PHARMACOLOGICAL EVALUATION OF *LEONOTIS LEONURUS* FOR ANTIEPILEPTIC ACTIVITY

By

Emile BIENVENU

Thesis submitted in partial fulfillment of the requirements for the degree of Magister Pharmaceuticae in the School of Pharmacy, Department of Pharmacology, at the University of the Western Cape.

Supervisor: Prof George J. Amabeoku

Department of Pharmacology

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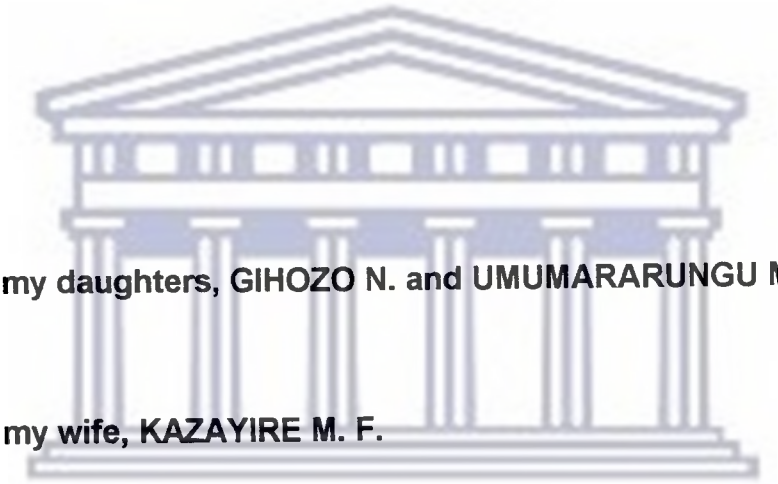
DEDICATION



To my daughters, GIHOZO N. and UMUMARARUNGU M. C.

To my wife, KAZAYIRE M. F.

To my late brother, AIMABLE NK.

CONTENTS

PHARMACOLOGICAL EVALUATION OF *LEONOTIS LEONURUS* FOR ANTIEPILEPTIC ACTIVITY

KEYWORDS

Traditional use

Medicinal plants

Scientific validation

Aqueous extract

Leonotis leonurus

Anticonvulsant properties

Seizures

Mice

Gabaergic and Glutaminergic systems

Phytochemical tests

ABSTRACT

PHARMACOLOGICAL EVALUATION OF *LEONOTIS LEONURUS* FOR ANTIEPILEPTIC ACTIVITY

Emile BIENVENU

M. Pharm thesis, School of Pharmacy, Department of Pharmacology, University of the Western Cape.

Purpose: The present study investigated the anticonvulsant properties of the aqueous extract of *Leonotis leonurus* (L.) R. BR. of the family, Lamiaceae and the possible mechanism of the antiepileptic activity of this plant. A preliminary phytochemical screening of the said plant extract was also carried out.

Methods: Seizures were chemically induced in mice with intraperitoneal injection of convulsant drugs such as pentylenetetrazole (90 mg/kg), picrotoxin (8 mg/kg), bicuculline (20 mg/kg) and N-methyl-DL-aspartic acid (400 mg/kg). The ability of the plant extract, intraperitoneally administered, to prevent or delay the tonic hind-limb extensor reflex was taken as an indication of anticonvulsant activity. Phenobarbitone and diazepam were used as standard antiepileptic drugs.

Results: *L. leonurus* extract in the doses of 200 and 400 mg/kg respectively protected 37.5% and 50% of animals used and significantly ($p<0.05$; Student's t-test) delayed pentylenetetrazole-induced tonic seizures. Similarly, the same doses of the plant extract significantly ($p<0.05$; Student's t-test) delayed the

onset of tonic seizures produced by picrotoxin and N-methyl-DL-aspartic acid. However, all the doses of aqueous extract of *L. leonurus* used did not alter the seizures induced by bicuculline (20 mg/kg) to any significant extent. High performance liquid chromatography (HPLC) and phytochemical tests respectively show a spectrum profile, characteristic of *L. leonurus* and the presence of alkaloids, saponins and tannins in the plant extract.

Conclusion: These data suggest that the aqueous extract of *L. leonurus* has anticonvulsant activity and may probably be acting through non-specific mechanisms, since it affects both gabaergic and glutaminergic systems.

May, 2001

STATEMENT

I declare that "*Pharmacological evaluation of Leonotis leonurus for antiepileptic activity*" 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 means of complete references.

Emile BIENVENU

May, 2001

Signed:.....

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LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

The use of plants as medicines has a long history. Plants have been a potential and primary source and continue to provide new remedies. In South Africa as in many other countries, traditional medicine is part of the culture of the people that use it. As a result, it is closely linked to their belief and has remained informal and successes based on oral traditions (Sofowora, 1982; Williamson et al., 1996).

Traditional medicine is accessible to most of the population in the third world. It is reported that 60-85 per cent of the population in every country of the developing world has to rely on traditional or indigenous forms of medicine (Sofowora, 1982). This is mainly because of shortage of hospitals and health centers, as well as of the lack of modern health care staff. Due to the socio-economic problems of developing countries, large numbers of people die daily of preventable or curable diseases because of the lack of simple health care. The common features in these countries are extremely limited resources, poor communication, vast distances, individual and community poverty, lack of education and so on (Sofowora, 1982). These particular conditions have resulted in traditional medicine becoming more accessible and cheaper than modern medicine.

An attempt to make modern health care more accessible to the rural population in developing countries often breaks down due to lack of spare parts for equipment. For example, countries in East Africa supplement their radio link system with a flying-doctor service, which only increases the cost of their modern health care (Sofowora, 1982). Additionally, the fact that many African countries have a proportion of nomads in their population creates logistical problems in making modern health care accessible to all. Traditional medical care does not encounter such difficulties as such people have their own traditional medicine practitioners. These factors, added to the fact that traditional medicine blends readily into the socio-cultural life of the people in whom culture is deeply rooted, makes traditional medicine enjoy a wider acceptability among the people of developing countries than does modern medicine. This is one of the reasons why traditional medicine practitioners could serve as an additional source of health manpower in developing countries, in their program of achievement of total health coverage of its people.

Traditional medicine presents other advantages. In terms of preparation and dosage of traditional remedies, there is greater likelihood of the body accepting the traditional medicine potions, because of their natural origin, than substances which have been invented in a laboratory. Also the concentration of active principle in the plant is usually small and it is further diluted when a decoction for traditional use is prepared (Sofowora, 1982). Likewise, it seems that an aqueous decoction of a drug has a greater bioavailability in the body than the many synthetic drug formulations used nowadays, even though only little documentation is available to

justify this (Sofowora, 1982). This fact can be rationalized on the grounds that the presentation of the active ingredient in the plant in a solubilized form would probably increase absorption by the body, even in the crudest forms of extraction such as teas and infusions. Everybody recognizes, however, the considerable improvement made in the modern formulations on the bioavailability of synthetic drugs. Another related advantage is that the development of resistance to synthetic chemotherapeutic agents occurring in modern medicine, for example, the resistance to chloroquine by some strains of plasmodium falciparum or vivax, the malaria parasites, or resistance to some antibiotics by certain strains of micro-organisms, is not encountered in traditional medicine. It is not known, however, if the non existence of such information is due to a lack of records (Sofowora, 1982).

However, one of the greatest arguments against traditional medicine today is the lack of scientific proof of its efficacy. Most of the claims are made by the traditional medicine practitioners themselves and many have not been thoroughly investigated scientifically. The imprecise diagnosis given by traditional medicine practitioners is also another point of criticism. For instance, a diagnosis of "stomach trouble" could mean indigestion, an ulcer, or cancer of the stomach. In fact, the pathology of certain diseases is not known to the traditional medicine practitioners. As a result, they tend to treat the symptom instead of treating the disease. Such a mistreatment leads sometimes to further complications. A lack of precision of dosage of medicaments used in traditional medicine is also criticized, even if some traditional medicine practitioners do specify dosage using such terms

as teaspoons-full and so on, and varying the dosage with the age of the patient. Another relevant point is that some of the plants constituents found in traditional medicine potions are toxic, even in minute doses. Examples to support this claim include eserine in *Physostigma venenosum* Balf., strychnine in *Strychnos* species and pyrrolizidine in *Symphytum officinale* L. (Comfrey) known to be highly hepatotoxic (Sofowora, 1982; De Smet, 1991).

Despite this, convergent sources confirm the irrevocable accessibility of traditional medicine to the majority of the population living in the rural areas especially in developing nations. In South Africa, for example, the westernization of the Zulus through urbanization and education did not affect the belief in traditional medicine remedies and healers. A dire shortage of western doctors in the rural areas, estimated in 1982 to be in the ratio of one medical practitioner for every 17500 people, has forced rural inhabitants to consult, by necessity, with traditional healers (De Smet, 1991; Bye et al., 1991). The reality is that rural population is in exponential growth, and the demand for traditional remedies is increasing. In view of the importance of traditional medicine practices, these should be improved by utilizing modified traditional medicine and retraining its practitioners. This needs an establishment of a self-regulating mechanisms within the groups of traditional practitioners themselves to ensure that quality care is given. Means for following up their patients and monitoring the methods used must be set up, as well as a scientific verification of various aspects, for example, physiological and pharmacological effects, reported about the traditional herbal remedies. Thus, the

present study aims to scientifically validate the claims of therapeutic successes of South African medicinal plants by pharmacologically evaluating *Leonotis leonurus* for antiepileptic activity.

The fact that there is a relationship between the use of medicinal plants and the psychological causes of illness in traditional medicine, a phytotherapeutic analysis of medicinal plants must work with a consciousness of the cultural concept of traditional medicine in which plant remedies are used.

CHAPTER 1

LITERATURE SURVEY

1.1 Traditional medicine

1.1.1 Definitions and terminology

Traditional medicine is the system of medicine based on past experience and cultural beliefs and practices handed down from generation to generation, verbally or in writing. The concept includes mystical and magical rituals, herbal therapy, psychiatry and other treatments which may not be explained by modern medicine. Studies suggest that this therapy is applied to conditions such as cancer, arthritis, chronic back pain, gastrointestinal problems, chronic renal failure, eating disorders, physical, mental or social disease, and so on (Sofowora, 1982; Shiffman Medical Library, 2001). Traditional medicine, however, uses technical terms found in botany, pharmacology and medicine. People often confuse or misuse some of these terms. In the available literature, effort is still being made to draw up a unanimous list of the key terms used.

A *traditional healer* is commonly described as a person who is recognized by the community to which he or she belongs as competent to provide health care. The person can use vegetable, animal and mineral substances and certain other methods which take inspiration from social, cultural and religious backgrounds. This description emphasizes the knowledge and beliefs that are prevalent in the community, regarding physical, mental, social well being and the cause of disease. A disagreement with certain aspects of this definition has appeared, based on the fact that a simple recognition by the community would include witch-doctors, diviners and spiritualists. Efforts are being made at present to avoid terminology which would present too many difficulties when reforms are introduced into the practice of traditional medicine as a whole. In this respect, at the third symposium organized by the Scientific, Technical and Research Commission of the Organization of African Unity in Abidjan, Ivory Coast in September 1979, it was agreed that the term used would be "traditional medical practitioner" (or traditional practitioner) for anglophone Africa and "praticien" for francophone Africa instead of "guerisseur" (Sofowora, 1982).

There are other terms used in traditional medicine practices. The term "*native medicine*" is a derogatory version of what should be termed traditional medicine. It is freely used to describe traditional medicine by many educated people. The native doctor uses his entire acquaintance with the structure of the human body and the anatomy of beasts to understand disease. He could not give an academic explanation of the functions of any one of the principal organs. For example, he

knows that the blood "runs" through the body. The connection between the circulation of blood and the beating of the heart is not a matter for him. The term "*folk medicine*" is a more acceptable reference to the knowledge of the mode of treatment or traditional beliefs which are common to a group of rural people. It doesn't involve a specific medical system, but uses tradition within any particular group or tribe of people. The term "*Juju*", is applied to that kind of traditional medicine which includes some supernatural or magical implication and the term "*ritual rites*" designs forms of procedure and sacrifice necessary to appease the gods in a particular situation for preventive or curative purposes (Sofowora, 1982).

The practice of "*incantation*" is also common in traditional medicine. Incantation is a form of a play on words (smiles) written or delivered orally in poetic form to conjure up efficacies into a medicine. Because of the name of the components of medicinal preparation repeated in the incantation, and by a play on words, that medicinal preparation is given the active effects desired of the medicine. This practice is not peculiar only to the African form of traditional medicine but also known throughout other parts of the world (Australia, Asia, Amerindian people). An incantation is believed to possess an evocative power, hence its efficacy. Nevertheless, the function of the incantation in producing a cure in traditional medicine can not be proved experimentally. For this reason, incantation has been dismissed from ethno-medical research to limit the occult power of herbs (Sofowora, 1982).

In traditional practice on health matters, the use of herbs is known to play a major role throughout the world. A traditional healer whose specialization lies in the use of herbs to treat various ailments is commonly called a "*herbalist*". A herbalist is expected to be highly knowledgeable in the vast array of herbs, particularly in the efficacy, toxicity, dosage and compounding of plants. They resemble actual pharmacists (Sofowora, 1982; Donker, 1989).

The World Health Organization (WHO) consultative group has defined a *medicinal plant* as any plant which, in one or more of its organs, contains substances (active ingredients) that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora, 1982). This definition includes medicinal plants studied scientifically to establish their therapeutic properties and active constituents, and plants known as medicinal, that have been used traditionally over several years, but which have not yet been subjected to a thorough scientific study. The term medicinal plant may not include crude drugs of natural or biological origin, mostly used by pharmacists and pharmacologists to describe whole plants or parts of plant which have medicinal properties. Medicinal plants include plants or plant parts used medicinally in galenical preparations such as decoctions and infusions, plants used for extraction of pure substances either for direct medicinal use or the hemi-synthesis of medicinal compounds, food, spice, and perfumery, microscopic plants, for example fungi and actinomycetes used for isolation of drugs, especially antibiotic and fibre plants, for example cotton, flax and jute, used for the preparation of surgical dressings (Sofowora, 1982). The WHO consultative

group has also recommended that the term "*vegetable drug*" indicates a part of a medicinal plant such as leaf, bark and root, used for therapeutic purposes (Sofowora, 1982).

Different galenical preparations used in traditional medicine are named by the appropriate terms. Thus a *concoction* means a preparation made usually from many ingredients. A *decoction* is a preparation obtained by decantation of filtration from a mixture of the plant medicine and water boiled beforehand. Many active constituents, for example some glycosides, are readily decomposed during such extraction because of the boiling. An *infusion* is a mixture prepared by pouring boiling water on a specified quantity of plant material and allowed to stand. A tea and an aqueous preparation made by decoction or infusion is called *tisane* (Sofowora, 1982).

1.1.2 The origin of herbal medicine

Different communities in the world, and especially in Africa have practiced the art of healing, based on herbal medicine. Traditional medicine practitioners have attempted to explain the acquisition of this knowledge by early man. The choice of plant materials for the treatment of various ailments was not based on knowledge of the plant constituents. It could well be, that it was influenced by religious thoughts, because the collection and administration were accompanied by magic

and religious rituals. Some plants are still used in the rituals of traditional religion in many parts of Africa today (Sofowora, 1982; Williamson et al., 1996).

Other origins have also been pointed out. A number of traditional medicine practitioners claim that knowledge of medicinal plants was communicated to their ancestors in various ways and not gained by accident. For example, some practitioners could have learnt some knowledge by inferring from the effect produced by various plants when eaten by domestic animals, that the preparation could be harmless to the patient and the dosage prescribed justifiable. Knowledge of traditional cures also comes from hunters. They noted specific plants which have an antidote effect for wounds or for relieving pain. Such knowledge could have been acquired when an animal shot, ran away chewing a plant and did not die (Sofowora, 1982). In South Africa, because of the lucrative trade by herbalists, some people have even decided to take up apprenticeships with established herbalists in order to learn about their medicines, especially their knowledge on a vast array of plants, and other substances (Donker, 1989). A great deal of such information on African medicinal and toxic plants has been transmitted orally from generation to generation. Even today, there are herbal preparations and remedies which have not been written down (Sofowora, 1982).

1.1.3 The basic concept of traditional and modern medicines

Traditional medicine, like modern medicine, aims at healing or preventing disease. The objective for both types of medicine is the same, but the concept of the cause of disease, the approach to healing, as well as the healing methods used are different for both types of medicines. For example, modern medicine uses the results of experiments and the disease is regarded as caused by physio-pathological agents, whereas traditional medicine still accepts the fact that disease can be due to supernatural causes or the intrusion of an object into the body. This system emphasizes psychological causes of disease and the linkage between traditional medicine and the culture and beliefs of the people that use it (Bryant et al., 1966; Sofowora, 1982; Gumede, 1990). Therefore, an investigation into the efficacy of herbal remedies must take into account the culture in which they are used.

1.1.4 Psychological causes of illness and the use of plant remedies

The cause of illness is one of the major features that illustrate a difference between traditional and modern medicines. In various societies, which are less technologically developed, the system of medical practice is dominated by traditional thoughts. The traditional medicine practitioner sees patients as an integral part of nature and believes that the state of illness arises from an

imbalance or a disharmony in the element that governs the integrity of the individual in his particular cultural environment (ancestors and gods, social worlds, spirit possession and so on). That is the reason why a treatment offered, is to restore the balance, using means of suitable sacrifices and ritual exposure. Thus, plants are selected on the basis of their perceived ability to restore harmony (Williamson et al., 1996). In this respect, a categorization of illness has been undertaken by Williamson E. M. et al. (1996), in order to bring out the underlying considerations in the use of plant remedies in African traditional medicine.

Traditional medical systems categorize illness into two broad groups. The first group is those illnesses which can be treated without religious invocations and for which most adults in the community would know a remedy and use it without a prescription. For example, aches, pain and minor injuries such as cuts and bruises. The second group is those illnesses, chronic or arising from serious accident or injury, thought to have a supernatural underpinning, which are serious enough to threaten the life of the patient. Treatment thus involves divination by specialists to find out what is the offence to gods or ancestorial spirits and what sacrifices they need to be appeased.

Supernatural explanations of illness are viewed with scepticism by medical scientists because of incompatibility between religious beliefs and biomedical scientific theories. Nevertheless, it is within such a context that a great number of traditional healers use plant remedies in the treatment of illness. On the basis of

the way in which plant preparations are used in African traditional medicine, some observations can be made. Firstly, plant remedies for minor ailments owe their therapeutic effect to physicochemical properties. For example, fresh leaves squeezed to stop bleeding from wounds contain tannins and other haemostatic properties. Similarly, plant remedies used in the treatment of fevers contain antipyretic properties (Williamson et al., 1996). However, the situation becomes different when plant remedies are used in the treatment of chronic illness. In this instance, traditional healers do not require the necessary technology for accurate diagnosis, and the underlying cause is simply attributed to supernatural intervention or witchcraft. Thus, the plant remedy is considered as a part of total ritual treatment regimen. Therefore, the plant preparation is selected and used not so much for its pharmacological properties as for its ritualistic significance (Sofowora, 1982; Williamson et al., 1996).

1.1.5 The status of the traditional use of medicinal plants

An investigation throughout the world into the status of traditional medicine generally, and the use of medicinal plants particularly, shows that in some areas, competition and confirmation with conventional health care remain, while in others collaboration and integration between the systems have occurred. World Health Organization reports different positions up to 1980 (Sofowora, 1982).

The people of Africa particularly have considered traditional medicine as part of their culture even though this form of medicine is not as well organized as, for instance, Indian or Chinese herbal medicine. Many countries in Africa have attached to their Ministries of health, a department dealing with traditional medicine and especially herbal medicine. Research in African traditional medicine and particularly pharmacopoeia has been started by the Organization of African Unity through the Scientific, Technical and Research Commission (OAU/STRC) since 1968 (Sofowora, 1982). Most African countries have at least one research group investigating medicinal plants. A number of research institutes on traditional medicine in Africa have herbalists on their staff. Research has developed from the screening of medicinal plants for bioactive agents for the development of drugs and dosage form for natural products of merit (Sofowora, 1982). The training of herbal medicine practitioners has started in some countries such as Niger, Tanzania, Ghana, Nigeria and so on. Some of them have associations of traditional medicine practitioners officially recognized by their governments. Other countries are still looking at the modalities to recognize them (Sofowora, 1982).

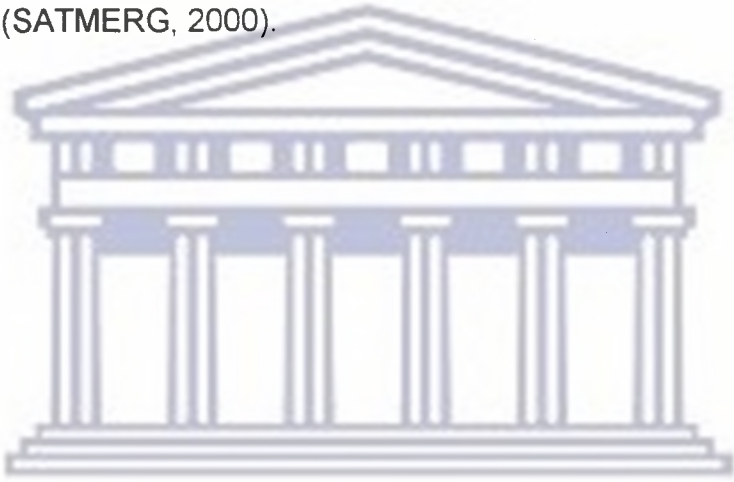
Besides, the World Health Organization has established a working group on traditional medicine in Geneva. The objectives are to foster a realistic approach to traditional medicine in order to promote it and further contribute to health care; to explore the merits of traditional medicine in the light of modern science in order to maximize useful and effective practices and discourage harmful ones; and to promote the integration of proven valuable knowledge and skills in traditional

medicine with Western medicine (Sofowora, 1982). South Africa is one of the countries which are in the process of integrating traditional medicine with modern sciences.

1.1.6 South African traditional medicine and modern medicine

The background of colonialism has widely influenced the South African medical field and the industrialization has particularly created a high modern medicine for which most of the rural population had no access. The services of the traditional healers, being numerous and plying their profession in the remotest quarters of South Africa, have been utilized to improve the health services for the entire population. A special effort has been undertaken to develop an alliance between these two types of medicine in order to promote complementary attitudes and methods. Researchers, medical doctors, traditional healers, authorities and the mass media are aware of the possibility and desirability of a cooperative accommodation between these two medical system and are still optimistic about coming to a definite kind of accommodation. The need to formalizing the use of traditional medicines in primary health care is seen as the first step for a multi-disciplinary and complementary approach between modern and alternative medicines. To this end, many efforts have been made in different ways. Thus, symposia, conferences and seminars on South African traditional medicine have taken place in the last couple of years (Bryant et al., 1966; Donker, 1989; Gumede,

1990). Actually, the South African traditional medicine is adopting a scientific approach through researches. Different scientists and several South African Universities are now widely involved in researches on medicinal plants used by local traditional practitioners. An example is the "The South African Traditional Medicines Research Group" (SATMERG) which seeks to promote the rational use of indigenous traditional medicines, as recommended by the World Health Organization (SATMERG, 2000).



1.2 Pathophysiology of epilepsy and anticonvulsant drugs

1.2.1 Definition

Epilepsy is the word used to describe a tendency to episodes, in which a variety of clinical phenomena may occur, which are caused by an abnormal discharge of brain electrical activity. This event is associated with a brief alteration in nervous system function (Wilkinson, 1989; Sudarsky, 1990). The clinical manifestations range from a brief disturbance of sensory or motor function to generalized seizures. Many people suffer an epileptic fit of one sort or another at some time in their lives and in the majority no underlying cause is found. This leads to the idea of a convulsive threshold; all people are potentially at risk of having an epileptic fit, but some do so with greater readiness than others. Individuals with a high convulsive threshold have an epileptic fit only under extreme conditions. People with a low convulsive threshold may have epileptic fits in the absence of any identifiable provocation (Wilkinson 1989; Martyn 1989).

1.2.2 Description of types of epilepsies

Seizures are classified into two principal categories: primary generalized seizures and partial seizures. A seizure disorder is said to be generalized if the whole brain participates in a disturbance of cerebral activity. It is a partial seizure if it is

restricted to a regional disturbance. Partial seizures are subdivided further into partial simple and partial complex, according to whether consciousness is disturbed. A partial simple seizure is a focal motor or focal sensory disturbance, with the patient awake and alert. Partial complex seizures cause a brief lapse of consciousness or a staring spell. An aura or warning is often present before a partial complex seizure. Familiarity or depersonalization, amnesia and confusion are common. A primary generalized seizure involves the whole brain. It is characterized by tonic-clonic seizures (grand mal epilepsy). The patient loses consciousness, falls as the body stiffens in a tonic contraction (tonic phase) and may salivate or lose control of the sphincters. This is followed by rhythmic contractions of the limbs for another clonic phase. After the seizure, the patient remains unresponsive, with a slow return of consciousness (Sudarsky, 1990; Westmoreland et al., 1994).

There are also various kinds of non-convulsive generalized seizures. The most common of which is the primary absence (petit mal epilepsy). It is characterized by brief generalized seizures lasting 5 to 30 seconds with impaired consciousness, but minimal movements. A variety of atonic and myoclonic seizure types occur, most typically in childhood. A partial seizure can spread to trigger a generalized seizure. This event is called a secondary generalized seizure. Epilepsy is a symptom of some underlying neurological disease and a diagnosis of epilepsy by itself is inadequate. Not only must the type of seizure be established, but the

reason why the patient is having seizures must also be understood (Walton, 1987; Sudarsky, 1990; Westmoreland et al., 1994).

1.2.3 Factors precipitating epilepsy

The factors that give rise to seizures must be understood through some precise observations. Firstly, the epileptic attack can be caused by biochemical insults to the brain, such as during hypoglycemia, anoxia, hypocalcemia, hyperventilation, water intoxication and sudden withdrawal of certain drugs such as barbiturates or alcohol (Biller, 1997). It is therefore permissible to infer that biochemical factors can promote conditions favorable for the occurrence of an attack. This kind of attack is qualified as non-recurrent seizures (Biller, 1997). Secondly, epilepsy can be subsequent to some previous active pathology, such as birth trauma to the brain, trauma to the skull and brain later in life, during or following meningitis, encephalitis or cerebral abscess, cerebral infarction, cerebral hemorrhage, or subarachnoid hemorrhage (Biller, 1997). Thirdly, primary generalized epilepsy (petit mal, grand mal, myoclonic jerks, photosensitive epilepsy) is familial. A significant number of patients develop it in childhood, adolescence or young adult life without any apparent cause. There may be a family history of epilepsy and it is assumed that the underlying reason for their seizures is that they have inherited a low convulsive threshold. These attacks which are acquired, inherited or arising from structural cortical lesions are qualified as recurrent seizures or recurrent

epilepsy (Frank, 1971; Wilkinson, 1989; Martyn, 1989; Biller; 1997). Fourthly, in some people, attack can be triggered by a sensory stimulus which is specific for the individuals. In fact, some factors can induce attacks at will and actually do so for secondary gain. This can be visual stimuli (for instance, flickering lights) or acoustic precipitants (for instance, a sudden loud noise). In addition, it is important to note that emotional stress can undoubtedly precipitate seizures through some as yet unexplained psychophysiological mechanism (Frank, 1971; Wilkinson, 1989).

Nevertheless, some non-epileptic events can be followed by an excitation or stimulation. These have to be distinguished from seizures arising from diseases of the brain. A cardiac inhibition, for instance, can cause the paroxysms of neurons, followed by a convulsion (Frank, 1971; Biller, 1997). To date, there is no single unifying explanation as to how these diverse factors determine the occurrence of seizures. Mechanisms that may cause the epilepsy processes remain difficult to clarify. However, it has been possible to investigate the physiological events which participate in the genesis of epilepsy (Sudarsky, 1990).

1.2.4 Approach of cellular mechanism of epilepsy

The widely accepted hypothesis shows that two primary factors produce seizures and these are an increased excitability of neurons and a synchronization of

neuronal populations (Walton, 1987; Sudarsky, 1990; Westmoreland et al., 1994). Under normal conditions, the neuron's membrane potential is determined by ion concentration gradients. Ionic changes in the environment of the epileptic focus can contribute to its instability (Sudarsky, 1990). Some neuroactive substances are involved, such as excitatory and inhibitory amino acids, as the most abundant neurotransmitters in the central nervous system and used for neurotransmission in most clinically relevant pathways (Conn et al., 1989; Westmoreland et al., 1994; Rang et al., 1999). The excitatory amino acids (EAAs) include mainly glutamate and aspartate and the inhibitory amino acids (IAAs) include gama aminobutyric acid (GABA), the major inhibitory neurotransmitter, and glycine.

The excitatory amino acid, glutamic acid (or glutamate) and the inhibitory amino acid, GABA are reported to widely play a major role in the genesis of epileptic seizures (Westmoreland et al., 1994; Rang et al., 1999).

1.2.4.1 Glutamic acid and epilepsy

1.2.4.1.1 General

Glutamate is the most important excitatory neurotransmitter in all rapidly conducting relay pathways of the motor and sensory systems of the outer tube of the central nervous system. It produces fast or prolonged synaptic excitation and triggers various calcium dependent processes in the target cells, including

production of nitric oxide. Glutamate also has a major role in synaptic plasticity during development and in the processes of learning and memory (Westmoreland et al., 1994). Glutamate is the neurotransmitter in the corticospinal, corticostriatal pathways, intrahemispheric and interhemispheric association pathways, hippocampal circuits, primary afferents and somatosensory and special sensory (visual, auditory) pathways, cerebellar afferents, and excitatory inter-neurons (Westmoreland et al., 1994).

1.2.4.1.2 Metabolism of glutamic acid

According to Rang et al. (1999), Krebs cycle is the main source of amino acid transmitters in the brain. There is a close relationship between the pathways for the synthesis of excitatory and inhibitory amino acids. Transaminase enzymes are responsible for the interconversion between krebs cycle intermediates and amino acids, or between amino acids themselves. Thus, glutamate is formed by intermediate metabolism of glucose via precursors of the Krebs cycle, particularly α-ketoglutarate. It may also be synthesized from glutamine. The synaptic effects of glutamate are terminated by its presynaptic reuptake via a sodium ion/ATP-dependent pump (Westmoreland et al., 1994).

1.2.4.1.3 Glutamate receptor mechanisms

On the basis of studies with selective agonists and antagonists, four main subtypes of EAAs receptors have been distinguished; namely N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA), Kainate (compound isolated from seaweed) and metabotropic receptors. The first three (often called ionotropic receptors) are ligand-gated ion channels. They are named according to their specific agonists and they have a pentametric structure. NMDA receptors are assembled from two types of subunit, NR_1 and NR_2, each of which can exist in different isoforms and splice variants, giving rise to many different receptor isoforms in the brain. The subunits comprising AMPA and kainate receptors, termed $GluR_{1-7}$ and $KA_{1,2}$, are closely related, but distinct from NMDA receptor subunits. AMPA receptors consist of combinations of $GluR_{1-4}$, each of which can be expressed in two splice variants, which vary in their physiological and pharmacological properties. The metabotropic receptors are monomeric G-protein-coupled receptors, linked to intracellular second messenger systems, and multiple subtypes have been detected (Smythies et al., 1978; Rang et al., 1999).

Applied to neurons, all four agonists mentioned above cause opening of cation-selective channels, and produce a rapid depolarization response. Single channel studies show that each receptor type predominantly opens one type of channel,

but there is considerable overlap and heterogeneity among the channels associated with the different receptors. The channels opened by NMDA clearly differ from AMPA and Kainate operated channels. The NMDA receptors are believed to be of considerable functional importance due to its special pharmacological properties and consequently more responsible for neuron excitability than other EAA receptors (Sudarsky, 1990; Rang et al., 1999).

1.2.4.1.4 Special features of NMDA receptor types of glutamate

NMDA receptors and their associated channels have been studied in more details than the other types, and show special pharmacological properties, which are postulated to play a role in physiological mechanisms. They are highly permeable to calcium ions, as well as to other cations. The NMDA receptor is coupled to an ion channel that allows influx of calcium, as well as sodium ion, and leads to an activation of NMDA receptors producing prolonged bursts of depolarization. NMDA receptors are readily blocked by magnesium ions; this block occurs when the cell is normally polarized, but disappears if the cell is depolarized. A study has shown NMDA receptors to facilitate the amino acid glycine, in activation of excitatory responses (normally glycine has inhibitory functions). Finally, NMDA receptors are selectively blocked by certain well known anaesthetic and psychotomimetic agents, such as Ketamine and phencyclidine. They act by blocking the activation of excitatory responses at the NMDA receptors (Rang et al., 1999).

1.2.4.1.5 Functional role of glutamate receptors

Studies on the effects of EAA antagonists on synaptic transmission in the central nervous system suggest that AMPA and Kainate receptors are mainly responsible for fast excitatory transmission in the central nervous system. NMDA receptors (which often coexist with AMPA receptors), on the other hand, are involved in the slower excitatory synaptic responses. Metabotropic glutamate receptors are linked either to IP3 production (inositol triphosphate) and release of intracellular calcium ion. NMDA and metabotropic glutamate receptors participate in various adaptive and patho-physiological events. Three such roles which are now generally accepted are the synaptic plasticity, the excito-toxicity and the pathogenesis of epilepsy (Rang et al., 1999).

1.2.4.1.6 Role of glutamic acid in epilepsy

During epileptic discharge, the membrane potential suddenly decreases by about 30 mV and remains depolarized for up to a few seconds before returning to normal. This event results from the abnormally exaggerated and prolonged action of an excitatory transmitter. The activation of glutamate at the NMDA receptors is believed to play an important role in this event. The activation of glutamate receptors of the NMDA type produces a voltage-dependent blocking action of magnesium ions, leading to depolarization. In fact, the enhancement of

glutaminergic neurotransmission is responsible for the discharge of neurons resulting in an epileptic attack whereas its reduction results in the antagonism of the seizures (Sudarsky, 1990; Rang et al., 1999).

1.2.4.1.7 Antagonists and agonists of glutamic acid

The search for selective glutamate antagonists helped for a better understanding of the physiological role of the different types of excitatory amino acid receptors and for potential therapeutic agents which can be useful for the treatment epilepsy and neuro-degenerative disorders. At the receptor level, selective antagonists exist for NMDA, AMPA and metabotropic glutamate receptors. They are only useful as experimental tools and not as therapeutic agents because of their inability to penetrate the blood-brain barrier (Rang et al., 1999).

The first antagonists for NMDA receptors were the phosphonate analogues, AP-5 (2-amino-5-phosphonopentanoic acid) and AP-7 (2-amino-7-phosphonopentanoic acid); later, more potent derivatives such as CPP (3-[2-carboxypirazin-4-yl]-propyl-1-phosphonic acid) and CGS 19755 (Selfotel or 1-[cis-2-carboxypiperidine-4-yl]-methyl-1-phosphonic acid) have been used in clinical trials. Selective antagonists for glutamate at the AMPA receptors are NBQX (2,3-dihydro-6-nitro-7-sulfamoyl-benzoquinoxaline) and CNQX (6-cyano-7-nitroquinoxaline-2,3-dione). Both NMDA and AMPA receptors are subject to facilitation by modulators that act at sites distinct from the glutamate binding site. In the case of NMDA receptors, channel

opening requires glycine, as well as glutamate .The binding site for glycine is distinct from the glutamate binding site, and both have to be occupied for the channel to open. This discovery appeared critical since glycine had been recognized as an inhibitory transmitter. The known competitive antagonists of glycine are kynurenic acid, chloro-kynurenate and HA-466. They block the action of glycine and thus, inhibit the action of glutamate. Compounds that facilitate the action of agonist at AMPA receptors include cyclothiazide (diuretic), which inhibit the fast desensitization produced by glutamate, aniracetam and other experimental compounds. Glutamic acid receptor agonists are compounds which act by mimicking the action of glutamic acid at the NMDA receptors, and then induce seizures. An example is the N-methyl-D-L-aspartic acid (NMDLA) which is a convulsant agent (Feldman et al., 1997; Rang et al., 1999).

1.2.4.2 Gama amino butyric acid and epilepsy

1.2.4.2.1 Storage

Gama amino butyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system. GABA occurs in the brain tissues, but not in other mammalian tissues, except in trace amounts. In the brain, it is particularly abundant (about 10 μmol/g tissue) in the nigrostriatal system, but occurs in lower

concentration (2-5 μmol/g) throughout the gray matter (Westmoreland et al., 1994; Rang et al., 1999).

1.2.4.2.2 Metabolism of GABA in the brain

The synthesis and metabolism of GABA are linked with those of glutamate and involve interactions between GABAergic neurons and astrocystes. In GABAergic terminals, GABA is synthesized from glutamate by action of glutamic acid decarboxylase (GAD); an enzyme found only in GABA-synthesizing neurons in the brain. GABA is destroyed by a transamination reaction, in which the amino group is transferred to α-oxoglutaric acid, to yield glutamate, with the production of succinic semialdehyde, and then succinic acid. This reaction is catalyzed by GABA-transaminase (GABA-T). GABAergic neurons have an active GABA uptake system, and it is this, rather than GABA-T, which removes the GABA from the synapse after it has been released (Westmoreland et al., 1994; Rang et al., 1999).

1.2.4.2.3 GABA receptors mechanisms

Receptors for the major inhibitory neurotransmitter, GABA, are divided into three main classes: $GABA_A$-receptors which are members of the ligand-gated ion channel superfamily, $GABA_B$-receptors which are members of the G-protein-linked

receptor superfamily and GABAc-receptors, recently discovered. They have been cloned and have shown many features in common with glutamate receptors.

$GABA_A$-receptors are located postsynaptically and they mediate fast postsynaptic inhibition. The associated channel is selectively permeable to chloride ions. The equilibrium membrane potential for chloride ion is usually somewhat negative to the resting potential of the cell, so that increasing chloride permeability hyperpolarizes the cell, thereby reducing its excitability or the depolarization produced by excitatory transmitter action (McKernan et al., 1996; Rang et al., 1999). $GABA_A$-receptors are pentameters, composed of three different subunits (α,β,γ) forming an array of α-helices around a central pore (the ion channel) with a large extracellular portion which incorporates the GABA binding site. The receptors subunits each exist in several subtypes, giving the familiar pattern of heterogeneity typical of neurotransmitter receptors (McKernan et al., 1996; Feldman et al., 1997; Rang et al., 1999).

$GABA_B$-receptors, on the other hand, are located on pre- and postsynaptic terminals. They closely resemble metabotropic glutamate receptors. They were cloned; and only two subtypes have been identified. They exert their effect by binding to voltage gated calcium channels, resulting in reducing transmitter release, and also by opening potassium channels, resulting in reducing

postsynaptic excitability (Smythies et al., 1978; Feldman et al., 1997; Rang et al., 1999).

A third class of receptors, $GABA_C$-receptors, recently came from studies in which GABA analogues, such as cis-4-aminocrotonic acid (CACA), produced inhibitory neuronal responses that were insensitive to both bicuculline and baclofen. Studies on GABA receptors suggest that CACA has a greater preference for $GABA_C$-receptors than $GABA_A$-receptors at lower concentrations, but activates $GABA_A$-receptors at higher concentrations. Thus, GABAc-receptors are selectively activated by the agonist CACA, by the nonselective agonists of GABA and by muscimol, which is a selective agonist for $GABA_A$-receptors. $GABA_C$-receptors show no modulation by benzodiazepines, barbiturates or neurosteroids. Like $GABA_A$-receptors, $GABA_C$-receptors are linked to chloride channels that are inhibited by picrotoxin or TBPS (t-butylbicyclophosphorothionate). The subunit structure of these two subtypes differs in that $GABA_C$-receptors appear to be formed from novel subunits, $\rho_1$and ρ_2. These are present in the retinal bipolar cells where $GABA_C$- and $GABA_A$-receptor-mediated responses can be observed (Bormann et al., 1996; Feldman et al., 1997).

1.2.4.2.4 Functions of GABA receptors

GABA is thought to function as an inhibitory transmitter in many different central nervous system pathways. The widespread distribution of GABA and the fact that

virtually all neurons are sensitive to its inhibitory effect, suggest that its function is ubiquitous in the brain. It has been estimated that GABA serves as a transmitter at about 30% of all the synapses in the central nervous system. It is thus believed to participate in the genesis of epileptic seizures (Westmoreland et al., 1994; Rang et al., 1999).

1.2.4.2.5 Role of GABA receptors in epilepsy

As discussed above, GABA mechanisms are inhibitory since the effect results in reducing the depolarization produced by excitatory transmitter action. They stabilize the membrane potential so that the membrane is unable to respond to stimuli (shunting inhibition phenomenon). Thus, GABA helps to contain discharges and consequently, drugs which block $GABA_A$-receptors produce seizures. In fact, the enhancement of GABAergic neurotransmission may antagonize epilepsy whereas its decrease may result in epileptic attack (Smythies et al., 1978; Sudarsky, 1990; Westmoreland et al., 1994; Rang et al., 1999).

1.2.4.2.6 Drugs affecting GABA receptors

$GABA_A$-receptors are targets for certain centrally acting drugs, such as benzodiazepines, barbiturates and neurosteroids. The principal example of $GABA_A$-receptor agonist is muscimol (derived from a hallucinogenic mushroom) which hyperpolarizes GABA-sensitive neurons. Bicuculline, a naturally occurring

convulsant compound, is a specific antagonist of $GABA_A$-receptors, by selectively blocking the action of GABA on $GABA_A$-receptors, which control chloride permeability. Pentylenetetrazole, another convulsant agent, acts by antagonizing the action of $GABA_A$-receptors. Picrotoxin is also a convulsant agent, which acts by blocking the chloride channel associated with the $GABA_A$-receptors, thus blocking the postsynaptic inhibitory effect of GABA. These compounds are useful in experimental studies, but have no therapeutic use (Frank, 1971; Rang et al., 1999).

Benzodiazepines selectively potentiate the effects of GABA on $GABA_A$-receptors; they thus have broad anticonvulsant properties. They bind with a high affinity to an accessory site (the "benzodiazepine receptor") on the $GABA_A$-receptors. The binding of GABA is then facilitated and its agonist effect is enhanced. Studies on recombinant $GABA_A$-receptors have shown that a small region of the γ-subunit confers benzodiazepine sensitivity, and mutations in this region affect the level of constitutive activity at this site, and its sensitivity to benzodiazepines. Sedative benzodiazepines, such as diazepam, are agonists by enhancing the action of GABA. Flumazenil, a convulsant analogue, is an antagonist of benzodiazepines (Rang et al., 1999). Diazepam has a well-defined role in the management of status epilepticus for which it is the drug of choice. Its relative short duration of action is a disadvantage (Gilman et al., 1991; Grahame-Smith et al., 1991).

Modulators which also enhance the action of GABA, include other central nervous system depressants such as barbiturates and neurosteroids. Neurosteroid hormones do not act on conventional intracellular steroid receptors, but include metabolites of progesterone and androgens which are formed in the nervous system and may have a physiological role (Rang et al., 1999). Barbiturates act by enhancing the inhibitory effect of GABA and facilitating the GABA-mediated opening of chloride channels. An example of the barbiturates is phenobarbitone. It is an effective agent for generalized tonic-clonic and partial seizures (Rang et al., 1999).

In searching for more lipophilic GABA analogues for controlling epilepsy and other convulsive states, baclofen was introduced in 1972. Unlike GABA, baclofen has little postsynaptic inhibitory effect, and its actions are not blocked by bicuculline. These findings, different from those related above, led to the recognition of the $GABA_B$-receptors, for which baclofen is a selective agonist. It is used to treat spasticity and related motor disorders (Rang et al. 1999). Competitive antagonists for $GABA_B$-receptors exist, such as saclofen and more potent compounds with improved brain penetration, such as CGP 35348 (3-aminopropyl-phosphinic acid). They are only used as experimental tools and produce only slight effects on central nervous system function. The main effect observed was an anticonvulsant action specifically in animal model of absence seizures, together with enhanced cognitive performance (Feldman et al., 1997; Rang et al., 1999).

1.2.5 Cellular mechanism of action of anticonvulsant drugs

The commonly used antiepileptic drugs are thought to act mainly by two general mechanisms in which they might abolish or attenuate seizures; by reducing electrical excitability of cell membranes, possibly by blocking sodium channels; and by enhancing GABA-mediated synaptic inhibition. This may be achieved by an enhanced postsynaptic action of GABA, by inhibiting GABA-transaminase, or by drugs with direct GABA-agonist properties. Additionally, a few drugs appear to act by a third mechanism, involving the inhibition of T-type calcium channels. A number of newer drugs act by blocking excitatory amino acid receptors; most of them are effective in experimental animal models and not used therapeutically because of their inability to penetrate the blood-brain barrier (Rang et al., 1999). However, those available for clinical use include lamotrigine which is thought to act by inhibiting the release of the excitatory neurotransmitter, glutamate, and topiramate which is suggested to act by multiple mechanisms, among other things through glutamate receptor antagonism (Gibbon, 2000; Mycek et al., 2000).

At a cellular level, many of the currently used antiepileptic drugs effect GABA-mediated synaptic inhibition in cortical structures. Many antiepileptic drugs such as phenobarbitone and benzodiazepines, enhance the activation of $GABA_A$-receptors, thus facilitating GABA-mediated opening of the chloride channels. Vigabatrin, a recently introduced antiepileptic drug, acts by inhibiting the enzyme GABA-transaminase which is responsible for inactivating GABA. Tiagabine inhibits GABA

uptake. All these lead to an enhancement of the action of GABA as an inhibitory transmitter (Feldman et al., 1997; Rang et al., 1999).

Other highly effective antiepileptic drugs such as phenytoin and carbamazepine affect membrane excitability by an action on voltage-dependent sodium channels, which carry the inward current necessary for the generation of an action potential. They block preferentially the excitation of cells that are firing repetitively; and the higher the frequency of the firing, the greater the block produced (Rang et al., 1999).

Pharmacologically, the effect of these drugs is to attenuate or suppress the seizure discharges. It is important to note that too much GABA-mediated inhibition in the brain can limit normal nervous system functions and these antiepileptic drugs possess the potential for causing sedation when present in excessive amounts (Sudarsky, 1990; Gilman et al., 1991). This is one of the several reasons why pharmacological and physiological effects reported about traditional herbal remedies need a scientific verification in terms of efficacy, mechanism of action and safety.

1.3 Description of the project

1.3.1 Introduction

The primary aim of this study was to assess the antiepileptic properties of the crude water extract of *Leonotis leonurus* (L.) R. Br. of the family Lamiaceae (Arnold et al., 1993; Hutchings et al.; 1996). *L. leonurus* is one of the plant species used by South African traditional medicine practitioners for the treatment of various ailments. It has a wide natural distribution over large parts of South Africa including the Western Cape Province (Van Wyk et al., 1997). It has been used by different communities in South Africa. It is known in Afrikaans as wilde dagga, in English as wild dagga, in Zulu as umunyane, in Sotho as lebake, in Xhosa as umfincafincane and in Shona as umhlahlampetu (Hutchings et al., 1996; Van Wyk et al., 1997). It is a shrub of two to five metres in height, with a thick, woody base and pale brown branches. All parts of the plant have a strong smell. The leaves are opposite each other on the stem, long and narrow, toothed in the upper half and distinctly hairy. Bright orange, tubular flowers are borne in characteristic rounded groups, which are neatly arranged along the branch ends (figure 1). The hairy flowers resemble lion's ears, hence the name "leonurus" (Van Wyk et al., 1997). The leaves of *L. leonurus* have traditionally been smoked for the relief of epilepsy. An infusion and

a decoction of the leaf and stem have been used internally for coughs, colds, influenza, bronchitis, high blood pressure and headaches. A tincture of the flower has also been used for the same purpose. Externally, decoctions have been applied to treat boils, eczema, skin disease, itching and muscular cramps (Watt et al., 1962; Hutchings et al., 1996; Van Wyk et al., 1997). This study, therefore, investigated the effect of water extract of *L. leonurus* on chemically induced seizures by pentylenetetrazole (PTZ), picrotoxin, bicuculline and N-methyl-D-L-aspartic acid (NMDLA) in mice. The study also tried to establish the chromatographic profile of the water extract using high performance liquid chromatography (HPLC) method, and to identify the various chemical constituents in the leaves using standard phytochemical tests.

Figure 1: *Leonotis leonurus* (L.) R. Br.; Lamiaceae (Arnold et al., 1993; Hutchings et al.; 1996).

1.3.2 The problem

As discussed previously, the use of traditional medicine through the use of medicinal plants in Africa and especially in South Africa, has long been considered an important characteristic of people's daily lives and socio-cultural heritage. The traditional medicine practice has remained less organized and based on cultural beliefs as to the cause of disease (Williamson et al., 1996). Moreover, the claims of therapeutic successes by traditional medicine practitioners are hardly subjected to scientific scrutiny. As a consequence, plants are often selected on the basis of their perceived ability to restore harmony and frequently used in a manner which does not permit a pharmacological interpretation of their efficacy. For example, the preparation is administered without regard for dosage, which would not be the case if the plant was prescribed on the basis of the pharmacological properties of the whole plant or its constituents (Sofowora, 1982; Williamson et al., 1996). *L. leonurus* is also used in this way for its potential antiepileptic properties. One major problem with this use is that nothing much is known about the mechanisms through which it produces its action and also its toxicity potential. This is supported by the fact that little or no scientific data exist about *L. leonurus*, particularly on its anticonvulsant activity.

1.3.3 Hypotheses and objectives

The efficacy of *L. leonurus* in epilepsy needs scientific proof in order to validate its use as an antiepileptic remedy in traditional medicine and also to establish its full medicinal effects. This project intended to investigate the antiepileptic properties of this plant.

It is widely accepted that the major inhibitory amino acid neurotransmitter in the brain, gamma aminobutyric acid (GABA) is highly implicated in the control of epilepsy. The enhancement of gabaminergic neurotransmission has been shown to antagonize seizures (Westmoreland et al., 1994; Amabeoku, 1998; Rang et al., 1999). On the other hand, the activation of glutamic acid, as an excitatory amino acid neurotransmitter in the brain, at the NMDA (N-methyl-D-aspartic acid) receptors, is believed to play a key role in the cellular mechanisms underlying epilepsy. A reduction of glutaminergic neurotransmission results in the antagonism of epilepsy (Smythies et al., 1978; Westmoreland et al., 1994; Rang et al., 1999). Accordingly, GABA-like or GABA-mimetics or glutamate antagonists appear to cause reduction of epileptic seizures. This project attempts to investigate the possible mechanism of antiepileptic action of *L. leonurus* extract based on the inhibitory neurotransmission and on the antagonism of excitatory neurotransmission.

The mechanism of action of the whole plant or plant part producing the physiological effect is better investigated if the plant is chemically characterized. Such an investigation also demonstrates that the reported physiological activity of the plant is real and leads to a better application of the possible drug based on the plant extract and a better formulation into appropriate dosage forms (Sofowora, 1982; Williamson et al., 1996). This project also aims to carry out a preliminary phytochemical analysis of *L. leonurus* extract in order to initiate much more pharmacological details of its effect and a possible use with reduced toxicity.

From the above, the aim of the present study is detailed in the following three objectives:

- To investigate the anticonvulsant properties of *L. leonurus*;
- To investigate the possible mechanism of the antiepileptic activity of this plant;
- To carry out a preliminary phytochemical screening of *L. leonurus* extract.

CHAPTER 2

MATERIALS AND METHODS

2.1 Plant material

2.1.1 Selection, collection, and identification of Leonotis leonurus

According to Lipp et al. (1989), Verproote (1989) and Williamson et al. (1996), the criteria for selecting plant species constitute an important step when a phytotherapeutic study must be carried out. *L. leonurus* has been selected on the basis of its popular use in South Africa by traditional healers and the reported antiepileptic activity for which little or no scientific data exist. The plant material was collected from Kirstenbosch Botanic Garden, Cape Town, South Africa and authenticated by Dr Gillian Scott, taxonomist of the "South African Traditional Medicine Research Group", School of Pharmacy, University of the Western Cape. A voucher specimen (number TRAD 10) was also deposited in the Herbarium in the Department of Botany, University of the Western Cape.

2.1.2 Preparation of aqueous extract of Leonotis leonurus

The leaves of the plant species collected were washed with distilled water, dried in a ventilated oven for 72 hours at 30°C and afterwards ground into fine powder (850 μg) using the waring commercial laboratory blender. A known quantity of the powder (20 g) was refluxed for 5 hours in boiled water (1 l), allowed to cool for 24 hours and centrifuged. The supernatant was freeze-dried for 72 hours to obtain the dried plant extract, which was kept in a dessicator (figure 2).

The *Leonotis leonurus* solution (LST) used in the test against the chemically-induced seizures, was freshly prepared on each day of the experiment by dissolving a given quantity of the dried extract in an appropriate volume of physiological saline solution.

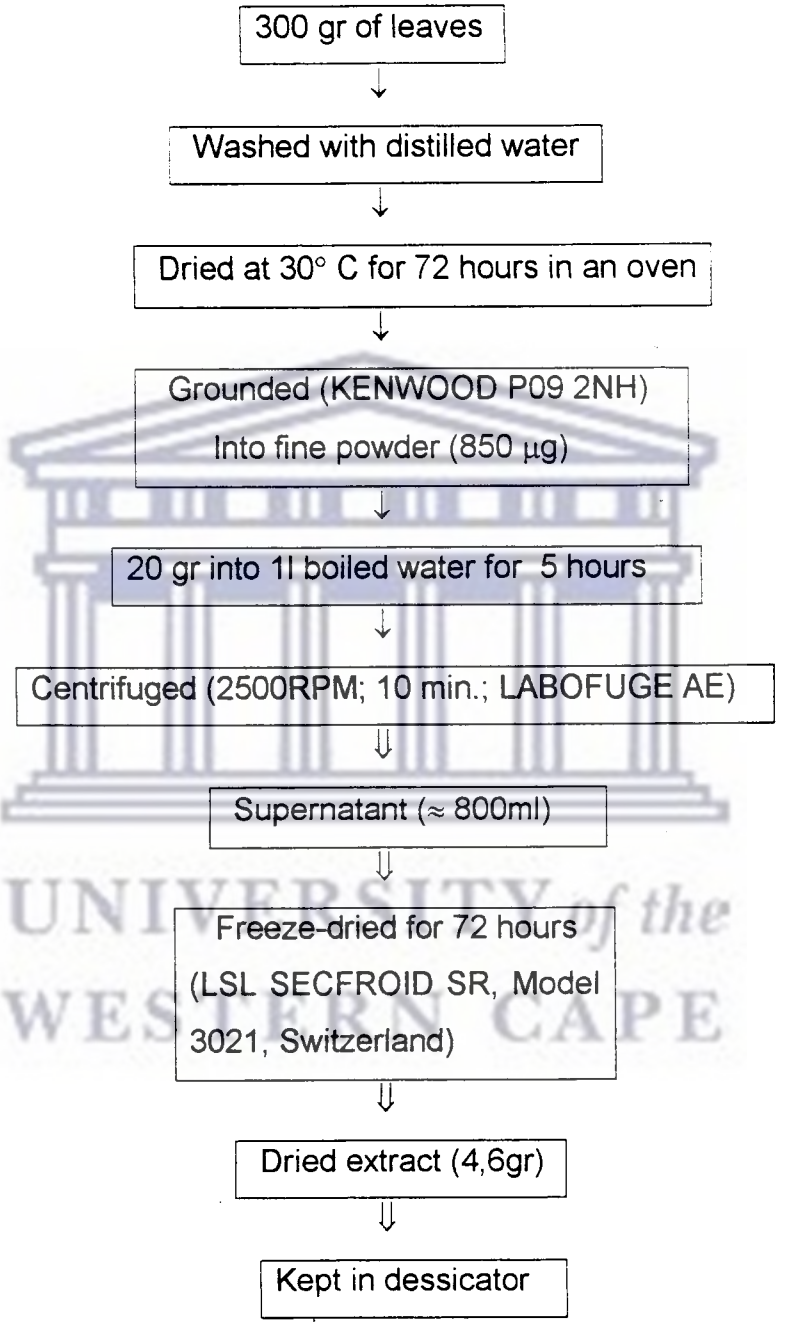
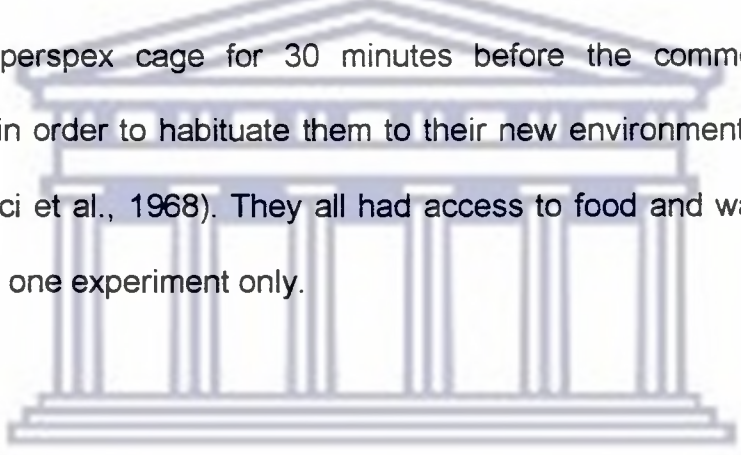


Figure 2: Preparation and storage of aqueous extract of *Leonotis leonurus*

2.2 Assessment of anticonvulsant properties

2.2.1 Experimental animals

Male albino mice bought from the University of Cape Town, Cape Town, Republic of South Africa, weighing 15-25 g each, were used in groups of eight per dose of drug or plant extract (Laurence et al., 1964). Each mouse was housed singly in a transparent perspex cage for 30 minutes before the commencement of the experiment, in order to habituate them to their new environment (Laurence et al., 1968; Tedesci et al., 1968). They all had access to food and water. Each mouse was used for one experiment only.



2.2.2 Drugs and chemicals

Pentylenetetrazole (PTZ; Sigma), picrotoxin (Sigma), N-methyl-DL-aspartic acid (NMDLA; Sigma) and phenobarbitone (Gardenal®, Rhone-poulenc Rorer, South Africa) were all dissolved in physiological saline. Bicuculline (Sigma) and diazepam (Valium®, Roche, South Africa) were suspended in a minimum amount of Tween 80 and Polyethylene glycol 400 (Fluka AG, Buchs) respectively, and adjusted to an appropriate volume with physiological saline.

Preliminary studies were carried out to establish the doses of convulsant drugs and plant extract that give a maximum response within a reasonable time. Thus, 90 mg/kg of pentylenetetrazole, 8 mg/kg of picrotoxin, 20 mg/kg of bicuculline and 400 mg/kg of N-methyl-DL-aspartic acid produced 100% seizures. Three different doses of LST, a lower (100 mg/kg), a medium (200 mg/kg) and a higher dose (400 mg/kg) were also chosen. The selected doses of the antiepileptic drugs, phenobarbitone and diazepam were respectively 10 mg/kg and 0.50 mg/kg. These were then used as the working doses for testing the effect of plant extract against seizures induced with the respective convulsant agents. All drugs and *L. leonurus* solutions were injected intraperitoneally (i.p.). The control animals received equal volume injections of the appropriate vehicles which included Tween 80 or polyethylene glycol 400, all dissolved in physiological saline. Fresh drug solutions were prepared on the days of the experiment.

2.2.3 Method

The investigation of anticonvulsant properties of aqueous extracts of *L. leonurus* was carried out using the method of Vellucci and Webster (1984), modified by Amabeoku and Chikuni (1993). Seizures were induced in animals with convulsant drugs such as pentylenetetrazole, picrotoxin, bicuculline and N-methyl-DL-aspartic acid. Mice were observed for 30 minutes for tonic convulsion episode. 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 onset of tonic convulsions and the proportion of mice presenting convulsions were recorded. Animals that did not convulse during the period of observation were considered not having convulsed (Akah et al., 1988; Amabeoku et al., 1993). Experiments were repeated with mice pretreated for 15 minutes with *L. leonurus*, the standard antiepileptic drug, phenobarbitone or diazepam, or the control vehicle, before the administration of the convulsant drug. Control experiments were also carried out concurrently with the test experiments.

2.2.4 Statistical analysis

The results on the onset of seizures were analyzed using the paired Student's t-test while the proportion of animals that exhibited tonic seizures was analyzed using the chi-squared test (Tallarida et al., 1981).

2.3 Phytochemical characterization of *L. leonurus*

2.3.1 Objectives

The aim was to detect the groups of compounds present in the aqueous extract of *L. leonurus* and to produce a fingerprint chromatogram characteristic to this plant. The constituents were detected chemically, while the method needed for the chromatogram was the high performance liquid chromatography (HPLC). (Harborne, 1984; Moffat, 1986).

2.3.2 Detection of chemical constituents

Using standard chemical tests, the water extract of *L. leonurus* was tested for compounds such as anthraquinones, alkaloids, cardiac glycosides, reducing sugars, saponins and tannins, reported to be bioactive (Sofowora, 1982; Harborne, 1984; Moffat et al., 1986).

2.3.3 HPLC analysis

The chromatographic system includes Beckman HPLC system consisting of double pump Programmable Solvent Module model 126; Diode Array detector

Module model 168; Samsung computer 386 with management System Gold (GoldV601) software supplied by Beckman; Column, C18 Bondapak 5 μm and dimensions (250 x 4.6 mm). The chromatographic conditions include mobile phase: solvent A: 1% acetic acid; solvent B: methanol; Mode: gradient; flow rate, 1 ml/min; injection volume, 10 μl; detector, UV at 360 nm; reference standard, Rutin (2.5 g dissolved in 100 ml of methanol). The HPLC operating conditions were programmed to give the following: at 0 min, solvent B: 20%; 5 min, solvent B: 40%; 15 min, solvent B: 60%; 20 min, solvent B: 80% and at 27 min, solvent B: 20%. The run rate was 30 min.

2.3.4 Data analysis

The characteristic feature of each compound present in *L. leonurus* extract was the retention time which is the time from injection of the sample to emergence of the peak.

CHAPTER 3

RESULTS

3.1 Anticonvulsant properties

3.1.1 Effect of aqueous extract of L. leonurus on pentylenetetrazole-induced seizures

Pentylenetetrazole (90 mg/kg) produced tonic seizures in all the animals used. A dose of 100 mg/kg of *L. leonurus* extract protected 25% of animals against pentylenetetrazole-induced seizures and did not affect the onset of the seizures to any significant extent. *L. leonurus* (200 mg/kg) protected 37.5% of mice against pentylenetetrazole-induced seizures, and significantly delayed the latency of the seizures. Similarly, 400 mg/kg of *L. leonurus* significantly prolonged the latency of the seizures produced by pentylenetetrazole, and protected 50% of animals against the seizures. The standard antiepileptic drugs, phenobarbitone (10mg/kg) and diazepam (0.50 mg/kg) profoundly antagonized the seizures produced by pentylenetetrazole (Table 1). The control vehicle did not affect the gross behavior

of mice or alter pentylenetetrazole-induced seizures to any significant extent (results not shown).

Table 1:

Effect of aqueous extract of *Leonotis leonurus* (LST) on pentylenetetrazole (PTZ)-induced seizures in mice

Dose (mg/kg)				No. convulsed/	Animals not	Onset of tonic
		Pheno-	Diaze-	No. used	convulsed (%)	convulsion (min.)
PTZ	LST	barbitone	pam			Mean ± S.E.M.
90	-	-	-	8/8	0	6.38 ± 0.88
90	100	-	-	6/8	25	9.67 ± 1.81
90	200	-	-	5/8	37.5	12.20* ± 2.73
90	400	-	-	4/8	50	16.00** ± 1.76
90	-	10	-	0/8♣	100	-
90	-	-	0.50	0/8♣	100	-

*p<0.05; **p<0.025 vs pentylenetetrazole control (90mg/kg; i.p.); Student's t-test.

♣p<0.001 vs pentylenetetrazole control (90mg/kg; i.p.); Chi-squared test .

3.1.2 Effect of aqueous extract of L. leonurus on picrotoxin-induced seizures

Picrotoxin (8 mg/kg) produced tonic seizures in all the animals used. *L. leonurus* (100 mg/kg) did not affect the incidence nor the latency of picrotoxin-induced seizures. *L. leonurus* (200 mg/kg) significantly delayed the latency, but did not alter the incidence of seizures produced by picrotoxin to any significant extent. A dose of 400 mg/kg of *L. leonurus* did not affect the incidence, but significantly prolonged the latency of picrotoxin-induced seizures. Similarly, the standard antiepileptic drug, phenobarbitone (10 mg/kg) did not alter the incidence, but profoundly delayed the onset of picrotoxin-induced seizures. Diazepam (0.50 mg/kg) significantly reduced the number of animals convulsing and significantly prolonged the latency of seizures produced by picrotoxin (Table 2). The control vehicle did not alter the seizures or affect the gross behavior of mice (results not shown).

Table 2:

Effect of aqueous extract of *Leonotis leonurus* (LST) on picrotoxin(PIRC)-induced seizures in mice

Dose (mg/kg)				No. convulsed/ No. used	Animals not convulsed(%)	Onset of tonic convulsion (min.)
PICR	LST	Pheno-barbitone	Diaze-pam			Mean ± S.E.M.
8	-	-	-	8/8	0	14.13 ± 0.40
8	100	-	-	8/8	0	15.38 ± 0.56
8	200	-	-	8/8	0	17.00* ± 0.87
8	400	-	-	8/8	0	19.37* ± 1.00
8	-	10	-	8/8	0	24.38** ± 1.32
8	-	-	0.50	2/8*	75	22.50** ± 1.75

*p<0.05; **p<0.025 vs picrotoxin control (8mg/kg; i.p.), Student's t-test.

*p<0.1 vs picrotoxin control (8mg/kg; i.p.); Chi-squared test.

3.1.3 Effect of aqueous extract of L. leonurus on bicuculline-induced seizures

Bicuculline (20 mg/kg) elicited tonic seizures in all the animals used. *L. leonurus* (100 - 200mg/kg) did not alter the incidence of bicuculline-elicited seizures significantly. Besides, not only *L. leonurus* (400 mg/kg) did not significantly alter the incidence of the seizures, but also it significantly shortened the latency of bicuculline-induced seizures. Both of the standard antiepileptic drugs, phenobarbitone (10 mg/kg) and diazepam (0.50 mg/kg) profoundly antagonized seizures produced by bicuculline (Table 3). The control vehicles did not affect the gross behavior of mice or the seizures produced by bicuculline significantly (results not shown).

Table 3:

Effect of aqueous extract of *Leonotis leonurus* (LST) on bicuculline(BIC)-induced seizures in mice

Dose(mg/kg)				No. convulsed/	Animals not	Onset of tonic
		Pheno-	Diaze-	No. used	convulsed (%)	convulsion (min.)
BIC	LST	barbitone	pam			Mean ± S..E.M
20	-	-	-	8/8	0	10.88 ± 0.48
20	100	-	-	8/8	0	9.88 ± 0.69
20	200	-	-	8/8	0	10.38 ± 0.75
20	400	-	-	8/8	0	6.63* ± 0.30
20	-	10	-	0/8♣	100	-
20	-	-	0.50	0/8♣	100	-

*p<0.025 vs bicuculline control (20 mg/kg; i.p.); Student's t-test.

♣p<0.001 vs bicuculline control (20mg/kg; i.p.);Chi-quared test.

3.1.4 Effect of aqueous extract of L. leonurus on N-methyl-D-L-aspartic acid- induced seizures

N-methyl-D-L-aspartic acid (400 mg/kg) elicited tonic seizures in all the animals used. *L. leonurus* (100 mg/kg) did not affect NMDLA-induced seizures significantly. *L. leonurus* (200 – 400 mg/kg) significantly prolonged the latency of the seizures. A dose of 200 mg/kg of *L. leonurus* did not affect the incidence of the seizures while 400 mg/kg protected 12.5% of the mice convulsing. However, the standard antiepileptic drugs, phenobarbitone (10 mg/kg) and diazepam (0.50 mg/kg) did not affect the seizures produced by NMDLA to any significant effect (Table 4). The control vehicle did not affect the gross behavior of the mice or alter the seizures significantly (results not shown).

Table 4:

Effect of aqueous extract of *Leonotis leonurus* (LST) on N-methyl-DL-aspartic acid (NMDLA)-induced seizures in mice

Dose (mg/kg)				No. convulsed/ No. used	Animals not convulsed (%)	Latency of tonic convulsion (min.)
NMDLA	LST	Pheno-barbitone	Diaze-pam			Mean ± S.E.M
400	-	-	-	8/8	0	3.50 ± 0.40
400	100	-	-	8/8	0	4.38 ± 0.26
400	200	-	-	8/8	0	5.75* ± 0.31
400	400	-	-	7/8	12.5	8.71* ± 1.42
400	-	10	-	8/8	0	2.25 ± 0.31
400	-	-	0.50	8/8	0	4.00 ± 0.42

*p<0.05 vs NMDA control (400mg/kg; i.p.); Student's t-test. The proportion of animals convulsing is compared using Chi-squared test.

3.2 Phytochemical characterization

3.2.1 Chemical constituents of aqueous extract of Leonotis leonurus

The standard chemical tests used showed the positive reactions for alkaloids, saponins and tannins, and the negative reactions for the rest of compounds tested.

3.2.2 HPLC fingerprint

According to the HPLC spectrum of water extract of *L. leonurus* obtained, the major peaks presented the following retention times (min): 17.69, 19.22 and 20.76 (figure 3). The peak for the reference standard, Rutin, appeared at the retention time (min) of 21.47 (figure 4).

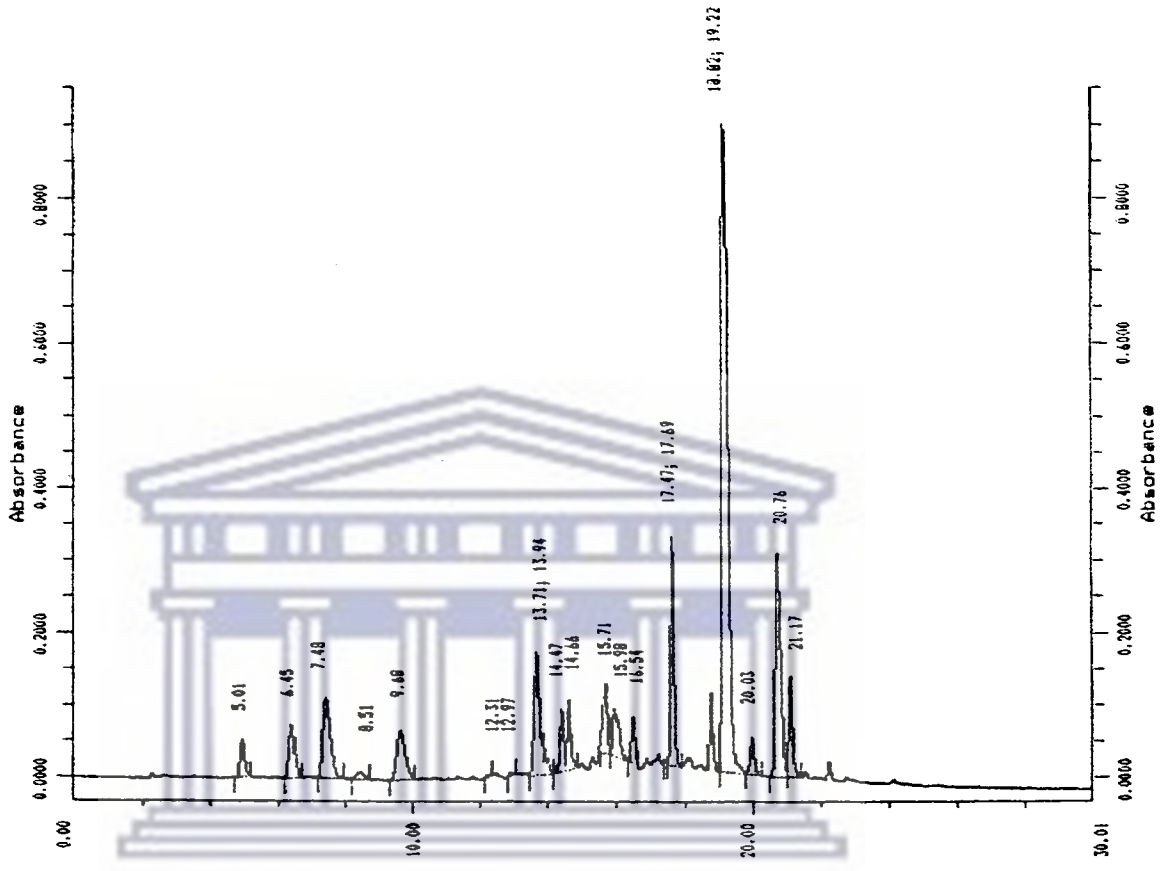


Figure 3: HPLC spectrum of aqueous extract of *Leonotis leonurus*

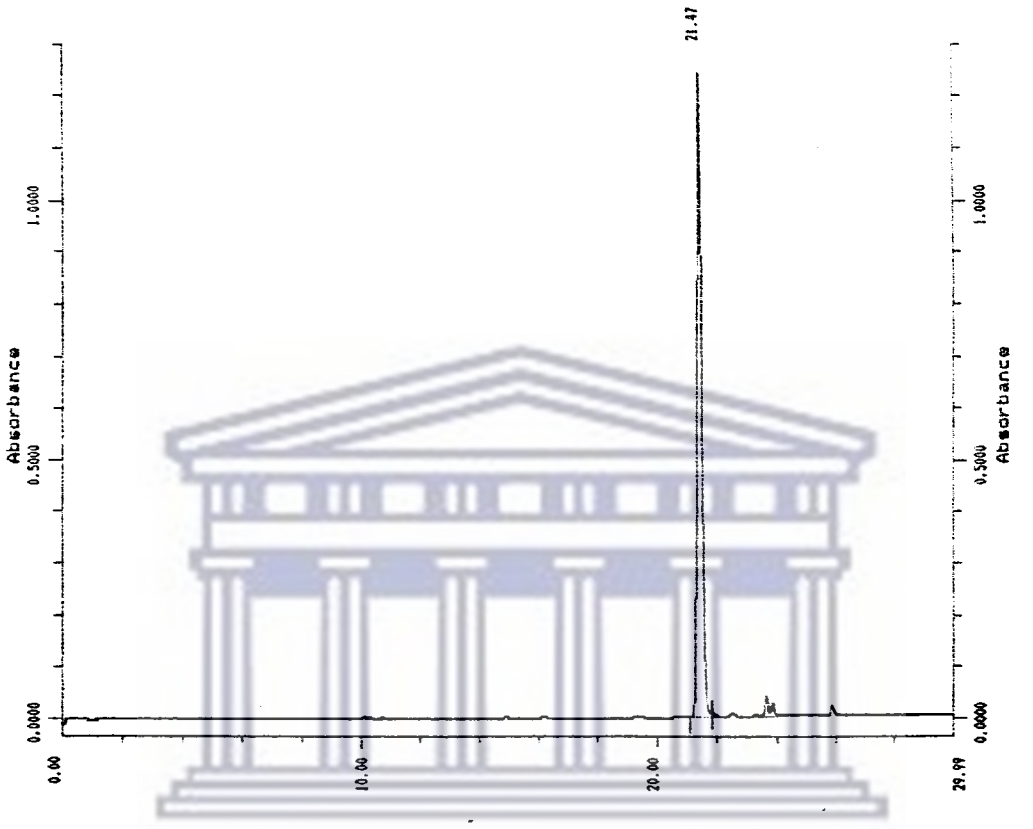


Figure 4: HPLC spectrum of Rutin, standard reference

CHAPTER 4

DISCUSSION

In the present study, both the antiepileptic activity of *L. leonurus* and the probable involvement of GABA (gamma amino butyric acid) and glutamic acid systems in this activity were investigated. GABA is the major inhibitory neurotransmitter in the brain whereas glutamic acid is an excitatory neurotransmitter in the brain. The inhibition of GABA neurotransmission and the enhancement of the action of glutamic acid have been shown to be the underlying factors in epilepsy (Westemoreland et al., 1994; Rang et al., 1999). Our study shows that the water extract of *L. leonurus* protected some of the animals against seizures induced by pentylenetetrazole (90 mg/kg), picrotoxin (8 mg/kg) and NMDLA (400 mg/kg), and also delayed the latency of the seizures. This is in conformity with the claims by traditional medicine practitioners that the plant is effective in the treatment of convulsion.

In this study, pentylenetetrazole was shown to induce seizures in all the mice used. The mechanism of pentylenetetrazole-induced seizures is uncertain (Rang et al., 1999). However, according to De Sarro et al. (1999), pentylenetetrazole may be

producing seizures by inhibiting gabaergic mechanisms. Standard antiepileptic drugs, phenobarbitone and diazepam are thought to produce their effects by enhancing GABA-mediated inhibition in the brain (Rang et al., 1999). It is therefore possible that the anticonvulsant effects shown in this study by the said drugs against seizures produced by pentylenetetrazole might be due to the activation of GABA neurotransmission. Since *L. leonurus* extract similarly antagonized seizures elicited by pentylenetetrazole in mice, it is probable therefore that it may also be exerting its anticonvulsant effects by affecting gabaergic mechanisms.

In the same study, picrotoxin also produced seizures in all the mice used. Picrotoxin has been shown to elicit seizures by antagonizing the effect of GABA by blocking the chloride channels linked to $GABA_A$-receptors. This prevents the conductance of chloride ions into the brain, thus inhibiting GABA-mediated inhibition and GABA neurotransmission (Westmoreland et al., 1994; Rang et al., 1999). In this study, phenobarbitone and diazepam were shown to antagonize the effect of picrotoxin and *L. leonurus* extract was also shown to delay the latency of picrotoxin-induced seizures. This suggests that *L. leonurus* may be affecting gabaergic mechanisms, probably by opening the chloride channels associated with $GABA_A$-receptors.

On the other hand, *L. leonurus* aqueous extract did not affect the seizures induced by bicuculline, a selective antagonist of GABA at the $GABA_A$-receptors. By antagonizing the onset of seizures elicited by bicuculline, phenobarbitone and

diazepam were shown in this study to affect $GABA_A$-receptors, which leads to the potentiation of the action of GABA. Since *L. leonurus* extract did not affect bicuculline-induced seizures, it is probable, therefore, that its effects on gabaergic mechanisms do not affect $GABA_A$-receptors.

NMDLA was also shown to elicit seizures in all the mice used. NMDLA, a specific agonist at the NMDA-receptors, mimics the action of glutamic acid and thus induces seizures by enhancing the glutaminergic system (Rang et al., 1999). Therefore, it is not surprising that the standard antiepileptic drugs, phenobarbitone and diazepam did not alter NMDLA-induced seizures to any significant extent. In this study, *L. leonurus* extract was shown to delay the latency of seizures induced by NMDLA, it may probably, therefore, be exerting its anticonvulsant effect partly by affecting glutaminergic mechanisms.

The phytochemical and the HPLC analyses showed the presence of alkaloids, saponins and tannins in the leaves of the plant, and distinct peaks which may characterize *L. leonurus* respectively.

CHAPTER 5

CONCLUSION

The present project was elaborated to verify the anticonvulsant properties of *L. leonurus* in order to prove and support its use as antiepileptic remedy by the traditional medicine practitioners. From the above, the results obtained in the experimental animal model of epilepsy are convergent to the confirmation that *L. leonurus* aqueous extract has anticonvulsant properties and may be acting by affecting both gabaergic and glutaminergic systems. This means that *L. leonurus* exerts its anticonvulsant effect through non-specific mechanisms. In accordance with the results of this study, *L. leonurus* might be clinically useful in the control of human epilepsies since it exhibited anti-seizure properties in mice.

As a result of the above mentioned, *L. leonurus* may be included with other *Lamiaceae* plants, such as *Leonurus cardiaca*, *Marrubium vulgare*, *Roylea elegans*, *Salvinia nemorosa* and so on, which have already been screened for anticonvulsant activity (Chauhan et al., 1988).

In conclusion, this project contributes not only to a scientific proof of the efficacy of *L. leonurus* in the treatment of epilepsy, but also to preliminary studies for a

phytochemical screening of this plant and for a suitable pharmaceutical formulation. My recommendation, however, is a call for more extensive pharmacological, toxicological and phytochemical screening of this plant, with a view to fully elucidate the mechanism of action, the active constituents and the safety of the plant as a medicinal remedy.

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