pathway originates from the periaqueductal grey (PAG) (inhibitory, activated by opioids) area of the midbrain (Bolay *et al.*, 2002). The PAG receives inputs from the thalamus, cortex and the hypothalamus. The descending serotogenic fibres from the PAG run first to the nucleus raphe magnus (NRM) (inhibitory, activated by opioids) of the brainstem, then through the dorsolateral funiculus (DLF) of the spinal cord which form synaptic connections on DH interneurons. The NRM receives inputs from spinothalamic neurons, via the adjacent nucleus reticularis paragigantocellularis (NRPG). The noradrenergic pathway from the locus coeruleus area of the reticular formation exhibits inhibitory effect on transmission in the DH (Attal *et al.*, 1999).

### *1.4.5 Animal models of nociception*

Currently, the commonly used behavioural tests of acute pain/nociception can be broadly considered based on the use of short-duration stimuli (in the order of seconds) or the use of long-duration stimuli (in the order of minutes) (Le Bars *et al.*, 2001). There are models of chronic pain in animals such as the rat with induced arthritis and rats that have had various lesions on the Central nervous system (CNS) or peripheral nervous system (PNS) (Colpaert, 1987) but due to ethical considerations and technical problems, the adjuvant-induced arthritis in rats is the most widely used in vivo model for the study of chronic pain (Le Bars *et al.*, 2001).

*The use of short-duration stimuli*

These tests involve a short period of stimulation. They can be classified by the nature of the stimulus, be it thermal (Tail-flick test, Tail immersion test and hot plate test), mechanical (Tail or paw pressure test) or electrical (stimulation of paw, tail or dental pulp) (Le Bars *et al.*, 2001).

*The use of long-duration stimuli*

The main types of behavioural test based on such stimuli use intradermal (formalin test) or intraperitoneal (Writhing test) injections. The rat formalin test (a valid model of clinical pain) was employed in this project to evaluate the analgesic properties of the extracts.

The formalin test is the most commonly used analgesic model involving intradermal injection of formalin solution (Le Bars *et al.*, 2001).This test is said to be most predictive of acute pain and simulates clinical pain (Costa-Lotufo *et al.*, 2004; Vasconcelos *et al.*, 2003; Vissers *et al.*, 2003). Injection of (0.5-15%) formalin solution (usually 37% solution of formaldehyde) into the dorsal surface of the hind paw of mice or rat provokes a painful behaviour, that could be assessed on a four-level scale related to the posture of the animal: 0, normal posture ; 1, with the injected paw remaining on the ground but not supporting the animal; 2, with the injected paw clearly raised; and 3, with the injected paw being licked, nibbled or shaken (Dubuisson *et al.*, 1977; Le Bars *et al.*, 2001). Intraplantar injection of formalin produces complex response patterns that last for approximately 1 hour. Two phases of nocifensive behaviour are typically described. These are initial or acute phase occurring about 5 minutes, with immediate and intense response that decreases within the first 3 minutes, followed by a quiescent period, between 5-10 minutes, and a second phase, occurring between 10-60 minutes and peaks at 20$^{th}$-

25th minutes (Dubuisson *et al.*, 1977; Kaneko *et al.*, 1997; Le Bars *et al.*, 2001). These nociceptive responses are evidenced by flinching, licking or biting the injected paw (Dubuisson *et al.*, 1977; Wheeler-Aceto *et al.*, 1990). It is generally agreed that the first phase results at least in part, from direct activation of primary afferent fibres, both low-threshold mechanoreceptive and nociceptive types sensitive to central analgesics (Le Bars *et al.*, 2001; Puig *et al.*, 1996; Szolcsanyi *et al.*, 2004) whereas the second phase is thought to involve inflammatory component with release of different pain mediating substances that possibly can activate small afferent hence sensitive to NSAIDs (Le Bars *et al.*, 2001; Malmberg *et al.*, 1992; Yashpal *et al.*, 1998) and corticosteroids (Vasconcelos *et al.*, 2003).

## *1.5 NEW AND INVESTIGATIONAL APPROACHES TO THE MANAGEMENT OF INFLAMMATION AND PAIN*

### *1.5.1 Peripherally and systemically active antinociceptives*

Current investigational approaches to treatment of pain and inflammation involve the use of peripherally and/or systemically active antinociceptives such as N-methyl-D-aspartase (*NMDA)* (e.g. MK-801) or *AMPA Antagonists* (Haeseler *et al.*, 2003), *COX-2 Inhibitors* (Stichtenoth *et al.*, 2003), *Nitrates* (Thomas *et al.*, 2002) and *Injectable steroids* (Nakashima *et al.*, 1998).

## *1.6 AIMS AND OBJECTIVES*

Majority of clinically important medicines belong to steroidal or non-steroidal anti-inflammatory agents for the treatment of inflammation and pain (Choi *et al.*, 2003). Though these drugs have potent therapeutic activity, they are expensive and may have severe adverse effects. Natural products are therefore being explored as substitutes for cure of diseases or as repository for the discovery of potent therapeutic compounds. Aqueous extract of stem bark of *T. monadelpha* is used in Ghana to treat various ailments including inflammation and pain (Abbiw, 1990; Irvine, 1961; Mshana, 2000) however there are no scientific data available to validate its use. Effects of alcoholic and petroleum ether extracts of the plant on animal or human subjects have not been investigated hitherto. Also, the safety with regard to the use of the plant has not been assessed.

The objectives of this project are to evaluate the anti-inflammatory and analgesic properties of aqueous, alcoholic and petroleum ether extracts of the stem bark of *T. monadelpha* and also assess the safety of the aqueous extract by using animal models.

Specific objectives include:

1. Evaluation of anti-inflammatory activity of the extracts using:

A). Carrageenan-induced foot edema in chicks (Roach and Sufka, 2003) as a model of acute inflammation.

B). Adjuvant-induced arthritis in rats (Pearson, 1956; Woode *et. al.*, 2006) as a model of chronic inflammation.

2. Evaluation of analgesic activity of the extracts using:

A). Formalin test as described by Dubuisson and Dennis (1977).

3. Evaluation of the safety of the aqueous extract (TWE) by using:

A). Sub acute toxicity studies as described by Ansah *et al.*, 2008; Veerappan *et al.*, 2007.



### *1.7 JUSTIFICATION OF THE OBJECTIVES*

A number of natural products are widely employed in the traditional medical system in many countries as alternative medicine for treatment of various diseases. These natural products have been recognized to possess analgesic, anti-inflammatory, anti-allergic, antioxidant etc properties (Auddy *et al.*, 2003; Eastwood, 1999). Some herbal preparations such as the boiled stem bark of *Newbouldea laevis* are used in Ghanaian society as alternative medicine for treatment of inflammation and pain due to lower incidence of severe adverse effect and lower cost (Woode *et al.*, 2008a). Also, phytochemicals from root bark of *Alstonia boonei* and seeds of *Pricralima nitida* have been used for the treatment of rheumatoid arthritis in Ghana and found to be effective anti-rheumatic agent in the adjuvant induced arthritis in rats (Woode *et al.*, 2006). Many medicinal plants provide relieve of symptoms comparable to that obtained from allopathic medicine. Despite this rapid growth, however, there is limited evidence about the effectiveness and safety or otherwise of these alternative medicines (Veerappan *et al.*, 2007). Though these drugs have very potent therapeutic activity, they are very expensive, sometimes may have severe adverse effects and upon continuous use, tolerance can develop.

Natural products are therefore being explored as substitutes for conventional medicines. Though *Trichilia monadelpha* like many other plants had been used over centuries in the traditional medical system as alternative medicine for the treatment of various ailments, the knowledge is based on its existence and application and there are no scientific data available to validate its use as anti-inflammatory and analgesic agents. Also, its safety has not been assessed. This project is justifiable because it aims at joining the global search for analgesic and anti-inflammatory agents. Furthermore, this project is the first of its kind to investigate on the effects of alcoholic and petroleum ether extracts of the plant on experimental animals and provides safety assessment of the plant.

*Chapter 2*

# MATERIALS AND METHODS

## *2.1 CHEMICALS AND DRUGS*

The following drugs and chemicals were used in the experiment; Carrageenan sodium salt (SIGMA, USA), formalin (BDH, Poole, England), liquid paraffin (BDH, Poole. England), sodium chloride (BDH, Poole, England), Diclofenac (TROGE, Humburg, Germany), dexamethasone (PHARM-INTER, Brussel, Belgium), morphine sulphate (PHYTO-RIKER, Accra, Ghana) and methotrexate (PHARM-INTER, Brussel, Belgium).

## *2.2 PLANT MATERIALS*

The stem bark of *Trichilia monadelpha* was collected from Bomaa in the Brong Ahafo region of Ghana in December, 2007. The plant was authenticated by Mr. Amissah, the curator of the Botanic Garden of KNUST. The bark was washed with water, chopped into pieces and air-dried for seven days. The dried pieces were pulverized mechanically to a coarse powder using a hammer mill. The powdered material (1800 g) was divided into three portions. 600 g of the powder was macerated with 2.0 L of 70% (v/v) ethanol in a glass-stopped flask for seventy-two (72) hours. The macerate was concentrated using rotary evaporator to obtain a dark-brown liquid which was dried in oven to give a solid gummy *Trichilia monadelpha* extract (referred to as *Trichilia* alcoholic extract, TAE in this project). 600 g of the powder was infused with 3.0 L of water and warmed for 60 minutes at 90°C. The infusion was filtered to obtain a dark-brown

filtrate which was evaporated over a hot water-bath and later in oven at 55°C until a constant weight was obtained. This was finally cooled in a desiccator to yield a dark-brown solid *Trichilia* water extract (TWE). The third portion (600 g) was extracted with 2.5 L of petroleum ether in a glass-stopped flask for seventy-two (72) hours. The filtrate was evaporated at room temperature (28°C) to yield a syrupy mass of *Trichilia* petroleum ether extract (TPEE). The percentage yield of the aqueous, ethanol and petroleum ether extracts were 18.02%, 15.2% and 1.06% respectively. All extracts were kept in a desicator.



### *2.3 ANIMALS*

Cockerels (*Gallus gallus*; strain Shaver 579), one-day post-hatch were obtained from Akropong Farms, Kumasi, Ghana. The day-old chicks were kept for six days in the animal house of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Chick mash, (GAFCO Tema, Ghana.) and water were available *ad libitum*. The room temperature was maintained at 29°C and overhead incandescent illumination was maintained on 12-hour light-dark cycle. On day six, the healthy chicks were selected and housed in stainless steel cages (34 x 57 x 40 $cm^3$) at population density of 6 chicks per cage. The chicks were brought to the laboratory 24 hours before the commencement of experiment.

Spraque-Dawley rats (120-320 g) and ICR mice (20-30 g) were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, and maintained in the Animal House of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. The animals were housed in groups of six in stainless steel

cages ($34 \times 47 \times 18$ $cm^3$) with soft wood shavings as bedding material. They were fed with normal commercial pellet diet (GAFCO, Tema), given water *ad libitum* and maintained under laboratory conditions.

### *2.4 PHYTOCHEMICAL SCREENING*

The presence of saponins, tannins, alkaloids, and glycosides in the extracts were tested by using simple qualitative methods described by Trease and Evans, (1989).

To test for saponins, 0.5 g each of TWE, TAE and TPEE was added to 10 ml of distilled water in a test tube. The mixture was shaken vigorously for five minutes. Persistent foam which lasted for more than 10 minutes indicated the presence of saponins.

To test for tannins, 0.5 g each of TWE, TAE and TPEE was added to 10 ml of distilled water in a test tube. The mixture was warmed and filtered. 1% aqueous $FeCl_3$ was added to the filtrate. Intense greenish colour indicated the presence of tannins.

For the detection of alkaloids, 0.5 g of the sample was added to 5 ml of 1% aqueous HCl. The mixture was stirred, warmed and filtered. Two drops of Dragendorff's solution were added to the filtrate. Appearance of orange colour suggested the presence of alkaloids.

Presence of glycosides was tested by adding 5 ml of diluted sulphuric acid to the test tube containing 0.5 mg of the sample. 2 ml of 20% NaOH was added followed by addition of few drops each of Fehling's solutions A and B. The mixture was warmed on water bath for two minutes. The brick-red precipitate that formed at the bottom of the test tube indicated the presence of glycosides.

Detection of steroids was performed by extracting 0.5 g of sample with 2.0 ml of chloroform in a test tube followed by filtration. Acetic anhydride was added to the filtrate. Concentrated sulphuric acid was carefully added at the side of the test tube. A blue colour that appeared at the interface suggested the presence of steroids.

For terpenoids, 0.5 mg of the sample was extracted with 2 ml of chloroform in a test tube followed by addition of 1 ml of concentrated sulphuric acid. The reddish-brown coloration showed the presence of terpenoids.

Presence of flavonoids were detected by adding 5 ml of ethyl acetate to 0.5 mg of the sample in a test tube and warmed. Few drops of dilute ammonia solution were added to the mixture. Appearance of yellowish colour at the bottom of the test tube indicated the presence of flavonoids

### *2.5 CARRAGEENAN INDUCED EDEMA IN CHICKS*

The Carrageenan induced foot pad edema in chicks (Roach *et al.*, 2003) was used with some modifications to evaluate the anti-inflammatory properties of TWE, TAE and TPEE. Dexamethasone (a steroidal anti-inflammatory drug) and diclofenac (a non-steroidal anti-inflammatory drug) were used as reference drugs. Each chick received injection of 0.1 ml of 1% carrageenan suspended in normal saline solution using a twenty-six (26) gauge needle connected to a micro syringe. Foot volumes were measured before carrageenan injection (0 hour) and at hourly intervals for five hours after injection by water displacement (Fereidoni *et al.*, 2000). The edema component of inflammation was quantified by measuring the difference

in foot volume before Carrageenan injection and at the various time points. Nineteen groups of chicks (n = 5) were used for the experiment. Groups were selected randomly to receive one of the following doses: extracts (10, 30, 100 and 300 mg/kg *p.o.),* diclofenac (10, 30 and 100 mg/kg *i.p.*) and dexamethasone (0.1, 0.3 and 1.0 mg/kg *i.p.).*

The control group received only distilled water. The extracts were suspended in 2% tragacanth mucilage and the reference drugs were suspended in normal saline solutions. The chicks received the extracts (*p.o.*) sixty minutes or the reference drugs (*i.p.*) ninety minutes after carrageenan injection.

All changes in injected foot volumes were presented as percentage increase from the pretreatment value obtained at time 0 (0 hour), which was calculated by using the formula;

$$\frac{Vt - Vo}{Vo} \times 100$$

Where $V_t$ is the foot volume (at different times) after carrageenan challenge and $V_0$ is the foot volume before carrageenan injection (i.e. time zero).

Data were presented as the effect of drugs on the time course and the total edema response for 5 hours using Graph Pad prism for windows version 5.0 (Graph Pad software, San Diego, CA, USA).

## *2.6 ADJUVANT INDUCED ARTHRITIS IN RATS*

Animals were weighed and assigned to groups (n = 5). Adjuvant arthritis was induced as previously described (Pearson, 1956) and modified by Woode *et al* (2006). Briefly, animals were given intraplantar inoculation with 0.1 ml of complete Freund's adjuvant (CFA) into the right hind paw. Diclofenac (NSAID), dexamethasone (SAID) and methotrexate (an immunosuppressant) were used as reference drugs. The CFA was prepared by suspending 3 mg/ml of heat killed *Mycobacterium tuberculosis* [strains C, DT and PN (mixed) obtained from the Ministry of Agriculture, Fisheries and Food, U.K] in paraffin oil. In order to achieve reproducibility, suspension of a fine aggregate of the *Mycobacterium* was prepared using mortar and pestle (Liyanage *et al.*, 1975).

Arthritic control group received intraplantar inoculation with CFA but no drug treatment, while non-arthritic control group received only intraplantar injection of 0.1 ml of incomplete Freund's adjuvant (IFA) (sterile paraffin oil). The drug treated animals received drugs (*i.p.* route for the reference drugs and oral route for the extracts) 10 days after inoculation with CFA. The extracts were suspended in 2% tragacanth mucilage and the reference drugs were dissolved in normal saline. The test drugs were prepared such that animal received not more than 1 ml of the extract orally or 0.5 ml of the reference drugs by *i.p.* injection. All drugs were freshly prepared.

Rats were assigned to one of the following study groups:

| | |
|---|---|
| Group 1 | Non-arthritic control /IFA (intraplantar injection of 0.1 ml IFA) |
| Group 2 | Arthritic control /CFA (intraplantar inoculation with 0.1 ml CFA) |
| Groups 3-6 | Treated daily with TWE (30-1000 mg/kg *p.o.*) |
| Groups 7-19 | Treated daily with TPEE (30-1000 mg/kg *p.o.*) |
| Groups 11-14 | Treated daily with TAE (30-1000 mg/kg *p.o.*). |
| Groups 15-17 | Treated every other day with dexamethasone (0.1, 0.3 and 1.0 mg/kg *i.p.*). |
| Groups 18-20 | Treated every other day with diclofenac (10, 30 and 100 mg/kg *i.p.*) |
| Groups 21-23 | Treated every four days with methotrexate (1.0, 0.3 and 1.0 mg/kg *i.p.*). |

Baseline values for joint thickness were taken by measuring the diameter of the rat hind paw joint (Murayama *et al.*, 1991) using an electronic digital caliper, for both ipsilateral and contralateral paws before intraplantar inoculation with CFA.

Thickness of the joints were measured every other day (day 0, 2, 4, 6, ...…28), and the edema component of inflammation was quantified by measuring the differences in joint thickness before CFA inoculation and at various time points.

All changes in joint thickness were presented as percentage increase from the pretreatment value obtained at day 0, which was calculated according to the formula

$$\frac{Tx - To}{To} \times 100$$

Where $T_x$ is the joint thickness after CFA inoculation and $T_0$ is the initial joint thickness taking on day zero (0) (i.e. before CFA inoculation).

Data were presented as the effect of drugs on the time course and the total edema response of adjuvant-induced arthritis for the 28 days.

### *2.7 FORMALIN INDUCED NOCICEPTION IN MICE*

The formalin test initially performed by Dubuisson and Dennis (1997) was carried out as described by Malmberg *et al.,* (1992). The animals (n = 5) were acclimatized to the test chambers (a plexiglass chamber each measured 15 x 15 x 15 $cm^3$) for thirty minutes before formalin injection. Diclofenac (an NSAID) and morphine (an opioid receptor agonist) were used as reference drugs. The mice were pre-treated with the test drugs (30 minutes for *i.p.* route and 1 hour for oral route) before intraplantar injection of 10 µl of 4% formalin into the ventral surface of the right hind paw, using a twenty-six (26) gauge needle connected to a micro syringe.

The animals were returned individually into the test chamber immediately after formalin injection. A mirror was placed at an angle of 45° beneath the chambers to allow an

unobstructed view of the hind paws. The behaviour of the animal was then captured (60 min) for analysis with a digital camera placed in front of the mirror.

Pain responses were scored for 60 min, starting immediately after formalin injection. A nociceptive score was determined for each 5-min time block by measuring the time spent biting/licking the injected paw (Hayashida *et al.*, 2003). Behavioural responses were scored from the videotapes with the aid of the public domain software JWatcher™ Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia). Average nociceptive score for each time block was calculated by multiplying the frequency and time spent in biting/licking the injected paw as considered indicative of pain following formalin injection (Mino *et al.*, 2004). The nociceptive scores were 0-10 minutes for the first phase and 10-60 minutes for the second phase (Hayashida *et al.*, 2003). The phases represented neurogenic and inflammatory pain responses respectively (Hunskaar *et al.*, 1987).

Groups of mice were randomly selected to receive one of the following treatments: Diclofenac (10, 30 and 100 mg/kg *i.p.*), morphine (1, 3 and 10 mg/kg *i.p.*) and the extracts (10, 30, 100, 300 mg/kg *p.o.*). Drugs were prepared such that no animal received more than 0.5 ml of the reference drug injected intraperitoneally or more than 1 ml of the extract given orally. All drugs were freshly prepared.

Data were presented as the mean ± s.e.m of pain scores between 0-10 and 10-60 min after formalin injection. The time-course curves were subjected to two-way (treatment x time) analysis of variance followed by Neuman-Keuls multiple comparison test

## *2.8 SUB ACUTE TOXICITY STUDIES*

Sub acute toxicity study was conducted following the procedures used by Veerappan *et al*., (2006) and Ansah *et al.*, (2008). Male Sprague-Dawley rats (150-300 g) were placed into 4 groups (n =5). Animals in the groups received 100, 300 or 1000 mg/kg (*p.o.*) of TWE once daily for two weeks. The control group received distilled water. During the period of administration, animals were observed and weighed daily to detect signs of toxicity. All visual observations made were systematically recorded.

### *2.8.1 Haematological analysis*

At the end of the 14 days period, the rats were anaesthetized with ether and blood was collected through a cardiac puncture into polystyrene tubes coated with Ethylenediaminetetraaccetic acid (EDTA) as anticoagulant. Red blood cells (RBC), total white blood cells (WBC), granulocytes, Platelets (PLT), Mean Corpuscular volume (MCV), Haemoglobin Concentration (HB), Mean Haemoglobin Concentration (MHC) and Mean Corpuscular Haemoglobin Concentration (MCHC) were determined using an automated analyzer, Cell Dyne: Model 331430 (Abbott Laboratories, IL, USA).

### *2.8.2 Biochemical analysis*

Blood was collected from the heart (without anticoagulant) for biochemical analysis. Serum was separated by centrifugation (750 g for 19 min). Serum determination of total proteins, total bilirubin, direct bilirubin, indirect bilirubin, albumin, globulin and albumin/globulin (A/G) ratios were performed using an automated analyzer, ATAC 8000 (Elan Diagnostics, CA,

USA). Levels of the liver enzymes like alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and γ-glutamyl aminotransferase (GGT) were also determined.

### *2.8.3 Determination of relative organ weights*

After blood collection, animals were sacrificed for histopathological studies. The following organs; heart, liver, kidney, and stomach were removed, washed and weighed immediately on an electronic balance. Organ-to-body weight ratios were calculated for statistical analysis.

## *2.9 STATISTICAL ANALYSIS*

Total foot volume for each treatment was calculated as area under the time course curve (AUC). The equation below was used to determine the percentage inhibition of edema.

$$\% \text{ inhibition of edema} = \left(\frac{AUC_{control} - AUC_{treatment}}{AUC_{control}}\right) x100$$

Differences in AUCs were analyzed by ANOVA followed by Newman-Keuls' *post hoc test*. $ED_{50}$ (dose responsible for 50% of the maximal effect) and inhibitory effects of drugs were analyzed by using an iterative computer least squares method, GraphPad Prism for Windows version 5.0 (GraphPad Software, San Diego, CA, USA).

The fitted midpoints ($ED_{50}$s) of the curves were compared statistically using *F* test (Motulsky and Christopoulos, 2003; Miller, 2003). GraphPad Prism for windows version 5.0 (GraphPad

Software, San Diego, CA, USA) was used for all statistical analysis and $ED_{50}$ determination. Levels of significance were determined by analysis of variance (ANOVA) followed by Student Newman-Keuls' post test using GraphPad Prism. All values were expressed as mean ± s.e.m. $P < 0.05$ were considered statistically significant.

# *Chapter 3*

# RESULTS

## *3.1 PHYTOCHEMICAL TEST*

The presence of alkaloids, tannins, saponins, steroids, terpenoids, flavonoids and glycosides in TWE, TAE and TPEE were determined by simple qualitative methods described by Trease and Evans (1989). The results were presented in Table 3.1

**Table 3.1** Phytochemical components of TWE, TAE and TPEE.

| | Alkaloids | Tannins | Saponins | Steroids | flavonoids | terpenoids | glycosides |
|---|---|---|---|---|---|---|---|
| TWE | + | + | + | — | + | — | + |
| TAE | + | + | + | — | + | + | + |
| TPEE | + | — | — | ++ | — | + | — |

Key: + = present; — = not present. ++ = present in high concentration.

### *3.2 CARRAGEENAN INDUCED EDEMA IN 7-DAY OLD CHICKS*

Intradermal injection of 10 µl of 1% Carrageenan induced a time-dependent edema response in 7-day-old chicks that peaked at 2-3 hours (Roach *et al.*, 2003). In this experiment, the effects of alcoholic (TAE), aqueous (TWE), and petroleum ether (TPEE) extracts of *T. monadelpha* on carrageenan induced pedal edema in chicks were investigated. Diclofenac and dexamethasone were used as reference drugs. Two-way ANOVA (treatment x time) revealed significant effects of drug treatment for TWE ($F_{4, 120} = 7.47$; $P < 0.001$), TPEE ($F_{4,120} = 10.67$; $P < 0.001$), Diclofenac ($F_{3, 96} = 10.74$; $P < 0.001$) and dexamethasone ($F_{3, 96} = 16.81$; $P < 0.001$) as shown in figures 3.1 and 3.2. There were no significant differences between the TAE (10, 30, 100 and 300 mg/kg) treated group and the control (Figure 3.2 **c** and **d**).

The maximal inhibitions of edema obtained from the area under the time course curves were 57.79±3.92% at 300 mg/kg for TWE (figure 3.2 **a** and **b)** and 63.83±1.28% at 300 mg/kg for TPEE (figure 3.2 **e** and **f)**. Diclofenac and dexamethasone also inhibited edema with maximal effects of 64.92±2.03% at 100 mg/kg and 71.85±1.53% at 1 mg/kg respectively (figure 3.1 **a** and **d**).

From the $ED_{50}$ (dose responsible for 50% of maximal response) values obtained from the dose response curves (figure 3.3), the order of potency was: 0.102±0.01 mg/kg for dexamethasone, 10.79±1.57 mg/kg for diclofenac, 28.90±2.67 mg/kg for TPEE, 55.78±6.27 mg/kg for TWE and 98.13±20.10 mg/kg for TAE.

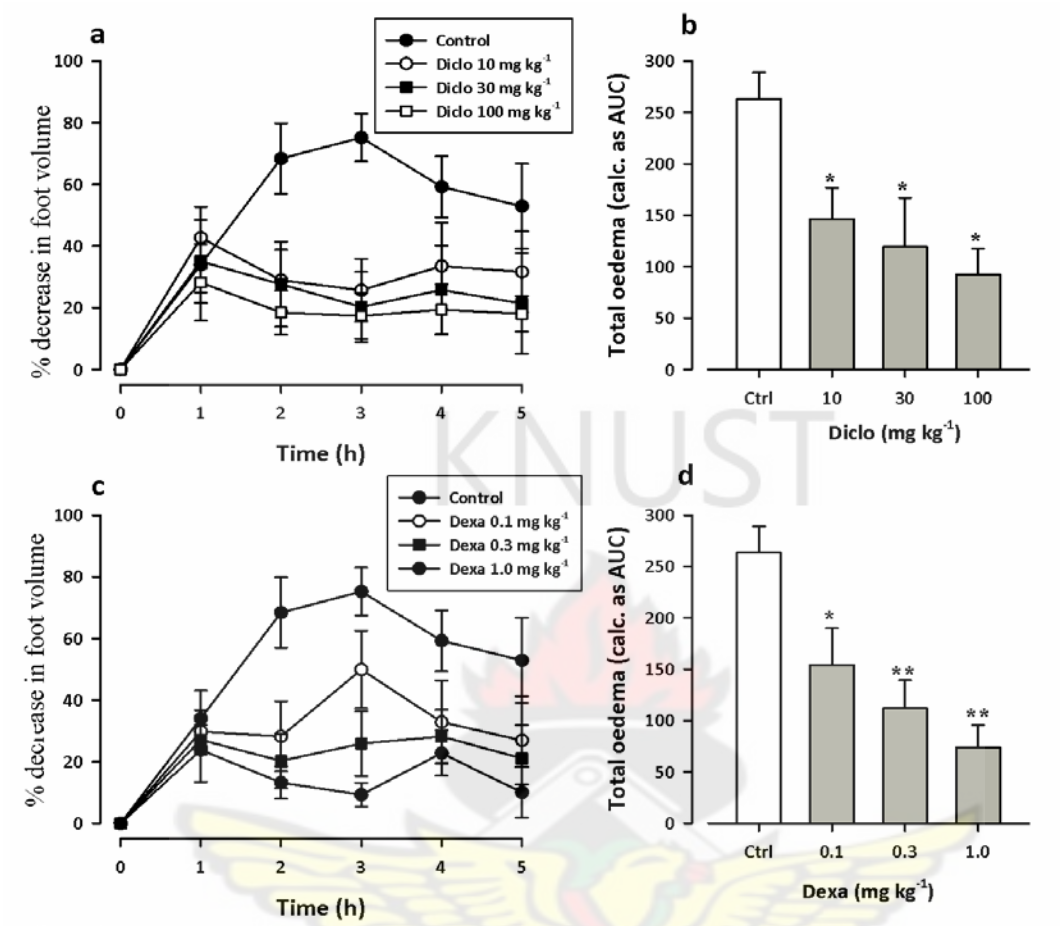



**Figure 3.1** Time course effects of (**a**) diclofenac (10-100 mg/kg *i.p.*) (**c**) and dexamethasone (0.1-1 mg/kg *i.p.*) on carrageenan induced edema in chicks and the total edema response for 5 hours defined as AUC for (**b**) diclofenac and (**d**) dexamethasone. Each point in **c** and column in **d** represents the mean ± s.e.m. (n = 5). $^{**}P < 0.01$, $^{*}P < 0.05$

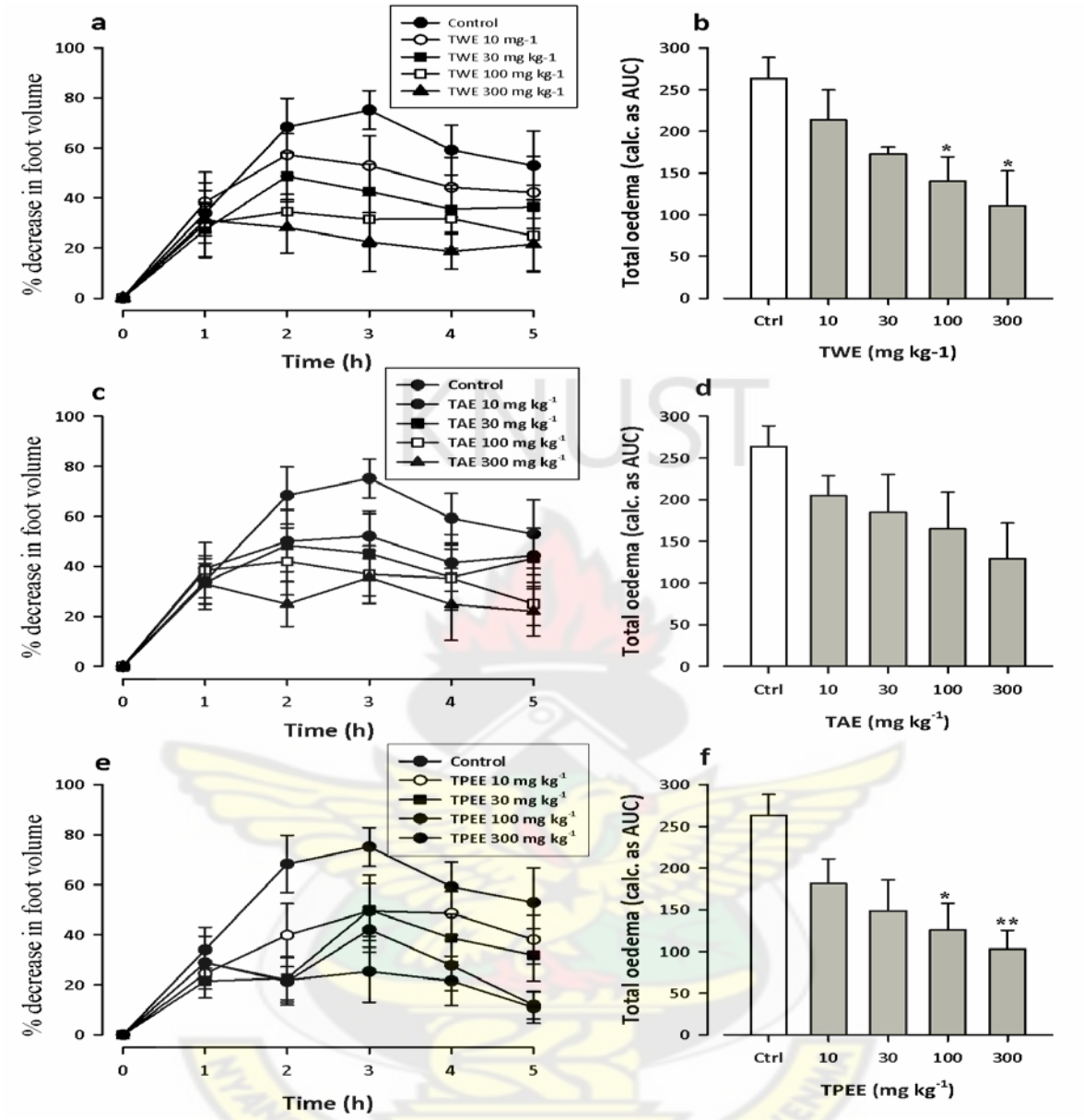



**Figure 3.2** Time course effects of (**a**) *TWE* (10-300 mg/kg *p.o.*), (**c**) *TAE* (10-300 mg/kg *p.o.*) and (**e**) *TPEE* (10-300 mg/kg *p.o.*) on Carrageenan-induced edema in 7-day-old chicks and the area under the time course curves (AUC) for (**b**) *TWE*, (**d**) *TAE* and (**f**) *TPEE*. Extracts were given 1 hour after Carrageenan challenge. Each point in **a, c,** and **e** and column in **b, d,** and **f** represents the mean ± s.e.m. (n = 5). $^{**}P < 0.01$, $^{*}P < 0.05$

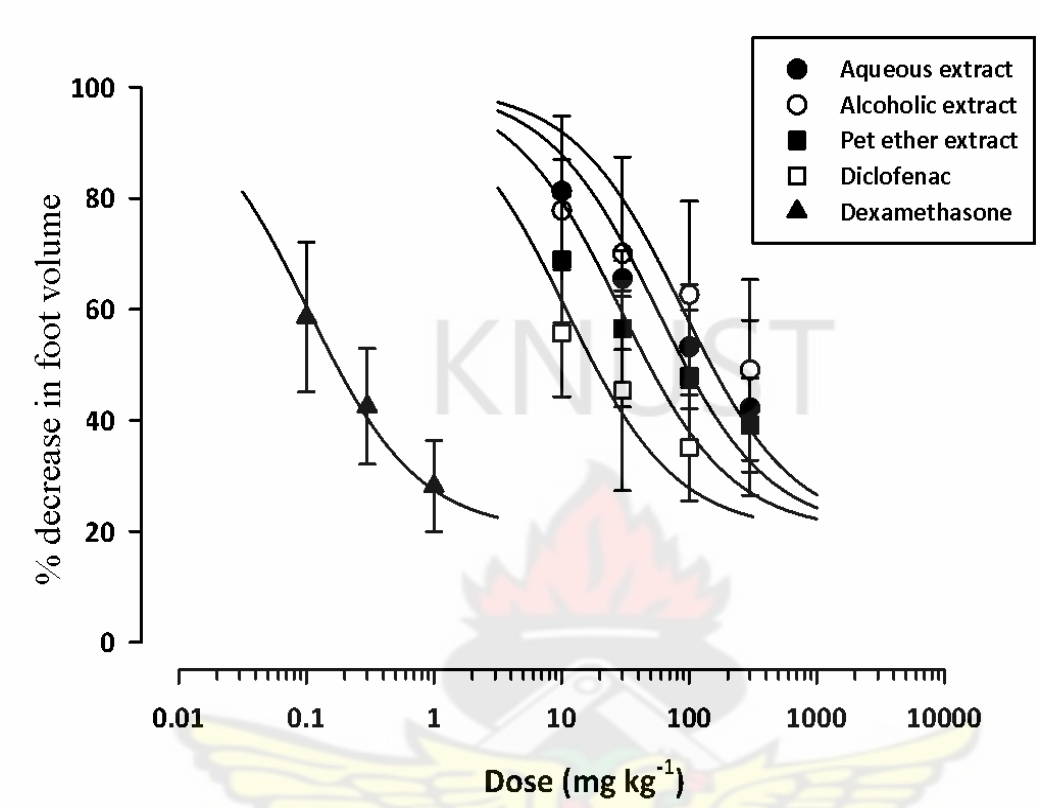



**Figure 3.3** Dose response curves for dexamethasone (0.1-1 mg/kg *i.p.*), diclofenac (10-100 mg/kg *i.p.*), *TWE* (10-300 mg/kg *p.o.*), *TAE* (10-300 mg/kg *p.o.*) and *TPEE* (10-300 mg/kg *p.o.*) on Carrageenan induced foot edema in 7-day old chicks. Animals were treated 1 hour after carrageenan challenge. Each point represents the mean ± s.e.m. (n = 5).

**Table 3.2** $ED_{50}$ (mg/kg) values of carrageenan induced edema in chicks

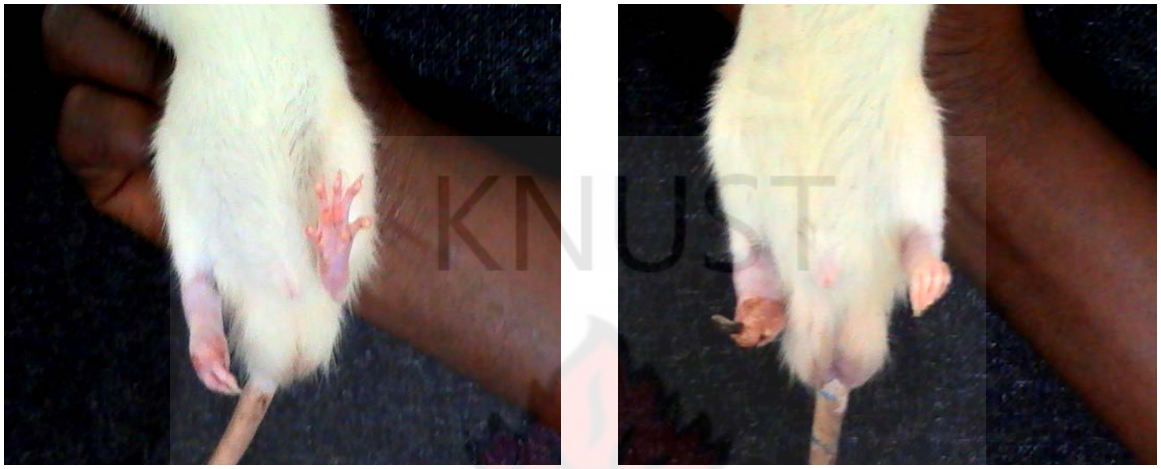
| **Drug** | **$ED_{50}$ (mg/kg)** |
|---|---|
| Dexamethasone | 0.10±0.01 |
| Diclofenac | 10.79±1.57 |
| TWE | 55.78±6.27 |
| TAE | 98.13±20.10 |
| TPEE | 28.90±2.67 |

### *3.3 ADJUVANT INDUCED ARTHRITIS IN RATS*

Intraplantar injection of CFA into the right foot pad of rats evoked a biphasic inflammatory response. These are the initial swelling of ipsilateral (injected) paw representing the acute inflammatory response followed by the chronic phase which is characterized by the spread of edema to the contralateral (non-injected) paw. These two phases correspond to days 0-10 and 11-28 respectively following CFA inoculation. In this investigation, inoculation of rats with 0.1 ml CFA (heat-killed mycobacterium tuberculosis, 3 mg/ml in paraffin oil) into the right hind paw resulted in inflammatory edema of the paw. All arthritic control animals (received CFA inoculation but no drug treatment) showed acute inflammatory edema characterized by unilateral inflammation of the ipsilateral paw that peaked around days 4-6 (figure 3.5) followed by polyarthritic phase which began on day 14 with the spread of edema to the contralateral paw (Figure 3.4).

In the current study, the effects of TWE (30, 100, 300 and 1000 mg/kg *p.o.*), TAE (30, 100, 300 and 1000 mg/kg *p.o.*), and TPEE (30, 100, 300 and 1000 mg/kg *p.o.*) on the polyarthritic/chronic phase were investigated. Diclofenac (10, 30 and 100 mg/kg *i.p.*), dexamethasone (0.1, 0.3 and 1.0 mg/kg *i.p.*) and methotrexate (0.1, 0.3 and 1.0 mg/kg *i.p.*) were used as reference drugs. Animals were treated daily with the extracts starting from day 10 up to day 28. Diclofenac and dexamethasone were given every other day while methotrexate was given every 4 days starting from day 10. From the results obtained (fig. 3.6), all the extracts significantly inhibited the polyarthritic phase edema; TWE ($F_{4,\ 180} = 14.90$, $P < 0.001$; figure 3.6 **a** and **b**), TAE ($F_{4,\ 180} = 10.36$, $P < 0.001$; figure 3.6 **c** and **d**) and TPEE ($F_{4,\ 180} =$

12.08, P < 0.001; figure 3.6 **e** and **f**). Dexamethasone ($F_{3,144}$ =37.07, P < 0.001), diclofenac ($F_{3,\ 144}$ = 43.36, P = 43.36) and methotrexate ($F_{3,144}$ = 30.03, P < 0.001) also significantly inhibited paw edema (figure 3.5 **a-f**).



**a** **b**

**Figure 3.4:** CFA induced arthritis in rats showing **a**) acute phase characterized by unilateral spread of inflammation of ipsilateral paw; and **b**) chronic phase characterized by contralateral spread of inflammation to the tail, genital and paws.

The maximal inhibitions of the extracts were 64.41±5.56% at 1000 mg/kg for TWE, 57.04±8.57% at 1000 mg/kg for TAE and 62.81±2.56% at 1000 mg/kg for TPEE (figure 3.6 **a-f**). The maximal inhibitions of the reference drugs were; 85.75±2.96% at 1 mg/kg for dexamethasone, 80.28±5.79% at 100 mg/kg for diclofenac and 74.68±3.03% at 1 mg/kg for methotrexate (figure 3.5 **a-f**).

The time course effect curves (figure 3.6 **c** and **e**) and the area under the time course curves (figure 3.6 **d** and **f**) of TAE and TPEE showed that the extracts were more potent at 30 mg/kg

than 100 mg/kg and 300 mg/kg. This resulted in the bell-shaped dose response curves (DRCs) of TAE and TPEE in figure 3.7.

The $ED_{50}$ values (in order of potency) obtained from the dose response curves (figure 3.7) were 0.031±0.002 mg/kg for methotrexate > 0.037±0.002 mg/kg for dexamethasone > 2.55±0.15 mg/kg for diclofenac > 79.232.67 for TWE > 93.24±3.43 mg/kg for TPEE > 98.03±2.49 mg/kg for TAE.

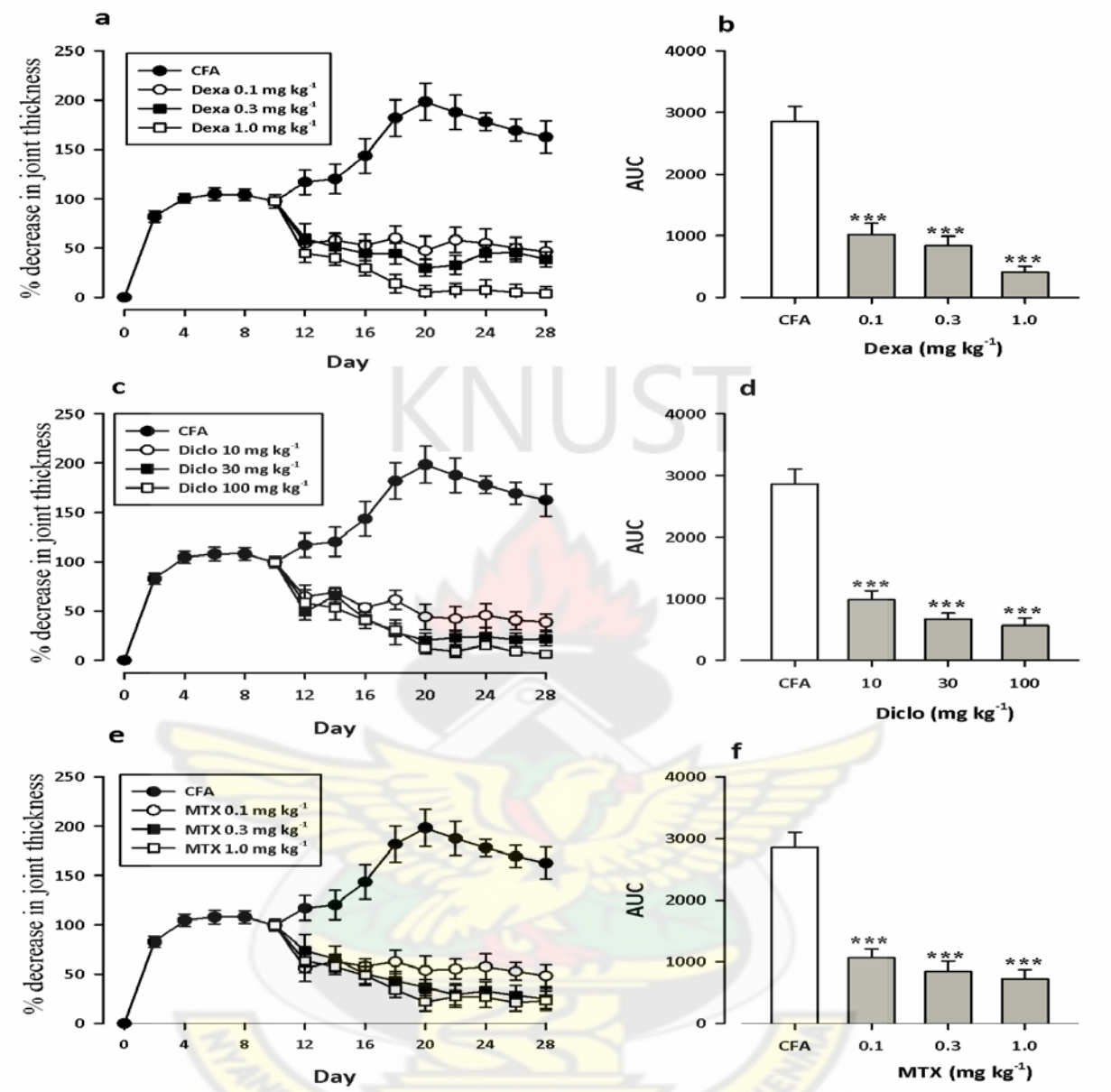



**Figure 3.5** Time course effects of (**a**) dexamethasone (0.1-1 mg/kg *i.p.*), (**c**) diclofenac (10-100 mg/kg *i.p.*) and (**e**) methotrexate (0.1-3 mg/kg *i.p.*) and total edema response presented as AUC of (**b**) dexamethasone, (**d)** diclofenac and (**f**) methotrexate of CFA induced arthritis in rats. Treatment started from day 10. Values are presented as mean± s.e.m. (n=5). ***P < 0.001, **P < 0.01, *P < 0.05.

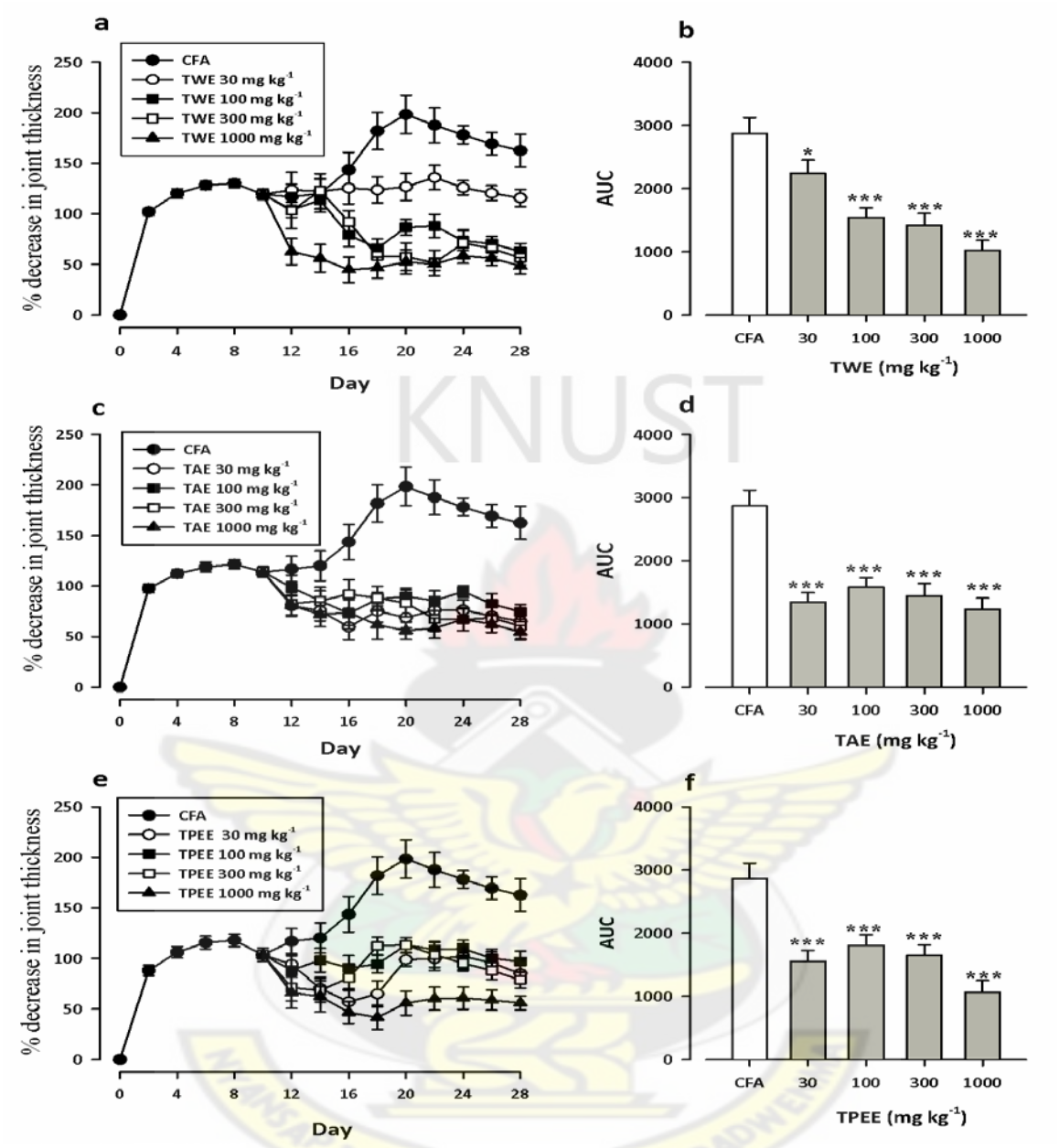



**Figure 3.6** Time course effects of (**a**) TWE (10-1000 mg/kg *p.o.*), (**c**) TAE (10-1000 mg/kg *p.o.*) and (**e**) TPEE (10-1000 mg/kg *p.o.*) on CFA induced arthritis in rats and the total edema response presented as AUC for (**b**) TWE, (**d**) TAE and (**f**) TPEE. Treatment started on day 10. Each point in (**a**), (**c**) and (**e**) and column in (**b**), (**d**) and (**f**) represents the mean ± s.e.m. (n = 5). ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$)

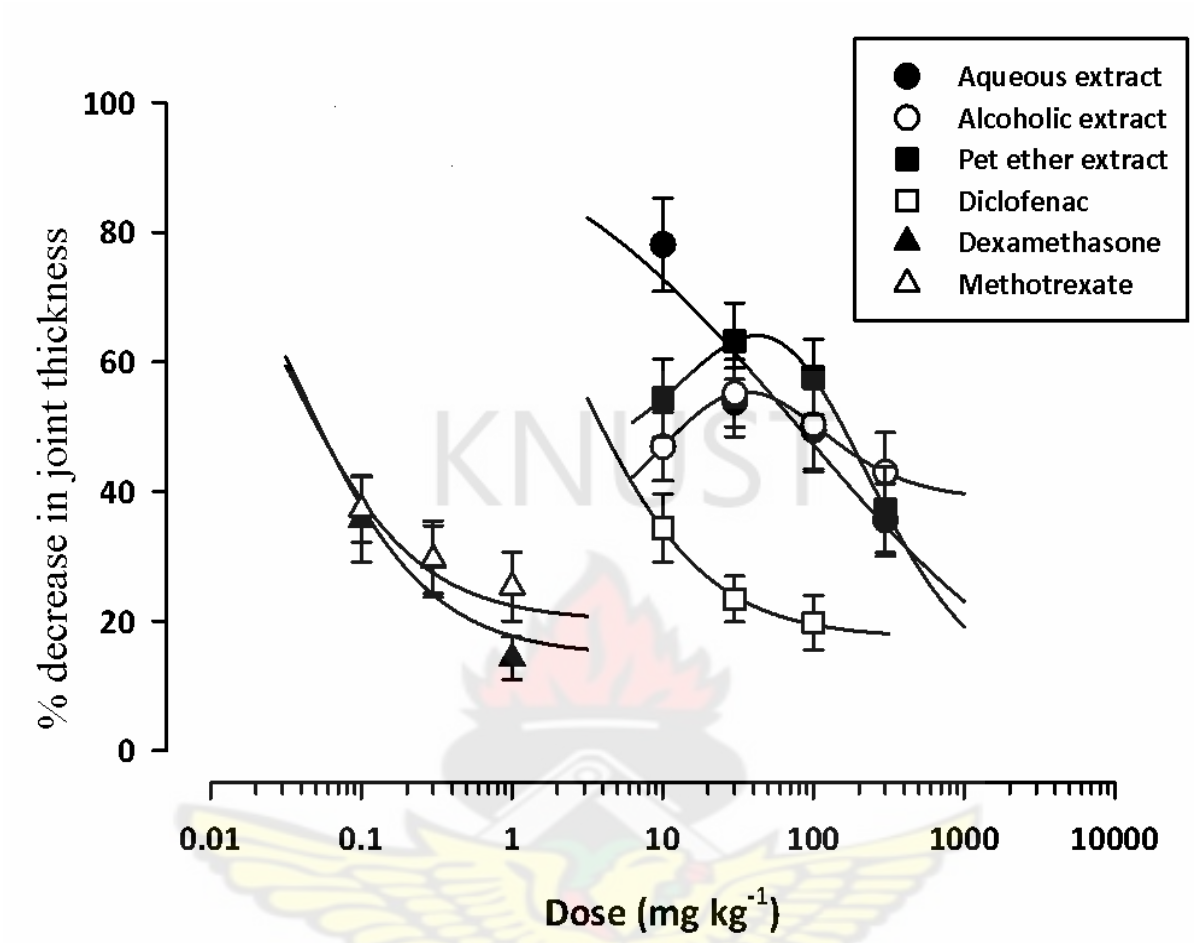



**Figure 3.7** Dose response curves for TWE (10-1000 mg/kg *p.o.*) (●), TAE (10-1000 mg/kg *p.o.*) (○), TPEE (10-1000 mg/kg *p.o.*) (■), dexamethasone (0.1-1mg/kg *i.p.*) (▲), diclofenac (10-100 mg/kg *i.p.*) (□) and methotrexate (0.1-1 mg/kg *i.p.*) (Δ) on CFA induced arthritis in rats. Each point represents mean ± s.e.m. (n = 5).

**Table 3.3** $ED_{50}$ values of diclofenac, dexamethasone, methotrexate and the extracts on CFA induced arthritis.

| DRUG | $ED_{50}$ |
|---|---|
| TWE | 79.23±2.70 |
| TAE | 98.03±2.49 |
| TPEE | 93.24±3.43 |
| Diclofenac | 2.55±0.149 |
| Dexamethasone | 0.037±0.002 |
| Methotrexate | 0.031±0.002 |

### *3.4 FORMALIN INDUCED NOCICEPTION IN MICE*

Intraplantar injection of 10 µl of 4% formalin evoked a characteristic biphasic response characterized by licking/biting of the injected paw (Dubuisson *et al.*, 1977; Wheeler-Aceto *et al.*, 1990). The two phases consisted of an initial intense response to pain beginning immediately (0-10 min) after formalin injection (first phase). This was followed by a slowly rising but longer lasting response from 10-60 minutes after formalin injection (second phase). These phases represented neurogenic and inflammatory pain respectively (Hayashida *et al.*, 2003; Hunskaar *et al.*, 1987) (figures 3.8-9).

In the current study, the analgesic effects of TWE (10, 30, 100 and 300 mg/kg *p.o.*), TAE (10, 30, 100 and 300 mg/kg *p.o.*) and TPEE (10, 30, 100 and 300 mg/kg *p.o.*) on formalin-induced nociception were investigated. Diclofenac (an NSAID, 10, 30 and100 mg/kg *i.p.)* and morphine (an opioid receptor agonist, 1, 3 and 10 mg/kg *i.p.*) were used as positive control drugs. In phase 1, TAE exhibited significant effect ($F_{4,\ 40}$ =3.19; $P < 0.035$) only at the dose of 300 mg/kg but did not show any significant effect ($F_{\ 4,\ 200}$ = 17.94; $P < 0.067$) in the second phase (figure 3.9). TWE and TPEE showed significant analgesic effect by reducing the licking or biting time of the animals in both phase 1 (TWE: $F_{4,\ 40}$ = 8.36; P < 0.004, TPEE: $F_{4,\ 40}$ = 14.03; P < 0.001) and phase 2 (TWE: $F_{4,\ 200}$ = 9.74; P < 0.001, TPEE: $F_{4,\ 200}$ = 8.66; P < 0.003). The reference drug, diclofenac showed significant analgesic effect in phase 1 only at 100 mg/kg ($F_{3,\ 32}$ = 3.62; P < 0.036, figure 3.8 **d**). However, it exhibited more significant effect in the inflammatory pain ($F_{3,\ 160}$ = 7.93; P < 0.0018; figure 3.8 **d**). Morphine was

effective in both the first phase ($F_{3, 36}$ = 26.26; P < 0.001) and the second phase ($F_{3, 160}$ = 19.41; P < 0.001) (figure 3.7 **b**).

The $ED_{50}$ values obtained from the dose response curves were 0.14±0.013 mg/kg for morphine > 5.02±0.17 mg/kg for TPEE > 6.80±0.22 mg/kg for TWE > 41.78±1.8 mg/kg for diclofenac > 96.15±2.2 mg/kg for TAE in phase one (figure 3.9) and 0.45±0.016 mg/kg for morphine > 1.97±0.53 mg/kg for TPEE > 2.69±0.22 mg/kg for TWE > 34.81±2.3 mg/kg for diclofenac > 88.33±4.9 mg/kg for TAE in the second phase (figure.3.9).

The maximal inhibition obtained from the area under the time course curves (AUCs) were; 95.60±2.35% at 10 mg/kg for morphine (figure 3.8 **a** and **b**) > 89.96±7.6% at 100 mg/kg for TPEE (figure 3.9 **e** and **f**) > 85.25±31.15% at 300 mg/kg for TWE (figure 3.9 **a** and **b**) > 64.03±18.67% at 100 mg/kg for diclofenac (figure 3.8 **c** and **d**) > 62.02±2.98% at 300 mg/kg for TAE (figure 3.9 **c** and **d**) in the first phase and 98.45±5.52% at 10 mg/kg for morphine > 96.24±2.98% at 300 mg/kg for TPEE > 94.91±4.88% at 300 mg/kg for TWE > 88.99±6.88% at 10 mg/kg for diclofenac > 47.17±15.40% at 300 mg/kg for TAE in the second phase.

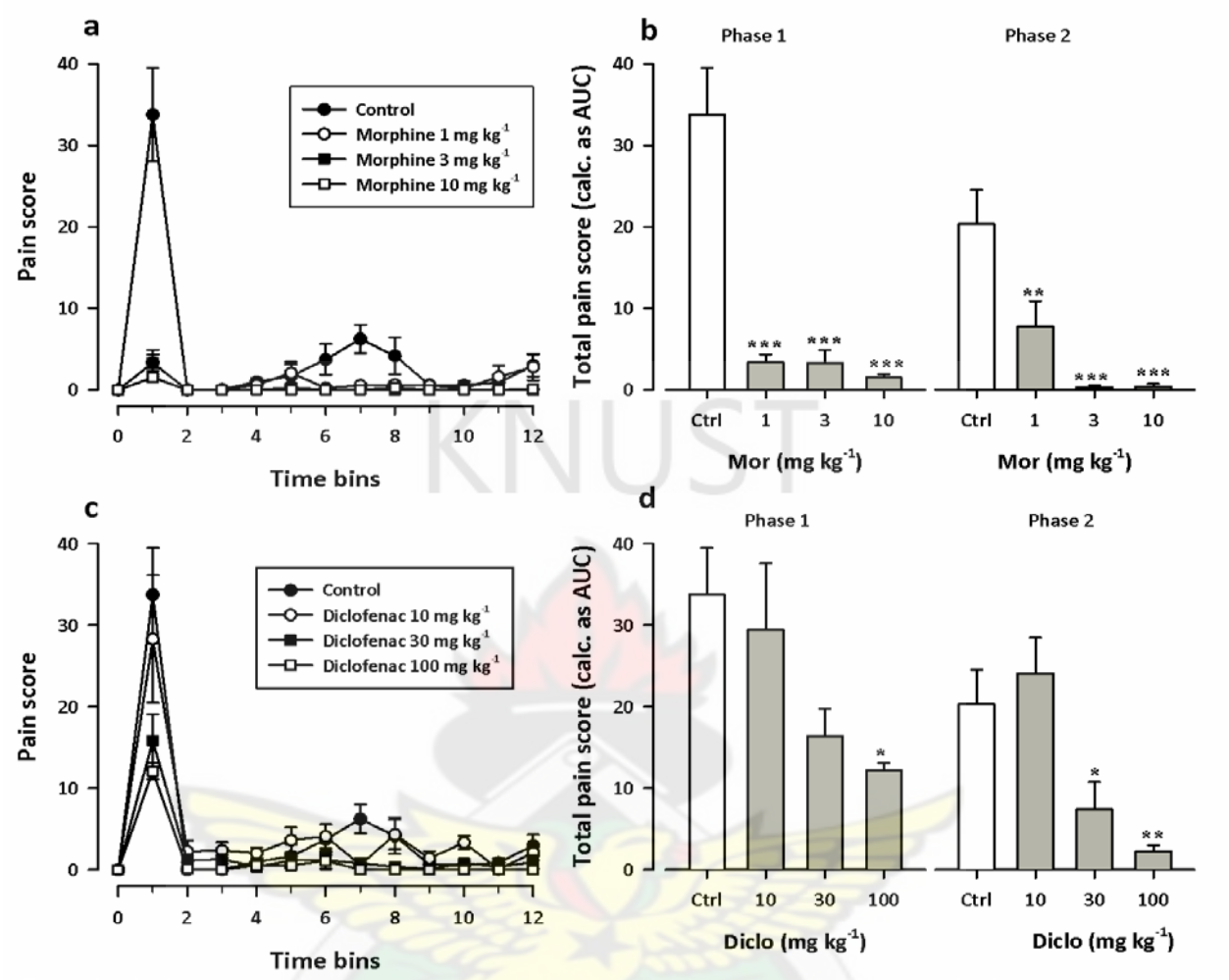



**Figure 3.8** Time course effects of (**a**) morphine (1-10 mg/kg *i.p.*) and (**c**) diclofenac (10-100 mg/kg *i.p.*) and the area under the time course curves (**b)** morphine, and (**d)** diclofenac on formalin test. Each point represents mean ± s.e.m. (n = 5). ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

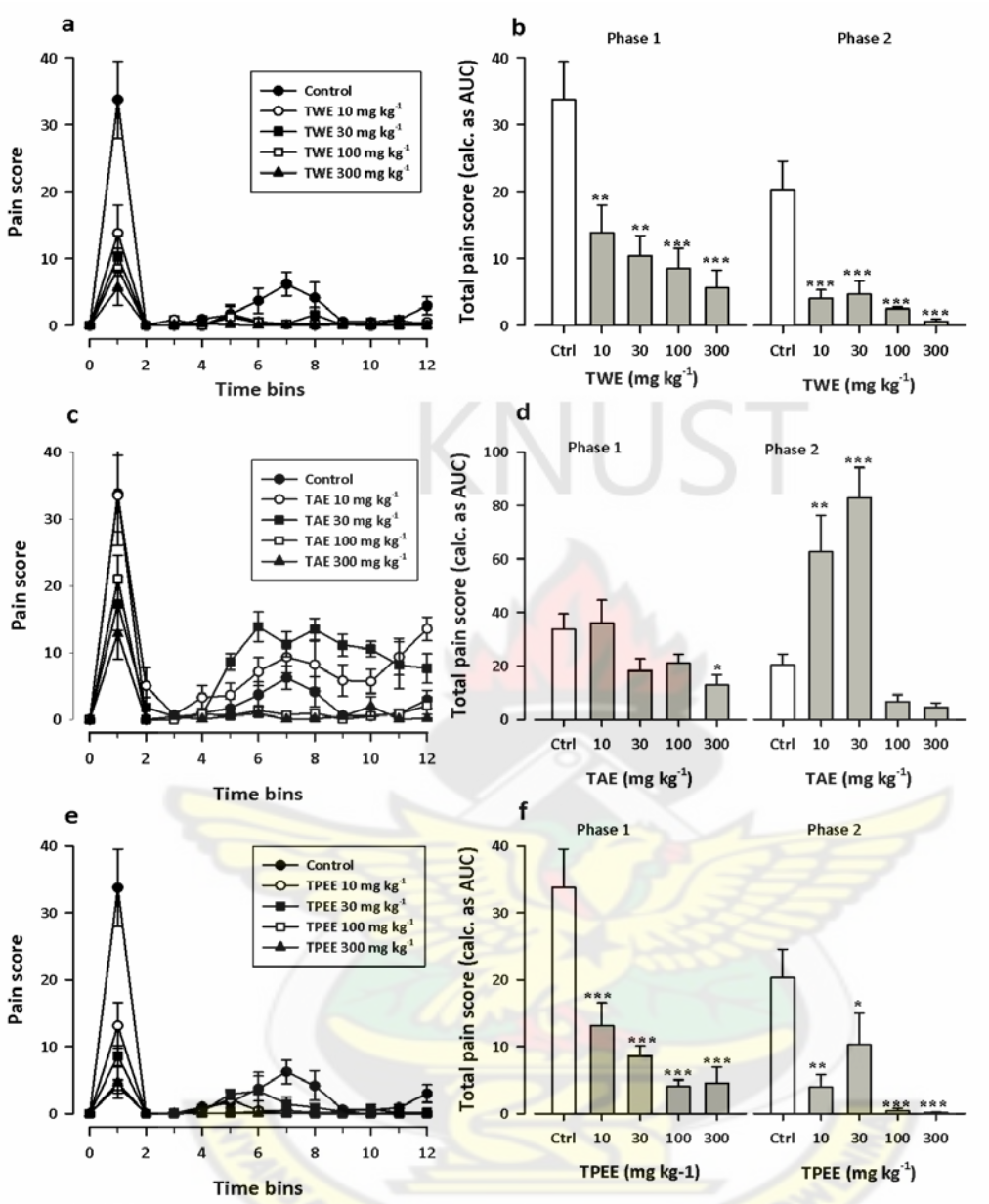



**Figure 3.9** Time course effects of (**a**) TWE (10-300 mg/k *p.o.*), (**c**) TAE (10-300 mg/kg *p.o.*) and (**e**) TPEE (10-300 mg/kg *p.o.*) and the area under the time course curves of the formalin test. Each point represents mean ± s. e. m. (n = 5). ***P < 0.001, **P < 0.01, *P < 0.05.

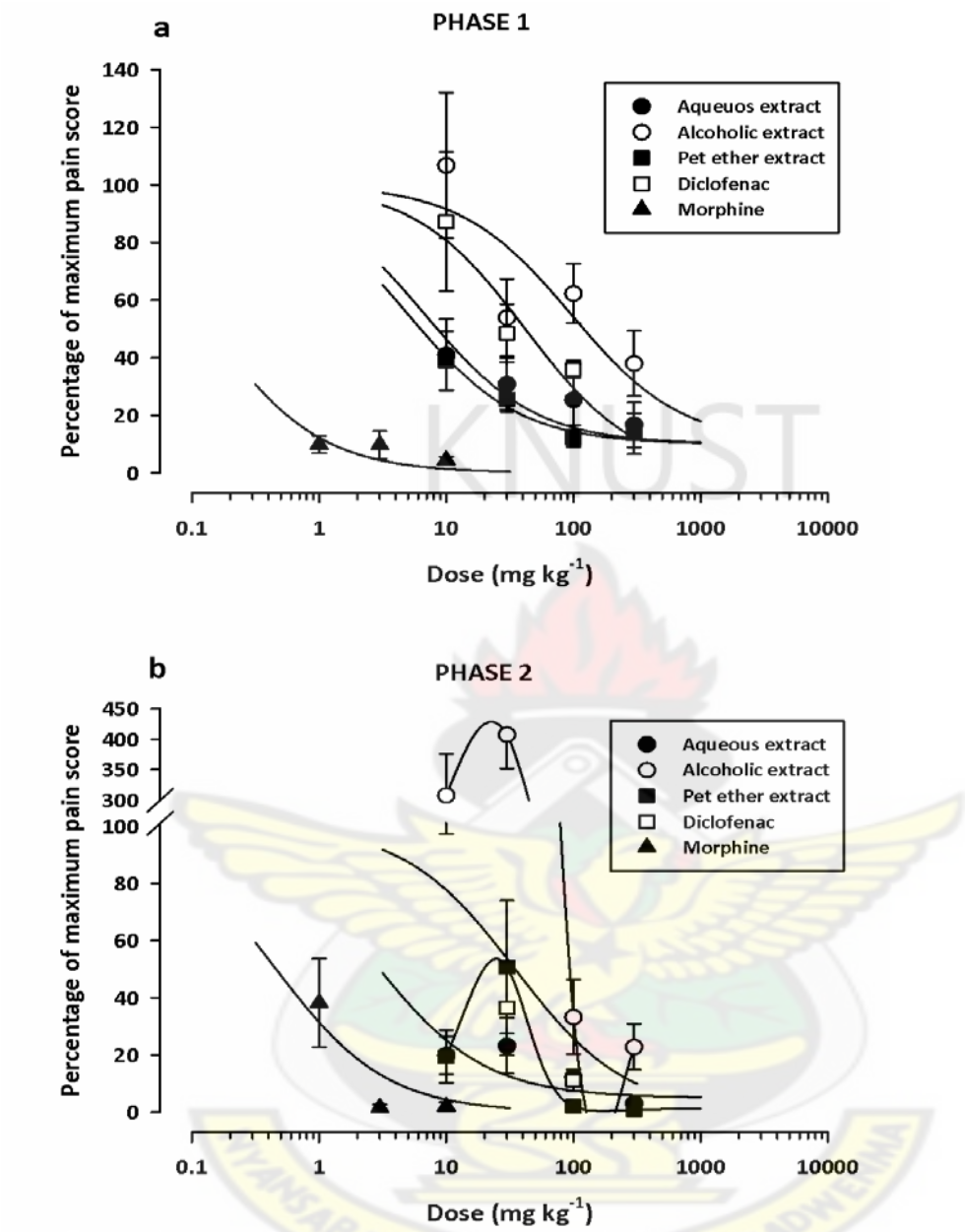



**Figure 3.10** Dose response curves of TWE (10-300 mg/kg *p.o.*) (●), TAE (10-300 mg/kg *p.o.*) (○), TPEE (10-300 mg/kg *p.o.*) (■), diclofenac (10-100 mg/kg *i.p.)* (□) and morphine (1-10 mg/kg *i.p.)* (▲) on the total nociceptive score for the first phase (**a**) and the second phase (**b**) of the formalin test. Each point represents mean ± s.e.m. (n= 5).

**Table 3.4** $ED_{50}$ values obtained from the dose response curves of the formalin-induced nociception in mice.

| **Drug** | **$ED_{50}$ (mg/kg)** | |
|---|---|---|
| | Phase 1 | Phase 2 |
| Morphine | 0.14±0.01 | 0.45±0.02 |
| Diclofenac | 41.78±1.8 | 34.81±2.3 |
| TWE | 6.80±0.22 | 2.69±0.22 |
| TAE | 96.15±2.2 | 88.33±4.9 |
| TPEE | 5.02±0.17 | 1.97± 0.53 |

### *3.5 SUB ACUTE TOXICITY STUDIES*

#### *3.5.1 Haematological analysis*

In the toxicity study, only TWE was used due to its wider usage in the traditional society. In this analysis, TWE (100-1000 mg/kg, *p.o.*) was administered to rats daily for two weeks. Levels of TWBC, RBC, PLT, HGB, MCV, MCH, and MCHC were determined at the end of day 14. TWE did not cause any significant change between the drug treated group and the control in all the parameters assessed (Table 3.5). At 1000 mg/kg, all the parameters tested except MCV and MCH were lower than those of the control group. However, the differences were not statistically significant. At 300 mg/kg, all the values except MCHC were insignificantly higher than those of the vehicle treated group.

**Table 3.5** Effects of aqueous *TWE* (100-1000 mg/kg *p.o.*) on hematological values of rats treated daily for two weeks. Values were expressed as mean ± s.e.m. (n = 5). Group means were compared using one-way analysis of variance followed by Newman-Keul's *post hoc* test. Mean values were not significantly different (P > 0.05) compared to the control group.

| Dose | TWBC ($x10^9$/L) | RBC ($x10^{12}$/L) | PLT ($x10^9$/L) | HGB (g/dL) | MCV (fl) | MCH (pg) | MCHC (g/dL) |
|---|---|---|---|---|---|---|---|
| Control | 4.40±0.34 | 6.63±0.29 | 431.50±65.96 | 12.50±0.84 | 55.43±1.45 | 19.90±0.25 | 36.15±0.52 |
| 100 mg/kg | 4.23±0.15 | 5.88±0.34 | 402.50±78.26 | 11.80±0.67 | 56.80±0.76 | 19.93±0.25 | 35.18±0.15 |
| 300 mg/kg | 4.90±0.43 | 6.63±0.43 | 440.00±42.68 | 13.23±1.04 | 56.72±0.90 | 20.48±0.29 | 35.85±0.69 |
| 1000 mg/kg | 4.03±0.64 | 5.90±0.65 | 369.00±60.70 | 11.87±1.40 | 55.83±1.13 | 19.97±0.42 | 35.83±0.23 |

### *3.5.2 Biochemical analysis*

At the end of day 14, blood biochemical values were analyzed. Levels of total protein (g/dL), albumin (g/dL), total bilirubin (mg/dL), direct bilirubin (mg/dL) and indirect bilirubin (mg/dL) were determined. Administration of TWE (100-1000 mg/kg *p.o.*) did not cause any significant differences ($P > 0.05$) between the control and the drug treated group in all the parameters tested (Table 3.6). Similarly, the extract did not cause any significant change in the levels of the liver enzymes tested as shown on table 3.7.

**Table 3.6** Effects of aqueous *T. monadelpha* extract (100-1000 mg/kg *p.o.*) on biochemical values of rats treated daily for two weeks. Values were expressed as mean ± s.e.m. (n = 5). Group means were compared using one-way analysis of variance followed by Newman-Keul's post hoc test. Mean values were not significantly different (P > 0.05) compared to the control.

| Dose | Total protein (g/dL) | Albumin (g/dL) | Total bilirubin (mg/dL) | Direct bilirubin (mg/dL) | Indirect bilirubin (mg/dL) |
|---|---|---|---|---|---|
| Control | 74.78±1.90 | 43.83±1.39 | 4.68±0.45 | 2.18±0.45 | 2.50±0.46 |
| 100 mg/kg | 72.05±0.64 | 43.28±1.12 | 4.70±0.46 | 2.15±0.53 | 2.53±0.41 |
| 300 mg/kg | 76.25±2.62 | 47.50±2.07 | 5.90±0.69 | 1.99±0.68 | 4.03±0.57 |
| 1000 mg/kg | 72.23±3.32 | 42.77±1.03 | 4.97±1.44 | 1.63±0.47 | 3.30±0.96 |

**Table 3.7** Effects of TWE (100-1000 mg/kg *p.o.*) on AST, ALT, ALP and GGT values of rats treated daily for two weeks. Values were expressed as mean ± s.e.m. (n = 5). Group means were compared using one-way analysis of variance followed by Newman-Keul's post hoc test. Mean values were not significantly different (P > 0.05) compared to the control.

| Dose | AST (U/L) | ALT (U/L) | ALP (U/L) | GGT (U/L) |
|---|---|---|---|---|
| Control | 6.10±0.76 | 17.05±1.67 | 18.35±1.20 | 14.63±2.33 |
| 100 mg/kg | 7.75±0.84 | 14.18±1.96 | 19.85±0.41 | 12.13±1.64 |
| 300 mg/kg | 8.47±0.79 | 11.55±0.97 | 17.70±1.43 | 10.55±0.56 |
| 1000 mg/kg | 8.90±2.34 | 10.80±1.42 | 17.83±2.64 | 12.13±1.63 |

### *3.5.3 Relative organ weights*

The TWE did not cause significant decrease in the relative weights of the organs at all dose levels compared to the control. Though the relative weight of the spleen increased at all dose levels, the differences were not significant compared to the control by Newman-Keuls multiple comparison test. There was no significant effect of the extract on the relative weight of the stomach, liver, spleen or the kidney (Table 3.8).

**Table 3.8** Effects of TWE (100-1000 mg/kg *p.o.*) on the relative weights of organs of rats treated daily for two weeks.

| Dose | Kidney (%) | Liver (%) | Stomach (%) | Spleen (%) |
|---|---|---|---|---|
| Control | 1.209±0.28 | 3.333±0.51 | 0.943±0.51 | 0.285±0.03 |
| 100 mg/kg | 1.011±0.29 | 3.147±0.10 | 0.920±0.06 | 0.326±0.01 |
| 300 mg/kg | 1.497±0.28 | 3.202±0.13 | 1.043±0.08 | 0.295±0.03 |
| 1000 mg/kg | 1.049±0.15 | 3.319±0.13 | 0.934±0.03 | 0.320±0.01 |

Values were expressed as mean ± s.e.m. (n = 5). Group means were compared using one-way analysis of variance followed by Newman-Keuls multiple comparison test. Mean values were not significantly different from the control group.

## *Chapter 4*

## DISCUSSION

The study was conducted to evaluate the anti-inflammatory and analgesic effects of the stem bark extracts of *Trichilia monadelpha.* The results of this study clearly demonstrated that extracts from the stem bark of *Trichilia monadelpha* has anti-inflammatory properties in both acute and chronic inflammation. The results also showed that the stem bark extract of the same plant has analgesic property.

Carrageenan-induced acute footpad edema in laboratory animals first introduced by Winter *et al.*, (1962) is a model of acute inflammation which has been widely used to evaluate non-steroidal anti-inflammatory drugs (NSAIDs) (Di Rosa and Willoughby, 1971). The mediators involved in carrageenan-induced footpad edema are released in three distinct phases, namely an initial release of histamine and 5-HT, a second phase mediated by kinnins and the third phase highly suspected to be mediated by prostaglandins (Di Rosa *et al.*, 1971). Inhibition of carrageenan-induced inflammation has shown to be highly predictive of anti-inflammatory drug activity in human inflammatory disease (Morris, 2003) and remains an acceptable preliminary screening test for anti-rheumatic activity. Also, studies have demonstrated that intraplantar injection of carrageenan in the 7-day old chick elicits a measurable, reliable and relatively short lasting state of edema that is differentially attenuated by the systemic administration of typical anti-inflammatory compounds (Roach and Sufka, 2003). The rat adjuvant arthritis on the other hand, is the most frequently used model of chronic inflammation

for screening NSAIDs, steroids and immunosuppressive drugs (Weichman, 1989; Crofford *et al.,* 1992; Aota *et al.,* 1996).

In this study, the reference anti-inflammatory drugs (diclofenac, dexamethasone and methotrexate) inhibited the edema associated with carrageenan-induced inflammation and the rat adjuvant arthritis, confirming the ability of the chick-carrageenan, and the rat adjuvant arthtritis to identify drugs with anti-inflammatory effects. Diclofenac, (an NSAIDs) is thought to inhibit inflammation mainly by inhibiting prostaglandin synthesis through inhibition of cyclooxygenase pathway (Vane Botting, 1987; Vane and Botting, 1996). Dexamethasone, a steroidal anti-inflammatory drug binds to specific glucocorticoid receptors (GRα and GRβ) in the cytoplasm and either induces or inhibits transcription of a particular gene (Funder, 1997). Inhibitory effect is brought about by switching off the various transcription factors (example NF-κB and AP-1) that activate genes for COX2, various cytokines (e.g.TNF-α, IL-1β, IL-6), adhesive factors as well as inducible nitric oxide synthase (Tak and Firestein, 2001). Activation of corticosteroid genes induces formation of specific mRNAs which direct the synthesis of lipocortin-1. The lipocortin-1 inhibits synthesis of phospholipase A2 and this prevents mobilization of arachidonic acids and synthesis of eicosanoids in the cell membrane (Cronstein and Weissmann, 1995; Vane and Botting, 1996). Methotrexate (a DMARD) on the other hand is folate antagonist and an immunosuppressant introduced for the treatment of malignancies (Crostein and Weissman, 1995). It is currently prescribed at lower doses for the treatment of rheumatoid arthritis (Furst and Kremer, 1988; Swierkot and Szechinski, 2006). Although various biochemical pathways may be involved in its anti-inflammatory effect in rheumatoid

arthritis, studies so far indicate that the most important actions of low-dose methotrexate are its effects in increasing adenosine level and reducing the pro-inflammatory while increasing the ant-inflammatory cytokine levels (Cronstein and Weissmann, 1995; Swierkot and Szechinski, 2006).

Inhibition of inflammatory edema by the extracts indicates the presence of compounds that are capable of inhibiting both acute and chronic inflammatory processes. The precise mechanism by which the *T. monadelpha* extracts inhibit inflammation was not investigated in this study, however, widespread research on natural products shows that plant constituents such as alkaloids, flavonoids, glycosides, steroids and many other secondary metabolites may exhibit anti-inflammatory effects (Calixto *et al.,* 2003; Darshan and Doreswamy, 2004). The anti-inflammatory properties of these plant constituents may be due to their modulatory effects on inflammatory mediators (such as arachidonic acid metabolites, peptides, glutamate, amines), the formation and/or action of second messengers (such as cGMP, cCAMP, various protein kinases and calcium), expression of gene transcription factors (such as AP-1, NF-κB), expression of key pro-inflammatory molecules such as inducible nitric oxide synthase (iNOS), cyclooxygenase, cytokines (such as IL-1β, TNF-α), neuropeptides and proteases (Calixto *et al.,* 2003; Calixto *et al.,* 2004). Phytochemical screening of the extracts indicated the presence of alkaloids, saponins, tannins, steroids, flavonoids, terpenoids and glycosides. The presence of such a large array of phytochemicals in the stem bark may suggest widespread phyto-pharmacological effects as indicated by its multipurpose uses in African traditional medicine. For instance, it is reported that medicinal herbs used for the treatment of rheumatoid arthritis

contain saponins of various kinds as the main constituent (Choi *et al.,* 2003; Kim *et al.,* 1992). Saponins are known to modulate the antioxidant systems in tissues to inhibit the spread of rheumatoid arthtritis and its concomitant tissue damage cause by toxicity of reactive oxygen species (Choi *et al.,* 2003). Also, flavonoids such as rutin, quercetin and biflavonoids have been found to produce significant antinociceptive and anti-inflammatory activities by inhibiting the synthesis and release of pain and / or inflammatory mediators (Calixto *et al.,* 2000; Damas *et al.,* 1985). Whereas it is clear that the *T. monadelpha* extracts were effective in reducing inflammation in adjuvant arthritis, it remains uncertain whether, this is translated into an improvement in indices of joint integrity such as bone and cartilage degradation. The ethanolic extract (TAE) did not show significant anti-inflammatory edema associated with adjuvant arthritis. This does not necessarily suggest the absence of compounds with anti-inflammatory effect in acute inflammation in the ethanolic extract. It is important to say that crude extracts contain several compounds and the different constituents may exhibit varying pharmacological effects in biological systems. Both ant-inflammatory and pro-inflammatory components may be present in the same plant part (Davicino *et al.*,2010). In such cases, the anti-inflammatory effect of the compounds may be masked as observed for ethanolic extract. It is also possible that the components that are capable of inhibiting acute inflammation were present in very small concentrations as the alcoholic extract is likely to contain many more phytochemicals compared to the aqueous and petroleum ether extracts.

Inflammation and pain often move hand in hand. There are several experimental models that are used to screen new analgesic drugs. The choice is subject to the duration (short term or long

term), the type of stimulus (thermal, electrical or chemical) or the mechanism and targeted site (peripheral or central) of action of the test drug. Among these models, the formalin test, previously described by Dubuisson and Dennis, (1977) has been widely employed as the most predictive of acute pain (Le Bars *et al.*, 2001) and a valid model of clinical pain (Costa-Lotufo *et al.*, 2004; Vasconcelos *et al.*, 2003). The formalin test postulates the mechanism and site of action of the test drug (Chau, 1989) as compared to other tests (hot plate, tail immersion acetic acid etc) that are suitable for testing only centrally acting analgesic drugs (Asongalem *et al.*, 2004a). The formalin test also gives an indication of the extract's potential on inflammation whereas the other tests are indicative of acute pain (Cowan, 1990). In this study, intradermal injection of 10 µl of 4% formalin evoked a biphasic response characterized by flinching, licking or biting of the injected paw as described by Duboisson and Dennis, (1977) and Wheeler-Aceto *et al.*, (1990). These were the first phase (0-5 min) corresponding to acute neurogenic pain and the second phase (15-30 min) corresponding to acute inflammatory pain (Asongalem *et al.*, 2004b; Mino *et al.*, 2004). It is suggested that the first phase is due to direct stimulation of nociceptive afferent fibres hence sensitive to drugs such as those that interact with the opioid system whereas the second phase is due to participation of inflammatory pain mediators (kinin, histamine and serotonin) that can possibly activate small afferent neurons hence sensitive to analgesic-anti-inflammatory drugs (Hunskaar *et al.*, 1987; Le Bars *et al.*, 2001; Malmberg *et al.*, 1992) and corticosteroids (Vasconcelos *et al.*, 2003). Generally, centrally acting analgesic drugs inhibit both phases but peripherally acting analgesic drugs inhibit only the second phase (Mino *et al.*, 2004).

From the results, both diclofenac and morphine exhibited analgesic effects. However, whereas morphine exhibited analgesic effect in both phases, diclofenac was effective only in the second phase. This peripheral analgesic effect of diclofenac (an NSAID) is said to be due to its ability to interfere with the cyclooxygenase pathway thereby inhibiting prostaglandin production. Decrease prostaglandin production decreases sensitization of the nociceptive nerve endings to inflammatory pain mediators such as bradykinin and 5-hydroxytryptamine (Malmberg *et al.*, 1992; Yashpal *et al.*, 1998). The analgesic effect exhibited by morphine (an opioid receptor agonist and analgesic-anti-inflammatory drug), is said to be due to activation of opioid receptors in the central(CNS) as well as the peripheral nervous systems (PNS) (Christie *et al.*, 2000). Morphine activates the opioid receptors in the descending inhibitory pathway neurons (in the periaqueductal grey matter, nucleus reticularis paragigantocellularis and nucleus raphe magnus) thereby inhibiting pain transmission in the brain (Benson and Chaouch,1987). At the spinal level, morphine activates the inhibitory glutamate, 5-HT, adenosine and enkephalin-containing neurons in the dorsal horn of the spinal cord as well as the peripheral terminals of nociceptive afferent neurons to inhibit pain transmission (Pasternak, 1993).

The extracts (TWE and TPEE) like morphine, significantly reduced the duration of the animals' response to pain in both phases. The analgesic effect of the extracts indicated that they contained compounds that were capable of inhibiting pain transmission in the central and peripheral nervous systems. The central analgesic effect of the extracts could be mediated by activating the opioid receptors in the descending inhibitory pathway in the brain or the nociceptive afferent neurons in the spinal cord as well as the peripheral terminals of

nociceptive afferent neurons to inhibit pain transmission. The peripheral analgesic effect of the extracts could be due to their ability to suppress synthesis and release of inflammatory pain mediators (such as kinin, histamine, nitric oxide, serotonin e.t.c.), interact with the inflammatory cells (e.g. mast cells and neurons) or inhibit peripheral cyclooxygenase activity to prevent release of prostaglandins as it might have occurred in acute and chronic inflammation tests. The analgesic effect of the extracts could be due to the presence of some phytochemical compounds. For instance, flavonoids are known to target prostaglandins which are implicated in the late phase of acute inflammation and pain perception (Rajnarayana *et al.*, 2001). Hence, the presence of flavonoids might have contributed to the anti-inflammatory and analgesic activities of the extracts. In general, the activities of TWE and TPEE on the formalin test indicated possible central analgesic activity as well as analgesic-anti-inflammatory effect likewise morphine and other centrally acting analgesic drugs. However, the mechanisms of action of the extracts were not investigated in this project. Further work on these extracts may investigate the mechanisms of actions responsible for their analgesic and anti-inflammatory activities.

Contrary to TWE and TPEE, TAE did not exhibit any significant effect in the second phase (pain due to inflammation) of the formalin test. The least anti-inflammatory effect of TAE in the second phase of the formalin test, as also observed in chick-carrageenan and the CFA-induced arthritis tests could be due to its inability to inhibit the cyclooxygenase activity and prevent release of inflammatory pain mediators. It could also be due to inability of the extract to interact with inflammatory cells such as mast cells, vascular endothelia cells, neurons e.t.c.

For instance, degranulation of mast cells releases histamine resulting to pain and inflammation. Therefore compounds that are capable of inhibiting mast cell degranulation could also be potential anti-inflammatory and analgesic compounds.

Assessment of blood is essential to the evaluation of risk since the haematological system carries a high percentage of toxicity in humans. Due to the exposure of the blood to numerous chemicals (both exogenous and endogenous), the cell components such as the erythrocyte, leukocytes and thrombocytes become most vulnerable to toxic compounds. In this investigation, oral administration of TWE for two weeks did not give any significant difference in the haematological parameters of extract treated groups and that of the control.

The liver is the largest gland in the human body and its main function is to maintain the internal body environment within a very limited range (homeostasis). To achieve this, the liver metabolizes, synthesizes and detoxifies a number of compounds including proteins, glucose, fats and natural products. Sometimes more toxic compounds are produced which may in turn cause damage to the liver. For liver function assessment, the levels of AST and ALT in the serum are determined. This is because the levels of these two enzymes are most often associated with hepatocellular damage (Lyoussi *et al.*, 2004). In general with liver diseases, the levels of ALT and AST rise and fall in parallel (Panthong *et al.*, 2003; Perharic *et al.*, 1995). AST is widely present in a number of different cells such as myocardium, skeletal muscles and brain.

ALP reaches spectacular height in biliary cirrhosis, hepatitis and in disease characterized by inflammation, regeneration and obstruction of intrahepatic bile ductules (Panthong *et al.*,

2003). Insignificant changes in the levels of ALP, ALT and AST indicated that, the extract had no marked adverse effect on the liver function. The liver synthesizes most of the plasma proteins such as albumin and globulin. Decrease in the levels of these proteins indicates liver damage. Bilirubin, the main pigment formed from the breakdown of haemoglobin in RBCs is conjugated in the liver and then secreted into bile. Increased bilirubin level in plasma may results from increase in its production, decrease in its conjugation, decrease in its secretion by the liver or obstruction of the bile ducts. When there is increased production or decreased conjugation, the unconjugated or indirect form of bilirubin increases. When the bile ducts are obstructed, there is a build up of direct bilirubin that escapes from the liver to increase the plasma level which can lead to hemolytic jaundice. Level of bilirubin in the serum is a determinant of the liver function and since there were no significant changes in the levels of the liver enzymes and other biochemical parameters assessed, it could be suggested that the extract did not have any marked adverse effect on the liver.

In the 14 days toxicity study, no death or any abnormal behaviour was recorded. The gross body weight changes of the treated rats were not significant as compared to the control group. The changes in the weights of the stomach, liver, kidney and spleen were also not significant. Since change in body weight is an indicator of adverse effect of drugs (Tofovic *et al.*, 1999), the current results postulated that continuous oral administration of the extract up to 1000 mg/kg for two weeks was non-toxic to rats.

To crown it all, this work suggests that the stem bark extract of *Trichilia monadelpha* is a potential source of analgesic and anti-inflammatory agents and thus provides the first

pharmacological evidence to support the use of the stem bark extract of the plant in the management of inflammation and pain conditions in African traditional medicicne.

## *Chapter 5*

# CONCLUSIONS

In conclusion, the results from this study have shown that the aqueous, alcoholic and petroleum ether extracts of the stem bark of *Trichilia monadelpha* have anti-inflammatory as well as analgesic effects in animal models. Consistent oral administration of the aqueous extract (up to 1000 mg/kg) for 14 days gave no evidence of toxicity in rats. Presence of tannin, alkaloids, saponins, flavonoids, steroids, terpenoids and glycosides in the extracts may have contributed to their pharmacological effects.

# REFERENCES


(IASP), IAftSoP (1983). Pain. **16:** 109-110.

Abbiw, D.K. (1990). Useful plants of Ghana. *KEW: Intermediate Technology Publications and the Royal Botanic Gardens*.

Abbott, F.V., Franklin, K.B., Westbrook, R.F. (1995). The formalin test: scoring properties of the first and second phases of the pain response in rats. *Pain* **60**(1)**:** 91-102.

Alam, M.S., Kaur, G., Hamid, H., Ali, A., Athar, M. (2004). Antiinflammatory evaluation of alcoholic extract of galls of *Quercus infectoria*. *Journal of Ethnopharmacology* **90:** 285-292.

Aota, S., Nakamura, T., Suzuki, K., Tanaka, Y., Okazaki, Y., Segawa, Y., Miura, M., Kikuchi, S. (1996). Effects of indomethacin administration on bone turnover and bone mass in adjuvant-induced arthritis in rats. *Calcif Tissue Int* **59**(5)**:** 385-391.

Asongalem, E.A., Foyet, H.S., Ekobo, S., Dimo, T., Kamtchouing, P. (2004a). Antiinflammatory, lack of central analgesia and antipyrectic properties of *Acanthus montanus* (Ness) T. Anderson. *Journal of Ethnopharmacology* **95:** 63-68.

Asongalem, E.A., Foyet, H.S., Ngongan, J., Folefoc, G., Dimo, T., Kamtchouing, P. (2004b). Analgesic and antiinflammatory activities of *Erigeron floribundus*. *Journal of Ethnopharmacology* **91:** 301-308.

Atkinson, D.C., Boura, A.L., Hicks, R .(1969). Observations on the pharmacological properties of inflammatory exudate. *Eur J Pharmacol* **8**(3)**:** 348-353.

Attal, N., Bouhassira, D. (1999). Mechanisms of pain in peripheral neuropathy. *Acta Neurol Scand Suppl* **173:** 12-24; discussion 48-52.

Auddy, B., Ferreira, M., Blasina, F., Lafon, L., Arredondo, F., Dajas, F., Tripathi, P.C., Seal, T., Mukherjee, B. (2003). Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. *J Ethnopharmacol* **84**(2-3)**:** 131-138.

Azuma, I., Kanetsuna, F., Kada, Y., Takashima, T., Yamamura, Y. (1972). Adjuvant-polyarthritogenicity of cell walls of mycobacteria, nocardia and corynebacteria. *Jpn J Microbiol* **16**(4)**:** 333-336.

Bako, S.P., Bakfur, M.J., John, L., Bala, E.L. (2005). Ethnomedicinal and phytochemical profile of some savanna plant species in Nigeria. *Int. J Bot* **1**(2)**:** 147-150.

Baliga, M.S., Jagetia, G.C., Ulloor, J.N., Baliga, M.P., Venkatesh, P., Reddy, R., Rao, K.V., Baliga, B.S., Devi, S., Raju, S.K., Veeresh, V., Reddy, T.K., Bairy, K.L. (2004). The evaluation of the acute toxicity and long term safety of hydroalcoholic extract of Sapthaparna (Alstonia scholaris) in mice and rats. *Toxicol Lett* **151**(2)**:** 317-326.

Banvolgyi, A., Pozsgai, G., Brain, S.D., Helyes, Z.S., Szolcsanyi, J., Ghosh, M., Melegh, B., Pinter, E. (2004). Mustard oil induces a transient receptor potential vanilloid 1 receptor-independent neurogenic inflammation and a non-neurogenic cellular inflammatory component in mice. *Neuroscience* **125**(2)**:** 449-459.

Bertolini, D.R., Nedwin, G.E., Bringman, T.S., Smith, D.D., Mundy, G.R. (1986). Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. *Nature* **319**(6053)**:** 516-518.

Besson, J.M., Chaouch, A. (1987). Peripheral and spinal mechanisms of nociception. *Physiol Rev* **67:** 67-186

Bhoola, K.D., Figueroa, C.D., Worthy, K. (1992). Bioregulation of kinins: kallikreins, kininogens, and kininases. *Pharmacol Rev* **44**(1)**:** 1-80.

Bolay, H., Moskowitz, M.A. (2002). Mechanisms of pain modulation in chronic syndromes. *Neurology* **59**(5 Suppl 2)**:** S2-7.

Burkill, H.M. (1985). The useful plants of West Tropical Africa. **4**.

Calixto, J.B., Beirith, A., Ferreia, J., Santos, A. (2000). Naturally occurring antinociceptive substances from plants. *phytotherapy research* **14:** 401-418.

Calixto, J, Otuki, MF, Santos, ARS (2003) Anti-Inflammatory Compounds of Plant Origin. Part I. Action on Arachidonic Acid Pathway, Nitric Oxide and Nuclear Factor kB (NF-kB). *Planta Med* **69:** 973-983.

Cassim, B., Mody, G., Bhoola, K. (2002). Kallikrein cascade and cytokines in inflamed joints. *Pharmacol Ther* **94**(1-2)**:** 1-34.

Chang, Y.H., Pearson, C.M., Abe, C. (1980). Adjuvant polyarthritis. IV. Induction by a synthetic adjuvant: immunologic, histopathologic, and other studies. *Arthritis Rheum* **23**(1)**:** 62-71.

Chau, T.T. (1989). Analgesic testing in animal models. In: *Pharmacological methods in the control of inflammation* J.Y. Chang, A.J. Lewis (eds), pp 195-212. New York: Alan R. Liss, Inc., 1989

Choi, E.M., Hwang, J.K. (2003). Investigations of anti-inflammatory and antinociceptive activities of Piper cubeba, Physalis angulata and Rosa hybrida. *J Ethnopharmacol* **89**(1)**:** 171-175.

Christie, M.J., Connor, M., Vaughan, C.W., Ingram, S.L., Bagley, E.E .(2000). Cellular actions of opioids and other analgesics: Implications for synergism in pain relief. *Clinical and experimental pharmacology and physiology* **27:** 520-523.

Colpaert, F.C. (1987). Evidence that adjuvant arthritis in the rat is associated with chronic pain. *Pain* **28**(2)**:** 201-222.

Connolly, K.M., Stecher, V.J., Danis, E., Pruden, D.J., LaBrie, T. (1988). Alteration of interleukin-1 production and the acute phase response following medication of adjuvant arthritic rats with cyclosporin-A or methotrexate. *Int J Immunopharmacol* **10**(6)**:** 717-728.

Costa-Lotufo, L.V., de Lucena, D.F., Andrade-Neto, M., Bezerra, J.N., Leal, L.K., de Sousa, F.C., Viana, G.S. (2004). Analgesic, antiinflammatory and central depressor effects of the hydroalcoholic extract and fractions from Aeolanthus suaveolens. *Biol Pharm Bull* **27**(6)**:** 821-824.

Cowan, A. (1990). Recent approaches in the testing of analgesics in animals. Modern methods in pharmacology. *Testing and evaluation of drugs of abuse* **6:** 33-42.

Crofford, LJ, Wilder, RL (1993) Arthritis and Autoimmunity in Animals, pp 523-539. London: Lea and Febiger.

Cronstein, BN, Weissmann, G (1995) Targets For Antiinflammatory Drugs. *Annu. Rev. Pharmacol. Toxicol.* **35:** 449-462.

Damas, J., Bourdon, V., Remacle-Velon, G., Lacomte, J. (1985). pro-inflammatory flavonoids which are inhibitors of prostglandin biosynthesis. *Prostaglandin Leukot Med* **19:** 11-24.

Darshan, S, Doreswamy, R ( 2004) Patented Antiinflammatory Plant Drug Development from Traditional Medicine. *Phytother. Res.* **18:** 343-357.

Di Rosa, M., Giroud, J.P., and, Willoughby, D.A. ( 1971). Studies of the mediators of the acute inflammatory response induced rats in different sites by carrageenan and turpentine. *Journal of pathology* **104:** 15-29.

Dokosi, O.B. (1998). Herbs of Ghana. *Accra: Ghana Universities Press.*

Doughetty, P.M., Sluka, K.A., Sorkin, L.S., Westlund, K.N., Willis, W.D. (1992). Neural changes in acute arthritis in monkeys.1.Parallel enhancement of responses in spinothalamic tract neurons to mechanical stimulation and excitatory amino acids. *Brain Res Rev* **17:** 1-13.

Dubuisson, D., Dennis., S.G. (1977). The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain* **4**(2)**:** 161-174.

Eastwood, M.A. (1999). Interaction of dietary antioxidants in vivo: how fruit and vegetables prevent disease? *QJM* **92**(9)**:** 527-530.

Erlinger, T.P., Platz, E.A., Rifai, N, Helzlsouer, K.J. (2004). C-reactive protein and the risk of incident colorectal cancer. *JAMA* **291**(5)**:** 585-590.

Feldmann, M., Maini, R.N. (2001). Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? *Annu Rev Immunol* **19:** 163-196.

Fereidoni, M., Ahmadiani, A., Samanian, S.A., Javan, M. (2000). An accurate and simple method for the measurement of paw edema. *J. Pharmacol. Toxicol. Methods* **43:** 11-14.

Funder, J.W.(1997). Glucocorticoid and mineralocorticoid receptors: biology and clinical relevance. *Ann Rev Med* **48**: 231-240.

Furst, D.E., Kremer, J.M. (1988). Methotrexate in rheumatoid arthritis. *Arthritis Rheum.* **31:** 305-314.

Govaert, R. (2001). How many species of plants are there? *Taxon* **50:** 1085-1090.

Haas, D.A., Nakanishi., O., MacMillan, R.E., Jordan, R.C., Hu, J.W. (1992). Development of an orofacial model of acute inflammation in the rat. *Arch Oral Biol* **37**(5)**:** 417-422.

Haeseler, G., Tetzlaff, D., Bufler, J., Dengler, R., Munte, S., Hecker, H., Leuwer, M. (2003). Blockade of voltage-operated neuronal and skeletal muscle sodium channels by S(+)- and R(-)-ketamine. *Anesth Analg* **96**(4)**:** 1019-1026, table of contents.

Harris, S.G., Padilla, J., Koumas, L., Ray, D.A., Phipps, R.P. (2002). Prostaglandings as modulators of immunity. . *Trends in immunology* **23:** 144-150.

Hayashida, K., Takeucht, T., Shimizu, H., Ando, K.A., Harada, E. (2003). Lactofering enhances opioid-mediated analgesia via nitric oxide in the rat spinal cord. *Am J physiol -Regul Inter and Comp Physiol* **285:** R306-R312.

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

Irvine, F.R. (1961). Woody plants of Ghana. *London: Oxford University Press.*

Issekutz, C.A., Issekutz, T.B. (1989). Quantitation 0f blood cell accumulation and vascular responses in inflammatory reactions. *In Pharmacological methods in the control of inflammation.*(eds Chang, J. Y. and Lewis, A.J. pp 129-150)**:** New York: Allan R. Liss inc.

Johnson, W.J., Muirhead, K.A., Meunier, P.C., Votta, B.J., Schmitt, T.C., DiMartino, M.J., Hanna, N. (1986). Macrophage activation in rat models of inflammation and arthritis. Systemic activation precedes arthritis induction and progression. *Arthritis Rheum* **29**(9)**:** 1122-1130.

Kaneko, M., Hammond, D.L. (1997). Role of spinal gamma-aminobutyric acidA receptors in formalin-induced nociception in the rat. *J Pharmacol Exp Ther* **282**(2)**:** 928-938.

Kavanaugh, A., Cohen, S., Cush, J.J. (2004). The evolving use of tumor necrosis factor inhibitors in rheumatoid arthritis. *J Rheumatol* **31**(10)**:** 1881-1884.

Kawaguchi, H., Pilbeam, C.C., Harrison, J.R., Raisz, L.G. (1995). The role of prostaglandins in the regulation of bone metabolism. *Clin Orthop Relat Res*(313)**:** 36-46.

Keam, S.J., Lyseng-Williamson, K.A., Goa, K.L. (2003). Pranlukast: a review of its use in the management of asthma. *Drugs* **63**(10)**:** 991-1019.

Kim, SH, Chung, J.M. (1992). An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain* **50**(3)**:** 355-363.

Kohashi, O., Tanaka, A., Kotani, S., Shiba, T., Kusumoto, S., Yokogawa, K., Kawata, S., Ozawa, A. (1980). Arthritis-inducing ability of a synthetic adjuvant, N-acetylmuramyl peptides, and bacterial disaccharide peptides related to different oil vehicles and their composition. *Infect Immun* **29**(1)**:** 70-75.

Kurth, T., Hennekens, C.H., Buring, J.E., Gaziano, J.M. (2004). Aspirin, NSAIDs, and COX-2 inhibitors in cardiovascular disease: possible interactions and implications for treatment of rheumatoid arthritis. *Curr Rheumatol Rep* **6**(5)**:** 351-356.

Laupattarakasem, P., Houghton, P.J., Hoult, J.R., Itharat, A. (2003). An evaluation of the activity related to inflammation of four plants used in Thailand to treat arthritis. *J Ethnopharmacol* **85**(2-3)**:** 207-215.

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

Leisten, J.C., Gaarde, W.A., Scholz, W. (1990). Interleukin-6 serum levels correlate with footpad swelling in adjuvant-induced arthritic Lewis rats treated with cyclosporin A or indomethacin. *Clin Immunol Immunopathol* **56**(1)**:** 108-115.

Liyanage, S.P., Currey, H.L., Vernon-Roberts, B. (1975). Influence of tubercle aggregate size on severity of adjuvant arthritis in the rat. *Ann Rheum Dis* **34**(1)**:** 49-53.

Lucas, S.M., Rothwell, N.J., Gibson, R.M. (2006). The role of inflammation in CNS injury and disease. *Br J Pharmacol* **147 Suppl 1:** S232-240.

Lyoussi, B., Hilaly, J.E., Israili, Z.H. (2004). Acute and chronic toxicological studies of *Ajuva iva* in experimental animals. *Journal of Ethnopharmacology* **91**(2004)**:** 43-50.

Ma, Q.P., Woolf, C.J. (1996). Progressive tactile hypersensitivity: an inflammation-induced incremental increase in the excitability of the spinal cord. *Pain* **67**(1)**:** 97-106.

Maggi, C.A., Giuliani, S. (1996). Evidence that tachykinin NK2 receptors modulate resting tone in the rat isolated small intestine. *Br J Pharmacol* **118**(5)**:** 1262-1268.

Malmberg, A.B., Yaksh, T.L. (1992). Antinociceptive actions of spinal nonsteroidal anti-inflammatory agents on the formalin test in the rat. *J Pharmacol Exp Ther* **263**(1)**:** 136-146.

Masu, Y., Nakayama, K., Tamaki, H., Harada, Y., Kuno, M., Nakanishi, S. (1987). cDNA cloning of bovine substance-K receptor through oocyte expression system. *Nature* **329**(6142)**:** 836-838.

Meller, S.T., Gebhart, G.F. (1997). Intraplantar zymosan as a reliable, quantifiable model of thermal and mechanical hyperalgesia in the rat. *Eur J Pain* **1**(1)**:** 43-52.

Merskey, H., Watson, G.D. (1979). The lateralisation of pain. *Pain* **7**(3)**:** 271-280.

Mino, J., Moscatelli, V., Hnatyszyn, O., Gorzalczany, S., Acevedo, C., Ferraro, G. (2004). Antinociceptive and antiinflammatory activities of Artemisia copa extracts. *Pharmacol Res* **50**(1)**:** 59-63.

Mohr, W., Wild, A. (1976). Adjuvant arthritis. *Arzneimittelforschung* **26**(10)**:** 1860-1866.

Moncada, S., Palmer, R.M.J., Higgs, E.A. (1991). Nitric oxide physiology, pathophysiology and pharmacology. *Pharmacological reviews* **43:** 109-142.

Mooijaart, S.P., Koeijvoets, K.M., Sijbrands, E.J., Daha, M.R., Westendorp, R.G. (2007). Complement Factor H polymorphism Y402H associates with inflammation, visual acuity, and cardiovascular mortality in the elderly population at large. *Exp Gerontol* **42**(11)**:** 1116-1122.

Morris, CJ (2003) Carrageenan-Induced Paw Edema in the Rat and Mouse. In: *Inflammation Protocols*, pp 115-121. Totowa, NJ: Humana Press Inc.

Mshana, N.R., Abbiw, K., Addae-Mensah, I., Adjanohoun, E., Ahyi, M.R.A., Ekpere, J.A., Enow-Orock, E.G.,Gbile, Z.O., Naomesi, G.K., Odei, M.A., Odunlami, H., Oten-Yeboah, A.A., Sarpong, K., Sofowora, A. and Tackie, A.N. (2000). Traditional medicine and Pharmacopoeia. Contribution to the Revision of Ethnobotanical and Floristic studies in Ghana. *Accra: Organization of African Unity/ Scientific, Technical and research commission*

Murayama, M., Mori, T., Bando, H.A., Amiya, T. (1991). Studies on the constituents of *Aconitum* species: IX. The pharmacological properties of the pyro-type aconitum alkaloids, components of processed aconite powder 'Kako-bushi-matsu': analgesic, anti-inflammatory and toxic activities. *Journal of Ethnopharmacology* **35:** 159-164.

Nakashima, Y.M., Todorovic, S.M., Covey., D.F., Lingle, C.J. (1998). The anesthetic steroid (+)-3alpha-hydroxy-5alpha-androstane-17beta-carbonitrile blocks N-, Q-, and R-type, but not L- and P-type, high voltage-activated Ca2+ current in hippocampal and dorsal root ganglion neurons of the rat. *Mol Pharmacol* **54**(3)**:** 559-568.

Neumann, S., Doubell, T.P., Leslie, T., Woolf, C.J. (1996). Inflammatory pain hypersensitivity mediated by phenotypic switch in myelinated primary sensory neurons. *Nature* **384**(6607)**:** 360-364.

Newbould, B.B. (1963). Chemotherapy of Arthritis Induced in Rats by Mycobacterial Adjuvant. *Br J Pharmacol Chemother* **21:** 127-136.

Nicoll, R.A., Schenker, C., Leeman, S.E. (1980). Substance P as a transmitter candidate. *Annu Rev Neurosci* **3:** 227-268.

Otsuka, M., Yoshioka, K. (1993). Neurotransmitter functions of mammalian tachykinins. *Physiol Rev* **73**(2)**:** 229-308.

Pasternak,G.W.(1993). pharmacological mechanisms of opioid analgesics. *Clin Neuropharmacol* **16:** 1-18

Panthong, A., Witthawaskul, P., Kanjanapothi, D., Taesothikul, T., Lertprasertsuke, N. (2003). Acute and subacute toxicities of the saponin mixture isolated from *Schefflera leucantha* Viguier. *Journal of Ethnopharmacology* **89:** 115-121.

Pearce, D.W., Puroshothaman, S. (1992). Protecting Biological Diversity: the economic value of pharmaceutical plants. *Global Environmental change working paper 92-27, Centre for Social and Economic Research on the lobal environment, University College.*

Pearson, C.M. (1956). Development of arthritis, periarthritis and periostitis in rats given adjuvants. *Proc Soc Exp Biol* **91:** 95-101.

Pearson, C.M. (1963). Experimental Joint Disease Observations on Adjuvant-Induced Arthritis. *J Chronic Dis* **16:** 863-874.

Perharic, L., Shaw, D., Leon, C., De-Smet, P.A., and Murray, V.S. (1995). Possible association of liver damage with the ues of Chinese herbal medicines for skin disease. *Vet. Hum. Toxicol.* **37**(6)**:** 562-566.

Principe, P.P. (1991). Valuing the Biodiversity of medicinal plants. In Akerele et al., (eds) The conservation of medicinal plants. *Proceedings of an international consultation,21-27 March1988, Chiang Mai, Thailand. Cambridge University press. 79-24*.

Prussin, C., Metcalfe, D.D. (2003). IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* **111**(2 Suppl)**:** S486-494.

Puig, S., Sorkin, L.S. (1996). Formalin-evoked activity in identified primary afferent fibers: systemic lidocaine suppresses phase-2 activity. *Pain* **64**(2)**:** 345-355.

Pushpangadan, P. (1995). Ethnobotany in India: a status report, GOI New Delhi.

Rainsford, K.D. (1982). Adjuvant polyarthritis in rats: is this a satisfactory model for screening anti-arthritic drugs? *Agents Actions* **12**(4)**:** 452-458.

Rajnarayana, K., Reddy M.S., Chaluvadi M.R. (2001). Bioflavonids classification, pharmacological, biochemical effects and therapeutic potential. *Indian J pharmacol* **33:** 2-16.

Ready, L.B. (1992). The acute pain service. *Acta Anaesthesiol Belg* **43**(1)**:** 21-27.

Riddle, R., Ryser, C.N., Morton, A.A., Sampson, J.D., Browne, R.H., Punaro, M.G., Gatchel, R.J. (2006). The impact on health-related quality of life from non-steroidal anti-inflammatory drugs, methotrexate, or steroids in treatment for juvenile idiopathic arthritis. *J Pediatr Psychol* **31**(3)**:** 262-271.

Roach, J.T., Sufka, K.J. (2003). Characterization of the chick carrageenan response. *Brain Res* **994**(2)**:** 216-225.

Robbins, C.S. (2000). Comparative analysis of management regimes and medicinal plants trade monitoring mechanism for American Gingseng and Goldseal. *Conserv. Bio.* **14:** 1422-1434.

Rosland, J.H., Tjolsen, A., Maehle, B., Hole, K. (1990). The formalin test in mice: effect of foermalin concentration. *Pain 1990* **42:** 235-242.

Salvemini, D., Wang, Z.Q., Wyatt, P.S., Bourdon, D.M., Marino, M.H., Manning, P.T., Currie, M.G. (1996). Nitric oxide: a key mediator in the early and late phase of carrageenin-induced rat paw inflammation. *British journal of pharmacology* **118:** 829-838.

Schaible, H.G., Schmidt, R.F., Willis, W.D. (1987). Enhancement of the responses of ascending tract cells in the cat spinal cord by acute inflammation of the knee joint. *Exp Brain Res* **66**(3)**:** 489-499.

Schiller, J., Fuchs, B., Arnhold, J., Arnold, K. (2003). Contribution of reactive oxygen species to cartilage degradation in rheumatic diseases: molecular pathways, diagnosis and potential therapeutic strategies. *Curr Med Chem* **10**(20)**:** 2123-2145.

Schmid, R.M., Whitcomb, D.C. (2006). Genetically defined models of chronic pancreatitis. *Gastroenterology* **131**(6)**:** 2012-2015.

Schultzberg, M., Hokfelt, T., Nilsson, G., Terenius, L., Rehfeld, J.F., Brown, M., Elde, R., Goldstein, M., Said, S. (1980). Distribution of peptide- and catecholamine-containing neurons in the gastro-intestinal tract of rat and guinea-pig: immunohistochemical studies with antisera to substance P, vasoactive intestinal polypeptide, enkephalins, somatostatin, gastrin/cholecystokinin, neurotensin and dopamine beta-hydroxylase. *Neuroscience* **5**(4)**:** 689-744.

Serhan, C.N. (2004). A search for endogenous mechanisms of anti-inflammation uncovers novel chemical mediators: missing links to resolution. *Histochem Cell Biol* **122**(4)**:** 305-321.

Shibata, M, Okubo, T, Takahashi, H, Inoki, R. (1989). Modified formalin test: characteristic biphasic pain response. *Pain* **76:** 337-347.

Simone, D.A., Sorkin, L.S., Oh, U., Chung, J.M., Owens, C., LaMotte, R.H., Willis, W.D. (1991). Neurogenic hyperalgesia: central neural correlates in responses of spinothalamic tract neurons. *J Neurophysiol* **66**(1)**:** 228-246.

Singh, H., Kumar, S., Dewan, S., Kumar, V.L. (2000). Inflammation induced by latex of Calotropis procera--a new model to evaluate anti-inflammatory drugs. *J Pharmacol Toxicol Methods* **43**(3)**:** 219-224.

Sluka, K.A., Westlund, K.N. (1993a). Behavioral and immunohistochemical changes in an experimental arthritis model in rats. *Pain* **55**(3)**:** 367-377.

Sluka, K.A., Westlund, K.N. (1993b). An experimental arthritis model in rats: the effects of NMDA and non-NMDA antagonists on aspartate and glutamate release in the dorsal horn. *Neurosci Lett* **149**(1)**:** 99-102.

Smith-Oliver, T., Noel, L.S., Stimpson, S.S., Yarnall, D.P., Connolly, K.M. (1993). Elevated levels of TNF in the joints of adjuvant arthritic rats. *Cytokine* **5**(4)**:** 298-304.

Sofowora, A. (1993). *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Ltd.: Nigeria.

Stichtenoth, D.O., Frolich, J.C. (2003). The second generation of COX-2 inhibitors: what advantages do the newest offer? *Drugs* **63**(1)**:** 33-45.

Swierkot, J., Szechinski, J. ( 2006). Methotrexate in rheumatoid arthritis. *Pharmacol Rep* **58** (4)**:** 473-492.

Szolcsanyi, J., Bolcskei, K., Szabo, A., Pinter, E., Petho, G., Elekes, K., Borzsei, R., Almasi, R., Szuts, T., Keri, G., Helyes, Z. (2004). Analgesic effect of TT-232, a heptapeptide somatostatin analogue, in acute pain models of the rat and the mouse and in streptozotocin-induced diabetic mechanical allodynia. *Eur J Pharmacol* **498**(1-3)**:** 103-109.

Tak, P.P., Firestein, G.S. (2001). NF-kappaB: a key role in inflammatory diseases. *J Clin Invest:* **107:** 7-11.

Taniyama, K., Makimoto, N., Furuichi, A., Sakurai-Yamashita, Y., Nagase, Y., Kaibara, M., Kanematsu, T. (2000). Functions of peripheral 5-hydroxytryptamine receptors, especially 5-hydroxytryptamine4 receptor, in gastrointestinal motility. *J Gastroenterol* **35**(8)**:** 575-582.

Thomas, S., Bruce, C., Birkhead, A., Wang, L. (2002). Effect of ecNOS polymorphisms and coronary artery disease upon exhaled nitric oxide.J. *J mol med.* **80:** 181-186.

Ticktin, T., Nantel, P., Ramirez, F., Johns, T. (2002). Effect of variation of harvest limits for non-timber forest species in Mexico. *Conserv. Bio.* **16:** 691-705.

Tofovic, S.P., Jackson, E.K. (1999). Effects of long-term caffeine consumption on renal function in spontaneously hypertensive heart failure prone rats. *J Cardiovasc Pharmacol* **33**(3)**:** 360-366.

Vane, J., Botting, R. (1987). Inflammation and the mechanism of action of anti-inflammatory drugs. *FASEB J.* **1:** 89-96.

Vane, J.R., Botting, R.M. (1996). Mechanism of Action of Anti-Inflammatory Drugs

*Scand J Rheumatol* **25:** 9-21.

Vasconcelos, S.M., Reboucas Oliveira, G., Mohana de Carvalho, M., Rodrigues, A.C., Rocha Silveira, E., Maria Franca Fonteles, M., Florenco Sousa, F.C., Barros Viana, G.S. (2003). Antinociceptive activities of the hydroalcoholic extracts from Erythrina velutina and Erythrina mulungu in mice. *Biol Pharm Bull* **26**(7)**:** 946-949.

Veerappan, A., Miyazaki, S., Kadarkaraisamy, M., Ranganathan, D. (2007). Acute and subacute toxicity studies of Aegle marmelos Corr., an Indian medicinal plant. *Phytomedicine* **14**(2-3)**:** 209-215.

Vissers, K., Hoffmann, V., Geenen, F., Biermans, R., Meert, T. (2003). Is the second phase of the formalin test useful to predict activity in chronic constriction injury models? A pharmacological comparison in different species. *Pain Pract* **3**(4)**:** 298-309.

Walz, D.T., Di Martino, M.J., Misher, A. (1971a). Suppression of adjuvant-induced arthritis in the rat by gold sodium thiomalate. *Ann Rheum Dis* **30**(3)**:** 303-306.

Walz, D.T., Dolan, M.M., DiMartino, M.J., Yankell, S.L., Friedman, H. (1971b). Effects of topical hydrocortisone and acetylsalicylic acid on the primary lesion of adjuvant-induced arthritis. *Proc Soc Exp Biol Med* **137**(4)**:** 1466-1469.

Wheeler-Aceto, H., Porreca, F., Cowan, A. (1990). The rat paw formalin test: comparison of noxious agents. *Pain* **40**(2)**:** 229-238.

Whicher, J.T, Thompson, D., Billingham, M.E.J. (1989). Acute phase proteins.In *pharmacological methods in the control of inflammation.* (eds) Chang, J.Y. & Lewis, A.J. pp 101-128. New York: Allan R. Liss, inc.

Whitehouse, M.W., Orr, K.J., Beck, F.W., Pearson, C.M. (1974). Freund's adjuvants: relationship of arthritogenicity and adjuvanticity in rats to vehicle composition. *Immunology* **27**(2)**:** 311-330.

WHO, (2002). Traditional medicine-growing needs and potential. *WHO Policy Perspectives Med.* **2:** 1-6.

Winter, C.A., Risely, E.A., Nuss, G.W, (1962). Carrageenan-induced oedema in hind paw of rats as an assay for antiinflammatory drugs. *Proceedings of the siciety of experimental Biology and medicine* **111:** 544-547.

Winter, C.A., Risley, E.A., Nuss, G.W. (1963). Anti-Inflammatory and Antipyretic Activities of Indomethacin, 1-(P-Chlorobenzoyl)-5-Methoxy-2-Methylindole-3-Acetic Acid. *J Pharmacol Exp Ther* **141:** 369-376.

Woode, E., Ainooson, G.K., Boakye-Gyasi, E., Ansah, C., Obiri, D.D., Kuffour, G.A., Mensah, A., Duwiejua, M. (2008a). Anti-arthritic and antioxidant properties of the ethanolic stem bark extract of Newbouldia laevis (P. Beauv.) Seaman ex Bureau (Bignoniaceae). *Journal of medicinal plants Research* **2**(8)**:** 180-188.

Woode, E., Boakye-Gyasi, E., Ainooson, G.K., Obiri, D.D., Ansah, C., Duwiejua, M., Donkoh, A. (2008b). Anti-inflammatory and antipyretic effects of an ethanolic extract of Palisota hirsuta roots. *African journal of pharmacy and pharmacology* **2**(9)**:** 191-199.

Woode, E., Obiri, D.D., Ansah, C., Duwiejua, M., and Kuffour, G.A. (2006). Total alkaloidal extract of *Picralima nitida* (fam. Apocynaceae) seeds has anti-inflammatory actions. *Journal of Ghana Science Association* **8:** 70-78.

Woolf, C.J., Salter, M.W. (2000). Neuronal plasticity: increasing the gain in pain. *Science* **288**(5472)**:** 1765-1769.

Yashpal, K., Coderre, T.J. (1998). Influence of formalin concentration on the antinociceptive effects of anti-inflammatory drugs in the formalin test in rats: separate mechanisms underlying the nociceptive effects of low- and high-concentration formalin. *Eur J Pain* **2**(1)**:** 63-68.

Yoshimura, N., Erdman, S.L., Snider, M.W., de Groat, W.C. (1998). Effects of spinal cord injury on neurofilament immunoreactivity and capsaicin sensitivity in rat dorsal root ganglion neurons innervating the urinary bladder. *Neuroscience* **83**(2)**:** 633-643.

Yu, X.M., Sessle, B.J., Vernon, H., Hu, J.W. (1994). Administration of opiate antagonist naloxone induces recurrence of increased jaw muscle activities related to inflammatory irritant application to rat temporomandibular joint region. *J Neurophysiol* **72**(3)**:** 1430-1433.