# ANTI-NOCICEPTIVE AND CENTRAL NERVOUS SYSTEM EFFECTS OF THE STEM BARK EXTRACTS OF *TRICHILIA MONADELPHA* (Thonn.) JJ De WILDE (MELIACEAE)

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In the

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Faculty of Pharmacy and Pharmaceutical Sciences

by

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## DECLARATION

The experimental work described in this thesis was carried out at the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, KNUST. This work has not been submitted for any other degree.

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## ABSTRACT

*Trichilia monadelpha* (Thonn.) JJ de Wilde (fam. Meliaceae) is used in Ghanaian and other African traditional medicine for the treatment of various painful and inflammatory conditions. This study therefore examined the anti-nociceptive and central effects of the petroleum ether, ethyl acetate and hydroalcoholic extracts of the stem bark of *Trichilia monadelpha* in various animal models. Preliminary phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, glycosides, saponins, sterols, tannins and triterpenes in the hydroalcoholic extract. The ethyl acetate extract showed the presence of alkaloids, glycosides, tannins and triterpenes whiles petroleum ether extract showed the presence of alkaloids, sterols and triterpenes. All three extracts (10-100mg $kg^{-1}$, *p.o.*), together with morphine and diclofenac (positive controls) showed significant dose-dependent anti-nociceptive activity in the chemical (acetic acid abdominal writhing and formalin tests), thermal (hot plate test) and inflammatory (carrageenan-induced mechanical hyperalgesia test) pain models in rodents. The anti-nociceptive effect of the systemic administration of hydroalcoholic extract was partly or wholly reversed by atropine (a non-selective muscarinic cholinergic receptor antagonist), naloxone (a non-selective opioid receptor antagonist), theophylline (a non-selective adenosine receptor antagonist) and glibenclamide (an ATP-sensitive $K^+$ channel inhibitor). The anti-nociceptive effects of the systemic administration of ethyl acetate extract and petroleum ether extract were partly or wholly reversed by atropine. Yohimbine, a selective $\alpha_2$-adrenoceptor antagonist however did not alter the anti-nociception produced by all three extracts. These results indicate that all three extracts produce dose-related anti-nociception in various animal models of chemical, thermal and inflammatory pain through mechanisms that involve an interaction with muscarinic cholinergic, adenosinergic, opioidergic pathways and ATP-sensitive $K^+$ channels. The petroleum ether extract however was the most potent. The central effects of the extracts were evaluated in various behavioural models-elevated plus-maze and the light-dark box tests. All three extracts (10-100 mg $kg^{-1}$

$^{1}$, *p.o.*) displayed possible central nervous system depressant activity (sedation) by decreasing the time spent by mice in the light compartment and the number of transitions in the light-dark box test. There was a decreased in open arm entries and risk assessment behaviours such as unprotected forms of stretch attend postures and head dips in the elevated plus-maze test. All three extracts (10-100 mg $kg^{-1}$, *p.o.*) did not modify the motor performance effects in the rotarod test but conversely decreased motor coordination in the beam traversal test, which is a more sensitive test able to identify motor deficits in mice. This effect in motor impairment may be ascribed to the reported central nervous system depressant (sedative effects) of the plant as having sedative effects in the hypno-sedative pentobarbitone sleeping time test. In summary, the study has shown that the petroleum ether, ethyl acetate and ethanolic extracts of *Trichilia monadelpha* stem bark have anti-nociceptive activity with some sedative effects.



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## ABBREVIATIONS

| | |
|---|---|
| 5-HT | 5-Hydroxytryptamine |
| ACC | Anterior cingulated cortex |
| Ach | Acetylcholine |
| AMPA | α-amino-3-hydroxy-5-methylsoxazole-4-propionic acid |
| ASIC | Acid sensing ion channels |
| ATP | Adenosine triphosphate |
| BK | Bradykinnin |
| CeA | Central nucleus of the Amygdala |
| CGRP | Calcitonin gene related peptide |
| CREB | cAMP response element-binding |
| CNS | Central nervous system |
| DH | Dorsal horn |
| DMARD | Disease modifying anti-rheumatic drug |
| EAE | Ethyl acetate extract of *Trichilia monadelpha* |
| $ED_{50}$ | Dose responsible for 50 % of the maximum response |
| GAFCO | Ghana Agro Food Company |
| HAE | Hydroalcoholic extract of *Trichilia monadelpha* |
| IASP | International Association for the Study of Pain |
| IL | Interleukin |
| i.p | Intraperitoneal |
| $IP_3$ | Inositol triphosphate |

| | |
|---|---|
| LmX1b | LIM homeobox transcription factor 1-beta |
| mGluR | Metabotropic Glutamate Receptor |
| NGF | Nerve growth factor |
| NK | Neurokinin |
| NMDA | N-methyl-D-aspartase |
| NRM | Nucleus raphe magnus |
| NSAIDs | Non-steroidal anti-inflammatory drugs |
| $P_2X_3$ | A member of the $P_2X$ family of ATP-gated ion channels |
| PAG | Periaqueductal grey |
| PEE | Petroleum ether extract of *Trichilia monadelpha* |
| PG | Prostaglandin |
| PKC | Protein kinase C |
| PLC | Phospholipase |
| *p.o.* | *Per os* |
| RVM | Rostral ventral medulla |
| SP | Substance P |
| TRP | Transient receptor potential |
| TRPV | Transient receptor potential vanilloid |

# CHAPTER 1

# INTRODUCTION

## 1.1 GENERAL INTRODUCTION

Traditional medicine (TM) is a widely used and rapidly growing health system of economic importance. In Africa up to 80 % of the population use some form of TM to help meet their health care needs. The widespread use is being fuelled by historical, socio-economic and cultural beliefs; this is peculiar to our part of the world. The use of traditional medicine is also becoming increasingly popular in developed countries due to the perception that they carry lesser side effects (WHO, 2002). Thorne *et al.*, (2002) also reported that the increased usage is also related to the increase in chronic diseases. In Ghana, the most predominant form of TM involves the use of medicinal plants.

Medicinal herbs have been used as a form of therapy in pain management throughout history (Almeida *et al.*, 2001). These plants have also proven to be an important source of new chemical substances with prospective therapeutic effects. It is therefore not amazing that several of the most important analgesic prototypes (e.g. salicylic acid and morphine) were originally derived from the plant sources (Shanmugasundaram and Venkataraman, 2005). The treatment of chronic pain is an area in which the practitioners of traditional medicine enjoy patronage and success (Akah and Nwambie, 1994).

Pain is a universal complain for most individuals who visit their physician, it usually tells of an ongoing damage to a tissue. There is evidence of the involvement of pain and inflammation in the pathogenesis of many diseases including cancers, atherosclerosis, cardiovascular diseases, arthritis, neurodegenerative diseases, diabetes mellitus and other life-threatening diseases. Pain is associated with significant morbidity and mortality. In individuals with acute and chronic pain, they become a burden on society and their quality of life

is greatly affected hence the association between pain and depression. These effects come at a great economic cost to the society (Merskey, 1994).

Standard medical practice has not been able to effectively control or treat chronic pain and inflammatory diseases. In cases where orthodox medicines have proven to be helpful the related untoward effects have been prohibitive. This inability and related toxicity from orthodox medicines is contributing greatly to the increased use of natural products especially herbal medicines in the management of chronic pain and inflammation (Kaboli *et al.*, 2001).

The increased reliance on traditional therapies for the management of chronic pain and inflammation calls for more research aimed at investigating the efficacy, safety and also standardising the use of the therapies (Chao *et al.*, 2009). The search for new anti-inflammatory and anti-nociceptive compounds with minimal side effects is continuing and at a greater pace, with researchers continuously and successfully isolating a variety of promising compounds from plant sources (Zakaria *et al.*, 2007; Sulaiman *et al.*, 2009).

Pharmacological investigations play a key role when it comes to researching medicinal plants. The benefits are multiple: first to the other fields or disciplines involved in the search for newer compounds toward development of better medications and more relevant to our setting, provision of information and data on scope of usage, therapeutic doses as well as the best methods of extraction and related toxicity (Golshani *et al.*, 2004). The latter goes a long way at improving the usage of herbal medicines in our communities where natural products remain a central component of healthcare delivery.

Consideration of these potential benefits for society was the most significant motivation for the research into the analgesic effects of *Trichilia monadelpha* in this study.

## 1.2 THE PLANT *TRICHILIA MONADELPHA*

**Botanical name**: *Trichilia monadelpha* (Thonn.) JJ De Wilde

**Family**: Meliaceae

**Synonyms**: *Trichilia heudelotii* Planch. ex Oliv. (1868)

**Local names**:

Twi: Otanduro; Opam;

Fante: Tanaduro;

Nzema:Tenuba.

**Figure 1.1 *Trichilia monadelpha* leaves (left) and stem bark (right)**

### 1.2.1 Description

*Trichilia monadelpha* is a tree that grows 12-20 m high, bearing a large open-spreading evergreen crown (Abbiw, 1990). The stem bark is pale grey to greenish brown or dark brown. It has a smooth surface with a pale brown to pink inner bark. It has alternate, imparipinnate compound leaves with 3-7 pairs of leaflets without stipules. The petiole is 4-13 cm long with leaflets being opposite, ovate to obovate or cuneate to obtuse at the base, acuminate at the apex, hairy below when young but glabrescent and pinnately veined. Its inflorescence has an axillary panicle 12-21 cm long, short and hairy.

Its flowers are unisexual: male and female flowers are very similar in appearance (greenish-yellow or greenish-white). The male flower has a rudimentary ovary

while the female has non-dehiscent anthers. The fruit is obovoid to globose in shape, with capsule 1.5-2.5 cm in diameter. The seeds are small and orange-red in colour with remaining the part being glossy black (Abbiw, 1990; Lemmens, 2008).

#### *1.2.2.1 Ecology*

*Trichilia monadelpha* is a common under storey tree in lowland, evergreen and semi-deciduous secondary forest (up to 650 m in altitude). It occurs along forest edges and sometimes in deciduous forest, but mostly along rivers and in other moist localities (Abbiw, 1990; Lemmens, 2008).

#### *1.2.2.2 Habitat and geographic distribution*

*Trichilia monadelpha* occurs from Guinea Bissau east to the Central African Republic and Democratic Republic of Congo and south to northern Angola (Cabinda). It is found in the lowlands, evergreen rain forests in Ghana and many parts of West Africa (Lemmens, 2008).

### 1.2.3 Traditional Uses

#### *1.2.3.1 Medicinal Uses*

**a) Pain and Inflammation:** A decoction of the stem barks of *T. monadelpha, Kigelia africana, Khaya ivorensis* and *Pilostigima thoningii;* roots of *Nauclea latifolia, Clausena anisata* and *Strophantus hispidus;* 0.5 g of *Xylopia aethiopica* and 0.5 g of *Monodora myristica* seeds is made. One table spoonful is taken three times daily to treat arthritis (Abbiw, 1990; Mshana *et al.*, 2000). The stem bark alone is boiled with water and the decoction is taken daily to treat the lumbago.

**b) Infections and skin disorders:** The stem bark of *T. monadelpha* is ground or crushed into powder and applied to wounds, yaws and any affected parts. The stem bark decoction is drunk daily to treat gonorrhoea and syphilis (Abbiw, 1990).

**c) Respiratory disorders:** The stem bark of *T .monadelpha*, rhizome of *Zingiber officinale*, and *Xylopia aethiopica* seeds are boiled together in water. The decoction is taken three times daily after meals to treat cough in adults and half the dose for children (Dokosi, 1998). A decoction of an unspecified part of the plant is used to treat pulmonary troubles (Burkhill, 1997).

**d) Gastrointestinal disorders:** The dried stem bark is ground into powder, boiled with water for one hour and then strained. The decoction is drunk as required in the treatment of dysentery and colicky abdominal pain. The stem barks of *T. monadelpha, Spathodea campanulata* and *Capara procera;* the root of *Maytenus senegalensis,* the fruit of *Piper guineense* and rhizome of *Zingiber officinale* are boiled together in water for 45 minutes. One table spoonful is taken three times daily for the management of dyspepsia, taken as an anti-helminthic and also used as an enema to treat gastro-intestinal complaints (Dokosi, 1998).

**e) Cardiovascular system disorders:** The leaf decoction of *T. monadelpha* is taken to treat palpitations and heart troubles (Burkhill, 1997).

**f) Central Nervous System disorders:** A decoction of the dried stem bark is known to cause sedation or drowsiness when taken (Burkill, 1985; Abbiw, 1990).

*1.2.3.2 Non-Medicinal Uses*

The flowering of the plant (which mainly occurs in August) serves as an indicator of the weather and season (Irvine, 1961; Burkhill, 1997). The trunk wood is used in making timber products, building materials, fuel and lighting, carving musical instruments, pit-props, and fences. It is used in building boats and canoes due to its ability to resist water. It is also used as firewood and for charcoal production. The seed oil is occasionally used in cooking. The reddish brown dye present in

the bark has been used for dyeing cloth and hides. *Trichilia monadelpha* is useful for soil protection and soil improvement (Abbiw, 1990).

### 1.2.4 Chemical Constituents of *Trichilia monadelpha*

There is a report on the chemical constituents of *T. monadelpha* in the Ghana Herbal Pharmaopoeia (2007) of the presence of reducing sugars, tannins (Pyrocatechuic acid), limonoids (dregeanin, heudelobin and related limonoids; Fig. 1.2) (Busia, 2007).

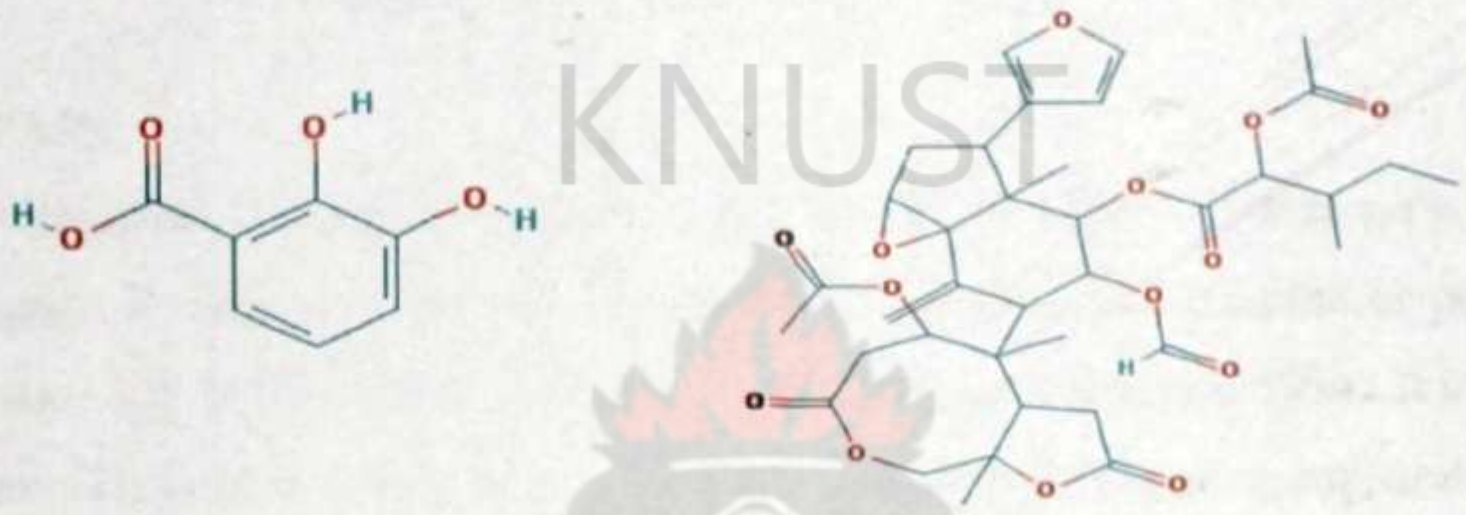

**Figure 1.2 Chemical structure of Pyrocatechuic acid (left) and Dregeanin (right)**

### 1.2.5 Work on *Trichilia monadelpha*

There is very little information on the pharmacological activities of *T. monadelpha* in established scientific literature.

### 1.2.6 Work on other *Trichilia* species

*Trichilia catigua,* A. Juss (Meliaceae), a native plant of Brazil popularly known as Catuaba or Catigua is widely used in Brazilian traditional medicine as a tonic for the treatment of fatigue, stress, impotence and deficits of memory. A few chemical studies have indicated that *T. catigua* extracts contain omega-phenyl alkanes, omega-phenyl alkanoic acids, omega-phenyl-gamma-lactones, alkyl-gamma-lactones, alkenyl-gamma-lactones and fatty acids besides β-sitosterol, stigmasterol, campesterol and also a mixture of flavalignans (Pizzolatti *et al.*, 2002; 2004).

Campos *et al.* (2004) reported on the pharmacological and neurochemical evidence for antidepressant-like effects of the herbal product Catuama. Further work showed the possible involvement of dopaminergic-mediated mechanisms (Campos *et al.*, 2005).

Previous work on other related species indicate that limonoids from these species have insect anti-feedant activities (Nakatani *et al.*, 1981). Aqueous leaf extracts of *T. dregeana* and *T. emetica*, which have similar traditional uses, exhibit antimicrobial activity (Dennis, 2002).



## 1.3 PAIN

The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"(Merskey, 1994). It involves anatomic structures, physiologic behaviours and psychological, cultural, social and cognitive factors. Pain can be a prepotent or overwhelming experience, often unsettling expected behaviour, and when severe, it demands and directs all of a person's attention. Pain is the most common symptom that motives a person to seek professional help. It sends those who suffer to a healthcare facility more often and with more speed than any other symptoms (Schim and Stang, 2004). Its location, radiation, duration, and severity provide important clues as to its cause. Despite its unpleasantness, pain can serve a useful purpose because it warns of impending tissue injury, motivating the person to seek relief.

Pain therefore is an important mechanism that helps to protect an organism by serving as a prompting alarm system to real tissue damage. It also induces learned avoidance behaviours, which may decrease the cause of the existing pain and as a result, may limit the (potentially) damaging consequences (Le Bars *et al.*, 2001).

The quality (sharp, burning or dull), intensity (mild, moderate or severe), duration (transient, intermittent, or persistent), and radiation (superficial or deep,

localized or diffused) of pain may vary from person to person and is peculiar to a particular disease condition. Since there are no consistent objective markers for pain, a patient is the only one to describe the characteristics of his pain (Clancy and McVicar, 1992). Pain can affect the quality of life of a person including sleep, thought, emotion, and daily activities.

### 1.3.1 Classification of Pain

Pain can be classified according to its duration: acute and chronic pain. Acute pain also known as adaptive pain serves to protect an individual from further injury and promote healing. However, chronic pain has been called maladaptive, a pathologic function of the nervous system or pain as a disease. Thus, it is reasonable to speak of 'pathological' verses 'physiological' for chronic verses acute or sub-chronic pain, respectively. It may be classified according to its pathophysiology: nociceptive, inflammatory, neuropathic and functional pain.

#### *1.3.1.1 Acute pain*

The classic definition of acute pain is pain that lasts less than six months. This reflects the notion that acute pain is the result of a tissue damaging event, such as trauma or surgery, and usually is self-limiting, ending when the injured tissues heal. The purpose of acute pain is to serve as a warning system and prompts a search for professional help. The location, radiation, intensity and duration aggravating or relieving factors of pain provide essential diagnostic clues. Inefficient management of pain can provoke physiologic responses that alter circulation and tissue metabolism and produce physical manifestations, such as tachycardia, reflective of increased sympathetic activity. Inadequately treated acute pain tends to cause anxiety, decrease mobility and respiratory movements such as deep breathing and coughing to the extent that it may complicate or delay recovery (Grichnick and Ferrante, 1991).

#### 1.3.1.2 *Chronic pain*

Chronic pain classically has been defined as pain lasting six months or longer. However, in practice one does not wait an arbitrary six months before deciding that the pain is chronic: rather, one considers the normal expected healing time for the underlying cause of the pain (DeLeo, 2006). The International Association for the Study of Pain defines chronic pain as "that which persists beyond the expected normal time of healing". Chronic pain associated with malignancy includes the cancer pain, migraine headaches, acquired immunodeficiency syndrome (AIDS), multiple sclerosis, sickle cell disease and end-stage organ system failure. Chronic pain can be quite variable. It may be unrelenting and extremely severe, as in metastatic bone pain. It can be relatively continuous with or without periods of escalation, as with some forms of back pain. Some conditions with recurring episodes of acute pain are particularly problematic because they have characteristics of both acute and chronic pain (Smith, 2003).

Unlike acute pain, persistent chronic pain usually serves no useful function (Merskey, 1994). In contrast, it imposes physiologic, psychological, familial, and economic stresses and may exhaust a person's resources. As painful conditions become prolonged and continuous, autonomic nervous system responses decrease. Decreased pain tolerance, which may result from the depletion of serotonin and endorphins, and depression are common in individuals with chronic pain. There is often loss of appetite, sleep disturbances and depression (Ruoff, 1996).

### 1.3.2 Types of Pain

#### 1.3.2.1 *Nociceptive pain*

Nociceptive pain is transient pain in response to a noxious stimulus at nociceptors that are located in cutaneous tissue, bone, muscle, connective tissue, vessels and viscera. Nociceptive stimuli are objectively defined as stimuli of such intensity that they cause or are close to causing tissue damage. The withdrawal

reflex (*e.g.* the reflexive withdrawal of a body part from a tissue-damaging stimulus) is used to determine when a stimulus is nociceptive (Smith, 2003).

Nociceptors, or pain receptors, are sensory receptors that are activated by noxious insults to peripheral tissues (Fig. 1.3). Structurally, the receptive endings of the peripheral pain fibres are free nerve endings. These receptive endings which are widely distributed in the skin, dental pulp, periosteum, meninges and some internal organs, translate the noxious stimuli into action potentials that are transmitted by a dorsal root ganglion to the dorsal horn of the spinal cord (Messeguer *et al.*, 2006; Suardiaz *et al.*, 2007).



*1.3.2.2 Inflammatory pain*

Inflammatory pain results from hypersensitivity of nociceptors to sensitization in an injured tissue. The body now changes focus from protecting against painful stimuli to protecting the injured tissue. The inflammatory response serves to prevent contact or movement of the injured part until healing is complete, thus reducing further damage (Anseloni and Gold, 2008; Harvey and Dickenson, 2008).

*1.3.2.3 Neuropathic pain*

When peripheral nerves are affected by injury or disease, it can lead to unusual and sometimes intractable sensory disturbances. These include numbness, paresthesias and pain. Depending on the cause, few or many axons could be damaged and the condition could be unilateral or bilateral. This leads to spontaneous firing of damaged neurons, generating action potentials that produce a form of neuropathic pain. Causes of neuropathic pain can be categorized according to the extent of peripheral nerve involvement. Conditions that can lead to pain by causing damage to peripheral nerves in a single area include nerve entrapment, nerve compression from a tumour mass and various types of neuralgias (trigeminal, post-herpetic and post-traumatic). Conditions involving pain caused by damage to peripheral nerves in a wide area include

diabetes mellitus, long-term alcohol use, hypothyroidism, renal insufficiency and drug treatment with neurotoxic agents (Vaillancourt and Langevin, 1999).

Neuropathic pain can also originate in the central nervous system (CNS) examples being that which occurs with spinal cord injury, multiple sclerosis and stroke (Baron and Treede, 2007; Garcia-Larrea and Magnin, 2008). Nerve injury can also lead to a multi-symptom, multi-system syndrome called complex regional pain syndrome (previously known as causalgia or reflex sympathetic dystrophy). Nerve damage associated with amputation is believed to be a cause of phantom limb pain. There may be allodynia or pain that is stabbing, jabbing, burning, or shooting. The pain may be persistent or intermittent. The diagnosis depends on the mode of onset, the distribution of abnormal sensations, the quality of the pain and other relevant medical conditions (*e.g.* diabetes, hypothyroidism, alcohol use, rash, or trauma) (Guevara-Lopez *et al.*, 2004).

#### *1.3.2.4 Functional pain*

This is new evolving concept in pain classification. There is pain sensitivity due to an abnormal processing or function of the nervous system in response to normal stimuli with no apparent neurologic insufficiency or peripheral abnormality detected. Conditions with features of such abnormal sensitivity or hyper-responsiveness include tension-type headache, fibromyalgia and irritable bowel syndrome (Nielsen and Henriksson, 2007).

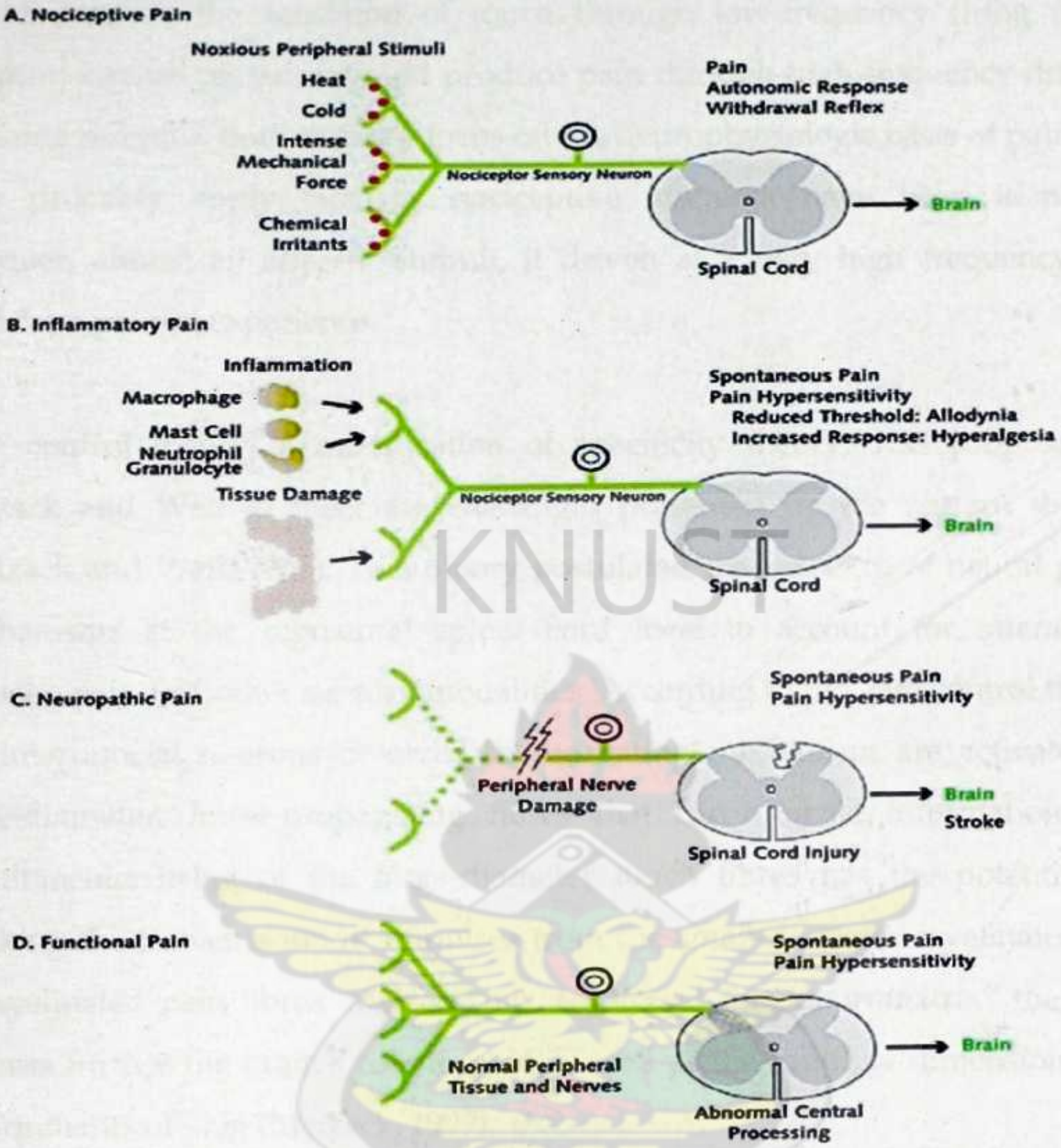


**Figure 1.3** **The four primary types of pains (Adapted from Woolf, 2004)**

### 1.3.3 Pain Theories

Traditionally, two theories have been offered to explain the physiologic basis for the pain experience. The first, specificity theory, regards pain as a separate sensory modality evoked by the activity of specific receptors that transmit information to pain centres or regions in the forebrain where pain is experienced (Bonica, 1991). The second theory includes a group of theories collectively referred to as pattern theory. It proposes that pain receptors share endings or pathways with other sensory modalities but that different patterns of activity (i.e., spatial or temporal) of the same neurons can be used to signal painful and

non-painful stimuli (Bonica, 1991). For example, light touch applied to the skin would produce the sensation of touch through low-frequency firing of the receptor; intense pressure would produce pain through high-frequency firing of the same receptor. Both theories focus on the neurophysiologic basis of pain, and both probably apply. Specific nociceptive afferents have been identified; however, almost all afferent stimuli, if driven at a very high frequency, can produce a painful experience.

Gate control theory, a modification of specificity theory, was proposed by Melzack and Wall to meet the challenges presented by the pattern theories (Melzack and Wall, 1965). This theory postulated the presence of neural gating mechanisms at the segmental spinal cord level to account for interactions between pain and other sensory modalities. According to the gate control theory, the internuncial neurons involved in the gating mechanism are activated by large-diameter, faster-propagating fibres that carry tactile information. The simultaneous firing of the large-diameter touch fibres has the potential for blocking the transmission of impulses from the small-diameter myelinated and unmyelinated pain fibres. Melzack has developed the "neuromatrix" theory to address further the brain's role in pain as well as the multiple dimensions and determinants of pain (Melzack, 1999).

### 1.3.4 Neurobiology of pain

The term *nociception,* which means "pain sense," comes from the Latin word *nocere* ("to injure"). It is the neural processes of encoding and processing noxious stimuli in the nervous system (Le Bars *et al.*, 2001). It involves the relay of noxious stimuli by nociceptive neurons, from the periphery through the spinal cord, brain stem, thalamus and cortex where it is perceived.

#### *1.3.4.1 Peripheral mechanisms*

Nociceptors, or pain receptors, are sensory receptors with free endings that are activated by noxious insults to peripheral tissues (Fig. 1.4). These receptive

endings, which are widely distributed in the skin, dental pulp, periosteum, meninges, and some internal organs, translate the noxious stimuli into action potentials that are transmitted by a dorsal root ganglion to the dorsal horn of the spinal cord. There are two types of afferent nerve fibres through which nociceptive action potentials are transmitted: myelinated Aδ-fibres and unmyelinated C-fibres.

The Aδ-fibre is large, with considerably greater conduction velocity, transmitting impulses at a rate of 10 to 30 m per second. It transmits fast, phasic pain of sharp, prickly quality and is typically elicited by mechanical or thermal stimuli. The C-fibre is the smallest of all peripheral nerve fibres and most abundant (Schaible, 2006). It transmits impulses at the rate of 0.5 to 2.5 m per sec. Pain conducted by C-fibres often is described as slow-wave pain because it is slower in onset and longer in duration. C-fibre nociceptors are polymodal (tonic pain with burning, itching, aching quality): they respond to all three types of stimuli (chemical, persistent mechanical or thermal stimuli) (Clark and Teisman, 2004). The slow-wave potentials generated in C-fibres are now believed to be responsible for central sensitization to chronic pain.

Silent nociceptors or mechano-insensitive nociceptors are neurons found in the joints, skin and viscera that contain Aδ and C-fibres. They are called silent because they are only active during inflammatory states when sensitized to mechanical and thermal stimuli.

C-fibres are known to have a number of polymodal receptors which can be activated by noxious thermal and mechanical stimuli. The transient receptor potential vanilloid receptor-1 (TRPV1) receptor-channel, one of such polymodal receptors, which responds to capsaicin, the extract of chilli peppers, may also be responsible for the generation of action potentials after application of noxious heat. The endogenous ligand for this receptor is unclear, although the

cannabinoid, anandamide, is one potential candidate (Caterina *et al.*, 1997; Melck *et al.*, 1999; D'Mello and Dickenson, 2008).

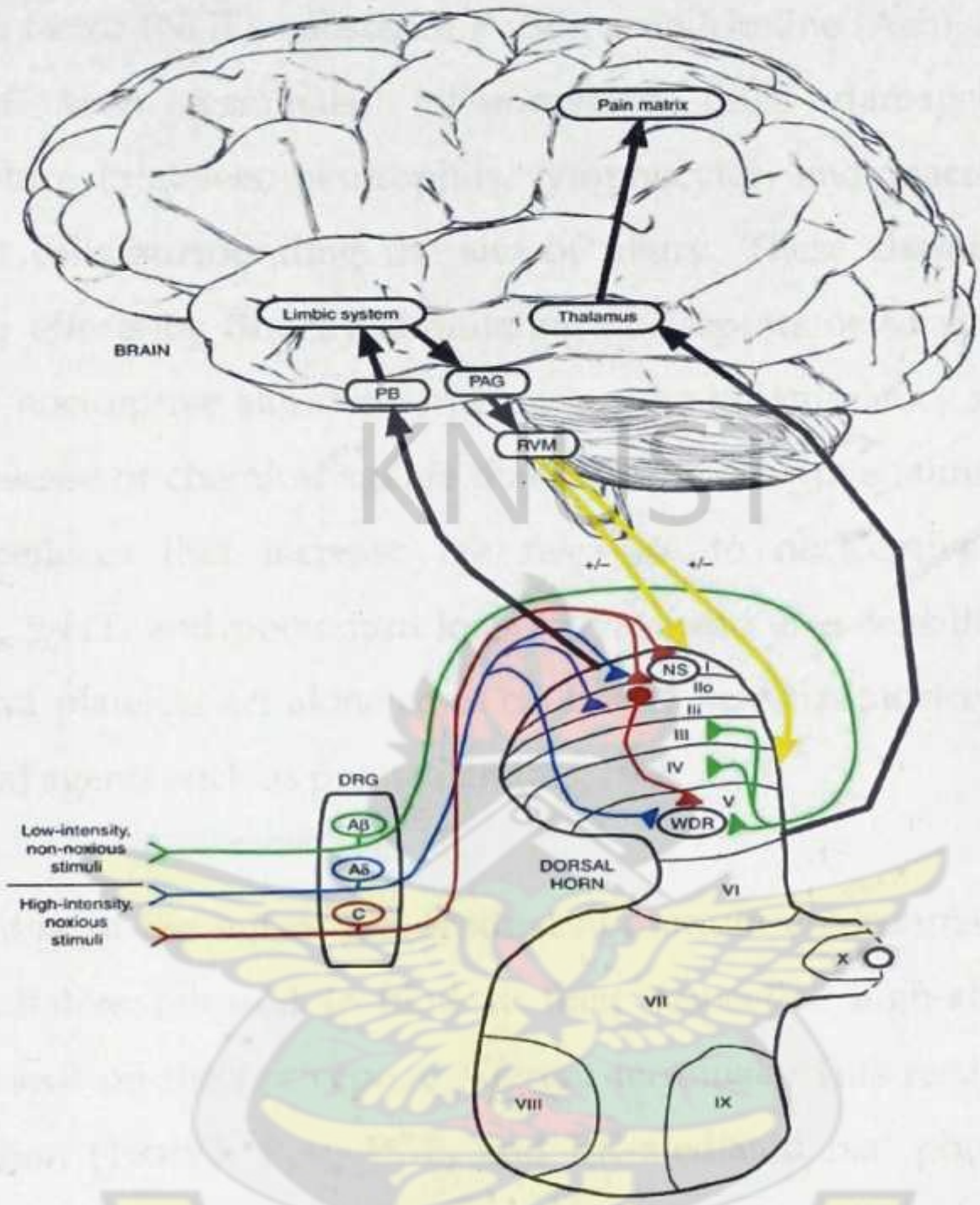


**Figure 1.4** **Pain pathways from periphery to brain. Primary afferent fibres (Aβ-, Aδ-, and C-fibres) transmit impulses from the periphery, through the dorsal root ganglion (DRG) and into the dorsal horn of the spinal cord. Nociceptive specific (NS) cells are mainly found in the superficial dorsal horn (laminae I–II), whereas most wide dynamic ranges (WDRs) are located deeper (lamina V). Projection neurones from lamina I innervate areas such as the parabrachial area (PB) and periaqueductal grey (PAG) and such pathways are affected by limbic areas. From here descending pathways (yellow arrows) from brainstem nuclei such as the rostral ventromedial medulla (RVM) are activated and modulate spinal processing. Lamina V neurones mainly project to the thalamus (spinothalamic tract), and from here the various cortical regions forming the 'pain matrix' (primary and secondary somatosensory, insular, anterior cingulate, and prefrontal cortices) are activated (Adapted from D'Mello and Dickenson, 2008)**

### 1.3.4.2 *Peripheral sensitization*

Tissue injury or inflammatory insults lead to the release of chemical mediators including hydrogen and potassium ions, Adenosine triphosphate (ATP), prostaglandins $E_2$ ($PGE_2$), leukotrienes, histamine, bradykinin (BK), epinephrine, nerve growth factor (NGF), substance P (SP), acetylcholine (Ach), and serotonin (5-HT) from axon terminals, inflammatory cells, damaged skin, the microvasculature (platelets, neutrophils, lymphocytes, and macrophages) and also by mast cells surrounding the site of injury. These chemical mediators produce their effects by directly stimulating nociceptors or sensitizing them to the effects of nociceptive stimuli; perpetuating the inflammatory responses that lead to the release of chemical agents that act as nociceptive stimuli; or inciting neurogenic reflexes that increase the response to nociceptive stimuli. For example, BK, 5-HT, and potassium ions activate and also sensitize nociceptors while Ach and platelets act alone or in concert to sensitize nociceptors through other chemical agents such as prostaglandins.

The high acidity of the injury site produces optimum environment for various chemical mediators released to bind to their respective high-affinity cognate receptors present on the nociceptive afferent terminals. This results in receptor phosphorylation (TRPV1, $P_2X_3$, $PGE_2$ and BK-mediated $Na^+$ phosphorylation), gene expression (NGF) and terminal sensitization. These ligands then activate different pathways such as elevating $Ca^{2+}$ (ASIC, TRPV1, $P_2X_3$), activating G-protein-coupled ($PGE_2$, BK, $\beta_2$), subsequently elevating cAMP and then PKA or elevating intracellular $Ca^{2+}$ via PLC or the Ras-MEK-ERK/MAP-kinase pathway (NGF).

### 1.3.4.3 *Central mechanisms*

During nociceptive transmission, the output of the spinal cord is dependent on various spinal mechanisms which can either increase or decrease the activity of dorsal horn neurons. Such mechanisms include local excitatory and inhibitory inter-neurons, N-methyl-D-aspartate receptor activation and descending

influences from the brainstem, which can be both inhibitory and excitatory in nature. Pain transmission therefore depends on a balance in these excitatory and inhibitory mechanisms which modulate spinal excitability.

*1.3.4.3.1 Spinal mechanisms*

The physicochemical properties of noxious stimuli, such as heat, extreme cold, pressure and chemicals are transformed into electrical activity by transient receptor potential-generating channels (TRP channels) and purinergic channels and amplified by sodium channels to elicit action potentials. Primary afferents carrying these peripheral inputs form glutamatergic synapses onto second-order neurons mostly in the superficial laminae (I, II and V) in the spinal dorsal horn (DH) (Fig. 1.4). There is then the organization of sensory inputs and the net output from spinal networks is carried by several pathways to distinct projection sites in the brain (Fig. 1.4).

There are two main types of intrinsic DH neurons. Nociceptive-specific (NS) neurons are mostly found superficially (laminae I and II) and synapse with Aδ- and C-fibres only. The wide dynamic range neurons (WDRs) lie more deeply (lamina V), receive input from all three types of sensory fibres (Aδ-, Aβ-, and C) and therefore respond to the full range of stimulation, from light touch to noxious pinch, heat, and chemicals (D'Mello *et al.*, 2008).

The lateral spinothalamic tract projects multimodal sensory inputs from spinal WDRs to the lateral thalamus and has been implicated in processing sensory and discriminative aspects of pain. The medial aspect of the spinothalamic tract and the spinoparabrachial tract project sensory inputs from NS neurons to the medial thalamus and limbic structures and are believed to mediate the emotional and aversive components of pain. The experience of pain is perceived in the cortex, and information is accordingly sent to the spinal cord to enable withdrawal from the noxious stimulus (Kuner, 2010) (Fig 1.5 a).

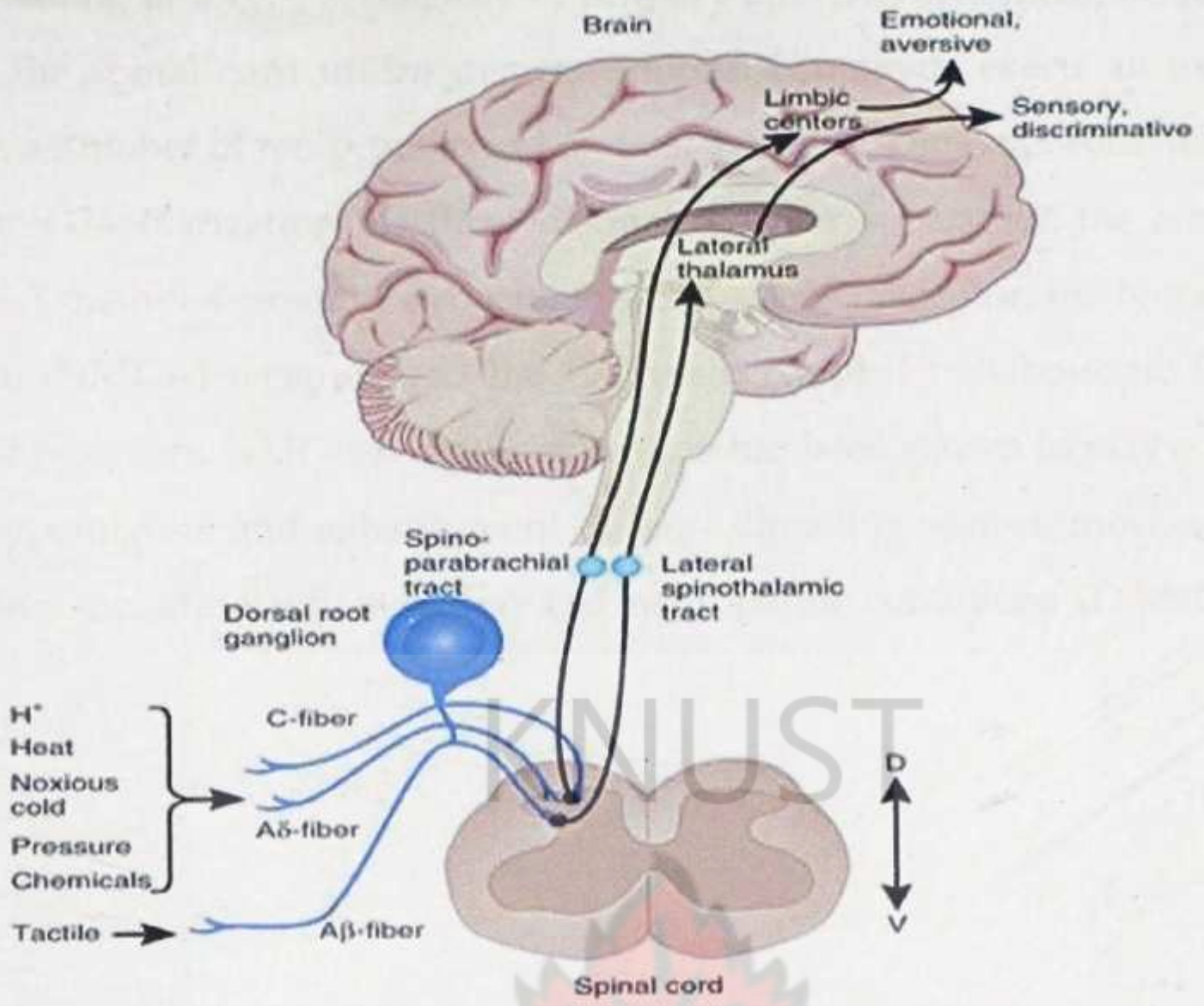


**Figure 1.5 Pain circuits: Schematic overview of the main circuits mediating physiological pain (Adapted from Kruner, 2010)**

*1.3.4.3.2 Central Sensitization*

After nerve injury or conditions of inflammation, shifts can occur in these excitatory and inhibitory mechanisms which modulate spinal excitability, often resulting in the heightened response of dorsal neurons to incoming afferent signals, and increased output to the brain, a phenomenon known as central sensitization. This process can be described as an amplification of low levels of afferent activity by spinal pharmacological means (Fig. 1.6) (D'Mello *et al.*, 2008).

Typical changes in the spinal cord neurons include facilitation, wind-up, action potential threshold reduction, receptive field expansion, oncogene encoding and long term potentiation.

Glutamate, an excitatory amino acid, is the major excitatory neurotransmitter found throughout the whole of the nervous system and is therefore essential for

pain signalling at levels. A majority of primary afferents synapsing in the dorsal horn of the spinal cord utilize this transmitter. Glutamate exerts an excitatory effect on a number of receptors found on post-synaptic spinal neurons, leading to membrane depolarization via three distinct receptor subclasses: the α-amino-3-hydroxy-5-methyl-4-isoxazeloproprionic acid (AMPA) receptor; the N-methyl-D-aspartate (NMDA) receptor and the G-protein coupled metabotropic (mGluR) family of receptors. NMDA receptor activation has been shown to play a key role in the hyperalgesia and enhancement of pain signalling seen in most persistent pain states including inflammation and neuropathic conditions (D'Mello *et al.*, 2008).

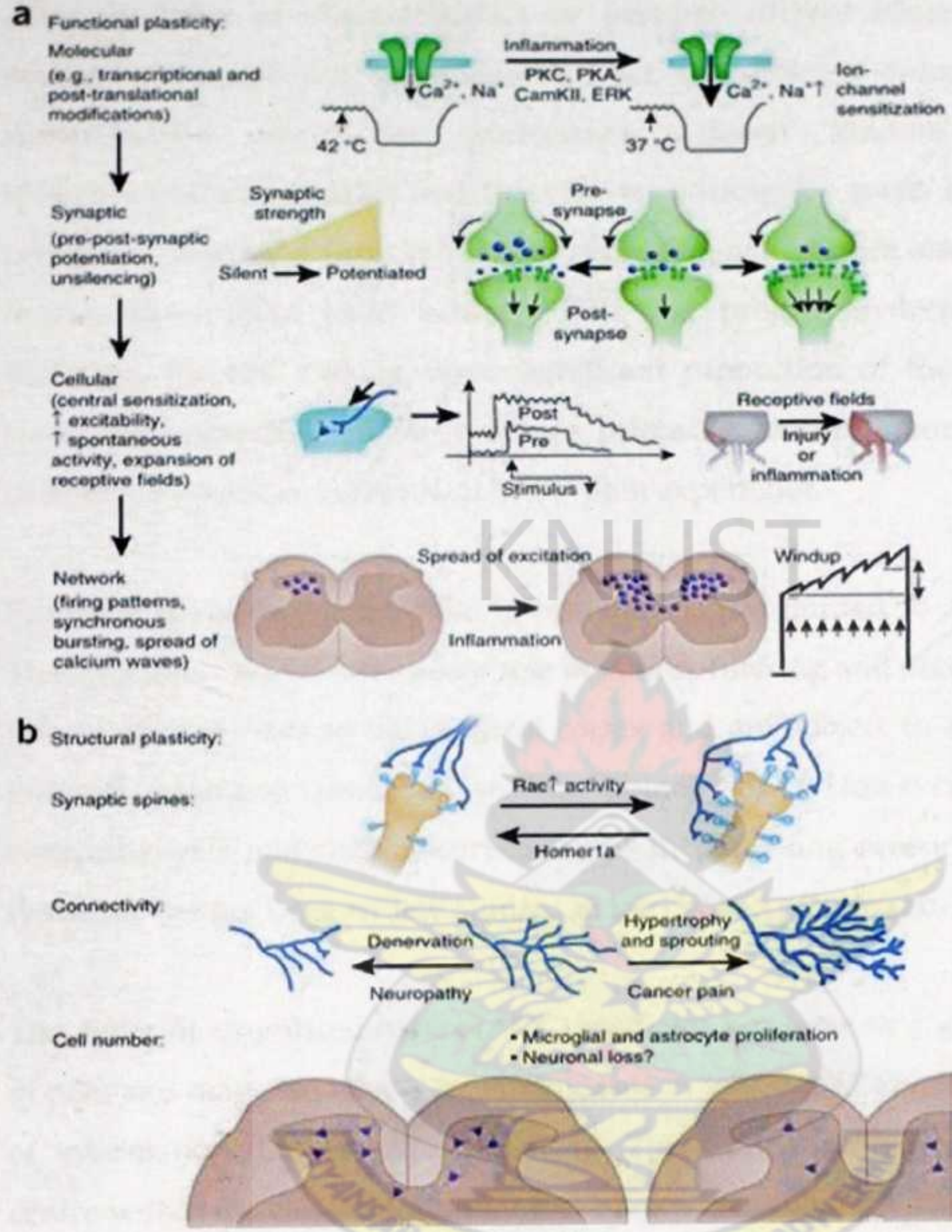


**Figure 1.6 Disease-induced functional and structural plasticity in neural substrates of pain. (a) Different levels of activity-dependent functional plasticity. Molecules may become functionally sensitized (top), synaptic transmission may become potentiated by presynaptic mechanisms (second row, arrow to the left) or by postsynaptic plasticity (arrow to the right), cells may respond to noxious stimuli with increased activity and expanded receptive fields after injury (third row) and network function may change so that more cell ensembles respond to noxious stimuli, collectively leading to a higher net spinal output after injury or inflammation (bottom). (b) Examples of nociceptive activity-induced structural plasticity. From the top, synaptic spines may increase in size and density; axons may sprout or degenerate; and cells may atrophy (for example, loss of inhibitory interneurons) or proliferate (for example, microglia and astrocytes) (Adapted from Kruner, 2010)**

*1.3.4.3.3 Supraspinal mechanisms*

After the relay of noxious inputs by primary afferent fibres, second-order neurons project to the spinothalamic tract, the different components of the spinoreticular tract, the postsynaptic dorsal column fibres, the spinocervicothalamic tract and the visceral nociceptive tracts that run in the posterior columns. A large number of projection neurons are also found deeper in the dorsal horn from lamina III–VI and project predominantly to the thalamus, thereby making up a significant proportion of the spinothalamic tract. This ascending pathway carries primarily sensory information and so provides the sensory component of the pain experience.



From the thalamus, nociceptive information is transmitted to cortical regions. Thalamic relay nuclei have a key role in gating, filtering and processing sensory information *en route* to the cerebral cortex and are subject to similar activity-induced plasticity processes as the spinal cord. However, the role of corticothalamic and thalamocortical loops in regulating sensory gating in the thalamus has not been widely studied in the context of pain (Hu, 2006).

The Anterior cingulated cortex (ACC) mediates key emotional-aversive aspects of pain and may also have a mnemonic role in which it allows transient storage of information during pain processing. There does not exist a specific pain centre within the cortex, but rather there are various cortical regions which may or may not be activated during a particular painful experience (D'Mello *et al.*, 2008). These regions constitute what is commonly referred to as a 'pain matrix' and include primary and secondary somatosensory, insula, anterior cingulate, and prefrontal cortices. Other sub-cortical structures (e.g. Hypothalamus, basal ganglia, cerebellum and the central nucleus of the amygdala- CeA) have also been implicated in the affective component of pain (Kuner, 2010).

Descending inhibitory systems block spinal transmission, leading to hyposensibility or a lack of pain, despite inputs coming in from the periphery.

Such inhibitory mechanisms have great value since they can enable the organism to ignore pain in crucial situations, such as flight or fight, and serve as a mechanistic basis for placebo-induced analgesia (Eippert, 2009). They may contribute to analgesia produced by a variety of non-pharmacological pain control approaches, such as acupuncture and hypnosis. There is compelling evidence to show that the axis of the periaqueductal gray (PAG) and rostral ventral medulla (RVM) can inhibit or facilitate sensory processing in the spinal dorsal horn (Porreca *et al.*, 2002). Descending control can also arise from the lateral and caudal dorsal reticular nucleus and the ventrolateral medulla. Owing to their therapeutic role and their contribution to opioidergic control of pain and placebo analgesia, much attention was initially focused on descending adrenergic and serotonergic pathways, originating from neurons in the locus coeruleus and nucleus raphe magnus, respectively, which finally lead to the activation of local encephalenergic neurons in the spinal dorsal horn.



### 1.3.5 Mediators of Pain

There is considerable evidence of the involvement of excitatory amino acids, glutamate and aspartate, and a number of peptides in the nociceptive transmission in the spinal cord. Some mediators involved in nociception include adenosine triphosphate (ATP), serotonin, histamine (5-HT), kinins (leucotrienes, BK, SP), prostanoids ($PGE_2$, $PGI_2$), cytokines and nerve growth factor (NGF).

#### *1.3.5.1 Kinins*

Bradykinin (BK) is a powerful nonapeptide algogenic substance formed in the kinin cascade from kininogens. It activates nociceptors in the presence of protein kinase C (PKC) and calcium ions. It then sensitises nociceptors by means of the activation of postganglionic sympathetic neurons which then produce $PGE_2$ (Dickenson, 1995).

The neuropeptide substance P (SP) is the first known "neurotransmitter" implicated in nociceptive transmission. Dorsal Root SP is believed to originate

from primary sensory neurons in the DRG. Out of the three sub-classes of neurokinin or tachykinin receptors: neurokinin-1, neurokinin-2 and neurokinin-3, SP has preference for neurokinin-1 receptor. TRPV1 activity is enhanced by various inflammatory mediators such as NGF, somatostatin, BK, PG, 5-HT, etc., implicating an essential role of TRPV1 in integrating different signal pathways for mediating nociceptor sensitization (Zhang *et al.*, 2007).

#### *1.3.5.2 Nerve Growth Factor*

Nerve growth factor has a key role not only in the development of sensory and autonomic neurones, but also in the processes of nociception (McMahon and Bennett, 1997). This factor, which is upregulated by the process of inflammation, is produced in the periphery by fibroblasts and schwann cells and then increases the excitability of nociceptors which leads to hyperalgesia. Various central and peripheral mechanisms have been postulated as a basis for these actions of nerve growth factor (Woolf, 2004). The production of antagonists for receptors of the tyrosine kinase family has the potential to provide a pharmacological target for the production of new analgesics to reduce the effects of nerve growth factor.

#### *1.3.5.3 Prostanoids*

Prostanoids (prostaglandins, leukotrienes and hydroxyacids) are among the most important mediators of inflammatory hyperalgesia and are generated from arachidonic acid by cyclooxygenase and lipooxygenase enzyme activity. Prostaglandins act via a number of receptors coupled with secondary messengers: $PGE_2$-EP receptors and $PGI_2$ (Prostacyclin)-IP3 receptors. Prostaglandins increase the level of cyclic AMP reducing their activating threshold for TTX-R sodium channels via protein kinase A (PKA) and enhancing their response to other stimuli e.g. BK. They are likely to be involved at multiple sites along the nociceptive pathway (Kassuya *et al.*, 2007).

#### 1.3.5.4 *Peptides*

Primary afferent fibres contain a number of peptides and their profile can be altered by sustained stimuli or by any form of damage to the nerve. Examples of peptides are galanin, somatostatin, cholecystokinin, vasoactive intestinal peptide, substance P and calcitonin gene related peptide (CGRP). Substance P and CGRP can be released into the periphery via the classic axon reflex. Substance P in neurogenic inflammation causes a degranulation of mast cells and thus the release of histamine, vasodilatation, and plasma extravasation with the subsequent release of other algogens (BK, serotonin) and the activation of other inflammatory cells (macrophages, monocytes, and lymphocytes). It is also able to induce production of nitric oxide, another vasodilator from the endothelial layer of blood vessels (Dray, 1995).

#### 1.3.5.5 *Adenosine Triphosphate*

This activates sensory neurons and increases their permeability to cations. It produces sharp, transient pain when administered intradermally. Adenosine, which is formed from the breakdown of ATP, also provokes pain and hyperalgesia. This is possibly due to the activation of adenosine $A_2$ receptors which are coupled with adenylate cyclise (Dray, 1995).

#### 1.3.5.6 *Histamine*

Histamine is released from mast cell degranulation by some inflammatory mediators (SP, IL-1 and NGF) and can act on sensory neurons to produce itching in low concentrations and pain in high concentrations. Sensory neurons express the $H_1$ receptors and its activation leads to increased calcium ion permeability (Dray, 1995).

### 1.3.6 Animal models of nociception

Animal models used for assessing analgesic activities have been designed for behavioural and non-behavioural manifestations of pain or nociception. A more practical use of animal models is in evaluating new chemical entities as

potential drugs for the treatment of a human disease state, such as hypertension or pain. They are therefore excellent in preliminary and in-depth screening of compounds with possible analgesic activity. In using animal models it is therefore important to create a test system that allows the basic mechanism of pathology to be systematically manipulated so as to obtain a better understanding of its biological basis.

Zimmermann (1986) re-interpreted the IASP definition of pain in relation to experimental animals: "an aversive sensory experience caused by actual or potential injury that elicits progressive motor and vegetative reactions, results in learned avoidance behaviour, and may modify species specific behaviour, including social behaviour".

It is important to note that in all biomedical research involving animals, pain research presents ethical problems. The importance of ethical clearance by those of international scientific review boards so as to ensure a given level of physiological well being in the animal cannot be underrated (Le Bars *et al.*, 2001).

### *1.3.6.1 Models of Acute pain*

Acute pain models can be classified into those with stimulus of short duration (phasic nociception) and those with stimulus of long duration (tonic/persistent nociception) (Le Bars *et al.*, 2001).

#### *1.3.6.1.1 Phasic Nociception*

These tests involve a short period (seconds) of stimulation and have somatic sites of stimulation. They can be classified by the nature of the stimulus: mechanical (Tail clip or Paw pressure test, Von-frey hair and tail clip test), electrical (stimulation of paw, tail or dental pulp) and thermal (Plantar/Hargreaves test, Tail-flick test, Tail immersion test and hot plate test) (Le Bars *et al.*, 2001).

#### 1.3.6.1.2 *Persistent/Tonic Nociception*

This type of behavioural test relies on the injection of a single neuroactive (alogenic) compound that will stimulate nociceptive fibres for a prolonged time. The main models based on such stimuli use intradermal (formalin test) or intraperitoneal (acetic acid-induced writhing test) injections.

### 1.3.6.2 *Models of Chronic pain*

These include pain models involving the injection of alogenic compounds into a particular site in experimental animals to produce persistent allodynia or hyperalgesia. Examples are carrageenan, Freud's adjuvant, iodoacetate and turpentine. Various neuropathic pain models have been developed to reflect the peculiar sensitivity of humans to thermal and mechanical stimuli. This leads to different pain pathways with different mediators, inhibitory and excitatory mechanisms. Examples are spinal nerve ligation (Chung model), diabetic neuropathy (Streptozocin-induced diabetic neuropathy) and inflammatory injury models (neuritis model).



## 1.3.7 Anxiety, Depression and Pain

The differential contributions of the noradrenergic and serotonergic components to opioid-induced analgesia have been a topic of much debate. Pharmacological manipulations that increase synaptic levels of serotonin and noradrenaline, such as the use of tricyclic antidepressants and other classes of antidepressant, have gained prominence in the clinical management of chronic pain, particularly in therapy-resistant states such as neuropathic pain and fibromyalgia (Porreca *et al.*, 2002; Heinricher *et al.*, 2009). The *in vivo* analgesic efficacy of selective serotonin reuptake inhibitors in clinical trials is lower than that of drugs that affect both serotonin and noradrenaline (for example, tricyclic antidepressants).

It has been suggested that the analgesic effects of antidepressant drugs occur mainly through the modulation of noradrenaline in the spinal dorsal horn. Research in mice lacking a LIM homeobox transcription factor called Lmx1b,

which lack serotonergic neurons in the adult CNS has shown markedly reduced analgesia in response to opioids and antidepressants. This suggests that central serotonergic neurons make up an important part of the descending pain modulatory circuitry that mediates analgesia induced by opioids and antidepressants (Zhao, 2007a; Zhao, 2007b; Kuner, 2010).

The comorbidity of anxiety disorders and depression with pain (Lepine and Briley, 2004) has implications for the outcome of pain and also possibly the outcome of depression and anxiety disorders (Mico *et al.*, 2006; Gureje, 2008). It is of great importance for clinicians to recognize and treat depression and anxiety, which affect 20 % to 50 % of patients with chronic pain (Banks *et al.*, 2011). These conditions affect patients' ability to participate in their own self care and recovery as well as altering the balance of neurotransmitters involved in modulating pain. Antidepressants as well as anticonvulsants are now in use in the management of chronic and neuropathic pain states which do not respond to conventional analgesics such as NSAIDS and opiates (Schaible and Richter, 2004).



### 1.3.8 Pharmacological Management of Pain

Acute pain should be aggressively managed and pain medication provided before the pain becomes severe. The current protocol for the management of acute pain pharmacologically relies on the use non-steroidal anti-inflammatory drugs (NSAIDs) and opioids. Opioid drugs are effective in treat nociceptive pain that has no coexisting nervous system pathology. In general, they are much less effective for treating neuropathic pain, because windup decreases opioid efficacy by a variety of mechanisms, including decreasing the number of mu- receptors (Woolf, 2004).

Adjuvant analgesics are useful in chronic pain syndromes and in cases where patients are not responding to the conventional analgesics. These include medications such as tricyclic antidepressants, anti-seizure medications, and neuroleptic anxiolytic agents. The fact that the pain suppression system has

non-endorphin synapses raises the possibility that potent, centrally acting, non-opiate medications may be useful in relieving pain. Serotonin has been shown to play an important role in producing analgesia. The tricyclic antidepressant medications block the removal of serotonin from the synaptic cleft to produce pain relief in some persons. These medications are particularly useful in some chronic painful conditions, such as post-herpetic neuralgia. Certain anti-seizure medications, such as carbamazepine, gabapentin and phenytoin, have analgesic effects in some pain conditions. These medications, which suppress spontaneous neuronal firing, are particularly useful in the management of pain that occurs after nerve injury. Other agents, such as the corticosteroids, may be used to decrease inflammation and nociceptive stimuli responsible for pain.



### 1.3.9 Non-Pharmacological Management of Pain

Helping patients learn to better manage the pain inhibits pain transmission and improves pain management (Galer and Dworkin, 2000). Exercise and psychological treatment are effective for treating chronic pain because these treatments may help retrain the nervous system to re-establish more normal neural connections.

### 1.3.10 Future Targets in Analgesia Research

A well defined understanding and targeting of the mechanisms that underlie spontaneous pain in pathological states is urgently required. Most of the key intracellular mediators of nociceptive plasticity, such as MAP kinases, PKC, PLC and CREB cycle can be explored for new drug discovery. Adenosine and ATP receptors and ion channels are also potential targets for the treatment of pathological pain.

## 1.4 JUSTIFICATION OF RESEARCH

*Trichilia monadelpha* (Meliaceae), a common plant used in traditional medicine in Ghana for treating a spectrum of diseases including inflammatory, pain-related conditions and CNS disorders such as schizophrenia and epilepsy. The leaves are

also used as a sedative. The management of pain and inflammation with anti-inflammatory and analgesic agents including NSAIDs, opioid analgesics, steroids, disease modifying anti-rheumatic drugs (DMARDs) and centrally acting drugs like anti-depressants and anti-convulsants though effective, have numerous life threatening side effects :respiratory depression, addiction and tolerance. The search for more effective analgesic agents with minimal or no side effects at therapeutic doses is therefore of great importance.

Previous work in our laboratory by Owusu (2009) on the ethanolic, aqueous and petroleum ether stem bark extracts of *T. monadelpha* showed great promise as an anti-nociceptive and anti-inflammatory agent. There is therefore the need for scientific validation of the pharmacological activities of *Trichilia monadelpha* stem bark extracts as anti-nociceptive agents in the management of pain and its mechanism of action as well as its CNS effects.

## 1.5 AIMS AND OBJECTIVES

This study is aimed at providing pharmacological evidence and basis for the traditional use of *Trichilia monadelpha* stem bark in the management of pain, its possible mechanism of action and CNS effects. The objective of this study was to evaluate the hydroalcoholic, ethyl acetate and petroleum ether extracts of the stem bark of *Trichilia monadelpha* using various animal models. Specific objectives included evaluating the three extracts for:

1) Analgesic activity using various *in vivo* models for different types of pain.

2) Possible mechanisms of analgesic activity.

3) Central effects using *in vivo* anxiety-related models and motor coordination models.

# CHAPTER 2

# MATERIALS AND METHODS

## 2.1 PLANT COLLECTION AND EXTRACTION

### 2.1.1 Plant Collection

The stem bark of *T. monadelpha* was collected from Bomaa, Brong-Ahafo Region, Ghana (7°05′06.60″N, 2°10′01.66″W), in the month of September, 2009 and authenticated by Mr. G.H. Sam of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. A voucher specimen (KNUST/FPP/079/10) was kept at the herbarium of the Faculty.

### 2.1.2 Plant Extraction

The stem bark was chopped into pieces and sun-dried. The dried bark was then pulverized through a 2 mm screen using a hammer mill. Fifteen kilograms of the powdered plant bark was serially extracted with 40-60 °C with petroleum ether, ethyl acetate and 70 % ethanol over 24-hour period using the Soxhlet apparatus. Resulting extracts were each concentrated into a syrupy mass under reduced temperature (60 °C for petroleum ether and ethyl acetate extracts and 70 °C for the ethanolic extract) and pressure using a rotary evaporator (Model: Rotavapor R-215, BÜCHI Labortechnik AG, Flawil, Switzerland). The extracts were further dried over a water bath and then kept in a desiccator for use. The yields were 0.9 % ($^{w}/_{w}$) for the petroleum ether extract (PEE); 0.7 % ($^{w}/_{w}$) for the ethyl acetate extract (EAE) and 9.6 % ($^{w}/_{w}$) for the hydro-ethanolic extract (HAE) of *T. monadelpha*.

## 2.2 DRUGS AND CHEMICALS

The following drugs and chemicals were used: acetic acid, formalin and theophylline (British Drug Houses Ltd, Poole, England); diclofenac (KRKA®, Novo Mesto, Slovenia); diazepam (INTAS®, Gujarat, India); morphine (Phytoriker®, Accra, Ghana); λ-carrageenan, atropine, naloxone, yohimbine, pentylenetetrazole (Sigma-Aldich Inc., St. Louis, MO, USA) and glibenclamide (Daonil®, Sanofi-Aventis, Guildford, UK).

## 2.3 EXPERIMENTAL ANIMALS

Male Sprague-Dawley rats (100-180 g), ICR mice (20-25 g) and C3H mice (20-25 g) were used in the experiments. The animals were purchased from Noguchi Memorial Institute for Medical Research, Accra, Ghana and kept at the Animal House of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology, (KNUST), Kumasi, Ghana. Each group consisted of five animals, housed in stainless cages (34 cm×47 cm×18 cm) with soft wood shavings as bedding. The animals were maintained under standard laboratory conditions (ambient conditions of temperature, light and relative humidity) with free access to commercial pellet diet (GAFCO, Tema, Ghana) and water. All experiments were conducted in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 85 - 23, 1985, revised 1996). All protocols used were approved by the Departmental Ethics Committee.

## 2.4 PHYTOCHEMICAL TESTS

The extracts were screened for the presence of alkaloids, flavonoids, glycosides, saponins, sterols, tannins and triterpenes.

### 2.4.1. Test for Alkaloids

An amount of 0.5 g of each extract was boiled with 10 ml of dilute hydrochloric acid (alcohol) in a test tube for 5 minutes. The supernatant liquid was filtered into another test tube and 1 ml of the filtrate was taken, into which 3 drops of

Dragendorff's reagent (potassium bismuth iodide solution) was added. The mixture was shaken and observed for the appearance of an orange spot and a precipitate formation (Sofowora, 1993).

### 2.4.2 Test for Flavonoids (Ammonia test)

An amount of 15 ml of 98 % ethanol was added to 0.5 g of each extract. A small piece of zinc metal was added to the resulting extracts followed by drop wise addition of concentrated hydrochloric acid. Colours ranging from orange to red indicated flavones, red to crimson indicated flavonols, crimson to magenta indicates flavonones (Evans, 2002).



### 2.4.3 Test for Glycosides

An amount of 0.5 g of each extract was warmed with 5 ml dilute $H_2SO_4$ on a water bath for 2 minutes. It was then filtered and the filtrate rendered distinctly alkaline with 2 to 5 drops of 20 % NaOH. 1 ml each of Fehlings solution A and B was then added to each filtrate and heated on the water bath for 2 minutes. A brick red precipitate indicates the presence of glycosides (Evans, 2002).

### 2.4.4 Test for Tannins

An amount of 0.5 g of each extract was boiled with 25 ml of water for 5 minutes. It was then cooled, filtered and the volume adjusted to 25 ml. To 1ml aliquot of each aqueous extract was added 10mls of water and 5 drops of 1 % lead acetate. The colour and amount of precipitate, if any, was noted and recorded. The procedure was repeated using 5 drops of 1 % ferric chloride (Odebiyi and Sofowora, 1978).

### 2.4.5 Test for Saponins

An amount of 0.5 g of each extract was shaken with a few millilitres of water in a test tube and the mixture observed for the presence of a froth which does not break readily upon standing (Sofowora, 1993).

### 2.4.6 Test for Sterols (Liebermann-Burchard's Test)

Two millilitres of chloroform was added to 0.5 g of each extract. 2 ml of acetic anhydride was then added to the chloroformic extract and few drops of concentrated $H_2SO_4$ were added along the sides of the test tube. A violet to blue coloration indicates the presence of sterols (Sofowora, 1993).

### 2.4.7 Test for Triterpenes (Salkowski Test)

An amount of 5 ml of 70 % ethanol was added to each extract and mixed with 5 ml chloroform. It was then warmed for 30 minutes. The chloroform solution was then treated with a small volume of concentrated sulphuric acid and mixed properly. A reddish brown coloration of the interface shows a positive result for the presence of triterpenes (Sofowora, 1993).



## 2.5 ANTI-NOCICEPTIVE EFFECTS

### 2.5.1 Acetic Acid-induced Writhing Test

This test was adopted from that described by Koster *et al.*, (1959) and Freitas *et al.*, (2009) and performed as described by Woode *et al.*, (2011). Mice were pre-treated with HAE, EAE or PEE (10, 30 and 100 mg $kg^{-1}$, *p.o.*), diclofenac (3, 10 and 30 mg $kg^{-1}$, i.p), or vehicle (10 ml 1 $kg^{-1}$, *p.o.*), 30 min (i.p) or 1 h (*p.o.*) before administration of acetic acid 0.6 % (10 ml $kg^{-1}$, i.p.). They were individually housed in a Plexiglas chamber (15 cm × 15 cm × 15 cm) atop a flat plain glass elevated 80 cm above the floor. A large mirror inclined at 45° below the floor of the chamber allowed a complete view of the mice (Woode and Abotsi, 2011).

The acetic acid administered induced writhing: a nociceptive behaviour indicated by exaggerated abdominal constriction with the outstretching of at least one hind limb. The nociceptive behaviour was videotaped using a camcorder (Everio™, model GZ-MG1300, JVC, Tokyo) for 30 min and analysed. Tracking of the behaviour was done using a public domain software JWatcher version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney,

Australia available at http://www.jwatcher.ucla.edu/). The total number of writhes per 5 min, starting 5 min after acetic acid administration was expressed in time course curves to indicate the maximal number of writhing induced. A dose-response curve was also plotted to determine the significant anti-nociceptive dose.

### 2.5.2 Carrageenan-induced Mechanical Hyperalgesia Test

Response to noxious mechanical stimulation was determined by measuring withdrawal thresholds to paw pressure. The method used was described by Randall and Sellito (1957) and modified by Villetti *et al.*, (2003). Continuous increasing pressure was applied to the dorsal surface of the inflamed hind paw using a blunt conical probe of an analgesimeter (IITC Life Science Inc., Woodland Hills, CA, USA). Mechanical pressure was increased until vocalization or withdrawal reflex occurred while rats were lightly restrained. A cut off point was set at 250 g to prevent tissue damage to the paw. Vocalization or withdrawal reflex thresholds were expressed in grams. A change in the hyperalgesic state was calculated as a percentage of the maximum possible effect (% MPE) from the formula: $[(P_2-P_1)/ (P_0-P_1) \times 100]$, where $P_1$ and $P_2$ were the pre- and post- drug paw withdrawal thresholds respectively, and $P_0$ was the cut-off.

Baseline paw withdrawal thresholds (PWTs) were taken on the experiment day before λ-carrageenan (100 µl of a 20 mg $ml^{-1}$ solution) was injected into the plantar surface of the rat's right hind paw (males; n=5). PWTs were determined again 2.5 h post-carrageenan to determine if mechanical hyperalgesia had developed. Threshold measurements were repeated three times and the values averaged. Rats were then treated with the test preparations (similar to that described for the writhing test above) 3h post-carrageenan administration. PWTs were taken again at 3.5, 4, 4.5, 5, 5.5, 6 and 6.5 h post-carrageenan.

### 2.5.3 Hot Plate Test

The test was carried out as described by Woolfe and MacDonald (1944). Mice were placed on a hot plate (Model 7280, Ugo Basile Inc., Milan, Italy) heated to 55±0.5 °C and the baseline reaction time of the animals to nociceptive responses such as licking/shaking of the paws or jumping were recorded. C3H mice (n=5) were treated with HAE, EAE or PEE (10, 30 and 100 mg $kg^{-1}$, *p.o.*), morphine (0.3, 1 and 3 mg $kg^{-1}$, i.p) or vehicle (10 ml $kg^{-1}$, *p.o.*). Immediately an animal signalled a pain reaction by licking the paws or jumping, it was removed from the plate. The reaction times were taken again at 0.5h, 1h and 2h after a latency period of 30 min (i.p.) or 1 h (*p.o.*) following the administration of the vehicle, drugs or extract. A cut-off reaction time was set at 30s to prevent damage to tissues of the foot.



### 2.5.4 Formalin-induced Nociception

The formalin test first described by Dubuisson and Dennis (1977) was carried out as described by Malmberg and Yaksh (1995), with a few modifications.

Each animal was assigned and acclimatized to one of 20 Perspex chambers (15 cm × 15 cm× 15 cm) for one hour prior to formalin injection. Rats were then pre-treated with PEE, EAE or HAE (10, 30 and 100 mg $kg^{-1}$, *p.o.*; morphine (0.3, 1 and 3 mg $kg^{-1}$, i.p) and vehicle (10 ml $kg^{-1}$, *p.o.*), 30 min for i.p. route and 1h for oral administration before intraplantar injection of 10 µl of 5 % formalin. The animals were immediately returned individually into the testing chamber. A mirror angled at 45° below the floor of the chamber allowed a complete view of the paws. The behaviour of the animals was then captured for 60 min for analysis by a camcorder (Everio™ model GZ-MG1300, JVC, Tokyo, Japan) placed in front of the mirror.

Pain response was scored for 60 min, starting immediately after formalin injection. A nociceptive score for each 5 min time block was determined by measuring the time spent biting/licking the injected paw (Hayashida *et al.*, 2003). Tracking of the behaviour was done using a public domain software JWatcher™

Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia available at http://www.jwatcher.ucla.edu/). Average nociceptive score was determined for each time block as the product of the frequency and duration of biting/licking per minute was used as nociceptive score. Data was expressed as mean±S.E.M of scores between 0-10 min (first phase/neurogenic) and 10-60 min (second phase/inflammatory) post formalin injection.

### 2.5.5 Analysis of the mechanism of action of the extracts in the formalin test

To investigate the possible mechanisms by which the extracts exerted their anti-nociceptive activity, further experiments were carried out with various antagonists in the formalin test. The doses of antagonists, agonists and other drugs were selected on the basis of previous literature data and in pilot experiments in our laboratory. The formalin test was chosen for this purpose because of the specificity and sensitivity in nociception transmission (Le Bars *et al.*, 2001).

#### *2.5.5.1 Involvement of the Opioidergic system*

Rats were pre-treated with naloxone (2 mg $kg^{-1}$, i.p, a non-selective opioid receptor antagonist). After 15 min, the rats received HAE, EAE or PEE (100 mg $kg^{-1}$, *p.o.*), morphine (1 mg $kg^{-1}$, i.p) or vehicle (10 ml $kg^{-1}$, *p.o.*). The nociceptive response to intraplantar injection of formalin was recorded 60 min after administration of extracts or vehicle and 30 min after morphine administration.

#### *2.5.5.2 Involvement of the Adenosinergic system*

Rats were pre-treated with theophylline (10 mg $kg^{-1}$, i.p, a non-selective adenosine receptor antagonist). After 15 min, the rats received HAE, EAE or PEE (100 mg $kg^{-1}$, *p.o.*), morphine (1 mg $kg^{-1}$, i.p) or vehicle (10 ml $kg^{-1}$, *p.o.*). The nociceptive response to intraplantar injection of formalin was recorded 60 min after administration of extracts or vehicle and 30 min after morphine administration.

#### 2.5.5.3 *Involvement of ATP-sensitive $K^+$ channels*

Rats were pre-treated with glibenclamide (8 mg $kg^{-1}$, *p.o.*, an ATP-sensitive $K^+$ channel inhibitor) and after 30 min received HAE, EAE or PEE (100 mg $kg^{-1}$, *p.o.*), morphine (1 mg $kg^{-1}$, i.p) or vehicle (10 ml $kg^{-1}$, *p.o.*). The nociceptive responses to formalin were recorded 60 min after administration of extracts or vehicle and 30 min after morphine administration.

#### 2.5.5.4 *Participation of the Muscarinic Cholinergic system*

Rats were pre-treated with atropine (5 mg $kg^{-1}$, i.p, a non-selective muscarinic receptor antagonist) and after 15 min received HAE, EAE or PEE (100 mg $kg^{-1}$, *p.o.*), morphine (1 mg $kg^{-1}$, i.p) or vehicle (10 ml $kg^{-1}$, *p.o.*). The nociceptive responses to formalin were recorded 60 min after administration of extracts or vehicle and 30 min after morphine administration.

#### 2.5.5.5 *Involvement of the $\alpha_2$-Adrenoceptors*

Rats were pre-treated with yohimbine (3 mg $kg^{-1}$, *p.o.*, a selective adrenoceptor antagonist,) and after 30 min received HAE, EAE or PEE (100 mg $kg^{-1}$, *p.o.*), morphine (1 mg $kg^{-1}$, i.p) or vehicle (10 ml $kg^{-1}$, *p.o.*). The nociceptive responses to formalin were recorded 60 min after administration of extracts or vehicle and 30 min after morphine administration.

## 2.6 ANXIOLYTIC EFFECTS

Comorbidity of depression and anxiety disorders with pain has implications for the outcome of pain and also possibly for the outcome of depression and anxiety disorders (Mico *et al.*, 2006; Gureje, 2008). This makes depression and anxiety management during pain treatment very important. Traditionally, the plant is used as a sedative and also in the management of Schizophrenia and epilepsy (Abbiw, 1990). The establishment of the stem bark extracts of *T. monadelpha* as having analgesic properties with possible CNS effects may help treat pain (even chronic and neuropathic) comorbid with anxiety/depression (Mico *et al.*, 2006).

In this study, therefore, some CNS effects of the stem bark extracts of *T. monadelpha* were investigated so as to establish its CNS activity. Experimental paradigms such as elevated plus-maze and light–dark box tests were used to study effects of the plant on anxiety. To rule out any motor impairment by the extracts, the beam traversal and rotarod tests were also used.

### 2.6.1 Elevated Plus-Maze Test

This test was adapted from the methods described by Pellow *et al.*, (1985) and Lister *et al.*, (1987). The apparatus was made of Plexiglas and consisted of two open arms (30 cm x 5 cm x 0.5 cm) and two enclosed arms (30 cm x 5 cm x 15 cm) extending from a central platform (5 cm x 5 cm) forming a plus-sign with like arms opposite each other (Fig. 2.1b). The maze was elevated 60 cm from the floor. A 25-W red fluorescent light 100 cm above the maze served as the source of illumination. Mice were randomly assigned to sixteen experimental groups (n=5) and treated with HAE, EAE or PEE (10, 30 and 100 mg $kg^{-1}$, *p.o.*), diazepam (0.1, 0.3 and 1.0 mg $kg^{-1}$, i.p), pentylenetetrazole (3, 10 and 30 mg $kg^{-1}$, i.p) and vehicle (10 ml $kg^{-1}$, *p.o.*) distilled water, 30 min after i.p injection and 1h after oral administration of the test preparations. Mice were placed individually in succession in the central platform of the maze for 5 minutes and their behaviour videotaped with a camcorder (Everio™ model GZ-MG1300, JVC, Tokyo, Japan) placed above the maze. Behavioural parameters were scored from the videotapes using a public domain software JWatcher, Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia available at http://www.jwatcher.ucla.edu/) as follows: (1) Number of entries into and time spent in each arm i.e. closed and open arms, (2) Number and duration of protected and unprotected stretch-attend postures, (3) Number and duration of protected and unprotected head dips.

Entry into an arm was defined as the animal placing all four paws into the arm. Protected head dipping was defined as the mouse stretching to dip its head into the open space and observing the environment with the body remaining in a

closed arm or the central platform while in unprotected head dipping, the mouse dips its head into the open space and observing the environment with the body being in an open arm. Protected stretch attend postures were defined as the mouse stretching forward and retracting without moving forward its feet whilst in the closed arm or central platform of the maze whereas unprotected stretch-attend postures were defined as the mouse exhibiting this behaviour whilst in the open arms.

### 2.6.2 Light-Dark Exploratory Test

The method used in this test has been described by Ardayfio *et al.*, (2006) with little modifications (Fig. 2.1a). The apparatus was a wooden box (36 cm x 33 cm x 30 cm) divided into two compartments by a wooden board with a small opening (8 cm x 8 cm) connecting the compartments. The larger compartment comprised two-thirds of the apparatus, painted white, open and illuminated by a 60-W lamp placed above the compartment. The smaller compartment was painted black and had a cover that was closed during testing. Mice were divided into sixteen groups (n=5) and treated with HAE, EAE or PEE, diazepam, pentylenetetrazole and vehicle as described above for the elevated plus-maze test. At the beginning of the experiment, mice were placed individually at a far corner of the dark compartment facing the light compartment, videotaped and scored in a manner similar to that described in the elevated plus-maze test. These parameters were assessed: (1) Total time spent in each compartment and (2) Total number of transitions between the compartments.

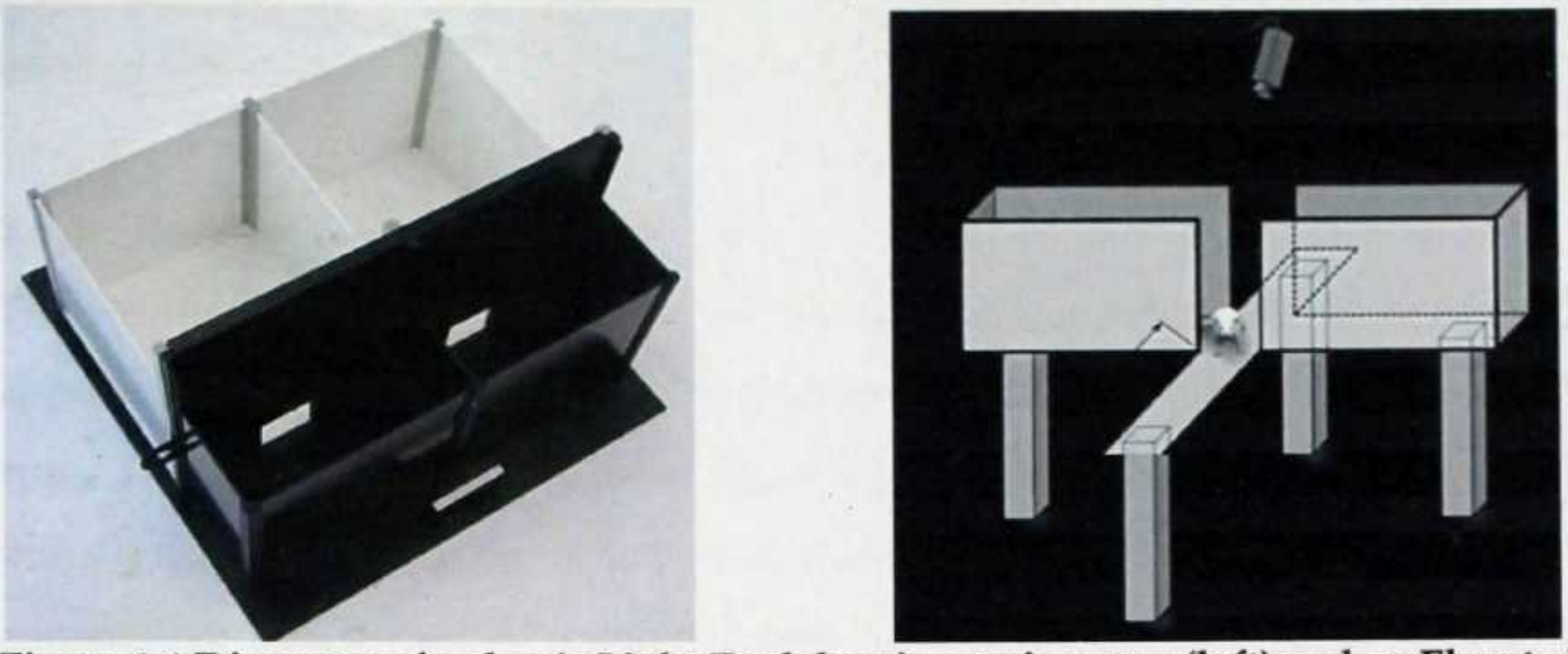

**Figure 2.1 Diagrams of a classic Light-Dark box in a twin setup (left) and an Elevated plus-maze setup (right)**



## 2.7 MOTOR COORDINATION EFFECTS

### 2.7.1 Beam Traversal Test

The beam walking task was adapted from the methods described by Carter *et al.*, (2001) and Meredith *et al.*, (2006). The apparatus consisted of a wooden beam 12 mm wide and 1m long elevated 50 cm above the floor on two narrow support stands (3 cm cross-section, 50 cm high) with one end on each stand (Fig. 2.2a). A goal box (20 cm on each side, with a 4 cm x 5 cm entrance hole) was secured on one of the narrow support stands. A 60-W desk lamp was positioned above the start end (the one without the goal box) of the beam to create an aversive stimulus (bright light). The mice were trained to traverse the beam (three consecutive trials each day for three days) to the goal box in less than 20s. Mice that could not traverse into the goal box within 20s were not used for the test. On the day of the experiment, mice were divided into groups (n=5) and treated with preparations: HAE, EAE or PEE (10, 30 and 100 mg $kg^{-1}$, *p.o*), diazepam (0.1, 0.3 and 1.0 mg $kg^{-1}$, i.p) or vehicle (10 ml $kg^{-1}$, *p.o.*) distilled water, 30 min after i.p. and 1h after oral administration of the test preparations. During testing, mice were placed individually at the start end of the beam and allowed up to 60s to traverse the beam. The test sessions were recorded with a video camera and later scored for total number of steps, stepping errors and time to traverse the beam.

Mice which did not cross the beam or fell down from the beam were given a score of 60s.

### 2.6.2 Rotarod Test

The effect of the extracts on motor co-ordination was assessed using the rotarod apparatus (Model 7600, Ugo Basile, Cormerio, Italy) rotating at a speed of 25 rpm. The method is as described by (Dunham and Miya, 1957). Mice were initially selected for their ability to remain on the rotarod for at least two consecutive 120s trials before the test day. On the test day (24h after selection), the latency to fall from the rotarod (one trial of 120s) was determined. Mice were randomly divided into thirteen groups (n=5) and received HAE, EAE or PEE (10, 30 and 100 mg $kg^{-1}$, *p.o.*), diazepam (0.1-1.0 mg $kg^{-1}$, i.p.) or vehicle (10 ml $kg^{-1}$, *p.o.*). Thirty minutes (i.p.) or 1 hour (*p.o.*) after the treatments, the latencies to fall from the rod were measured. Mice that stayed on the rotarod for more than 120s were given the maximum score, 120s (Fig. 2.2b).


**Figure 2.2 Diagrams of the Beam traversal test setup (left) and Rotarod test (right)**

## 2.8 STATISTICAL ANALYSIS

All data are presented as mean±S.E.M. The time-course curves were subjected to two-way (*treatment* × *time*) repeated measures analysis of variance (ANOVA) with Bonferroni's *post hoc* test. Total nociceptive score for each treatment was calculated in arbitrary unit as the area under the curve (AUC). To determine the percentage inhibition for each treatment, the following equation was used:

$$\%\, inhibition = \left( \frac{AUC_{control} - AUC_{treatment}}{AUC_{control}} \right) \times 100$$

Differences in AUCs were analyzed using one-way ANOVA with drug treatment as a between-subjects factor. Further comparisons between vehicle and drug-treated groups were performed using the Newman-Keuls test.

Doses for 50 % of the maximal effect ($ED_{50}$) for each drug were determined by using an iterative computer least squares method, with the following nonlinear regression (three-parameter logistic) equation:

$$Y = \frac{a + (b - a)}{\left(1 + 10^{(LogED_{50} - X)}\right)}$$

Where, X is the logarithm of dose and Y is the response. Y starts at *a* (the bottom) and goes to *b* (the top) with a sigmoid shape.

The fitted midpoints ($ED_{50}$s) of the curves were compared statistically using *F* test. GraphPad Prism for Windows, Version 5 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses and $ED_{50}$ determinations. $P \leq 0.05$ was considered statistically significant in all analysis.

# CHAPTER 3

# RESULTS

## 3.1 PHYTOCHEMICAL TESTS

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, saponins, sterols, tannins and triterpenes in HAE. EAE had alkaloids, glycosides, tannins and triterpenes whiles PEE showed the presence of alkaloids, sterols and triterpenes (Table 1).

**Table 1 Phytochemical constituents of the HAE, EAE and PEE of the stem bark of *T. monadelpha***

| CONSTITUENTS | INFERENCE | | |
|---|---|---|---|
| | **HAE** | **EAE** | **PEE** |
| **ALKALOIDS** | + | + | + |
| **FLAVONOIDS** | + | - | - |
| **GLYCOSIDES** | + | + | - |
| **SAPONINS** | + | - | - |
| **STEROLS** | + | - | + |
| **TANNINS** | + | + | - |
| **TRITERPENES** | + | + | + |

+: present, - : not detected

## 3.2 ANTI-NOCICEPTIVE EFFECTS

### 3.2.1 Acetic Acid-induced Writhing Test

Acetic acid injection intraperitoneally produced the writhing response exhibited as contractions of the abdominal muscle combined with the stretching of the at least a hind limb in the vehicle-treated group. Figure 3.1 shows the effect of the

extracts and diclofenac on acetic acid-induced writhing during the 30 min observation period. HAE (10-100 mg $kg^{-1}$, *p.o.*) dose-dependently and significantly ($F_{3,16}$=38.09, $P$<0.0001) reduced the number of abdominal writhes with a maximum inhibition of 91.58±7.13 % at 100 mg $kg^{-1}$ (Fig. 3.1b). EAE (10-100 mg $kg^{-1}$, *p.o.*) caused a significant ($F_{3,16}$=15.77; $P$<0.0001), dose-dependent reduction in the number of writhes with a maximum inhibition of 84.20±11.67 % at 100 mg $kg^{-1}$ (Fig. 3.1d). PEE (10-100 mg $kg^{-1}$, *p.o.*) also significantly ($F_{3,16}$=68.63; $P$<0.0001) reduced the number of writhes in a dose-dependent manner with a maximum inhibition of 96.55±6.47 % at 100 mg $kg^{-1}$ (Fig. 3.1f). Additionally, diclofenac, (3-30 mg $kg^{-1}$, i.p) profoundly inhibited ($F_{3,16}$=51.59, $P$<0.0001) abdominal writhing by a maximum of 92.98±2.70 % (Fig. 3.1h).

From the $ED_{50}$ values obtained by non-linear regression, PEE (2.11±0.46 mg $kg^{-1}$) was comparable in potency to HAE ($ED_{50}$: 2.54±1.22 mg $kg^{-1}$; $F_{2,26}$=2.48, $P$=0.1035) but more potent than EAE (6.29±3.65 mg $kg^{-1}$; $F_{2,26}$=7.79, $P$=0.0022). However, diclofenac was the most potent (1.02±0.26 mg $kg^{-1}$; Fig. 3.2).

### 3.2.2 Carrageenan-induced Mechanical Hyperalgesia Test

All three extracts of *T. monadelpha*, as well as morphine increased significantly the paw withdrawal latency, calculated as a percentage of the maximum possible effect (% MPE). HAE, EAE and PEE (30-300 mg $kg^{-1}$, *p.o.*) administered 3h after carrageenan administration produced a significant [HAE: $F_{3,16}$=43.84, $P$<0.0001; EAE: $F_{3,16}$=9.15; $P$=0.0009; PEE: $F_{3,16}$=15.88, $P$<0.0001; Fig. 3.2a-f] and dose-dependent increase in the paw withdrawal latencies with maximal effect at 300 mg $kg^{-1}$ (Fig. 3.3a-f). Morphine (0.3-3mg $kg^{-1}$, i.p) also profoundly and significantly ($F_{3,16}$=55.10; $P$<0.0001; Fig. 3.2g-h) reversed completely the carrageenan-induced mechanical hyperalgesia with the highest effect at 3 mg $kg^{-1}$ (Fig. 3.2g-h). The $ED_{50}$ values obtained were 57.13±25.44 mg $kg^{-1}$, 74.63±21.75 mg $kg^{-1}$ and 46.97±25.33 mg $kg^{-1}$ for PEE, HAE and EAE respectively (Table 3.1). Morphine however was the most potent (1.70±0.59 mg $kg^{-1}$, Fig. 3.4).

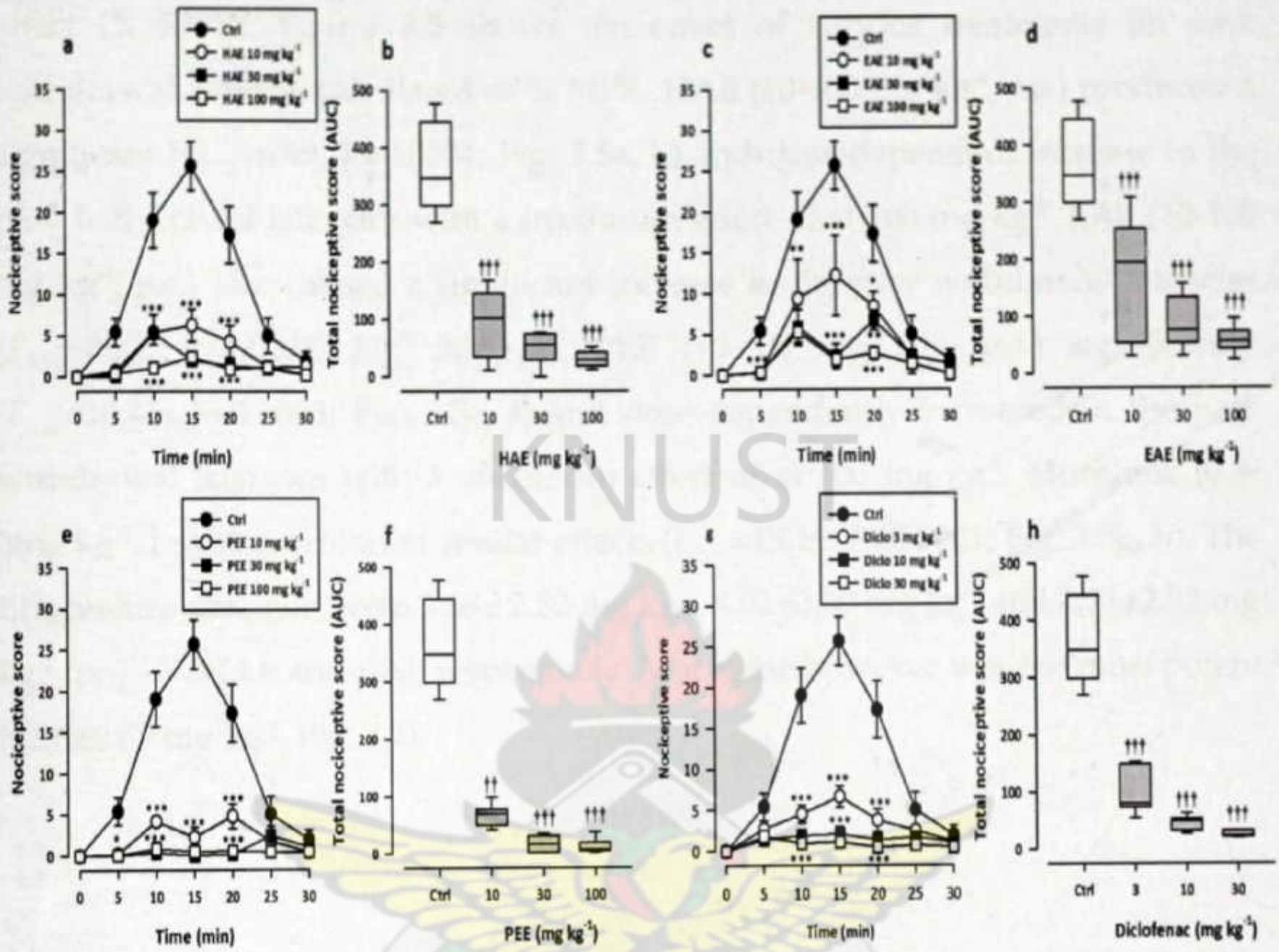


**Figure 3.1 Effects of HAE (10-100 mg kg$^{-1}$, *p.o.*), EAE (10-100 mg kg$^{-1}$, *p.o.*), PEE (10-100 mg kg$^{-1}$, *p.o.*) and diclofenac (3-30 mg kg$^{-1}$, i.p) on the time course curves (a, c, e, g) and the total nociceptive score (calc. as AUCs) (b, d, f, h) of acetic acid-induced writhing in mice. Nociceptive scores are shown in 5 min time blocks up to 30 min for the time course curves. Data are presented as mean±S.E.M (n=5). The lower and upper margins of the boxes (b, d, f, h) represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box.*P≤0.05, **P≤0.01, ***P≤0.001 compared to control group (ctrl) (Two-way repeated measures ANOVA followed by Bonferroni's post hoc). †P≤0.05, ††P≤0.01, †††P≤0.001 compared to control group (ctrl) (one-way ANOVA followed by Newman-Keuls post hoc)**

### 3.2.3 Hot Plate Test

All three extracts of *T. monadelpha*, as well as morphine increased significantly the paw withdrawal latency, calculated as a percentage of the maximum possible effect (% MPE). Figure 3.5 shows the effect of various treatments on paw withdrawal latency, calculated as % MPE. HAE (10-100 mg $kg^{-1}$, *p.o.*) produced a significant ($F_{3,16}$=6.89, $P$=0.0034; Fig. 3.5a, b) and dose-dependent increase in the paw withdrawal latencies with a maximum effect of at 100 mg $kg^{-1}$. EAE (10-100 mg $kg^{-1}$, *p.o.*) also caused a significant increase in the paw withdrawal latencies ($F_{3,16}$=7.22; $P$=0.0028; Fig. 3.5c, d). PEE (10-100 mg $kg^{-1}$, *p.o.*) significantly ($F_{3,16}$=10.45; $P$=0.0005; Fig. 3.5e, f) and dose-dependently increased in the paw withdrawal latencies with a maximum effect of at 100 mg $kg^{-1}$. Morphine (0.3-3mg $kg^{-1}$, i.p) also exhibited similar effects ($F_{3,16}$=15.16; $P$<0.0001; Fig. 3.5g, h). The $ED_{50}$ values obtained were 3.68± 2.82 mg $kg^{-1}$, 4.92±3.60 mg $kg^{-1}$ and 2.91±2.93 mg $kg^{-1}$ for PEE, HAE and EAE respectively. Morphine however was the most potent (0.16±0.09 mg $kg^{-1}$, Fig. 3.7).

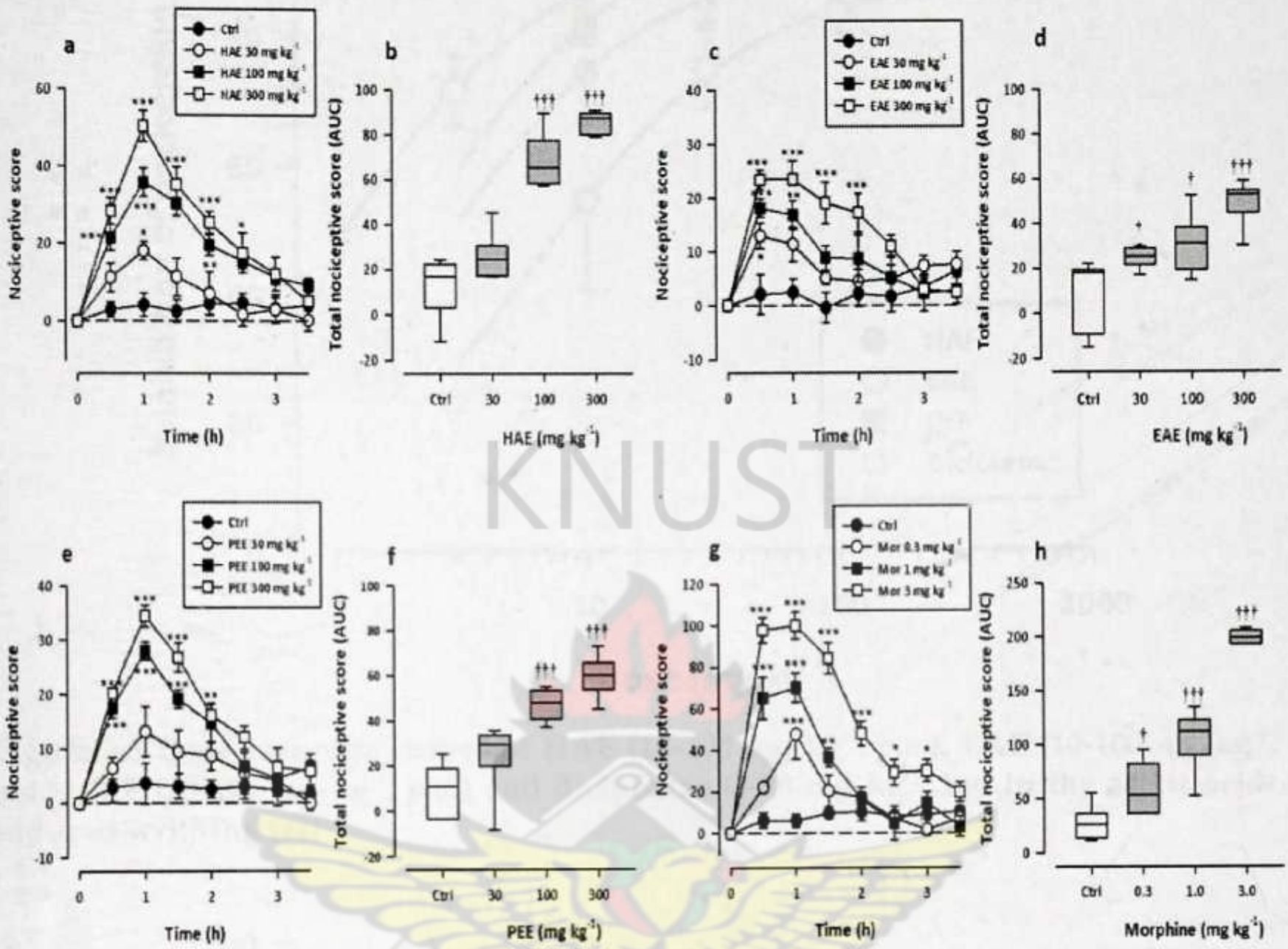


**Figure 3.2 Effects of HAE (10-100 mg kg$^{-1}$, *p.o.*), EAE (10-100 mg kg$^{-1}$, *p.o.*), PEE (10-100 mg kg$^{-1}$, *p.o.*) and morphine (0.3-3.0 mg kg$^{-1}$, i.p) on the time course curves (a, c, e, g) and the total nociceptive score (AUCs) (b, d, f, h) of carrageenan-induced mechanical hyperalgesia in rats. Data was presented as mean±S.E.M. (n=5). The lower and upper margins of the boxes (b, d, f, h) represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box.*P≤0.05, **P≤0.01, ***P≤0.001 compared to control group (ctrl) (Two-way repeated measures ANOVA followed by Bonferroni's post hoc). †P≤0.05, ††P≤0.01, †††P≤0.001 compared to control group (ctrl) (one-way ANOVA followed by Newman-Keuls post hoc)**

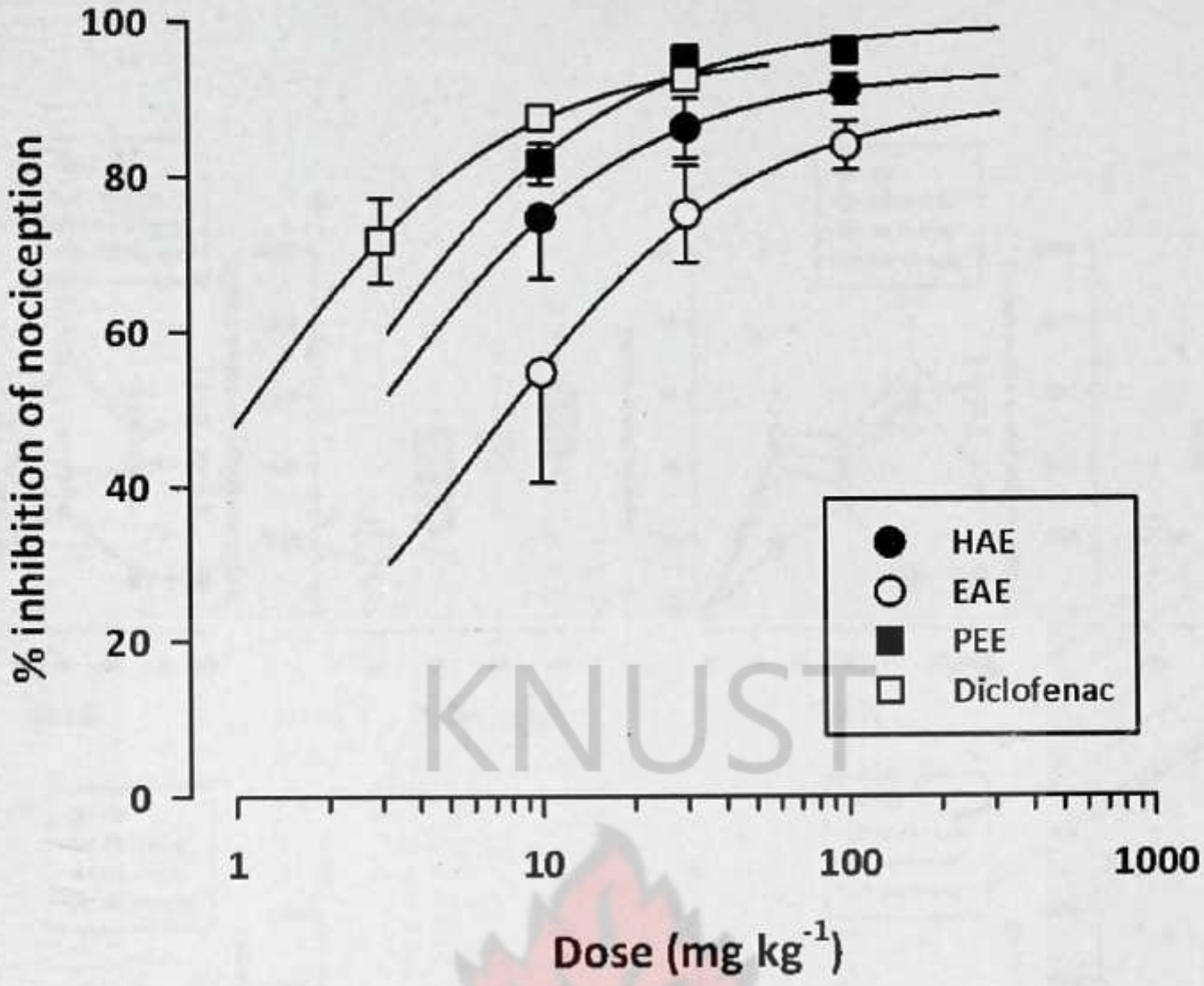


Figure 3.3 Dose response curves of HAE (10-100 mg $kg^{-1}$, *p.o.*), EAE (10-100 mg $kg^{-1}$, *p.o.*), PEE (10-100 mg $kg^{-1}$, *p.o.*) and diclofenac (3-30 mg $kg^{-1}$, i.p) in the acetic acid-induced writhing test

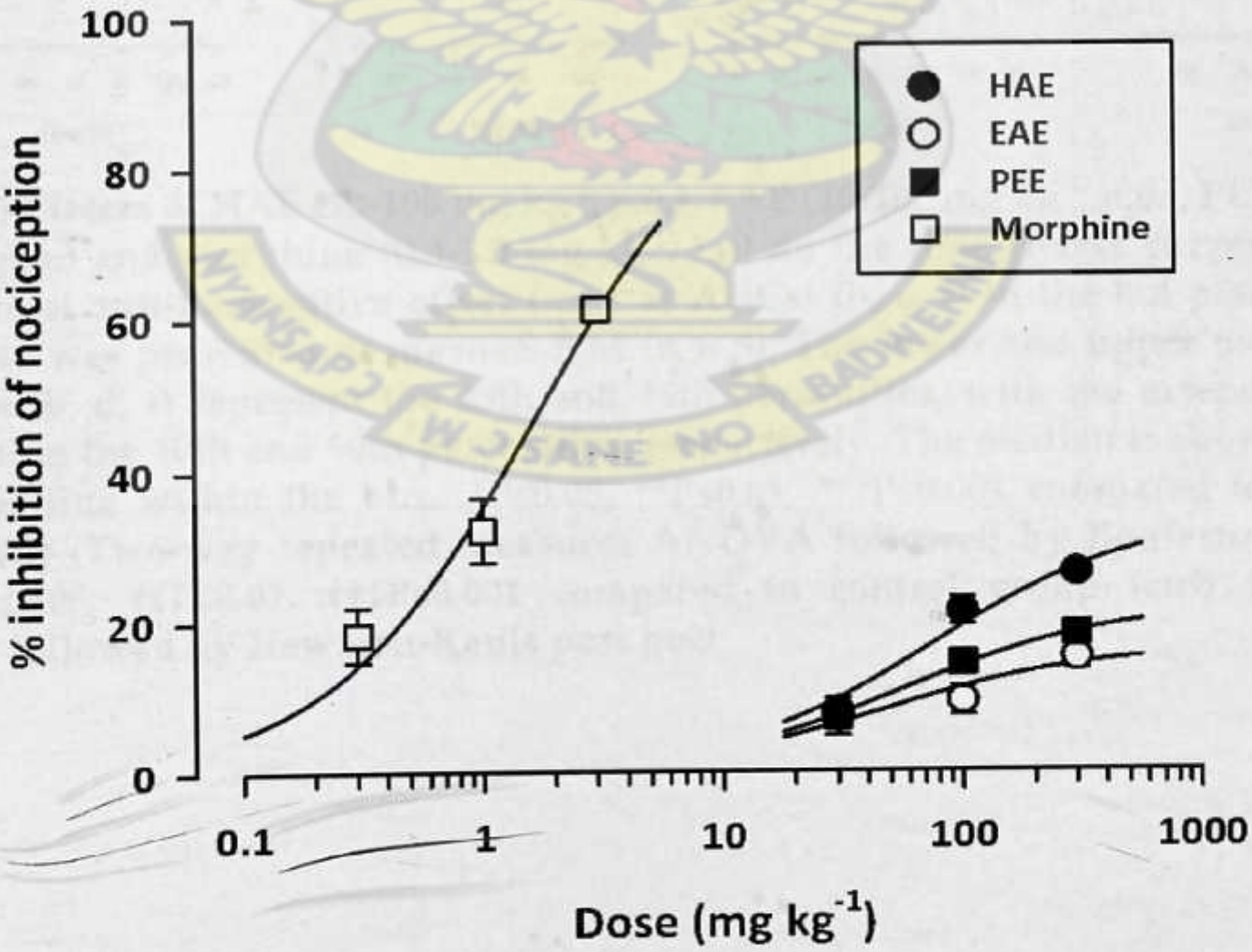


Figure 3.4 Dose response curves of HAE (10-100 mg $kg^{-1}$, *p.o.*), EAE (10-100 mg $kg^{-1}$, *p.o.*), PEE (10-100 mg $kg^{-1}$, *p.o.*) and morphine (0.3-3.0 mg $kg^{-1}$, i.p) in the carrageenan-induced hyperalgesia test

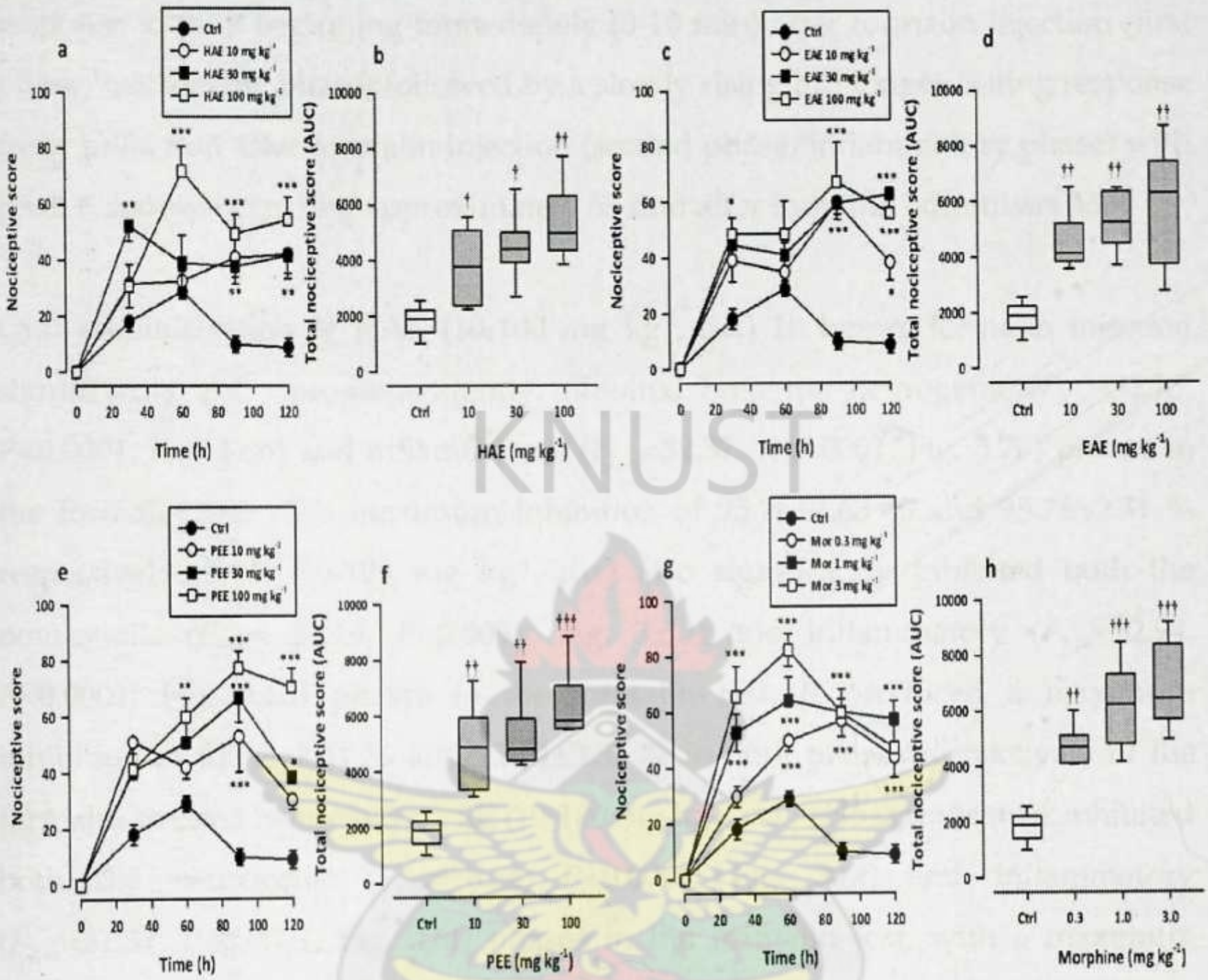


**Figure 3.5 Effects of HAE (10-100 mg kg$^{-1}$, *p.o.*), EAE (10-100 mg kg$^{-1}$, *p.o.*), PEE (10-100 mg kg$^{-1}$, *p.o.*) and morphine (0.3-3.0 mg kg$^{-1}$, i.p) on the time course curves (a, c, e) and the total anti-nociceptive effect (calc. as AUCs) (b, c, f) in the hot plate test in mice. Data was presented as mean±S.E.M (n = 5). The lower and upper margins of the boxes (b, d, f) represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box. .*P≤0.05, **P≤0.01, ***P≤0.001 compared to control group (ctrl) (Two-way repeated measures ANOVA followed by Bonferroni's post hoc). †P≤0.05, ††P≤0.01, †††P≤0.001 compared to control group (ctrl) (one-way ANOVA followed by Newman-Keuls post hoc)**

### 3.2.4 Formalin Test

Intraplantar injection of the right hind paw with 5 % formalin evoked a characteristic biphasic response in rats. This consisted of an initial intense response to pain beginning immediately (0-10 min) after formalin injection (first phase/neurogenic phase) followed by a slowly rising but longer lasting response from 10-60 min after formalin injection (second phase/inflammatory phase) with peak response occurring approximately 35 min after formalin administration.

Oral administration of HAE (10-100 mg $kg^{-1}$, *p.o.*) 1h before formalin injection significantly and dose-dependently inhibited both the neurogenic ($F_{3,16}$=32.75, $P$<0.0001; Fig. 3.6b) and inflammatory ($F_{3,16}$=32.37; $P$<0.0001; Fig. 3.7b) phases in the formalin test with maximum inhibition of 95.76±1.63 % and 95.76±2.31 % respectively. EAE (10-100 mg $kg^{-1}$, *p.o.*) also significantly inhibited both the neurogenic ($F_{3,16}$= 24.16, $P$<0.0001; Fig. 3.6d) and inflammatory ($F_{3,16}$=22.94, $P$<0.0001; Fig. 3.6d) phases in the formalin test. It produced a maximum inhibition of 91.17±2.41 % and 92.54±3.35 % in both phases respectively of the formalin-evoked behaviours. PEE (10-100 mg $kg^{-1}$, *p.o.*) also significantly inhibited both the neurogenic ($F_{3,16}$=18.16; $P$<0.0001; Fig. 3.6f) and inflammatory ($F_{3,16}$=21.31, $P$<0.0001; Fig. 3.6f) phases in the formalin test, with a maximum inhibition of 97.72±1.50 % and 92.12±3.60 % respectively. Morphine (0.3-3.0 mg $kg^{-1}$, i.p) significantly and dose-dependently inhibited both the neurogenic ($F_{3,16}$=25.28, $P$<0.0001; Fig. 3.6h) and inflammatory ($F_{3,16}$=27.69, $P$<0.0001; Fig. 3.6h) phases in the formalin test, with a maximum inhibition of 88.54±2.64 % and 91.28±2.22 % respectively.

The $ED_{50}$ values obtained were 8.15±2.30 mg $kg^{-1}$, 3.23±0.94 mg $kg^{-1}$ (PEE); 6.43±1.14 mg $kg^{-1}$, 3.16±0.37 mg $kg^{-1}$ (HAE) and 7.19±1.45 mg $kg^{-1}$, 2.61±0.83 mg $kg^{-1}$ (EAE). Morphine was the most potent in both phases (0.26±0.05 mg $kg^{-1}$, 0.18±0.02 mg $kg^{-1}$, Fig. 3.8).

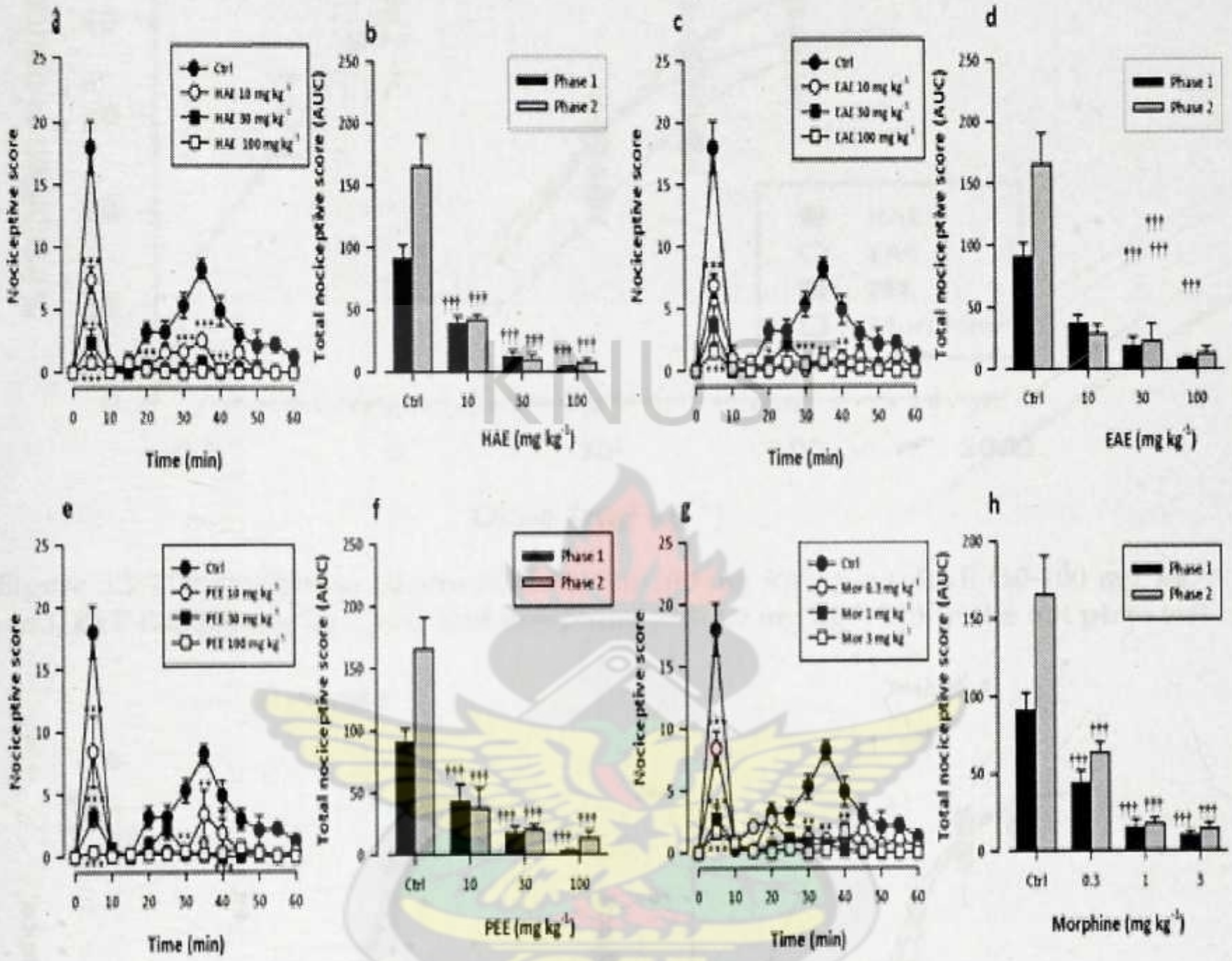


**Figure 3.6 Effects of HAE (10-100 mg kg$^{-1}$, *p.o.*), EAE (10-100 mg kg$^{-1}$, *p.o.*), PEE (10-100 mg kg$^{-1}$, *p.o.*) and morphine (0.3-3.0 mg kg$^{-1}$, i.p) on the time course curves (a, c, e) and the total nociceptive score (calc. as AUCs) (b, d, f) of formalin-induced nociception in rats. Nociceptive scores are shown in 5 min time blocks up to 60 min for the time course curves. Data are presented as mean±S.E.M (n=5). *P≤0.05, **P≤0.01, ***P≤0.001 compared to control group (ctrl) (Two-way repeated measures ANOVA followed by Bonferroni's post hoc). †P≤0.05, ††P≤0.01, †††P≤0.001 compared to control group (ctrl) (one-way ANOVA followed by Newman-Keuls post hoc)**

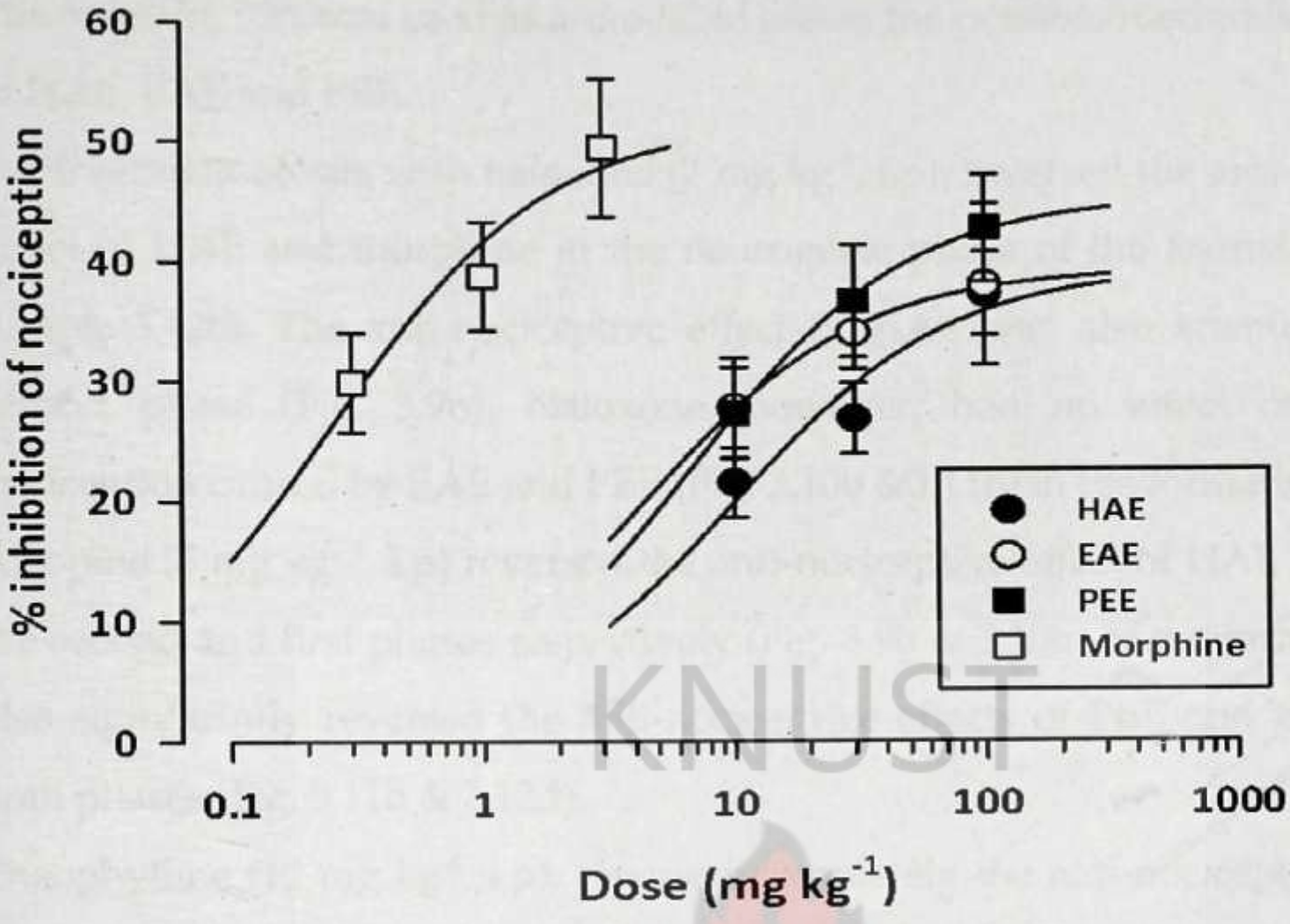


Figure 3.7 Dose response curves of HAE (10-100 mg $kg^{-1}$, *p.o.*), EAE (10-100 mg $kg^{-1}$, *p.o.*), PEE (10-100 mg $kg^{-1}$, *p.o.*) and morphine (0.3-3.0 mg $kg^{-1}$, i.p) in the hot plate test

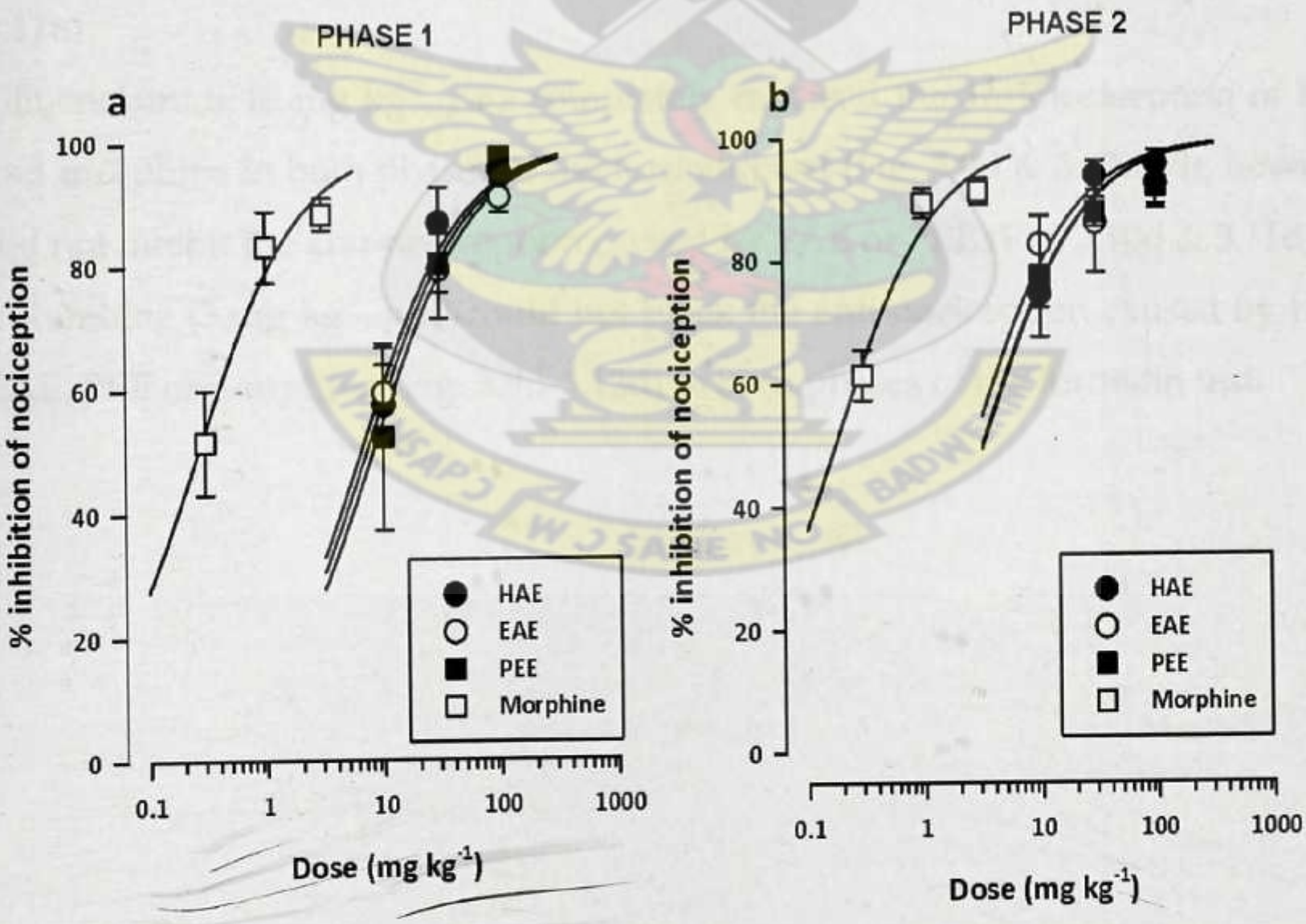


Figure 3.8 Dose response curves of HAE (10-100 mg $kg^{-1}$, *p.o.*), EAE (10-100 mg $kg^{-1}$, *p.o.*), PEE (10-100 mg $kg^{-1}$, *p.o.*) and morphine (0.3-3.0 mg $kg^{-1}$, i.p) in phase1 (a) and 2 (b) of the formalin test

### 3.2.5 Assessment of the possible mechanism of action of HAE, EAE and PEE

The formalin test was used as a model to assess the possible mechanism of action of HAE, EAE and PEE.

Pre-treatment of rats with naloxone (2 mg $kg^{-1}$, i.p), reversed the anti-nociceptive effect of HAE and morphine in the neurogenic phase of the formalin test (Fig. 3.9b & 3.12b). The anti-nociceptive effect of HAE was also attenuated in the second phase (Fig. 3.9b). Naloxone, however, had no effect on the anti-nociception caused by EAE and PEE (Fig. 3.10b &3.11b) in the formalin test.

Atropine (5 mg $kg^{-1}$, i.p) reversed the anti-nociceptive effect of HAE and EAE in the second and first phases respectively (Fig. 3.9b & 3.10b) of the formalin test. It also significantly reversed the anti-nociceptive effects of PEE and morphine in both phases (Fig. 3.11b & 3.12d).

Theophylline (10 mg $kg^{-1}$, i.p), reversed completely the anti-nociception of HAE and morphine in both phases of the formalin test (Fig. 3.9b & 3.12b). It, however, had no effects on the anti-nociception caused by EAE and PEE (Fig. 3.10b & 3.11b).

Glibenclamide (8 mg $kg^{-1}$, *p.o.*) completely reversed the anti-nociception of HAE and morphine in both phases of the formalin test (Fig. 3.9d & 3.12d). It, however, did not inhibit the anti-nociception caused by EAE or PEE (Fig. 3.10d & 3.11d).

Yohimbine (3 mg $kg^{-1}$, *p.o.*) could not block the anti-nociception caused by HAE, EAE, PEE or morphine (Fig. 3.9d-3.12d) in both phases of the formalin test.

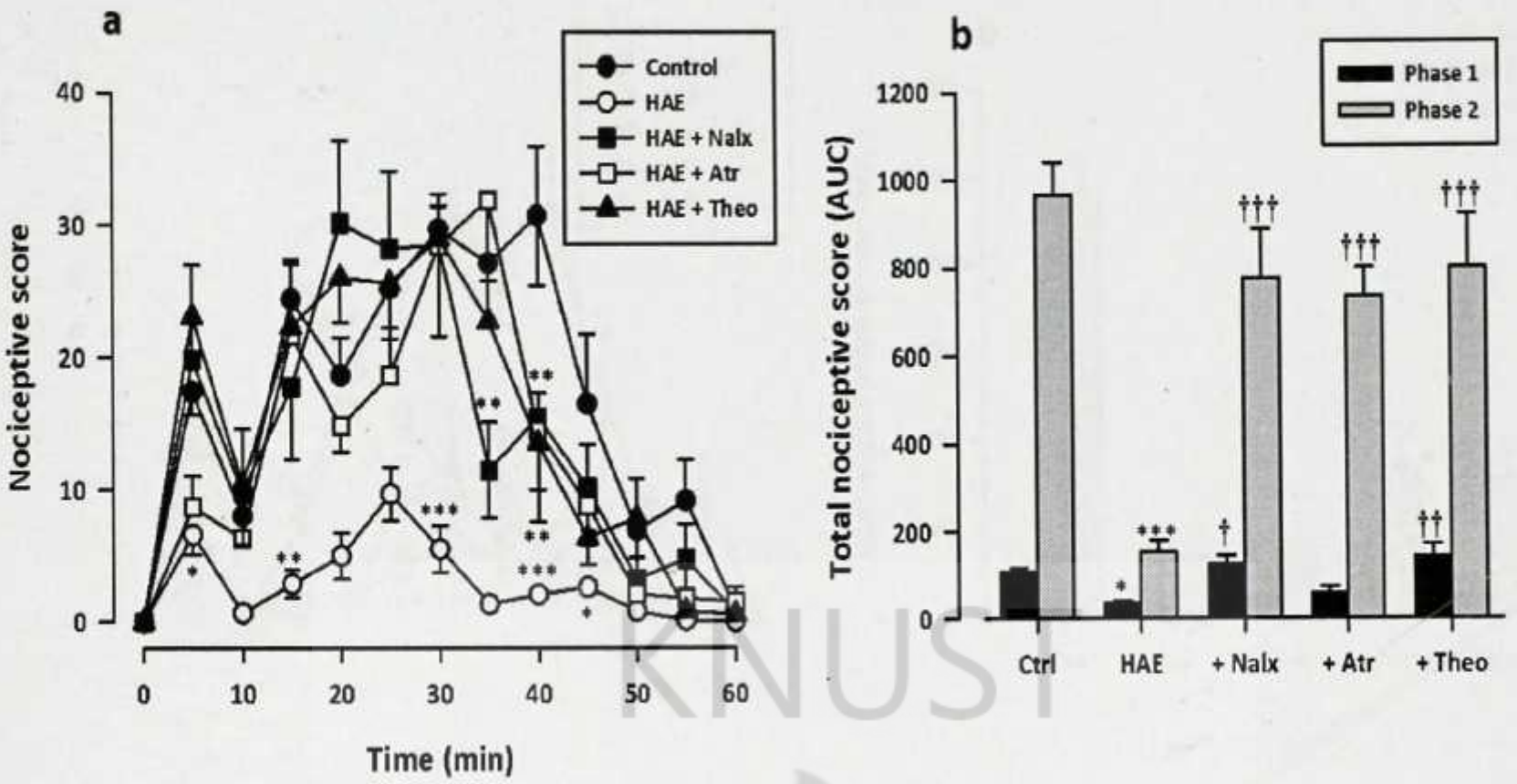


Figure 3.9 Effects of pre-treatment of rats with atropine (5 mg $kg^{-1}$, i.p), naloxone (2 mg $kg^{-1}$, i.p), theophylline (10 mg $kg^{-1}$, i.p), glibenclamide (8 mg $kg^{-1}$, *p.o.*) and yohimbine (3 mg $kg^{-1}$, *p.o.*) on the anti-nociceptive effects of HAE (100 mg $kg^{-1}$, *p.o.*) on the time course curves (a, c) and the total nociceptive score (calc. as AUCs) (b, d) in the formalin test. Nociceptive scores are shown in 5 min time blocks up to 60 min for the time course curves. Data are presented as mean±S.E.M (n=5). *P≤0.05, **P≤0.01, ***P≤0.001 compared to control group (ctrl) (Two-way repeated measures ANOVA followed by Bonferroni's post hoc). †P≤0.05, ††P≤0.01, †††P≤0.001 compared to control group (ctrl) (one-way ANOVA followed by Newman-Keuls post hoc)

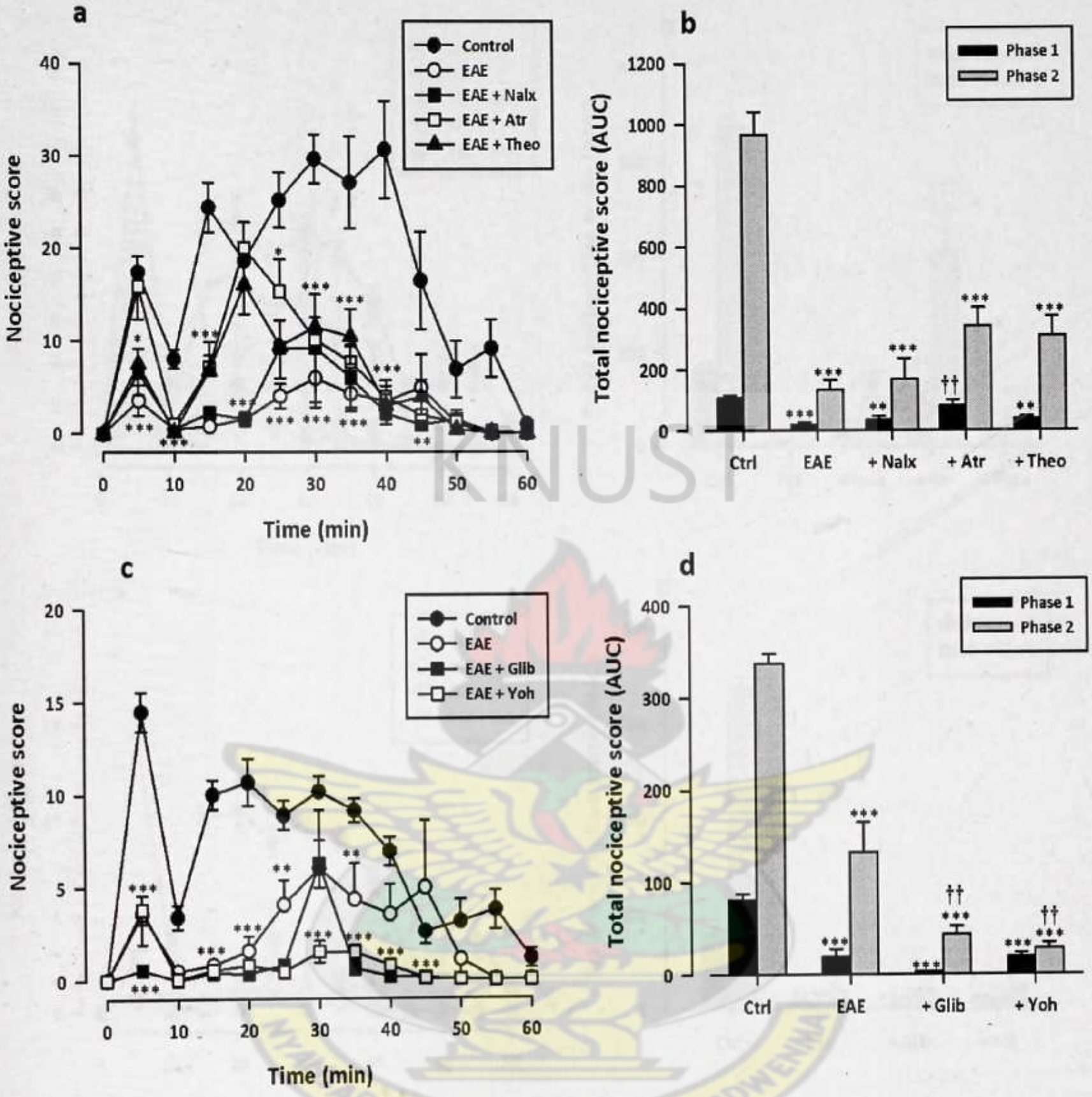


Figure 3.10 Effects of pre-treatment of rats with atropine (5 mg $kg^{-1}$, i.p), naloxone (2 mg $kg^{-1}$, i.p), theophylline (10 mg $kg^{-1}$, i.p), glibenclamide (8 mg $kg^{-1}$, *p.o.*) and yohimbine (3 mg $kg^{-1}$, *p.o.*) on the anti-nociceptive effects of EAE (100 mg $kg^{-1}$, *p.o.*) on the time course curves (a, c) and the total nociceptive score (calc. as AUCs) (b, d) in the formalin test. Nociceptive scores are shown in 5 min time blocks up to 60 min for the time course curves. Data are presented as mean±S.E.M (n=5). *P≤0.05, **P≤0.01, ***P≤0.001 compared to control group (ctrl) (Two-way repeated measures ANOVA followed by Bonferroni's post hoc). †P≤0.05, ††P≤0.01, †††P≤0.001 compared to control group (ctrl) (one-way ANOVA followed by Newman-Keuls post hoc)

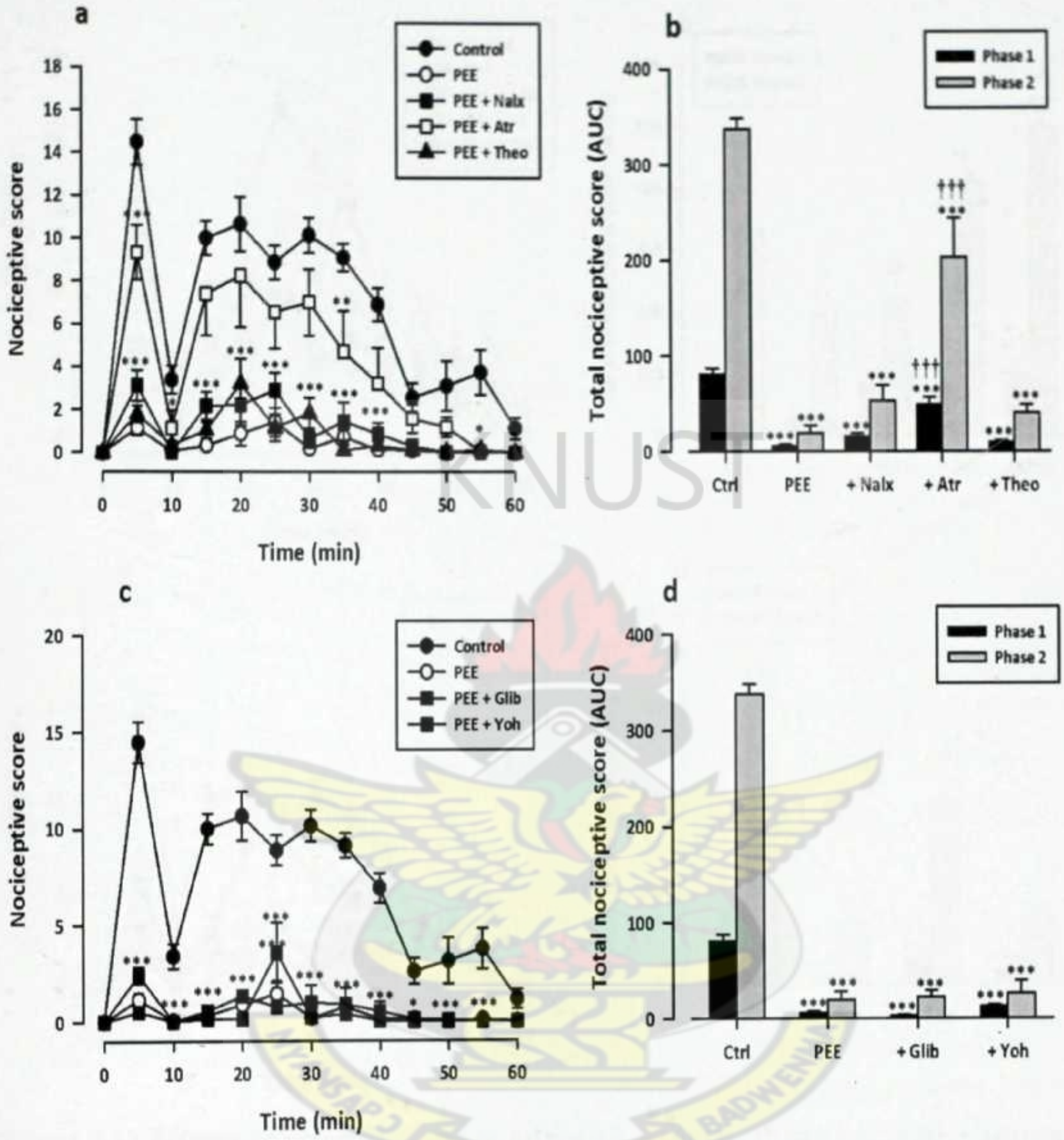


Figure 3.11 Effects of pre-treatment of rats with atropine (5 mg $kg^{-1}$, i.p), naloxone (2 mg $kg^{-1}$, i.p), theophylline (10 mg $kg^{-1}$, i.p), glibenclamide (8 mg $kg^{-1}$, *p.o.*) and yohimbine (3 mg $kg^{-1}$, *p.o.*) on the anti-nociceptive effects of PEE (100 mg $kg^{-1}$, *p.o.*) on the time course curves (a, c) and the total nociceptive score (calc. as AUCs) (b, d) in the formalin test. Nociceptive scores are shown in 5 min time blocks up to 60 min for the time course curves. Data are presented as mean±S.E.M (n=5). *P≤0.05, **P≤0.01, ***P≤0.001 compared to control group (ctrl) (Two-way repeated measures ANOVA followed by Bonferroni's post hoc). †P≤0.05, ††P≤0.01, †††P≤0.001 compared to control group (ctrl) (one-way ANOVA followed by Newman-Keuls post hoc)

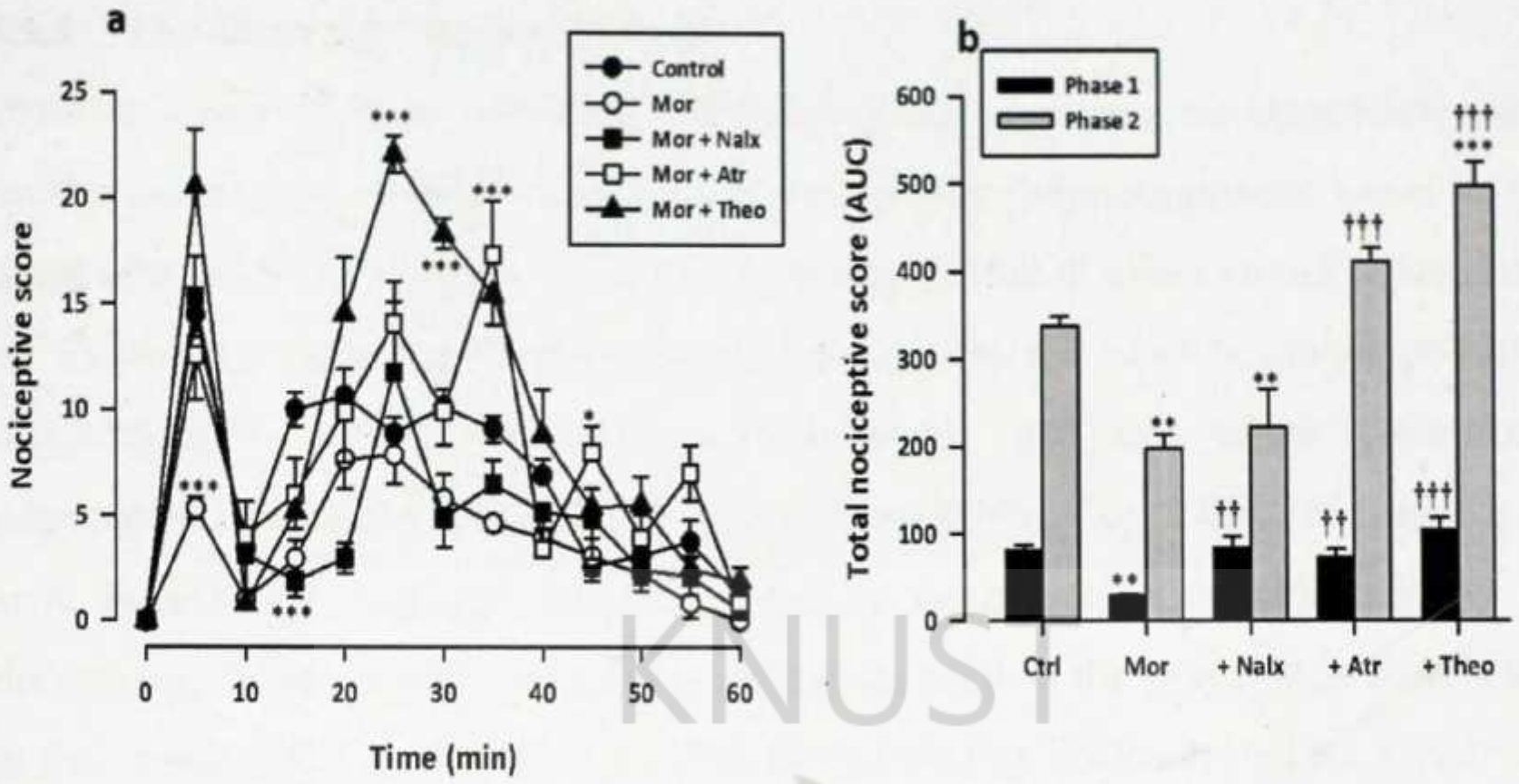


Figure 3.12 Effects of pre-treatment of rats with atropine (5 mg $kg^{-1}$, i.p), naloxone (2 mg $kg^{-1}$, i.p), theophylline (10 mg $kg^{-1}$, i.p), glibenclamide (8 mg $kg^{-1}$, *p.o.*) and yohimbine (3 mg $kg^{-1}$, *p.o.*) on the anti-nociceptive effects of morphine (1 mg $kg^{-1}$, i.p) on the time course curves (a, c) and the total nociceptive score (calc. as AUCs) (b, d) in the formalin test. Nociceptive scores are shown in 5 min time blocks up to 60 min for the time course curves. Data are presented as mean±S.E.M (n=5). *P≤0.05, **P≤0.01, ***P≤0.001 compared to control group (ctrl) (Two-way repeated measures ANOVA followed by Bonferroni's post hoc). †P≤0.05; ††P≤0.01; †††P≤0.001 compared to control group (ctrl) (one-way ANOVA followed by Newman-Keuls post hoc)

## 3.3 ANXIOLYTIC EFFECTS

### 3.3.1 The Elevated Plus-Maze Test

Oral treatment of mice with HAE (10-100 mg $kg^{-1}$, *p.o.*) had no significant effect on the percentage number of entries as well as the percentage time spent in the open arm of the EPM ($F_{3,20}$=1.233; $P$=0.3238; Fig. 3.13a). It affected risk assessment by increasing significantly the percentage unprotected stretch attend postures ($F_{3,20}$=3.896; $P$=0.0242; Fig. 3.14a). It however did not affect percentage unprotected head dip measures ($F_{3,20}$=0.498; $P$=0.6878; Fig. 3.15a) from the open arm. EAE(10-100 mg $kg^{-1}$, *p.o.*) significantly decreased open arm activity by decreasing the percentage number of entries as well as the percentage time spent in the open arm of the EPM ($F_{3,20}$=5.212; $P$=0.0080; Fig. 3.13b). It had no significant effect on risk assessment of both the percentage unprotected stretch attend postures ($F_{3,20}$=2.829; $P$=0.0646; Fig. 3.14b) and percentage unprotected head dip measures ($F_{3,20}$=1.198; $P$=0.3361; Fig. 3.15b). PEE (10-100 mg $kg^{-1}$, *p.o.*) decreased open arm activity by decreased the percentage number of entries as well as the percentage time spent in the open arm of the EPM ($F_{3,20}$=3.399; $P$=0.0392; Fig. 3.13c). It had no significant effect on risk assessment of both the percentage unprotected stretch attend postures ($F_{3,20}$=0.6231; $P$=0.6092; Fig. 3.14c) and percentage unprotected head dip measures ($F_{3,20}$=2.174; $P$=0.1228; Fig. 3.15c).

Diazepam (0.1-1 mg $kg^{-1}$, i.p) significantly increased the percentage entries and time spent in the open arm of the EPM ($F_{3,20}$=6.067; $P$=0.0041; Fig. 3.13d). Percentage unprotected stretch attend postures ($F_{3,20}$=5.758, $P$=0.0052; Fig. 3.14d) and percentage unprotected head dips ($F_{3,20}$=7.569; $P$=0.0014; Fig. 3.15d) were also significantly increased, a confirmation of its known anxiolytic activity. Pentylenetetrazole (3-30 mg $kg^{-1}$, i.p), an anxiogenic agent significantly decreased open arm avoidance by decreasing the percentage entries and time spent ($F_{3,20}$=7.818; $P$=0.0012; Fig. 3.13e) in the open arm of the EPM. It however decreased significantly the percentage unprotected stretch attend postures ($F_{3,20}$=7.376; $P$=0.0013; Fig. 3.14 e) and percentage unprotected head dip measures

($F_{3,20}$=5.204; $P$=0.0081; Fig. 3.15e), an indication of an increase in risk assessment behaviour.

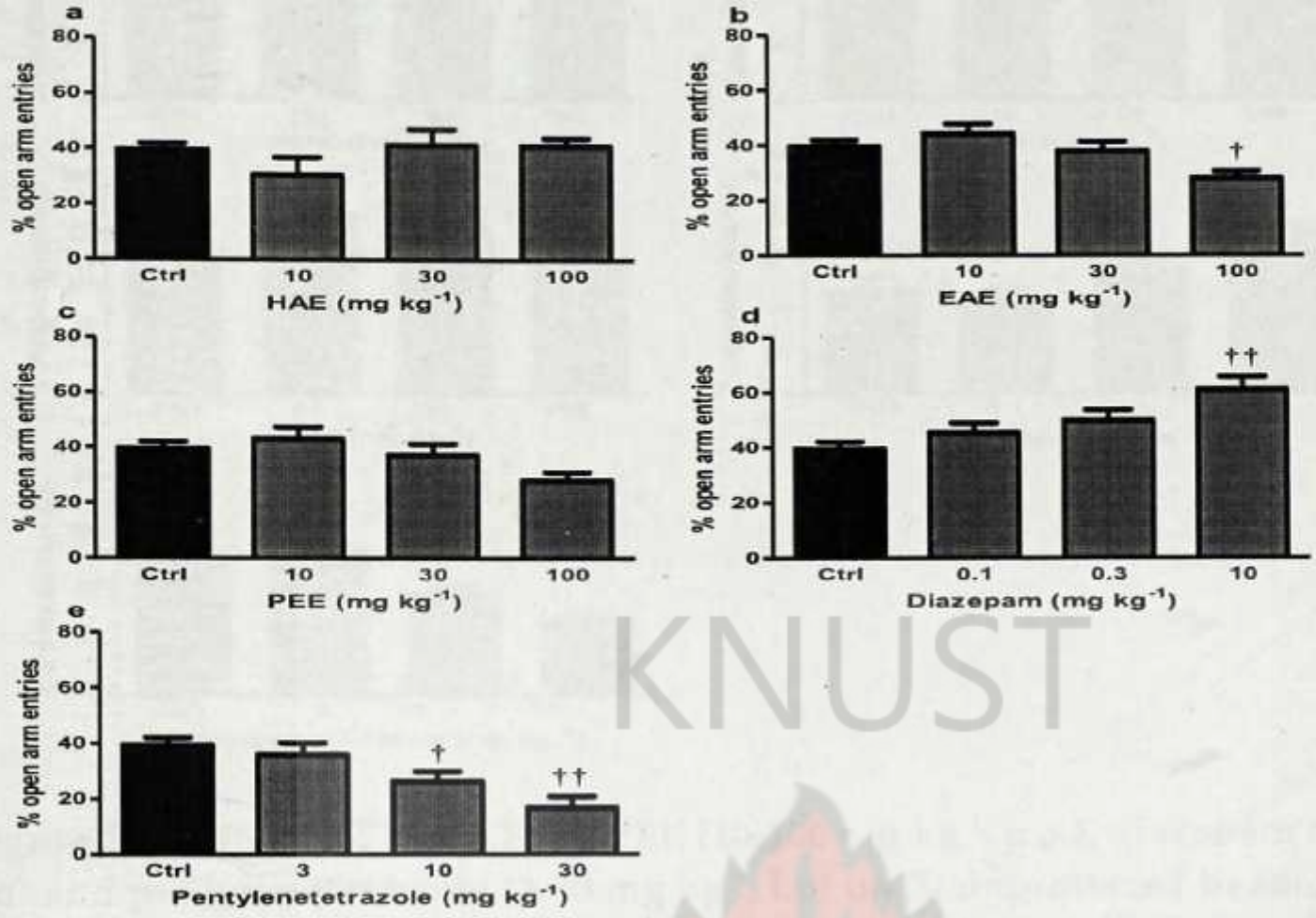


**Figure 3.13** Effects of HAE, EAE, PEE (10-100 mg kg$^{-1}$, *p.o.*), diazepam (0.1-1.0 mg kg$^{-1}$, i.p) and pentylenetetrazole (3-30 mg kg$^{-1}$, i.p) on % unprotected open arm entries in mice on the EPM. Each point represents mean±S.E.M (n = 6). †P≤0.05; ††P≤0.001 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keuls post hoc test)

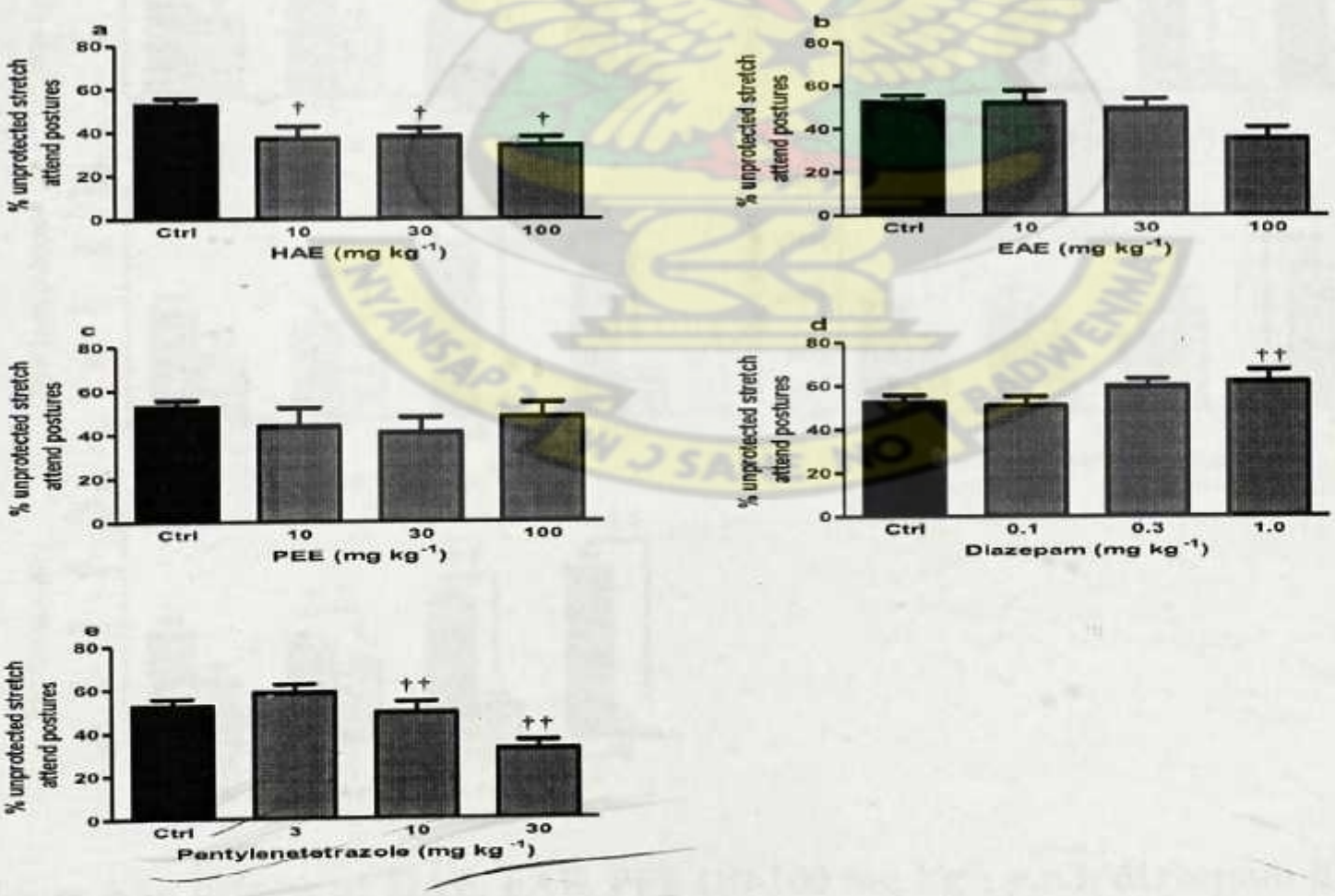


**Figure 3.14** Effects of HAE, EAE, PEE (10-100 mg kg$^{-1}$, *p.o.*), diazepam (0.1-1.0 mg kg$^{-1}$, i.p.) and pentylenetetrazole (3-30 mg kg$^{-1}$, i.p) on % unprotected stretch-attend postures in mice on the EPM. Each point represents mean±S.E.M (n=6). †P≤0.05; ††P≤0.001 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keuls post hoc test)

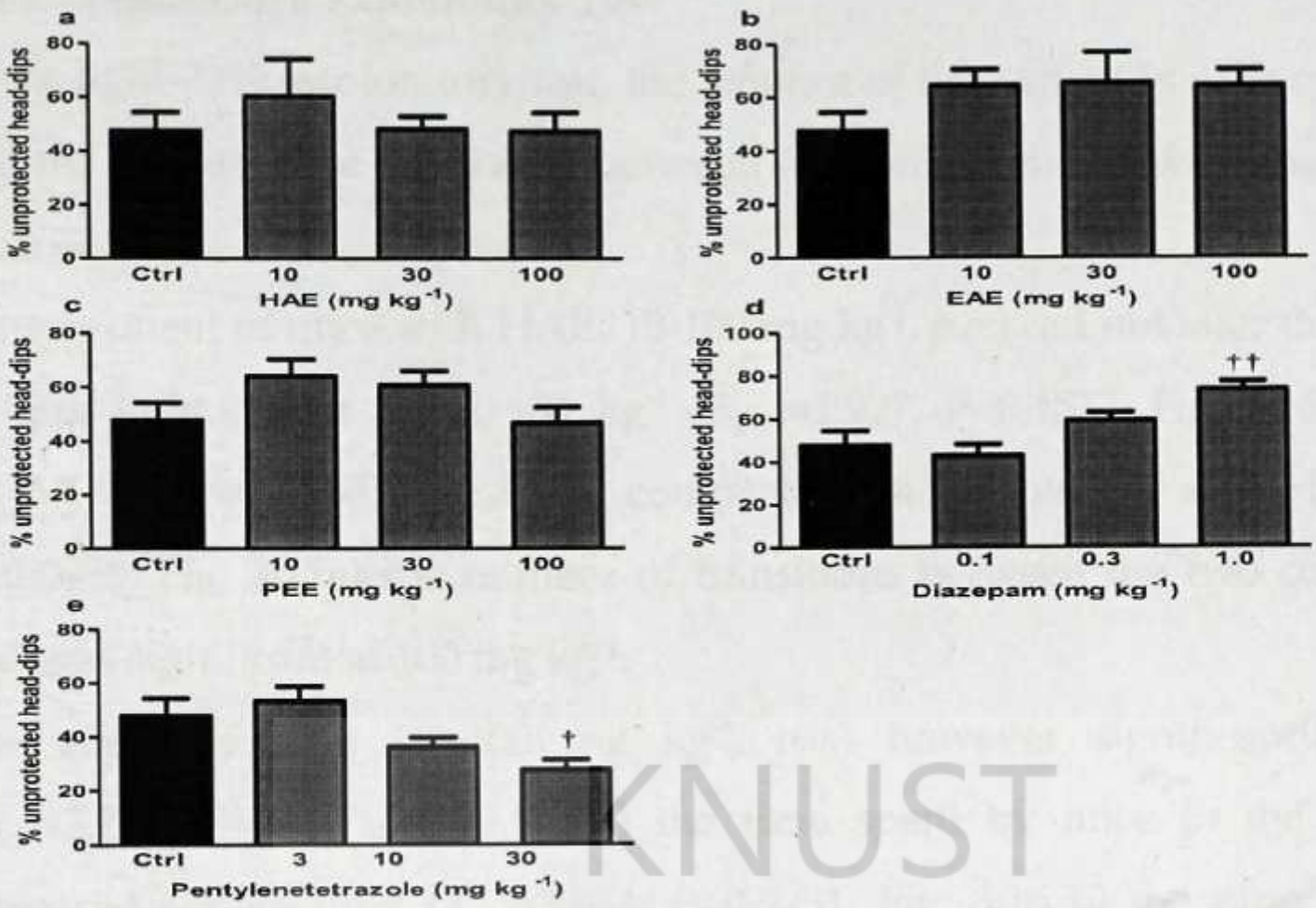


Figure 3.15 Effects of HAE, EAE, PEE (10-100 mg $kg^{-1}$, *p.o.*), diazepam (0.1-1.0 mg $kg^{-1}$, i.p) and pentylenetetrazole (3-30 mg $kg^{-1}$, i.p) on % unprotected head-dips in mice on the EPM. Each point represents mean±S.E.M (n=6). †P≤0.05; ††P≤0.001 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keuls post hoc test)

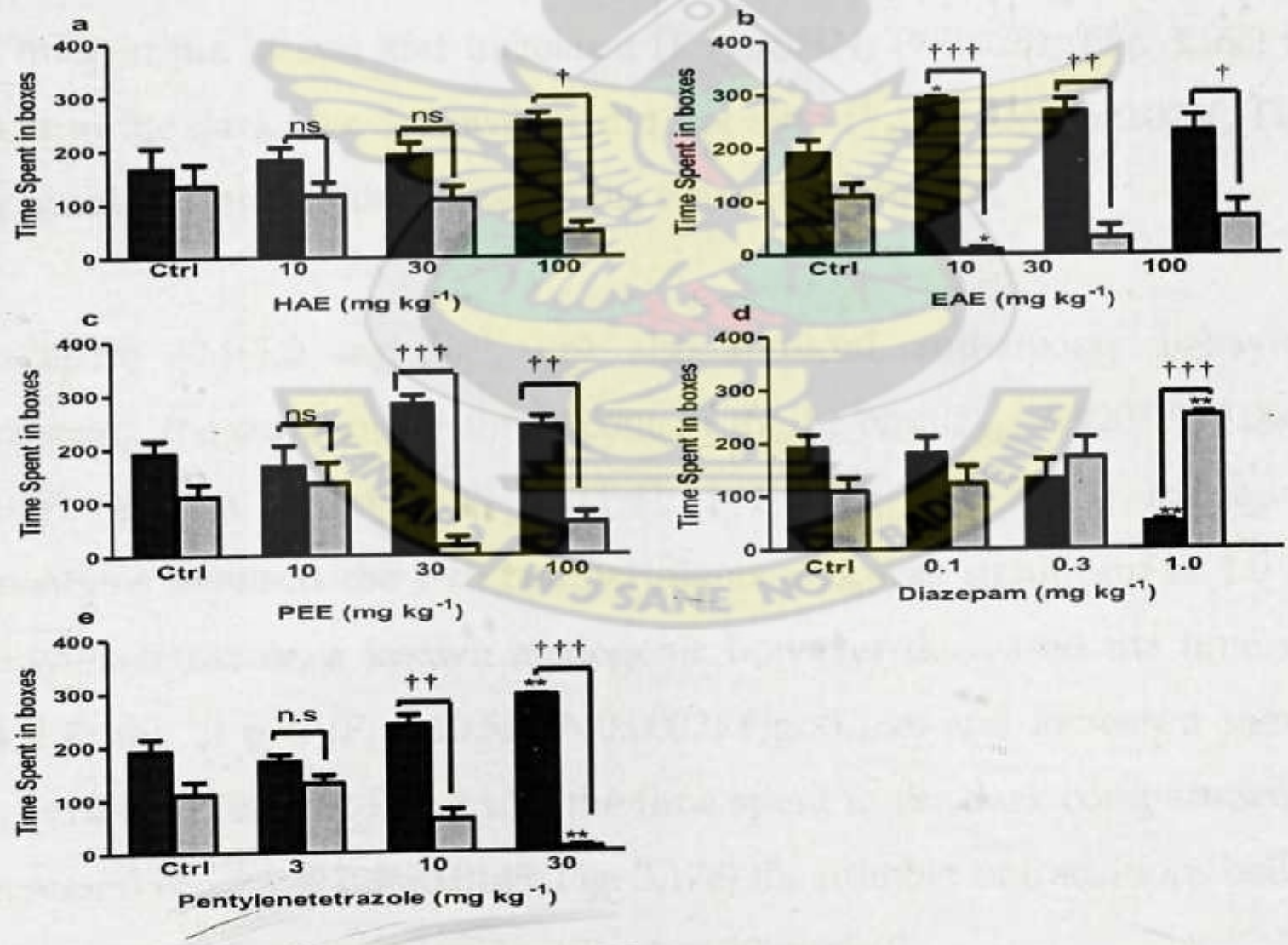


Figure 3.16 Effects of HAE, EAE, PEE (10-100 mg $kg^{-1}$, *p.o.*), diazepam (0.1-1 mg $kg^{-1}$, i.p) and pentylenetetrazole (3-30 mg $kg^{-1}$, i.p) on mice behaviour and on the time spent in the light-dark compartment in the light-dark exploratory test. Each point represents mean±S.E.M (n=6) *P≤0.05; **P≤0.001; ***P≤0.0001 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keuls post hoc test). ††P≤0.001; †††P≤0.0001 compared to treated group

### 3.3.2 Light-Dark Exploratory Test

In the light-dark exploratory test, the amount of time spent in each compartment and the frequency of transitions between the compartments were used as indices of anxiety.

Pre-treatment of mice with HAE (10-100 mg $kg^{-1}$, *p.o.*) did not alter the time spent in both light except at 100 mg $kg^{-1}$ ($F_{3,20}$ =1.927, $P$=0.1577; Fig. 3.16a) and dark ($F_{3,20}$=1.939; $P$=0.1558; Fig. 3.16a) compartments. It however altered ($F_{3,20}$=5.732; $P$=0.0096; Fig. 3.17a) the number of transitions between the two compartments and was significant at 100 mg $kg^{-1}$.

The effect of EAE (10-100 mg $kg^{-1}$, *p.o.*) however significantly decreased ($F_{3,20}$=3.766; $P$=0.0271; Fig. 3.16b) the time spent by mice in the lit box and increased significantly ($F_{3,20}$=3.854; $P$=0.0251; Fig. 3.16 b) the time spent in the dark box. It did also decreased significantly ($F_{3,20}$=15.57; $P$<0.0001; Fig. 3.17b) the number of transitions between the two compartments. Likewise, PEE (10-100 mg $kg^{-1}$, *p.o.*) significantly decreased ($F_{3,20}$=3.854; $P$=0.0256; Fig. 3.16c) the time spent by mice in the lit box and increased ($F_{3,20}$=3.834; $P$=0.0251; Fig. 3.16c) the time spent in the dark box. It however did not alter ($F_{3,30}$=3.536; $P$=0.0236; Fig. 3.17c) the number of transitions between the two compartments.

Diazepam (0.1-1.0 mg $kg^{-1}$, i.p) also induced anti-anxiety behaviours by increasing the duration of time spent in the lit box ($F_{3,20}$=5.420; $P$=0.00628; Fig. 3.16d). It also increased ($F_{3,20}$=11.62; $P$=0.0001; Fig. 3.17d) the number of transitions between the two compartments and was significant at 1.0 mg $kg^{-1}$. Pentylenetetrazole, a known anxiogenic however decreased the time spent by mice in the lit box ($F_{3,20}$=10.52; $P$=0.0002; Fig. 3.16e) and increased significantly ($F_{3,20}$=10.46; $P$=0.0002; Fig. 3.16e) the time spent in the dark compartment. It also decreased ($F_{3,20}$=4.492; $P$=0.0145; Fig. 3.17e) the number of transitions between the two compartments and was significant at 30 mg $kg^{-1}$.

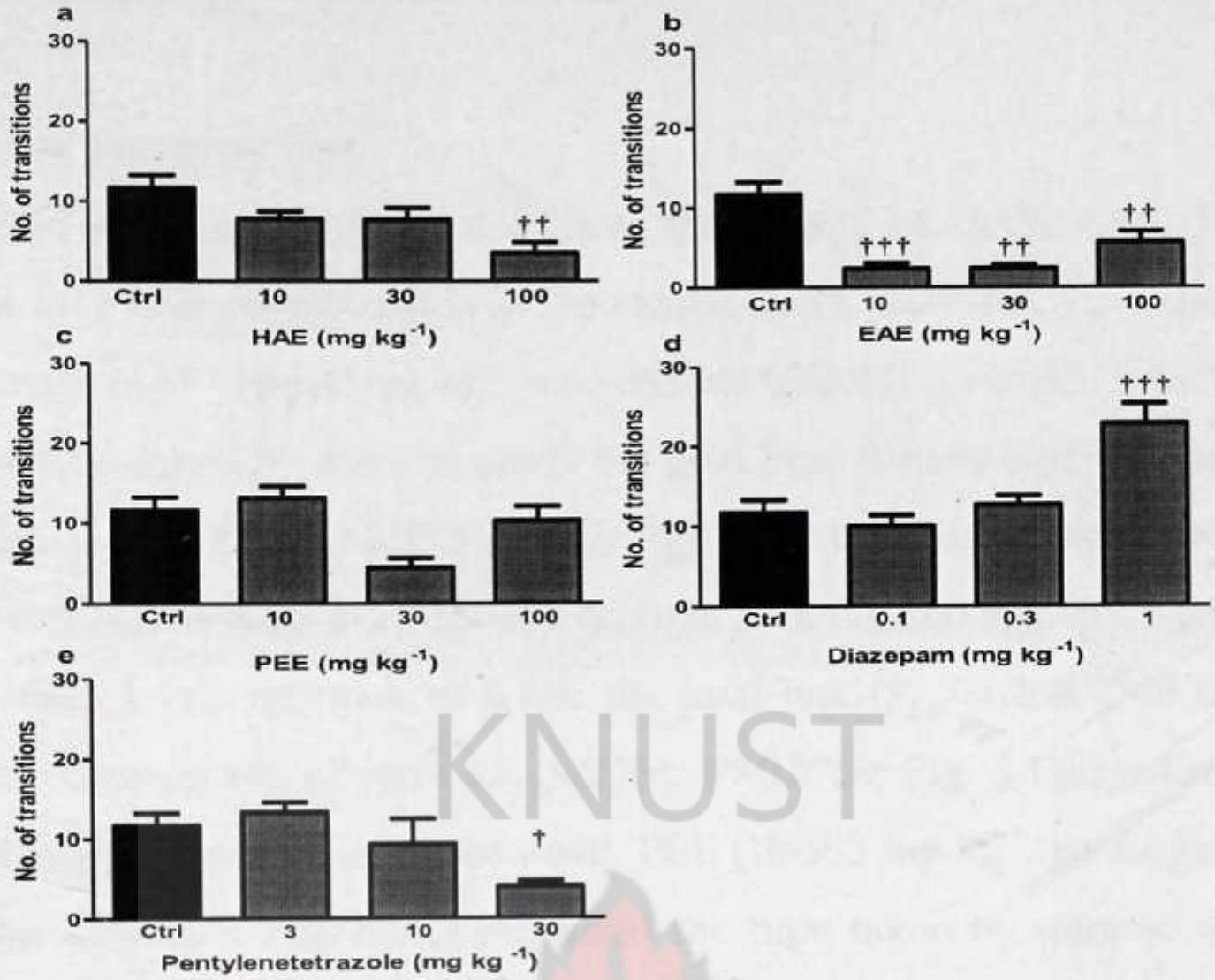


Figure 3.17 Effects of HAE, EAE, PEE (10-100 mg $kg^{-1}$, *p.o.*), diazepam (0.1-1 mg $kg^{-1}$, i.p) and pentylenetetrazole (3-30 mg $kg^{-1}$, i.p) on mice behaviour on the number of transitions in the light-dark exploratory test. Each column represents the mean±S.E.M (n=6). †P≤0.05; ††P≤0.001; †††P≤0.0001. (One-way ANOVA followed by Newman-Keuls test)

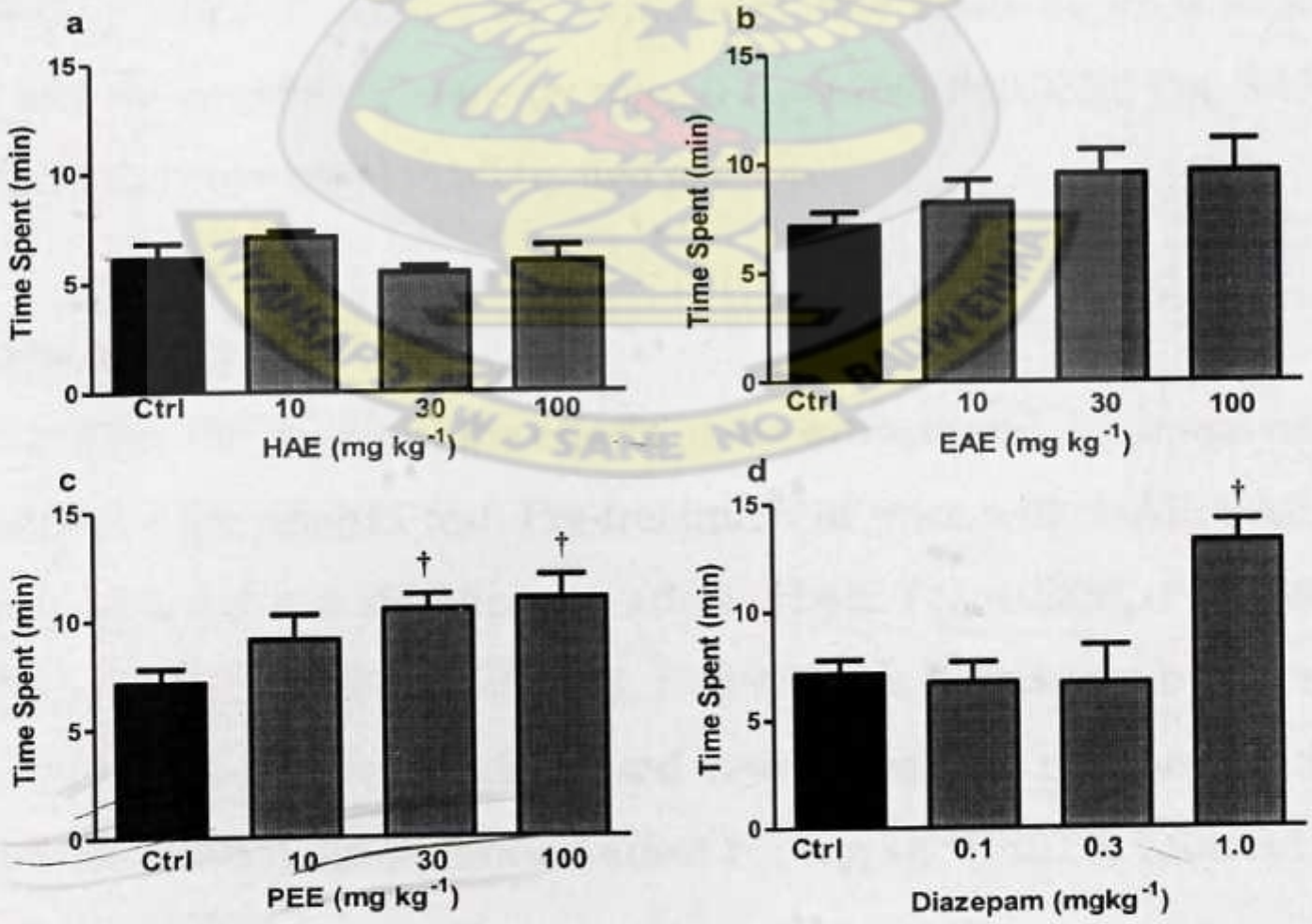


Figure 3.18 Effects of HAE, EAE, PEE (10-100 mg $kg^{-1}$, *p.o.*) and diazepam (0.1-1 mg $kg^{-1}$, i.p) on the time spent in the beam traversal task (a-d). Each column represents the mean±S.E.M (n=6). †P≤0.05 (One-way ANOVA followed by Newman-Keuls test)

## 3.4 MOTOR COORDINATION EFFECTS

### 3.4.1 Beam Traversal Test

Figures 3.18-3.19 represent the results of the effects of HAE, EAE, PEE and diazepam on motor-co-ordination in the mouse beam walk test. Pre-treatment of the mice with HAE (10-100 mg $kg^{-1}$, *p.o.*) did not affect ($F_{3,16}$=1.635; $P$=0.2209; Fig. 3.18a) the time taken by mice to reach the goal box. It increased the number of steps taken by mice ($F_{(3, 16)}$=3.852; $P$=0.03; Fig. 3.19a) to traverse the beam with no stepping errors observed in all treated groups. EAE (10-100 mg $kg^{-1}$, *p.o.*) did not alter the time taken by mice to reach the goal box ($F_{3,16}$=1.208; $P$=0.3388; Fig. 3.18b) and the number of steps ($F_{3,16}$=2.751; $P$=0.0768; Fig. 3.19b) taken by the mice. No stepping errors were observed. PEE (10-100 mg $kg^{-1}$, *p.o.*) significantly ($F_{3,16}$=3.566, $P$=0.0379; Fig. 3.18c) increased the time taken by mice to reach the goal box and the number of steps ($F_{3,16}$=10.26; $P$=0.0005; Fig. 3.19c) taken by the mice to traverse the beam. No stepping errors were observed in all treated groups.

Diazepam (0.1-1 mg $kg^{-1}$, i.p) at 1 mg $kg^{-1}$ significantly and dose dependently increased ($F_{3,16}$=7.839; $P$=0.0019; Fig. 3.18d) the time taken by mice to reach the goal box and the number of steps by mice ($F_{3,16}$=3.700; $P$=0.0339; Fig. 3.19d) with no stepping errors observed in all treated groups.

### 3.4.2 Rotarod Test

Figure 3.20 show the results of the effects of the extracts and diazepam on motor-co-ordination in the rotarod test. Pre-treatment of mice with HAE, EAE or PEE (10-100 mg $kg^{-1}$) did not significantly affect (HAE: $F_{3,16}$=0.8889, $P$=0.4680; EAE: $F_{3,16}$=0.2756, $P$=0.8421; PEE: $F_{3,16}$=0.1234, $P$=0.945) the time spent by mice on the rotarod. Diazepam, however, decreased latency to fall off the rotating rod ($F_{3,16}$=5.365, $P$=0.0096) with significant effect at 1 mg $kg^{-1}$ ($P \leq 0.05$, Table 3.1)

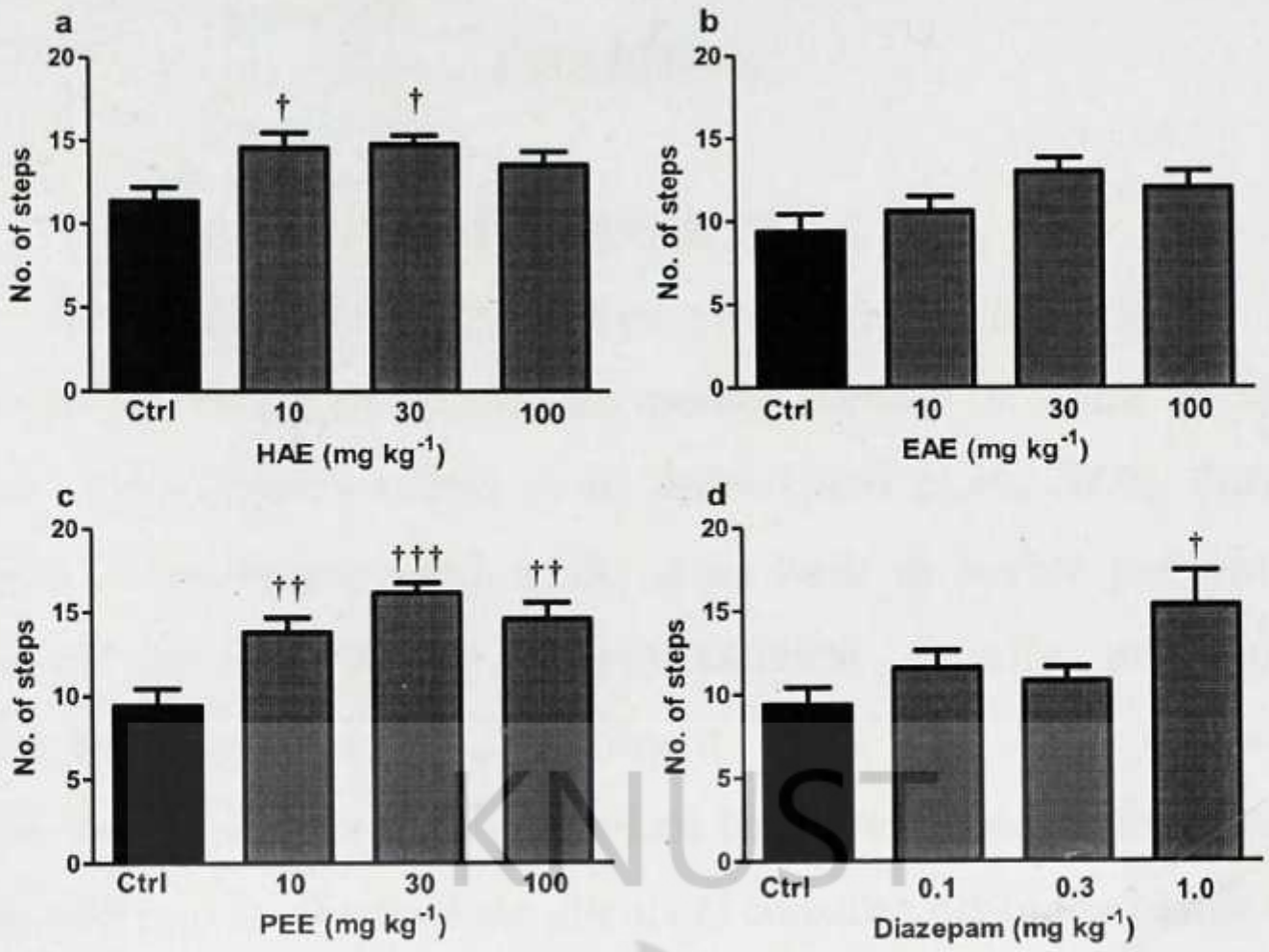


Figure 3.19 Effects of HAE, EAE, PEE (10-100 mg $kg^{-1}$, *p.o.*) and diazepam (0.1-1 mg $kg^{-1}$, i.p) on the number of footsteps to traverse beam. Each column represents the mean±S.E.M (n=6). †P≤0.05; ††P≤0.01; †††P≤0.001. n=5. (One-way ANOVA followed by Newman-Keuls test)

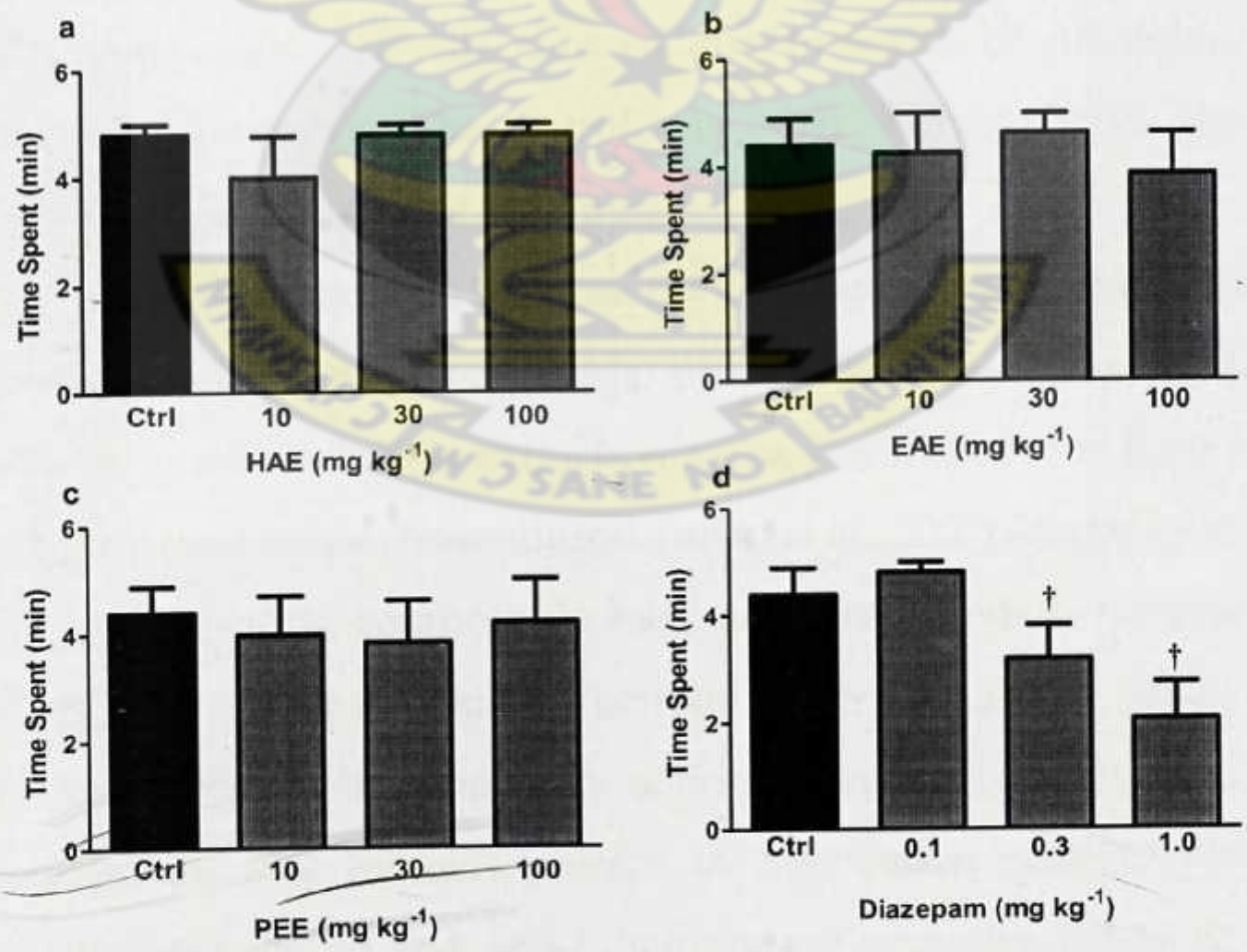


Figure 3.20 Effects of HAE, EAE, PEE (10-100 mg $kg^{-1}$, *p.o.*) and diazepam (0.1-1 mg $kg^{-1}$, i.p) on mice on the rotarod. Each point represents mean±S.E.M (n=6). †P≤0.05 compared to control groups (one-way ANOVA followed by Newman Keuls test)

# CHAPTER 4

## DISCUSSION

Separation and determination of active chemical constituents are generally recommended for standardization and quality control of herbal products and herb-related investigations (Zhao *et al.*, 2006; Chao *et al.*, 2009). Furthermore, identification of the major compounds in an herb or herbal preparation may prove helpful in elucidating pharmacological activity and underlying mechanisms for potential drug development (Tseng *et al.*, 2007). It was based on this premise that three extracts of the plant bark were used in this study , to be able to characterise the effects of the chemical constituents of the plants into polar (HAE-hydroalcoholic extract), mid-polar (EAE-ethyl acetate extract) and non-polar fractions (PEE- petroleum ether extract).

Preliminary phytochemical screening of the stem bark extracts of *T. monadelpha* showed the presence of all seven test constituents in the ethanolic extract (HAE). The ethyl acetate extract (EAE) showed the presence of alkaloids, tannins, terpenoids and glycosides. The petroleum ether extract (PEE) showed the presence of alkaloids, sterols and triterpenes.

It has been reported by several authors that the presence of many biologically active phytochemicals such as terpenoids, flavonoids, alkaloids, steroids, tannins and glycosides in various plant extracts may be responsible for their respective pharmacological properties (Narendhirakannan *et al.*, 2007; Maganha *et al.*, 2010). Flavonoids and phenolic compounds have been reported to possess multiple biological effects such as antioxidant activity (Bors and Saran, 1987), analgesic activity *in vivo* and anti-inflammatory action. Many flavonoids such as rutin, quercetin, betalains and luteolin present in *Amaranthus spinosus* also possess significant anti-nociceptive and anti-inflammatory activities (Chao *et al.*, 2009). Though the individual secondary plant metabolites have not yet been isolated

and tested pharmacologically, the presence of these metabolites may justify the traditional use of *T. Monadelpha* in the management of painful conditions.

This study has demonstrated that oral administration of HAE, EAE and PEE extracts of *Trichilia monadelpha* stem bark have potent anti-nociceptive activity in the chemical (acetic acid and formalin test), thermal (hot plate test) and inflammatory (carrageenan-induced mechanical hyperalgesia test) in rodents.

The abdominal writhing test is used for detecting both central and peripheral analgesia hence very useful in screening for the anti-nociceptive effects of compounds. All the extracts produced marked, dose-related anti-nociception in this model. Intraperitoneal administration of acetic acid is known to release endogenous substances such as prostaglandins ($PGE_2$ and $PGF_{2\alpha}$), sympathomimetic amines, bradykinin, serotonin and pro-inflammatory cytokines such as substance P, interleukins-IL- β and IL-8 (Deraedt *et al.*, 1980). The writhing response (abdominal constriction) is related to the sensitization of nociceptive receptors to the release of prostaglandins e.g. $PGE_2$ and $PGF_{2\alpha}$, (Bose *et al.*, 2007)at peripheral sites, which contribute to inflammatory pain. (Deraedt *et al.*, 1980; Vogel and Vogel, 1997; Dhara *et al.*, 2000) An important limitation of the acetic acid-induced abdominal writhing test however is that non-analgesic drugs, such adrenergic blockers, antihistamines and muscle relaxants can lead to possible false-positive results (Le Bars *et al.*, 2001). Non-steroidal anti-inflammatory drugs (NSAIDs), opioids and centrally acting analgesics inhibit the anti-nociception produced by this model. It is therefore possible that the extracts exert their anti-nociceptive effects probably by inhibiting the synthesis of prostaglandins, and/ suppressing nociceptors to the action of prostaglandins, and/ by interfering with activation of nociceptors by one of these endogenous mediators.

The results from this study also show that all three extracts exhibited significant anti-nociceptive effect in the hot plate test. This model is known to be more

sensitive to centrally acting analgesics (Santos *et al.*, 2005). It has been well documented that the hot plate test is a central anti-nociceptive test in which opioid agents exert their analgesic effects via supraspinal and spinal receptors (Nemirovsky *et al.*, 2001; Silva *et al.*, 2010). Such analgesic agents elevate pain threshold of animals towards heat and pressure and as such, some amount of central activity (spinal and supraspinal mechanisms), can be conferred on the extracts since they exhibited significant activity in this pain model (Jain *et al.*, 2001). The two behavioural components that were measured in terms of their reaction times namely paw licking and jumping, are considered to be supraspinally integrated responses (Le Bars *et al.*, 2001) hence there is the involvement of central mechanisms in the anti-nociception of the extracts.



The carrageenan-induced mechanical hyperalgesia test is useful in elucidating centrally mediated anti-nociceptive responses, which focuses mainly on changes above the spinal cord level (Vongtau *et al.*, 2004). All the three extracts exhibited significant inhibition of mechanical analgesia, albeit less effectively compared to the effect in other pain models. This confirms involvement of central pain pathways. The application of blunt tip into the inflamed hind paw is likely to activate slowly-adapting mechanoceptors with decreased thresholds, which are predominantly C-fibres (in cutaneous and subcutaneous structures), which normally require greater stimulus intensities for activation (Birder and Perl, 1994; Lewin and Moshourab, 2004).

It is known that central pain modulation occurs via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems (Pasero *et al.*, 1999; Salawu *et al.*, 2008; Chindo *et al.*, 2010). The analgesic effect produced by the extracts therefore may be via central mechanisms involving opiate, dopaminergic, descending noradrenergic and serotonergic systems and/ via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes, and other endogenous substances that are key players in inflammation and pain. The results obtained also corroborate with the

observed activity of the extracts in the second phase (inflammatory pain) of the formalin test.

The formalin-induced paw pain is the most predictive *in vivo* model of acute pain (Le Bars *et al.*, 2001) and also a valid model of clinical pain (Vasconcelos *et al.*, 2003; Costa-Lotufo *et al.*, 2004). It produces a distinctive biphasic nociceptive response: the first phase (neurogenic pain), which starts almost immediately after formalin injection and continues for 10 min, reflects centrally mediated pain through the direct chemical sensitisation of peripherally localised TRPA-1 containing nociceptors (McNamara *et al.*, 2007). The second phase (inflammatory pain) 10-60 min after formalin injection is due to inflammation with firstly, an increase in primary afferent drive, a release of inflammatory mediators such as serotonin, histamine, bradykinin and prostaglandins (Tjolsen *et al.*, 1992) and at to some degree, glutamate-dependent sensitization of spinal nociceptive neurons (Coderre and Melzack, 1992; Tjolsen *et al.*, 1992). Centrally acting drugs such as opioids inhibit both early and late phases almost equally (Vogel *et al.*, 1997). Most NSAIDs, non-narcotic analgesics such as acetyl salicylic acid and corticosteroids such as hydrocortisone, and dexamethasone which are primarily peripherally acting, only inhibit the late phase (Hunskaar and Hole, 1987; Santos *et al.*, 2005).Diclofenac, an NSAID which blocks the synthesis of prostaglandins inhibits mostly the late phase and to some extent the early phase (Ortiz *et al.*, 2008). The inhibition of both phases of formalin-induced nociception by HAE, EAE and PEE in this study implies that both central and peripheral mechanisms are involved in their anti-nociception.

The formalin test was used in the assessment of the mechanism of action of the extracts because of its ability to postulate the mechanism and site of action of a test drug (Chau, 1989) as compared to other tests (hot plate, tail immersion, acetic acid writhing etc) that are suitable for testing only centrally acting analgesic drugs (Asongalem *et al.*, 2004). It was chosen because of its specificity and its ability to screen test drugs for both central and peripheral involvement. Some

specific antagonists of the nociceptive pathway were used to assess the mechanisms of the extracts in nociception: atropine, naloxone, theophylline, glibenclamide and yohimbine.

All extracts interacted with the muscarinic cholinergic pathway to exert anti-nociception. Atropine, a non-selective muscarinic cholinergic antagonist reversed partly or wholly the anti-nociception of PEE in both phases and that of EAE and HAE in the second/inflammatory phase. This suggests peripheral and central muscarinic cholinergic involvement ($M_1$-$M_4$ receptors) in the anti-nociception of PEE and central muscarinic cholinergic involvement in the anti-nociception of HAE and EAE. It has been reported that activation of muscarinic receptors induce anti-nociception in thermal, inflammatory and neuropathic pain (Jones and Dunlop, 2007; Sanders and Maze, 2007)and are involved in mediating the analgesic effects of muscarinic agonists at the spinal and supraspinal level (Honda *et al.*, 2000; Sawynok and Liu, 2003; Wess *et al.*, 2003).

Naloxone, a non-selective opioid antagonist reversed partly or wholly the anti-nociception of HAE and morphine in the second/inflammatory phase suggesting a possible central opioidergic involvement.

Theophylline, a non-selective adenosinergic antagonist reversed partly or wholly the anti-nociception of morphine in both phases and of HAE in the second/inflammatory phase suggesting a possible adenosinergic pathway involvement. It however had no effect on the anti-nociception of EAE and PEE. It is well known that theophylline, an adenosine antagonist blocks $A_1$ and $A_2$ receptor subtypes. The activation of $A_1$ receptor peripherally and spinally has been found to produce significant anti-nociception (Sawynok, 1998; Sawynok *et al.*, 2003). The anti-nociception of HAE therefore may be due to activation of $A_1$ receptors and/or an increment in endogenous adenosine either centrally or peripherally.

Glibenclamide, an ATP-sensitive $K^+$ channel blocker reversed partly or wholly the anti-nociception of HAE in the first/neurogenic phase suggesting its possible interaction with the ATP-sensitive $K^+$ channels.

Yohimbine, a selective $\alpha_2$-adrenergic antagonist however did not alter the anti-nociception of the extracts suggesting that their anti-nociception may not involve an interaction with $\alpha_2$-adrenoceptors.

The central analgesic property of this plant has been established in this study in the hot plate test as well as the first phase of the formalin test. Traditional use of this plant includes its use as a sedative and in the treatment of CNS conditions (Abbiw, 1990). Also, considering the increasing evidence of the comorbidity of anxiety and depression with pain, the central effects of these extracts were investigated. Tricyclic anti-depressants and other medications with serotonergic and noradrenergic effects have been shown to relieve a variety of chronic pain syndromes and neuropathic pain, lending credence to the theory that chronic pain and depression share a common biologic pathway (Lepine *et al.*, 2004). In this study, some neurobehavioural effects of the stem bark extracts of *Trichilia monadelpha* were investigated to establish the CNS activity of this plant.

Experimental paradigms such as the light-dark exploratory and elevated plus-maze tests were employed in evaluating the extracts. These tests are widely used for identifying possible candidates for new treatment obtained from natural sources for anxiety disorders. The effects of the extracts on motor coordination and balance in rodents were also elicited using the rotarod and the beam traversal tests.

The light-dark exploratory test relies on the rodent's natural aversion to brightly lit, open areas compared to dark areas, and their innate exploratory behaviour (File *et al.*, 2004). When mice are initially placed in the dark compartment, clinically effective anxiolytics have been shown to cause a decrease in the latency to emerge from the small, dark compartment into the large, brightly lit and open

area whereas stress and anxiogenic treatments increase emergence latency and time spent in the dark area (Shimada *et al.*, 1995).

In the present study, HAE showed no significant change in the number of transitions between the two compartments and the amount of time spent in the lit box by mice at all doses except at 100 mg $kg^{-1}$, where it was decreased. EAE and PEE however significantly decreased the number of transitions between the two compartments and the amount of time spent in the lit box by mice at all doses. It was however significant only in PEE-treated groups.

Diazepam which is a classical anxiolytic caused significant anxiolytic activity by decreasing the emergence latency of mice into the lit compartment and also greatly increasing the amount of time spent in the lit box especially at 1.0 mg $kg^{-1}$. Pentylenetetrazole, a known anxiogenic agent caused opposite effects to that of diazepam at all doses used in this study. This suggests that HAE, EAE and PEE may contain compounds that have anxiogenic-like or CNS depressant activity (sedation) at the doses used. The best explanation for these findings can be attributed to the CNS depressant/ sedative effects of the plant that was reported by the work of Owusu (2009) of the aqueous stem bark extract of *Trichilia monadelpha* in the pentobarbitone-sleeping time test in rats. This result may therefore confirm its traditional use as a sedative.

The elevated plus-maze test is the most widely used behavioural animal model of anxiety used in the screening of putative anxiolytic drugs (Carobrez and Bertoglio, 2005; Wei *et al.*, 2007). It affords an excellent example of a model that is sensitive to unconditioned behaviour. It derives from some early work on exploratory patterns (Montgomery, 1955), the basic premise of which was the environmental novelty simultaneously evokes fear and curiosity, thereby creating a typical approach-avoid conflict. The EPM is known to be sensitive to both anxiolytic drugs especially benzodiazepines and anxiogenic drugs (Handley and Mithani, 1984; Pellow *et al.*, 1985; Lister, 1987). The primary indices of EPM

anxiety comprise of spatio-temporal measures of arm entries and time spent in open arm with anxiolytics e.g. diazepam generally increasing and anxiogenics e.g. pentylenetetrazole decreasing these measures (Chen *et al.*, 2006). HAE did not alter the time spent in the open arm. EAE and PEE decreased the time spent in the open arm of the EPM, with PEE being significant at 100 mg $kg^{-1}$, $P$=0.0012).

Another important index, the risk assessment behaviours such as unprotected stretch attend postures and head dips have been validated and shown to be a more predictive determinant of anxiety (Rodgers and Johnson, 1995; Rodgers and Dalvi, 1997). All three extracts decreased the percentage unprotected forms of both stretch attend postures and head dips. EAE and PEE decreased percentage open arm entries whiles HAE had no effect on open arm entries. This indicates that these extracts may contain compounds that have sedative effects at the doses used. Diazepam increased the percentage unprotected forms of the risk assessment behaviours and also increased open arm entries. This indicates a reduction of anxiety/fear related behaviours. Pentylenetetrazole decreased the unprotected forms of the risk assessment behaviours and also open arm entries.

Some drugs, though not anxiolytics (e.g., amphetamines) may appear anxiolytic (false positive) in this test if it stimulates motor activity. It may affect the spatio-temporal parameters of anxiety such as increasing the time spent in the open arms and number of open arm entries etc. On the contrary, an agent that causes motor impairment or sedation may appear anxiogenic (e.g., buspirone). It may decrease the time spent in the open arms, because it clearly reduces locomotor activity. To eliminate possible impairment of motor activity and coordination by the extracts, the rotarod test and beam traversal task were used in this study.

The rotarod test is used to assess motor coordination and balance in rodents. The latency, which measures the time an animal can stay on the rotating rod before falling off, indicates the animal's gross motor ability. All three extracts did not cause any alteration in the amount of time mice spent on the rotating rod

suggesting absence of impaired motor coordination. Pre-treatment of mice with diazepam caused significant impaired motor coordination at 1.0 mg $kg^{-1}$ as reflected by the decreased time spent on the rod. Diazepam produced motor impairment at the higher doses because benzodiazepines such as diazepam act as anxiolytics at low doses and can produce myorelaxant effect at higher doses. The results from the rotarod test showed clearly that the extracts did not have any effect on motor performance of the animals used.

The beam traversal task is designed to challenge an animal's fine motor balance and coordination skills (Carter *et al.*, 1999; Meredith and Kang, 2006). This test measures skilled walking and increased errors in experimental groups reflect impaired coordination (Meredith *et al.*, 2006). Its ability to measure more than one parameter (stepping errors, time spent to reach the goal box and number of falls) affords this test a greater advantage over the rotarod, which measures only the latency to fall off the rotating rod. Contrary to results in the rotarod test, all three extracts showed some impairment in motor coordination. This finding is not surprising since it has been shown that the beam traversal walk is more sensitive than the rotarod test in detecting motor deficits in mice (Stanley *et al.*, 2005).

Diazepam at 1.0 $mgkg^{-1}$ caused significant increase in the time to traverse the beam. This is expected since benzodiazepines at high doses have sedative effects and cause ataxia (Woods and Winger, 1995). These findings of possible motor impairment in this study may be attributed to the sedative effects of the aqueous extract of *T. monadelpha* stem bark as reported by Owusu (2009) where the hypno-sedative effects of the extract at 100 mg $kg^{-1}$-1000 mg $kg^{-1}$ were investigated. Further studies however must be conducted to assess the exact mechanism of action of the plant extracts in their CNS activity.

It can be inferred from this study the presence of multiple compounds with analgesic activity in the stem bark of *T. monadelpha*. These act via different mechanisms that, together, produce the anti-nociception seen with the use of the plant extract in traditional medical practice. In all the pain models used in this

study, the three extracts exhibited significant analgesic activity with possible sedation that cannot be attributed to a decreased motor function.

# CHAPTER 5

## CONCLUSIONS & RECOMMENDATIONS

This study demonstrates that the hydroalcoholic, ethyl acetate and petroleum ether extracts of the stem bark of *Trichilia monadelpha* have peripheral and central anti-nociceptive activity in thermal, chemical and inflammatory pain models of nociception in rodents. The petroleum ether extract was found to be the most potent.



The hydroalcoholic extract produces analgesic effects through mechanisms that involve an interaction with the opioidergic, adenosinergic, muscarinic cholinergic pathways and ATP-sensitive $K^+$ channels while that of the ethyl acetate and petroleum ether extracts involve an interaction with the muscarinic cholinergic system. All three extracts possess some sedative effects.

The following are recommended:

1. The active compounds in the extracts responsible for their anti-nociceptive effects should be isolated and characterized.

2. Further work on other mechanisms underlying the analgesic effects of the extracts should be done.

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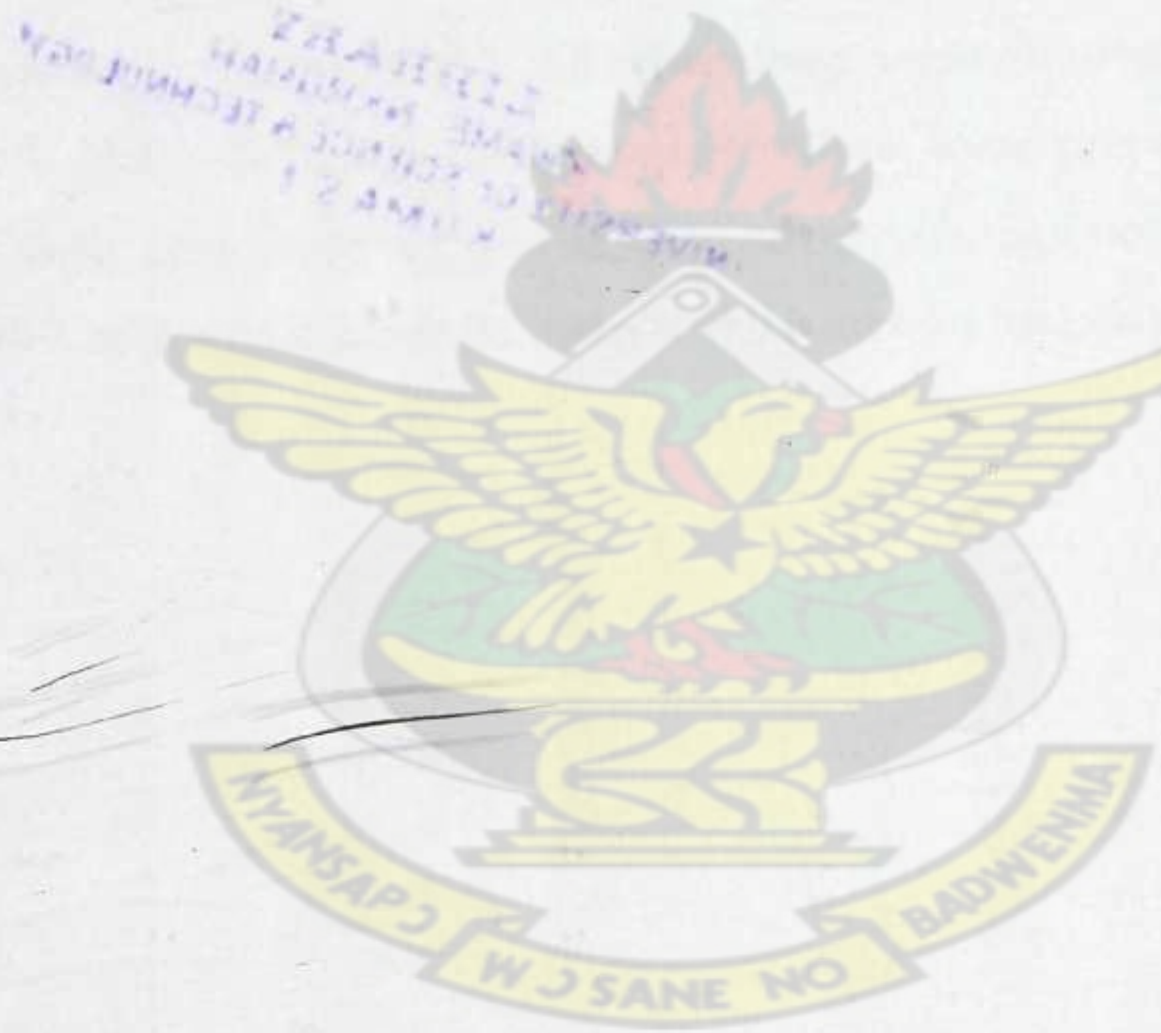

# Appendix

## 7.1 PHARMACOLOGICAL METHODS

### 7.1.1 PREPARATION OF λ-CARRAGEENAN SUSPENSION

A 2 % Carrageenan suspension was prepared by sprinkling 200 mg of λ-Carrageenan evenly over the surface of 10 ml 0.9 % NaCl solution. This was then used 10 min after preparation.

### 7.1.2 DRUG PREPARATION AND ADMINSTRATION

The plant extracts were triturated with 1-2 drops of 80 % Tween solution and then dissolved in 10 ml of NaCl. All other drugs were diluting the stock solution with 0.9 % NaCl. Generally, drug concentrations were prepared such that the required dose was always given in equivalent volumes not exceeding a total volume of 1 ml for oral administration and 0.5 ml for intraperitoneal route.