Neuropharmacological profile of *Mentha longifolia*: Effects on convulsion, nociception and pentobarbitone-induced sleep in mice

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A thesis submitted in partial fulfillment of the requirements for the degree of Magister Pharmaceuticiae in the Discipline of Pharmacology, School of Pharmacy, University of the Western Cape.

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Keywords

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Analgesic effect

Collection

Identification

Extraction

Chemo-shock method

Pentobarbitone sleeping time

Acetic-acid Writhing method

Phytochemical analysis

HPLC fingerprinting

Mice

Abstract

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M.Pharm thesis, Discipline of Pharmacology, School of Pharmacy,

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Mentha longifolia Huds., subspecies, capensis Briq, a plant species used for the treatment of epilepsy, painful conditions such as headache and toothache, and insomnia amongst other ailments, was investigated for anticonvulsant, analgesic and central nervous system depressant activities using chemically-induced seizures, acetic acid-induced writhing and hot-plate thermal stimulation, and pentobarbitone sleeping tests respectively in mice. The parameters used for the measurement of the anticonvulsant activity included the onset of seizures and/or the incidence of the seizures. For the analgesic activities, the parameters of measurement were, the number of writhes for the acetic acid test and reaction time of animals to thermal stimulation for the hot-plate test. For the central nervous system depressant activity, akin to the anti-insomniac activity, the parameter of measurement was the duration of sleep elicited by pentobarbitone. All the data obtained were analysed using the paired Student's t-test with the exception of that on the incidence of seizures, which was analysed using Chi-squared test.

Aqueous extract of *M. longifolia* significantly delayed the onset of pentylenetetrazole-induced seizures, profoundly antagonised the seizures elicited by picrotoxin and had no effects against seizures induced by either bicuculline or N-methyl-DL-aspartic acid. *M. longifolia* completely antagonised acetic acid-induced writhing and profoundly delayed the reaction times of the animals to hot-plate thermal stimulations in similar manner to the standard drugs, paracetamol and morphine respectively. Like the standard drug, diazepam, *M. longifolia* significantly prolonged the duration of sleep induced by pentobarbitone.

The phytochemical analysis carried out on the leaves of *M. longifolia* showed the presence of saponins, tannins, reducing sugars, cardiac glycosides, flavonoids and triterpene steroids. The HPLC spectrum of *M. longifolia* showed major peaks at the following retention times (minutes): 20.52, 22.37, 23.15, 24.87 and 26.93.

The data obtained show that *M. longifolia* has anticonvulsant, analgesic and anti-insomniac activities, thus justifying the claim by traditional health practitioners of its use in epilepsy, painful conditions and insomnia.

May 2004

Declaration

I declare that "Neuropharmacological profile of *Mentha longifolia*: Effects on convulsion, nociception and pentobarbitone-induced sleep in mice" is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Shaun Jerome Erasmus May 2004

Signed:.....

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Contents

List of figures

List of tables

Chapter 1

Introduction

The world is endowed with a wealth of medicinal plants. Plant medicine has been used from time immemorial and is still very much relied upon especially among the rural communities (Watt and Breyer-Brandwijk, 1962; Van Wyk *et al.*, 1997). It is estimated that around 70,000 plant species, from lichens to towering trees, have been used at one time or another for medicinal purposes (Das Prajapati *et al.*, 2003).

Medicinal plants or herbs are providing the starting material for the isolation or synthesis of conventional drugs. Herbs are becoming popular throughout the developed world, as people strive to stay healthy in the face of chronic stress and pollution, and to treat illness with medicines that work in concert with the body's own defenses (Das Prajapati *et al.*, 2003).

According to Das Prajapati *et al.* (2003), medicinal plants have curative properties due to the presence of various complex chemical structures of different composition, which are found as secondary plant metabolites in one or more parts of the plant. Plant metabolites are grouped according to their composition as alkaloids, glycosides, corticosteroids, essential oils and so on.

Currently, there is a worldwide interest in medicinal plants as well as in traditional health systems (Marrin-Betollo, 1980; Bouldin *et al.*, 1999; Amos *et al.*, 2001; WHO, 2002). In most cases, the claims of therapeutic successes of medicinal plants are only from oral or personal communications from traditional medicine practitioners. Another aspect of plant medicine that is always questioned is the safety. Many a time, little or no scientific data exist to verify the therapeutic success and the safety of these agents.

The aim of this project was to attempt to scientifically validate some of the claims by traditional healers of therapeutic successes of the plant under investigation. Also an attempt was made to suggest possible mechanism of action of the said plant in some of the areas under test. All these will contribute to the safe use of the plant.

The neuropharmacological profile of *Mentha longifolia*, known to be used in various ailments (Watt and Breyer-Brandwijk, 1962; van Wyk *et al.*, 1997), involving three actions namely anti-epileptic, analgesic and effects on pentobarbitone-induced sleep were investigated in mice.

Chapter 2

Literature review

2.1 Traditional medicine

Many people, especially in the developing countries, are making use of traditional herbal medicine to cure their ailments. This is evident in South Africa, as a large number of South Africans consult traditional healers, mostly in addition to medical practitioners. It is estimated that there are approximately 200,000 traditional healers in the country. This by far outnumbers the conventional health practitioners. This work force, represented by traditional healers, is a potentially important resource for primary health care, since they are more accessible to the population. Partnership building between traditional health practitioners and conventional health practitioners will increase health care coverage. Bringing together the two systems of medicine will enable the traditional health practitioners and the conventional health practitioners to complement each other, and thereby promote and enhance management of diseases and disorders (Sambo, 2003). The World Health Organization (WHO) defines traditional medicine as the total combination of knowledge and practices, whether explicable or not, used in diagnosing, preventing or eliminating physical, mental or social diseases and which may rely exclusively on past experience and observation handed down from generation to generation, verbally or in writing. Traditional medicine is indeed Africa's culture, future and heritage because the region has a rich bio-resource base: about 6.377 plant species are used in tropical Africa, more than 4,000 of these as medicinal plants (Chatora, 2003). Medicinal plants are important for pharmacological research and drug development, not

only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds (WHO, 1999; 2002).

2.2 *Mentha longifolia* Huds., subspecies, *capensis* Briq.

2.2.1 Description

Family: Lamiaceae

Common names: wild mint (English)

kruisement, balderjan (Afrikaans)

Koena-ya-thaba (Southern Sotho?)

inixina, inzinziniba (Xhosa)

ufuthana lomhlanga (Zulu)

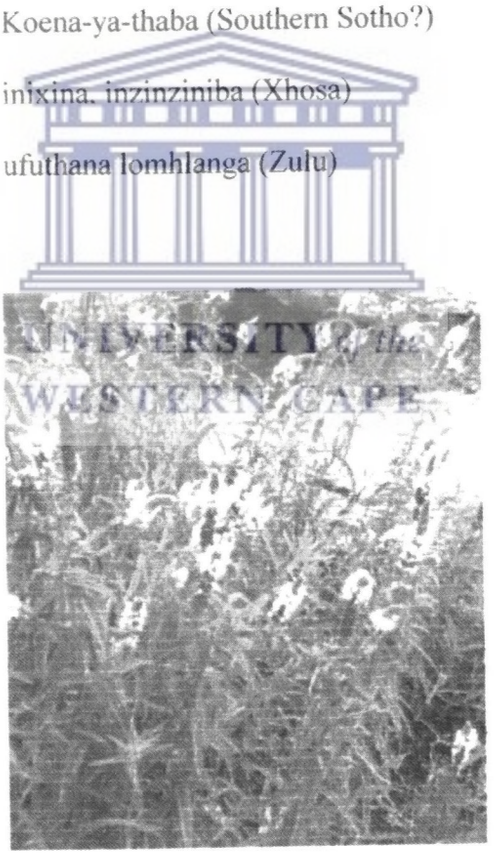


Figure 1. *Mentha longifolia* (from Van der Walt, 2004)

Mentha longifolia is a fast-growing, perennial herb (Figure 1). It creeps along an underground rootstock. It can reach up to 1.5 m high in favourable conditions, but is usually between 0.5-1 m high and even shorter in dry conditions (Van der Walt, 2004). *M. longifolia* is strongly aromatic, giving it its characteristic menthol smell.

The leaves are formed in pairs opposite each other along the square-shaped stem. The soft, lanceolate leaves (long and narrow with a sharp point) are between 45-100 mm long and 7-20 mm wide (Van der Walt, 2004). The leaves are coarsely hairy; the edges sparsely toothed and vary in colour from light and dark green to grey.

The small flowers of *M. longifolia* are crowded into spikes at the tip of the stems. They vary in colour from white to mauve. The plant flowers throughout the summer months from November to April (Van der Walt, 2004).

2.2.2. Distribution

M. longifolia is found in marshes and along streams, from the Cape through Africa and Europe.

In South Africa, three different subspecies of the plant are recognized.

- *M. longifolia* subsp. *wissi* (Cape velvet mint) is found only in two places, the one near Brandberg in Namibia and the other near Garies in Namaqualand. The long and thin, grey-green leaves of subsp. wissi are said to be unpleasantly aromatic.
- *M. longifolia* subsp. *capensis* (balderjan), with a strong peppermint scent, is the most widespread and occurs from Calvinia down to the Cape Peninsula through

the Eastern Cape, Lesotho, Orange Free State, KwaZulu-Natal to Gauteng and Limpopo, (former Northern Province).

- The distribution of *M. longifolia* subsp. *polyadena* (spearmint) is along two disjunct areas, the first from Gauteng, Swaziland, northern KwaZulu Natal, eastern Free State and northern Lesotho and then with a long jump down to the southern Cape, it is found again between Humansdorp and the Swartberg (Van der Walt, 2004).

2.2.3 Uses

Mentha longifolia is found in most parts of the country and is easy to harvest. This results in it being a popular traditional medicine. It is mainly used for respiratory ailments but many other uses have also been recorded. The leaves are used, usually to make a tea that is drunk for coughs, colds, stomach cramps, asthma, flatulence, indigestion, fever and headaches (Van der Walt, 2004). In the Cape, an infusion has been used to treat hysteria and insomnia and the infusion of the dry roots used to treat epilepsy (Van Wyk and Gericke, 2000).

2.3 Epilepsy

The term "epilepsy" applies to a group of disorders that are characterized by sudden and transient episodes (seizures) of motor (convulsions), sensory, autonomous or psychic origin (Leonard, 2000). It is one of the most common central nervous system (CNS) disorders, occurring in one of every 100 people (Burnham, 1998). During a seizure, which is a self-sustaining episode of neural hyperactivity, the neurons of the brain cease

their normal activities and begin to fire in massive, synchronized bursts. The seizures can be visualized on the electroencephalogram (EEG) as spikes and are usually correlated with abnormal and excessive discharges in the brain. After a few seconds or minutes, the inhibitory mechanisms of the brain regain control, the seizure stops and the person returns to normal. Epilepsy is estimated to affect 20-40 million individuals worldwide. It is more common in children than in adults (Leonard, 2000).

2.3.1. Classification of epilepsy

Epilepsy can be classified into two groups:

- primary or idiopathic epilepsy
- secondary or symptomatic epilepsy

Primary epilepsy refers to epilepsies for which no specific cause can be identified. Secondary epilepsy arises when the symptoms are associated with trauma, neoplasm, infection, cerebrovascular disease or some other physically induced lesion of the brain (Leonard, 2000).

For the purpose of drug treatment, the epilepsies are classified according to the seizure type. The main groups are:

1. Partial (focal) seizures, or seizures initiated locally in the brain. These include:
    a) Simple partial seizures, which encompass focal motor attacks and seizures

with somatosensory signs or psychic symptoms.

b) Complex partial seizures, including temporal lobe or psychomotor seizures where consciousness is impaired; these may begin as simple partial seizures.

c) Secondary generalized seizures, which commence as simple partial seizures or complex partial seizures but later develop into generalized tonic-clonic, clonic or tonic seizures.

2. Generalized seizures, including bilateral symmetrical seizures or seizures without local onset. These include:

a) Clonic, tonic and tonic-clonic seizures

b) Myoclonic seizures

c) Absence and atypical absence seizures

d) Atonic seizures

This classification does not take into account the frequency, duration or causes of precipitation of the seizure (Leonard, 2000). Status epilepticus is the term used to describe any type of attack that is maintained for more than one hour and may qualify as focal or generalized.

2.3.2. Pathological basis of epilepsy

The pathophysiology of epilepsy is poorly understood and so far there is no clear association between the abnormal function of a specific group of neurons and the genesis of seizures (Leonard, 2000). Epileptogenesis involves the complex interaction of multiple

factors. Lesions arising from traumatic haemorrhage can cause secondary seizures, whereas other forms of brain damage like ischaemic stroke are less likely to cause seizures. Microscopic changes involving glial proliferation and loss of neurons have been identified in epileptic patients, and a loss of those neurons containing inhibitory neurotransmitters has been particularly implicated in the aetiology of the disease. It is uncertain whether such changes are the cause or the consequence of the seizures. An important area of research for the future is the controversy regarding the possible genetic basis of the epilepsies. It has been calculated that there are at least 100 different trait markers that may predispose some individuals to the disease, and it has been shown that identical seizure disorders may be present in patients who either have or do not have a particular genetic marker (Leonard, 2000).

2.3.3. Animal models used in epilepsy

Animal models have been developed not only in an attempt to screen potential anti-epileptic drugs but also to define more precisely the possible aetiology of the condition. There are at least 12 single locus mutations that produce neurological syndromes with spontaneous seizures in mice. According to Leonard (2000), the "tottering mouse" shows spontaneous seizures, which resemble absence attacks, both in terms of the behavioural and the EEG changes. In this model, the only specific cellular pathology is a selective outgrowth of axons from the locus ceruleus, which results in an increase in the noradrenaline content of the neocortex, hippocampus, cerebellum and thalamus. The seizures are attenuated in this species by the local injection of the

neurotoxin 6-hydroxydopamine, which destroys the noradrenergic terminals. This neurotoxin usually results in the lowering of the seizure threshold in most species of animal. Thus, the precise relevance of these findings in the "tottering mouse" to the human condition is unclear.

Strong sensory stimuli (e.g. 90dB) can precipitate tonic-clonic seizures in some strains of mice, the DBA/2 strain being particularly susceptible, while posturally induced seizures in "epileptic-like" mice have been extensively studied and have been shown to be associated with abnormalities in both the adenosine triphosphatases (ATPases) and various biogenic amine neurotransmitters (Leonard, 2000). The nearest model to human epilepsy is photically induced seizures in the Senegalese baboon.

Rodent models in which seizures are induced by electroshock or by convulsant drugs such as pentylenetetrazole, picrotoxin or bicuculline are mainly used in screening procedures to identify potential anticonvulsants (Leonard, 2000).

2.3.4. Biochemical hypotheses of epilepsy

There have been a number of hypotheses concerning the biochemical flaw in epilepsy. The most popular hypotheses relate to the amino acid transmitters gamma-aminobutyric acid (GABA), which is inhibitory and glutamate, which is excitatory. Normal brain function depends upon a balance of excitation and inhibition. If excitation exceeds inhibition, the brain tissue becomes hyperexcitable and if the imbalance is sufficiently large, a seizure occurs (Leonard, 2000).

In the brains of epileptic patients and animals, membrane-bound enzymes (particularly

the ATPases involved in the ionic pumps for calcium, sodium and potassium) have been found to function abnormally. A reduction in Na^+/K^+-ATPase activity has been reported in human focal epileptogenic tissue; in experimental animals following the localized application of alumina cream; and in DBA/2 mice that exhibit sound-induced seizures. A reduction in ATPase activity is consistent with the hypothesis that a defect in ion channels may occur in epilepsy.

Endogenous epileptogenic compounds may be produced in the brains of epileptic patients. Both tetrahydroisoquinolones and beta-carbolines have been detected in the human brain, as has the tryptophan analogue quinolinic acid, which all have convulsant and excitotoxic properties (Leonard, 2000).

In chronic epileptics and in patients with febrile seizures the concentration of GABA has been found to be reduced. The central role of GABA in epilepsy is further suggested by the observation that drugs that reduce the GABA concentration are epileptogenic, while those that raise the GABA concentration are generally anticonvulsants (Leonard, 2000).

2.3.5. Anticonvulsants

2.3.5.1. Mechanisms of anticonvulsant drug action

Three major mechanisms are proposed:

a) Drugs that bind to the voltage-dependent sodium channel

Three important anticonvulsant drugs, phenytoin, carbamazepine and lamotrigine

bind to the voltage-dependent sodium channel, which initiates neuronal action potentials. Each time the neuron fires, the sodium channel cycles through its "active", "inactive" and "resting" states. It is believed that these three drugs hold the channel a little longer in its inactive state. This means that the neuron can fire at moderate rates (for example, those involved in thinking) but not at very rapid rates (those involved in seizures).

a) Drugs that enhance activity in the $GABA_A$ system

The barbiturates, benzodiazepines, valproate and vigabatrin are thought to increase activation in the $GABA_A$ receptor system. This enhances $GABA_A$-mediated Cl^- influx, which stabilises the membrane near its resting potential and results in decreased neuronal excitability.

b) Drugs that bind to T-type voltage-dependent calcium channels

Two drugs active against absence seizures, trimethadione and ethosuximide are thought to bind to T-type voltage-dependent calcium channels. These channels are particularly important in the thalamus, where they are thought to contribute to the genesis of absence attacks. Trimethadione and ethosuximide decrease activity in T-type calcium channels (Burnham, 1998).

2.3.5.2. Anticonvulsants in clinical use

The eight classes of anticonvulsants that are used clinically are:

1. Barbiturates e.g. phenobarbitone and primidone
2. Hydantoins e.g. phenytoin

3. Dibenzapines e.g. carbamazepine
4. Oxazolidinediones e.g. trimethadione
5. Succinimides e.g. ethosuximide
6. Benzodiazepines e.g. diazepam, clobazam and clonazepam
7. Sulphonamides e.g. acetazolamide
8. Short chain fatty acids e.g. sodium valproate

The newer drugs include vigabatrin, gabapentin and lamotrigine. These compounds are fairly broad-spectrum, have relatively mild side effects and are associated with few drug interactions (Burnham, 1998).

2.4. Insomnia

Humans spend approximately one third of their life asleep. The clinical importance of sleep is reflected in the frequency and severity of complaints about insomnia, a condition that signifies unsatisfactory or insufficient sleep (Leonard, 2000). Insomnia is the most common sleep-related complaint and the second most common overall complaint (after pain) reported in primary care settings (Attarian, 2000). It affects 35% of the general population during the course of a year but, despite its high prevalence, 69% of patients suffering from sleep disorders never report it to healthcare providers (Balter and Uhlenhuth, 1992). Insomnia is a symptom and is not a diagnosis or disease (Sellers *et al.*, 1998). The prevalence of insomnia increases with age, is more common among females, women in minority groups, the unemployed and those with medical or psychiatric disorders (Holbrook, 2000). Patients may complain about difficulty in getting to sleep, disturbing dreams, early wakening and daytime drowsiness due to poor sleep at night.

2.4.1. Classification of insomnia

Insomnia can be subdivided into categories based on duration and aetiology of symptoms. Insomnia may be classified into three major types:

1. Transient insomnia
2. Short-term insomnia
3. Long-term insomnia

Transient insomnia occurs in normal sleepers who experience acute stress lasting for a few days and is typically due to factors that are readily understood as being disruptive of sleep (e.g. acute illness, social stress, jet-lag, hospitalisation). Short-term insomnia is associated with situational stress and last a few weeks. Short-term insomnia can be caused by grief, emotional stress or the use of stimulants or other medications that can cause sleep disturbance (Steele, 2003). Long-term insomnia is associated with underlying psychiatric illness and last for more than three weeks.

2.4.2. Physiology of sleep

Although there is no evidence for a specific sleep "center" in the brain, it is generally accepted that the level of consciousness is located in the diffuse network of nerve cells that comprise the reticular formation (Leonard, 2000). Normal sleep consists of at least two phases:

1. Slow wave sleep

   The EEG shows predominantly high-voltage synchronous activity. The tonus if skeletal muscle is maintained.

2. Rapid-eye-movement (REM) sleep

   The EEG shows an arousal pattern, the eyes move rapidly and irregularly, skeletal muscle relax completely and dreaming may take place.

These two phases alternate throughout the total sleep period, REM sleep making up about 25% of the total (Sellers et al., 1998). The alternation between slow wave sleep and REM sleep depends upon a balance of serotonin (5-HT) and catecholamine influences on the reticular formation.

The sleep produced by hypnotics differs from normal sleep, in that short wave sleep patterns are altered and shortened by the appearance of EEG spindles, REM sleep is suppressed and total sleep time is prolonged (Sellers et al., 1998).

2.4.3 Hypnotic drugs

Hypnotic drugs provide only symptomatic treatment for insomnia. Although often efficacious in the short term, they do little to alter the underlying cause which should be sought and treated where possible (Ashton, 2003).

The hypnotic drugs commonly in use include

a) Benzodiazepines e.g. diazepam
b) Non-benzodiazepines e.g. zopiclone
c) Antihistamines e.g. promethazine

2.5. Pain

2.5.1. Pain transmission

The majority of tissues and organs contain special sensory receptors (nociceptors) connected to primary afferent nerve fibres of different diameters. Small myelinated Aδ fibres and unmyelinated C fibres are responsible for the transmission of painful stimuli. These afferent primary fibres terminate in the dorsal horn of the spinal grey matter (Woolfrey and Kapur, 1998).

According to Kadar (1998), pain is essential for survival. Pain serves as a warning of impending or actual tissue or organ injury. Pain sensations originate from the stimulation of naked nerve endings found in all parts of the body. Pain-producing substances such as histamine or kinins stimulate the naked nerve endings directly while prostaglandins lower the pain threshold by increasing the sensitivity of the receptors to the stimulus (Kadar, 1998).

The sensation of pain is transmitted from the periphery through the spinal cord to higher integrative centers in the CNS by "fast" myelinated Aδ fibers at 10-30 m/s, and by non-myelinated "slow" C fibers at 0.5-2 m/s.

2.5.2. Neurotransmitters and pain

Various neurotransmitters found in the dorsal horn of the spinal cord may be involved in pain modulation. These include amino acids such as glutamate and GABA, monoamines such as noradrenaline and 5-hydroxytryptamine (5-HT) and certain peptide molecules, of which the opioid peptides are the most important. Opioid receptors are found in both the CNS and the periphery. In the CNS they are found in high concentrations in the limbic system, the brainstem and the spinal cord. The natural ligands (molecules that bind to the receptor) for opioid receptors are a group of neuropeptides known as endorphins. Opioid analgesics mimic the actions of these natural ligands and exert their effect through the μ, δ and to a lesser extent, the κ receptors. These receptors mediate the analgesic effect of morphine-like drugs (Woolfrey and Kapur, 1998).

Chapter 3

Materials and Method

3.1 Plant material

3.1.1 Selection, collection and identification of *Mentha longifolia*

Mentha longifolia was selected based on the reported use by traditional healers for epilepsy, insomnia and pain. The plant material was collected from Kirstenbosch Botanical Gardens, Cape Town, South Africa and identified by Dr. F. Weitz, a taxonomist in the Department of Biodiversity and Conservative Biology. A voucher specimen (Harris 02) was deposited in the University's Herbarium.

3.1.2 Preparation of aqueous extract of *Mentha longifolia*

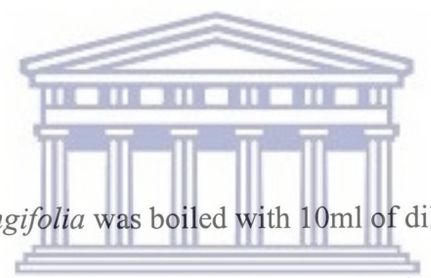
The leaves of the plant under study were washed with distilled water and dried in a ventilated oven at 30-35°C for about 72 hours. The dried leaves were ground into a fine powder (850μm) using the Waring commercial laboratory blender. A known quantity of the powder was refluxed in previously boiled water (one liter) and allowed to cool. The cooled solution was filtered, the filtrate frozen at -80° C and freeze-dried for 72 hours to obtain the dried plant extract, which was used for the experiments.

The *Mentha longifolia* solution that was used in the different experiments was freshly prepared on each day of the experiment performed by dissolving a given quantity of the dried extract in an appropriate volume of 0.9% w/v sodium chloride solution.

3.2. Phytochemical analysis of *Mentha longifolia*

Phytochemical analysis was performed using the methods of Harborne (1984) and Ikhiri *et al*. (1992) to determine the groups of chemical compounds present in the aqueous extract of *Mentha longifolia*.

3.2.1. Alkaloids



0.5g of the powdered *M. longifolia* was boiled with 10ml of dilute hydrochloric acid (alcoholic) in a test tube for 5 minutes. The mixture was cooled and the debris was allowed to settle. The supernatant liquid was filtered into another test tube. 1ml of the filtrate was taken and three drops of Dragendorffs' reagent added, shaken and observed for precipitate formation.

3.2.2. Saponins

A little of the powdered *M. longifolia* was shaken with water. The mixture was observed for frothing.

3.2.3 Tannins

0.2g of the powdered *M. longifolia* was boiled in 5ml of water. The "tea" was cooled and filtered. A few drops of 5% ferric chloride solution were added to the filtrate and observed for precipitate formation.

3.2.4. Reducing sugars

0.2g of the powdered *M. longifolia* was boiled in 5ml of water. The "tea" was cooled and filtered. An equal quantity (5ml) of Fehlings A and B solutions were added to the filtrate and heated on a water-bath, and then observed for precipitate formation.

3.2.5. Anthraquinones

0.1g of the powdered *M. longifolia* was shaken in 10ml of ferric chloride solution (15%) and 5ml of hydrochloric acid, and immersed in a water bath for 10 minutes. The mixture was filtered immediately. The filtrate was cooled and extracted with 10ml of carbon tetrachloride. The carbon tetrachloride layer was separated, washed with 5ml of water and shaken with 5ml of dilute ammonia solution. The resultant mixture was observed for a colour change in the ammoniacal layer.

3.2.6. Cardiac glycosides

0.5g powdered *M. longifolia* was boiled in 5ml of alcohol (70%) for 2 minutes. The

mixture was filtrated and 10ml of water and 5ml of chloroform were added to the filtrate. It was then shaken. The lower chloroform layer was separated off and evaporated to dryness on a water-bath. The cooled residue was dissolved in 3ml of glacial acetic acid containing 0.1ml of ferric chloride. The solution was carefully transferred to the surface of 2ml of sulphuric acid and observed for colour change at the interphase.

3.2.7. Flavonoids

The powdered *M. longifolia* (10g) was boiled for 2 to 3 minutes in a water-bath (100ml). To 3ml of the filtrate, 3ml of acid-alcohol, solid magnesium and 1ml of t-amyl-alcohol were added. The mixture was then observed for colour change.

3.2.8. Triterpene Steroids

The powdered *M. longifolia* was extracted in ether. The extract was evaporated to dryness and the residue was redissolved by adding several drops of acetyl anhydride and sulphuric acid. The mixture was then observed for colour change.

3.3. Pharmacological assessment

3.3.1. Experimental animals

Male albino mice bred in the Animal house, Discipline of Pharmacology, School of Pharmacy, University of the Western Cape, were used in groups of eight per dose of drug

or plant extract. The animals weighed between 15 and 25g. All the mice had free access to food and water before the commencement of the experiments. Each mouse was used for only one experiment.

3.3.2. Drugs and chemicals

Pentylenetetrazole (PTZ, Sigma Co.), picrotoxin (Sigma Co.), N-methyl-DL-aspartic acid (Sigma Co.), phenobarbitone (Gardenyl®, Rhone-poulenc Rorer, South Africa), pentobarbitone (Kyron Lab. Ltd., RSA) and morphine (Bodene) were all dissolved in physiological saline to appropriate volumes. Bicuculline (Sigma Co.) was suspended in 0.5ml of Tween 80, and diazepam (Valium®, Roche, South Africa) was suspended in polyethylene glycol 400 (Fluka AG, Buchs). Both suspensions were separately adjusted to appropriate volumes with physiological saline. Acetic acid (Merck) was dissolved to an appropriate strength in physiological saline. Paracetamol (4-Acetamidophenol, Sigma Co.) was dissolved in a minimum amount of propylene glycol (Heynes Matthew) and made up to the appropriate volume with physiological saline. Preliminary studies were carried out to establish the doses of plant extract and drugs as well as the pretreatment times to be used. All drugs and plant extract solutions were injected intraperitoneally (ip) in a volume of 1ml/100g of animal. Fresh drug solutions were prepared on the days of the experiment.

3.3.3. Anti-epileptic effect

The method of Vellucci and Webster (1984) and modified by Amabeoku and Chikuni

(1993) was used to assess the anticonvulsant effect of the plant extract. The mice were housed

singly in a transparent Perspex cage for 30 minutes before commencement of the experiment in order to habituate them to their new environment. Standard convulsant agents such as pentylenetetrazole (PTZ), bicuculline, picrotoxin and N-methyl-DL-aspartic acid (NMDLA) were used to induce convulsions in animals. The animals were observed for 30 minutes for tonic convulsion in each experiment. Seizures were manifested as tonic hind-limb extension. The ability of the plant extract to prevent this feature or prolong the latency or onset of the tonic hind limb extension was taken as an indication of anticonvulsant activity (Navarro-Ruiz *et al.*, 1995; Navarro-Ruiz *et al.*, 1996; Amabeoku *et al.*, 1998). The time of onset of seizures and proportion of animals convulsing or not convulsing were obtained during this period of observation. Animals that did not convulse during the period of observation were considered as not having convulsed (Akah *et al.*, 1988; Amabeoku *et al.*, 1998). Experiments were repeated with other groups of animals pretreated for 15 minutes with plant extract or standard anticonvulsant drug (phenobarbitone or diazepam) prior to the administration of either of the convulsant agents.

3.3.4. Effect on pentobarbitone-induced sleep

The methods of Kitano *et al.* (1994) and Williamson *et al.* (1996) were used to assess the effect of the plant extract on pentobarbitone-induced sleep. Pentobarbitone, a barbiturate, was used to induce sleep in the animals. The time of onset and duration of sleep were noted.

The animals are said to be asleep when they lose their righting reflex. The period between loss and recovery of righting reflex was noted as sleeping time (Miya *et al.*, 1973; Wambebe, 1985 and Rolland *et al.*,1991). Experiments were repeated with animals pretreated for 15 minutes with either plant extract or standard hypnotic drug, diazepam (Rang *et al.*, 2000), prior to administration of pentobarbitone. The interval between loss and recovery of righting reflex has also been used as the index of hypnotic effect (Fujimori and Cobb, 1965).

3.3.5. Analgesic effect

3.3.5.1. Acetic acid Writhing test

The methods of Koster *et al.* (1959) and Williamson *et al.* (1996) were used to assess the analgesic effect of the plant extract. 3% acetic acid was used to induce writhing reflex in the animals. 0.2 ml of 3% acetic acid was injected intraperitonally and 5 minutes after injection, the writhes produced by the animals were counted for 15 minutes. Experiments were repeated with animals pretreated for 15 minutes with the plant extract or standard drug, paracetamol (500 mg/kg), prior to the administration of 3% acetic acid.

3.3.5.2. Hot plate method

The methods of Eddy and Leimback (1953) and Williamson *et al.* (1996) were used. Pain was induced by thermal stimulation.

The control animals were placed singly into a 2L beaker placed on top of a thermostatically controlled hot plate at 50 to 55°C. The pain threshold is reached when the animal lifts and licks its paws or attempts to jump out of the beaker. The time taken for the animals to exhibit the above characteristics was noted by means of a stopwatch. Experiments were repeated with animals pretreated for 15 minutes with the plant extract or standard drug, morphine (10 mg/kg), prior to thermal stimulation.

3.4. Statistical analysis

All the data obtained from the pharmacological tests, with the exception of the proportion of animals convulsing, were analyzed using the paired Student's t-test. The proportion of animals convulsing was analyzed using the Chi-squared test (Tallarida and Murray, 1981; Amabeoku *et al.*, 2001 and Bienvenu *et al.*, 2002).

3.5. HPLC analysis

Using standard chromatographic methods, the spectral profile of the plant extract was analyzed. The equipment and parameters used were as follows:
Chromatographic system: Beckman HPLC system consisting of a double pump Programmable Solvent Module 126, Diode Array detector Module 168, with 32 carat Gold software supplied by Beckman; Column C18 Bondapak 5μm and dimensions (250 x 4.6mm).

Chromatographic conditions: Mobile phase, solvent A: methanol (MeOH); solvent B: 5% acetic acid (CH_3COOH); Mode: gradient, increasing the organic phase (MeOH) from 20% to 90% over 18 minutes; flow rate: 1ml/min; reference standard, Rutin (2.5g dissolved in 10ml MeOH). The run time was 25 minutes.

Chapter 4

Results

4.1. Phytochemical analysis

The plant tested positive for saponins, tannins, reducing sugars, cardiac glycosides, flavonoids and triterpene steroids. *M. longifolia* tested negative for alkaloids and anthraquinones (Table 1).

Table 1. Phytochemical analysis of *Mentha longifolia*

Compound	Results
Alkaloids	Negative
Saponins	Positive
Tannins	Positive
Reducing sugars	Positive
Cardiac glycosides	Positive
Flavonoids	Positive
Triterpene steroids	Positive
Anthraquinones	Negative

4.2. Anti-epileptic effect

4.2.1. Effect of aqueous extract of *M. longifolia* on pentylenetetrazole-induced seizures

Pentylenetetrazole (95 mg/kg) produced tonic seizures in all the animals used.

A dose of 100 mg/kg of *M. longifolia* did not protect any of the animals since they all convulsed. A dose of 200 mg/kg of *M. longifolia* protected 12.5% of mice against pentylenetetrazole-induced seizures, but did not significantly reduce the onset of seizures. *M. longifolia* (400 mg/kg) protected 50% of the animals against pentylenetetrazole-induced seizures and significantly delayed the onset of seizures.

The standard anti-epileptic drugs, phenobarbitone (20 mg/kg) and diazepam (0.5 mg/kg), profoundly antagonized the seizures produced by pentylenetetrazole (Table 2).

Table 2. Effect of aqueous extract of *M. longifolia* (ML) on pentylenetetrazole (PTZ)-induced seizures in mice

Dose (mg/kg)				No. convulsed / No. used	Animals not convulsed (%)	Onset of tonic convulsion (min)
PTZ	ML	Pheno-barbitone	Diaze-pam			Mean ± SEM
95	-	-	-	8/8	0	3.38 ± 0.75
95	100	-	-	8/8	0	4.50 ± 1.34
95	200	-	-	7/8	12.5	5.71 ± 1.67
95	400	-	-	4/8	50	10.25* ± 1.88
95	-	20	-	0/8$^{\Phi}$	100	0
95	-	-	0.5	0/8$^{\Phi}$	100	0

*$p<0.005$ vs. pentylenetetrazole control (95 mg/kg; ip.), Student's t-test.

$^{\Phi}p<0.001$ vs. pentylenetetrazole control (95 mg/kg; ip.), Chi-squared test (n = 8).

4.2.2. Effect of aqueous extract of *M. longifolia* on picrotoxin-induced seizures

Picrotoxin (10 mg/kg) produced tonic seizures in all the animals used. A dose of 100 mg/kg of *M. longifolia* protected 12.5% of mice and significantly delayed the onset of picrotoxin-induced seizures. A dose of 200 mg/kg of *M. longifolia* protected 50% of mice and also significantly delayed the onset of picrotoxin-induced seizures. Similarly, *M. longifolia* (400 mg/kg) protected 25% of the animals and significantly delayed the onset of picrotoxin-induced seizures. The standard anticonvulsant drug, phenobarbitone (20 mg/kg) protected 37.5% of mice and also significantly delayed the onset of tonic convulsion. Diazepam (0.5 mg/kg) significantly reduced the number of animals convulsing and also significantly delayed the onset of picrotoxin-induced seizures in mice (Table 3).

Table 3. Effect of aqueous extract of *M. longifolia* (ML) on picrotoxin (PCT)-induced seizures in mice

Dose (mg/kg)				No. convulsed / No. used	Animals not convulsed (%)	Onset of tonic convulsion (min)
PCT	ML	Pheno-barbitone	Diaze-pam			Mean ± SEM
10	-	-	-	8/8	0	12.38 ± 0.68
10	100	-	-	7/8	12.5	18.00* ± 1.80
10	200	-	-	4/8	50	16.00** ± 0.76
10	400	-	-	2/8$^{\Phi}$	75	21.50*** ± 1.80
10	-	20	-	5/8	37.5	25.20*** ± 1.45
10	-	-	0.5	1/8$^{\Phi\Phi}$	87.5	25.60*** ± 0

*p<0.02; **p<0.01; ***p<0.001 vs. picrotoxin control (10 mg/kg; ip.); Student's t-test.

$^{\Phi}$p<0.01; $^{\Phi\Phi}$p<0.005 vs. picrotoxin control (10 mg/kg, ip.), Chi-squared test (n = 8).

4.2.3. Effect of aqueous extract of *M. longifolia* on bicuculline-induced seizures

Bicuculline (30 mg/kg) induced seizures in all the animals used. A dose of 100 mg/kg of *M. longifolia* protected 12.5% of mice, but did not significantly alter the onset of bicuculline-induced seizures. *M. longifolia* (200 mg/kg) protected 25% of the animals used but did not alter the onset of bicuculline-induced seizures in mice. A dose of 400 mg/kg of *M. longifolia* protected 12.5% of animals against seizures induced by bicuculline (30 mg/kg). However, this dose significantly delayed the onset of bicuculline-induced seizures. The standard anti-epileptic drugs, phenobarbitone (20 mg/kg) and diazepam (0.5 mg/kg) profoundly antagonised seizures produced by bicuculline (Table 4).

Table 4. Effect of aqueous extract of *M. longifolia* (ML) on bicuculline (BIC)-induced seizures in mice

Dose (mg/kg)				No. convulsed / No. used	Animals not convulsed (%)	Onset of tonic convulsion (min)
BIC	ML	Pheno-barbitone	Diaze-pam			Mean ± SEM
30	-	-	-	8/8	0	5.25 ± 0.41
30	100	-	-	7/8	12.5	5.71 ± 0.44
30	200	-	-	6/8	25	5.50 ± 0.70
30	400	-	-	7/8	12.5	26.57$^{\#}$ ± 0.57
30	-	20	-	0/8*	100	0
30	-	-	0.5	0/8*	100	0

The data on onset of tonic seizures were compared using Student's t-test.

$^{\#}p<0.001$ vs bicuculline control (30 mg/kg; ip), Student's t-test (n = 8).

*$p<0.001$ vs. bicuculline control (30 mg/kg; ip.), Chi-squared test (n = 8).

4.2.4. Effect of aqueous extract of *M. longifolia* on N-methyl-DL-aspartic acid-induced seizures

NMDLA (400 mg/kg, ip.) elicited seizures in all the mice used. Aqueous extract of *M. longifolia* (100-800 mg/kg, ip.) did not affect the onset or incidence of the seizures induced by NMDLA (400 mg/kg, ip.). Similarly, phenobarbitone (20 mg/kg, ip.) or diazepam (0.5 mg/kg) did not affect the onset or incidence of NMDLA (400 mg/kg, ip) – induced seizures (Table 5).

Table 5. Effects of aqueous extract of *M. longifolia* (ML) on N-methyl-DL-Aspartic acid (NMDLA)-induced seizures in mice

Dose (mg/kg)				No. convulsed / No. used	Animals not convulsed (%)	Onset of tonic convulsion (min)
NMDLA	ML	Pheno-barbitone	Diaze-pam			Mean ± SEM
400	-	-	-	8/8	0	5.24 ± 0.67
400	100	-	-	8/8	0	6.19 ± 0.80
400	200	-	-	8/8	0	5.43 ± 0.41
400	400	-	-	8/8	0	5.95 ± 0.36
400	800	-	-	8/8	0	5.52 ± 0.54
400	-	20	-	8/8	0	6.33 ± 0.82
400	-	-	0.5	8/8	0	6.41 ± 0.77

The data on the onset of seizures induced by NMDLA was analysed by paired Student's t-test, while the incidence of the seizure was analysed using the Chi-squared test (n=8).

4.3. Pentobarbitone-induced sleep test

Pentobarbitone (35 mg/kg, ip.) –induced sleep, which lasted for 20 minutes in animals pretreated with physiological saline. Aqueous extract of *M. longifolia* (100-200 mg/kg, ip.) did not alter the sleep induced by pentobarbitone (35 mg/kg, ip.). However, there was a dose-dependent increase in the sleeping time by *M. longifolia* (400-800 mg/kg, ip.). A dose of 400 mg/kg (ip.) of aqueous extract of *M. longifolia* weakly prolonged pentobarbitone (35 mg/kg, ip.) –induced sleep. *M. longifolia* (600-800 mg/kg, ip.) significantly prolonged the sleep induced by pentobarbitone (35 mg/kg, ip.). Similarly, the standard drug, diazepam (0.5 mg/kg, ip.) significantly prolonged the pentobarbitone-induced sleep (Table 6). Aqueous extract of *M. longifolia* (400-800 mg/kg, ip.) markedly sedated the animals.

Table 6. Effect of aqueous extract of *M. longifolia* (ML) on pentobarbitone (PB) – induced sleep in mice

Dose (mg/kg)			Duration of sleep (min)
PB	ML	Diazepam	Mean ± SEM
NS	-	-	20.00 ± 4.03
35	100	-	17.25 ± 2.83
35	200	-	19.38 ± 3.72
35	400	-	27.38 ± 6.62
35	600	-	40.38* ± 1.76
35	800	-	54.88* ± 2.36
35	-	0.5	51.63* ± 3.06

*$p<0.001$ vs. pentobarbitone (35 mg/kg) control, Student's t-test (n=8)

4.4. Analgesic effect

4.4.1. Acetic acid Writhing test

3% Acetic acid (0.2 ml) produced a large number of writhes in control mice pre-treated with physiological saline. Aqueous extract of *M. longifolia* (6.25 mg/kg) significantly inhibited the writhes produced by 3% acetic acid. *M. longifolia* (12.5-100 mg/kg) completely inhibited the writhes induced by 3% acetic acid. Similarly, paracetamol (500 mg/kg) completely inhibited 3% acetic acid-induced writhes in mice (Table 7). During the pre-treatment period, the gross behaviour of the animals was not altered.

Table 7. Effect of aqueous extract of *M. longifolia* on acetic acid-induced writhing in mice

Treatment group	Dose (mg/kg)	Writhing Mean ± SEM
Normal saline	-	20.00 ± 0.52
M. longifolia	6.25	3.50* ± 1.09
	12.5	0*
	25	0*
	50	0*
	100	0*
Paracetamol	500	0*

*$p<0.001$ vs. acetic acid control, Student's t-test (n=8).

0.2 ml of 3% acetic acid was administered intraperitoneally to animals throughout the experiments.

Writhing is expressed as number/count per 15 minutes.

4.4.2. Hot plate test

The threshold of pain was considered to have been reached when the mice lifted and licked their paws or attempted to jump out of the beaker placed on the hotplate at 50-55 °C. The reaction time of the animals to thermal stimulation was obtained before (0 minutes) and 15, 30 and 60 minutes after pre-treatment with physiological saline, aqueous extracts of *M. longifolia* or the standard drug, morphine. Aqueous extract of *M. longifolia* (25-400 mg/kg) significantly prolonged the reaction time of the animals to hot-plate thermal stimulation 15 minutes after treatment. 30-60 minutes after treatment, the aqueous extract of *M. longifolia* significantly delayed the response of the mice to hot-plate thermal stimulation in a dose-dependent manner. Similarly, morphine (10 mg/kg) significantly delayed the response of the animals to hot-plate thermal stimulation 15, 30 and 60 minutes after treatment (Table 8).

Table 8. Effect of aqueous extract of *M. longifolia* on hotplate-induced nociception in mice

Treatment group	Dose (mg/kg)	Response time (s) 0	15	30	60
Normal saline	-	3.13 ± 0.23	3.00 ± 0.27	2.63 ± 0.26	2.50 ± 0.38
M. longifolia	25	3.28 ± 0.29	7.81*± 0.97	7.56* ± 1.11	6.85*± 0.92
	50	3.13 ± 0.23	7.18*± 0.97	7.34* ± 0.71	7.02*± 0.57
	100	3.13 ± 0.23	14.60*± 2.05	12.75* ± 1.84	12.33*± 1.21
	200	3.13 ± 0.23	11.69*± 1.30	14.73* ± 2.37	15.23*± 1.50
	400	3.13 ± 0.23	17.44*± 2.14	17.98* ± 1.63	21.92*± 2.06
Morphine	10	3.28 ± 0.29	11.68*± 4.03	12.41* ± 1.78	13.37*± 2.16

*$p<0.001$ vs. saline control, Student's t-test (n = 8)

The response time in seconds is expressed as means ± SEM

4.5.HPLC analysis

The HPLC analysis of *M. longifolia* showed major characteristic peaks at the following retention times (minutes): 20.52, 22.37, 23.15, 24.87 and 26.93 (Fig. 2).

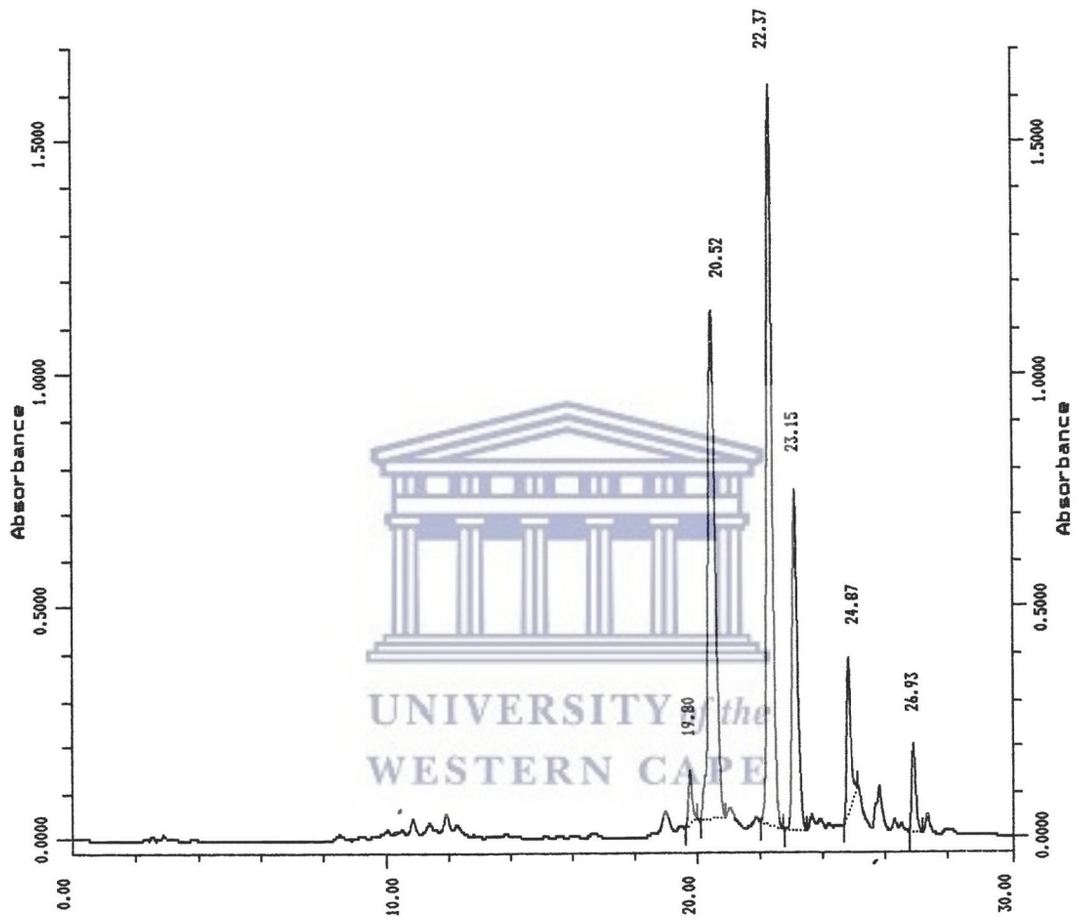


Fig. 2. HPLC chromatogram of *M. longifolia*

Chapter 5

Discussion

Mentha longifolia has been used to treat epilepsy, toothache, headache, hysteria and insomnia among other disorders (Van Wyk *et al.*, 1997; Van Wyk and Gericke, 2000). Little or no scientific information exists about the central effects of the plant. This study therefore, investigated some of the central effects of *M. longifolia*, which relate to its use in convulsion, painful conditions and insomnia.

The data obtained in this study, show that the aqueous extract of *M. longifolia* (400 mg/kg) attenuated the seizures produced by pentylenetetrazole (95 mg/kg). The standard anticonvulsant agents, phenobarbitone (20 mg/kg) and diazepam (0.5 mg/kg) also completely protected the animals against pentylenetetrazole-induced seizures. Pentylenetetrazole (PTZ) has been reported to elicit seizures by inhibiting gamma aminobutyric acid neurotransmission (Okada *et al.*, 1989; De Sarro *et al.*, 1999). Gamma aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain, which is widely implicated in epilepsy. The enhancement of the GABAergic neurotransmission has been shown to attenuate seizures while the inhibition of the neurotransmission promotes seizures (Meldrum, 1975; Olsen, 1981; Gale, 1992; Leonard, 2000). In line with this, the complete protection of mice against PTZ-induced seizures by both the standard anticonvulsant drugs, phenobarbitone and diazepam is not unexpected, since they have been shown to exert their anticonvulsant activities by enhancing GABA-

mediated inhibition (Olsen, 1981; Leonard, 2000; Rang *et al.*, 2000). It is therefore possible that the aqueous extract of *M. longifolia* may be attenuating PTZ-induced seizures by interfering with GABAergic neurotransmission.

Aqueous extract of *M. longifolia* and the standard anticonvulsant agents, phenobarbitone and diazepam, antagonised the seizures elicited by picrotoxin in the present study. Postsynaptic $GABA_A$-receptors are functionally linked to benzodiazepine receptors, barbiturate receptors and chloride ion channels to form GABA-chloride ionophore complex, which is involved in the modulation of GABAergic inhibitory transmission (Olsen, 1981; Bennett and Brown, 2003; Rang *et al.*, 2000). According to Rang *et al.* (2000) and Nicoll (2001), picrotoxin, an antagonist of $GABA_A$-receptors, produces seizures by blocking the chloride ion channels linked to $GABA_A$-receptors, thus preventing the entry of chloride ions into the neuron. This in turn, inhibits GABA mediated inhibition and hence GABA neurotransmission. Phenobarbitone and diazepam are believed to enhance GABAergic inhibition and neurotransmission by increasing chloride flux through chloride ion channels at $GABA_A$-receptor sites (Olsen, 1981; Trevor and Way, 2001; Rang *et al.*, 2000). This may explain the antagonistic effects of phenobarbitone and diazepam on picrotoxin-induced seizures in the mice used. It is possible, therefore, that the *M. longifolia* extract antagonises picrotoxin-induced seizures by opening the chloride ion channels associated with $GABA_A$-receptors. This supports further the hypothesis that the *M. longifolia* extract may be interfering with GABAergic neurotransmission.

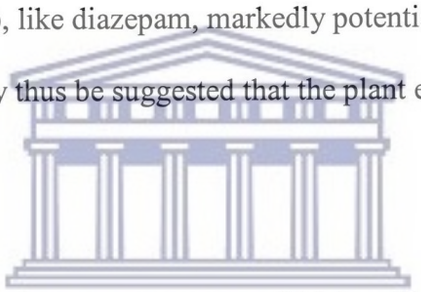
According to Rang *et al.* (2000) bicuculline, a potent selective $GABA_A$-receptor

antagonist, produces seizures by blocking the effect of GABA at central $GABA_A$-receptors, which have been associated with epilepsy (Gale, 1992). In the present study, the standard anticonvulsant drugs, phenobarbitone and diazepam, which act by enhancing GABA neurotransmission, antagonised bicuculline-induced seizures. However, *M. longifolia* did not significantly affect bicuculline-induced seizures. This observation therefore suggests that the aqueous extract of *M. longifolia* may be interfering with GABA neurotransmission but not at $GABA_A$-receptors.

The involvement of glutamic acid receptor (NMDA-receptor) synaptic functions in the pathogenesis of epilepsy is widely accepted. According to Chapman and Meldrum (1993) and Leonard (2000), activation of NMDA-receptors is thought to underlie seizures. NMDLA, an agonist at the NMDA-receptor (Watkins and Evans, 1981), elicited seizures in mice in this study. However, the aqueous extract of *M. longifolia* and the standard anticonvulsants, phenobarbitone and diazepam, did not alter NMDLA-induced seizures. This suggests that glutaminergic mechanisms may not be involved in the anticonvulsant activity of the plant extract.

In the present study, pentobarbitone induced sleep in mice. Aqueous extract of *M. longifolia* at doses between 600 and 800 mg/kg significantly potentiated pentobarbitone-induced sleep. Similarly, diazepam, a standard central nervous system depressant drug with hypnotic activity (Waller *et al.*, 2001; Bennett and Brown, 2003) significantly potentiated pentobarbitone-induced sleep. It is important to note that the *M. longifolia* extract like diazepam, profoundly sedated the animals during the pre-treatment period.

In animal experiments, the term "hypnotic" has been applied to a much deeper stage of central depression of drug-induced unconsciousness associated with loss of muscle tone and of righting reflexes. Many of the pharmacological tests, even though non-specific, are based on the potentiation of sleeping time induced by barbiturates or other sedative agents (Vogel and Vogel, 1997). Furthermore, according to Fujimori and Cobb (1965), the interval between loss and recovery of righting reflex in a CNS depressant drug-induced sleep test has been used as an index of the hypnotic effect. Kitano *et al.* (1994) reported that potentiation of pentobarbitone-induced sleep could be used to show CNS depressant activity of drugs. As shown in the present study, the aqueous extract of *M. longifolia* (600 – 800 mg/kg), like diazepam, markedly potentiated pentobarbitone-induced sleep in mice. It may thus be suggested that the plant extract possesses a hypnotic effect.



The present study shows that the aqueous extract of *M. longifolia* (6.25 – 100 mg/kg) exerted an inhibitory activity on the acetic acid-induced writhing response. Similarly, paracetamol, a standard peripherally acting analgesic drug (SAMF, 2003), antagonised the acetic acid-induced writhing. In the hot-plate test, *M. longifolia* (25 – 400 mg/kg) significantly prolonged the reaction time of the animals to hot-plate thermal stimulation over the period of observation. Similarly, morphine, a standard centrally acting analgesic drug (SAMF, 2003) delayed the reaction time to the hot-plate thermal stimulation. According to Eddy and Leimback (1953), Koster *et al.* (1959) and Williamson *et al.* (1996), acetic acid writhing and hot-plate tests are normally used to study the peripheral and central analgesic effects of drugs

respectively. Since like paracetamol, the aqueous extract of *M. longifolia* inhibited acetic acid-induced writhing and also like morphine, the extract delayed the reaction time to hot-plate thermal stimulation, it is probable that this plant extract could be producing its analgesic effects both peripherally and centrally.

The phytochemical analysis shows that the leaves of *M. longifolia* contain saponins, tannins, reducing sugars, cardiac glycosides, flavonoids and triterpene steroids. The therapeutic benefits of traditional medicines might depend upon a combination of constituents. Some of these components found in *M. longifolia* might have contributed to the observed effects. According to Chauhan *et al.* (1988), Rahman and Iqbal (1999) and iHumans (inc Website, 2001), terpenoids have been shown to have strong anticonvulsant activity in different types of medicinal plants. Terpenoids have been shown to include saponins and triterpene steroids (Williamson *et al.*, 1996; Bruneton, 1999). It could, therefore, be suggested that the saponins and triterpene steroids may contribute to the activity of *M. longifolia* in seizures. Furthermore, the saponins and triterpene steroids have been shown to have analgesic properties (Bruneton, 1999). This may also contribute to the analgesic activity of the *M. longifolia* extract. Saponins have been reported to have potent sedative activity (Wagner *et al.*, 1983; Dubois *et al.*, 1986). During the pre-treatment of the animals before the commencement of the pentobarbitone-induced sleep experiment, *M. longifolia* (600 – 800 mg/kg) sedated the animals. It is, therefore, likely that saponins found in the plant may have contributed to this sedative effect, which could also have partly accounted for the potentiation of the pentobarbitone-induced sleep.

The spectral profile of *M. longifolia* showed major peaks at the following retention times (minutes): 20.52, 22.37, 23.15, 24.87 and 26.93 (Fig. 2.).

Chapter 6

Conclusion

The aqueous extract of *M. longifolia* has been shown to significantly delay the onset of seizures induced by pentylenetetrazole and to significantly antagonise picrotoxin-induced seizures. This indicates that the *M. longifolia* extract has anticonvulsant activity. Since pentylenetetrazole- and picrotoxin-induced seizures have been shown to occur as a result of attenuation of GABA neurotransmission, it is tempting to suggest that *M. longifolia* extract may be producing its anticonvulsant activity by enhancing GABA neurotransmission. The extract did not alter the seizures produced by NMDLA, suggesting that it was not interfering with glutaminergic systems in the brain.

The *M. longifolia* extract potentiated pentobarbitone-induced sleep in mice. This model, even though non-specific, has been used to test drugs for hypnotic activity. It has also been used to test drugs for CNS depressant activity, which contributes to the hypnotic activity. The potentiation of barbiturate-induced sleep has also been used as the index of hypnotic activity. The *M. longifolia* extract may, therefore, be said to have a hypnotic activity.

The aqueous extract of *Mentha longifolia* significantly antagonised acetic acid-induced writhing and significantly delayed the reaction time to hot-plate thermal stimulation. Both models have been used to test for peripherally and centrally acting analgesic drugs respectively.

It can therefore be suggested that the *M. longifolia* extract has peripheral as well as central analgesic activity.

The various constituents found in the plant extract such as saponins and triterpene steroids might have contributed to all the observed effects of *M. longifolia*.

The spectral profile obtained for the *M. longifolia* extract will help in easy identification of this species of plant.

Finally, since *M. longifolia* is said to have anticonvulsant, hypnotic and analgesic effects, the findings from this study could provide a rationale for the use of the plant by traditional medicine practitioners in epilepsy, insomnia and painful conditions such as headache and toothache.

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