Evaluation of the anticonvulsant activity of *Elytropappus rhinocerotis* (L.f) Less

OSARO IYAMU

A thesis submitted in partial fulfillment of the requirements for the degree of Magister Pharmaceuticiae Scientiae in the school of pharmacy, University of the Western Cape.

SUPERVISOR: PROFESSOR GEORGE J. AMABEOKU

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DECLARATION

I declare that the thesis Evaluation of the anticonvulsant activity of *Elytropappus rhinocerotis* (L.f) Less is my own work, that it has not been submitted before any degree examination in any other university and that all the source used or quoted here by me have been indicated and acknowledged by complete reference.

Osaro Iyamu **November 2016**

Signed.....

DEDICATION

I dedicate this thesis to my late grand fathers Honourable Benjamin IKponwonsa Ogbeiwi and Mr. Isaac Iyamu for their undying love and sacrifice towards me and my sisters. Especially for their love and passion for the education of the African girl child which has pushed me to where I am today and to go further.

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Key words

Elytropappus rhinocerotis

Anticonvulsant activity

Acute toxicity

Methanol leaf extract

Hplc fingerprinting

Traditional medicines

Phytochemical analysis

Gabaergic and glutaminergic mechanisms

Mice

Antiepileptic

Neurotransmission

Antagonized

Induced Tonic convulsions

ABBREVIATIONS

AEDs	antiepileptic drugs
AIDS	Acquired immune deficiency syndrome
AMPA	α-amino-3-hydroxy-5-methyl Isoxazole-4-proprionic acid
BIC	bicuculline
Ca^{2+}	Calcium ions
Cl^-	chloride ions
CNS	central nervous system
DMSO	dimethylsulfoxide
DZ	diazepam
EEG	electroencephalogram
ER	*Elytropappus rhinocerotis*
E. Rhinocerotis	*Elytropappus rhinocerotis*
GABA	gamma-aminobutyric acid
$GABA_A$	gamma-aminobutyric $acid_A$ receptors
$GABA_B$	gamma-aminobutyric $acid_B$ receptors
$GABA_c$	gamma-aminobutyric $acid_c$ receptors
GAD	glutamic acid decarboxylase
GlyRs	glycine receptors
Hcl	hydrochloric acid

HPLC	high performance liquid chromatography
K^+	potassium ions
Mg^{2+}	Magnesium ions
MS	muscimol
Na^+	Sodium ions
NMDA	N-methyl-D-aspartate
NMDLA	N-methyl-DL-aspartate
NOAEL	no-observed-adverse-effect-level
PB	phenobarbitone
PHY	phenytoin
PIC	picrotoxin
PTZ	pentylenetetrazole
QC	quality control
STN	strychnine
Uv	ultraviolet
TM	traditional medicines
WHO	world health organization
Zn^{2+}	zinc ions

ABSTRACT

Evaluation of the anticonvulsant activity of *Elytropappus rhinocerotis* (L.f) Less.

O.IYAMU

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

Epilepsy is a worldwide neurological disorder which is also prevalent in South Africa. Herbal medicines, besides orthodox medicines, have been used from time immemorial for the treatment of epilepsy even though, generally, there is want of scientific evidence to substantiate their effectiveness.

People with epilepsy have used different types of plants and herbs now known as *herbal therapies* over thousands of years although no clinical benefit is implied by this term. Moreover, the use of traditional medicine to treat CNS disorders such as epilepsy in South Africa still lacks a lot of scientific data although a study that was conducted in the Western Cape Province of South Africa revealed that the people in the Bredasdorp community use *Elytropappus rhinocerotis* to treat convulsions.

Hence, the main objective of this current study was to evaluate the anticonvulsant activity of *Elytropappus rhinocerotis.* Other objectives were: to determine the safety profile by performing acute toxicity test; determination of chemical components through phytochemical analysis and the determination of HPLC fingerprint of the plant sample.

E. rhinocerotis belongs to the family, Asteraceae. It is an erect bushy shrub which grows up to 1 to 2m in height; with minute, grayish-green leave. It is known in Afrikaans as "renosterbos".

To realize the objectives of this study, the dried leaves of *E. rhinocerotis* were milled into fine powder. The dried powder was then extracted in methanol. The resultant filtrate was evaporated to a semi-solid extract which was then freeze-dried to obtain a dried leaf methanol plant extract.

The anticonvulsant effects of the methanol leaf extract of *E. rhinocerotis* was studied by chemical induction of seizure in experimental mice using standard convulsant agents such as, pentylenetetrazole (PTZ), bicuculline, picrotoxin, N-methyl-DL-aspartic acid and strychnine.

All of the experimental mice manifested tonic convulsions induced by Pentylenetetrazole (PTZ) (100 mg/kg, i.p), bicuculline (30 mg/kg, i.p), picrotoxin (20 mg/k, i.p), N-methyl-DL-aspartic acid (NMDLA) (500 mg/kg, i.p) or strychnine (2 mg/kg, i.p). Tonic convulsions induced in experimental mice by PTZ, bicuculline, picrotoxin or strychnine was significantly antagonized by leaf methanol extract of *E.rhinocerotis* (200 and 400 mg/kg, i.p), muscimol (2 mg/kg, i.p), phenobarbitone (12 mg/kg, i.p) or diazepam (0.5 mg/kg, i.p). The tonic convulsions induced in mice by NMDLA (500 mg/kg, i.p) was significantly antagonized by leaf methanol extract of *E.rhinocerotis* (400 mg/kg, i.p). Strychnine induced tonic convulsions in mice was significantly antagonized by leaf methanol extract of *E.rhinocerotis* (400 mg/kg, i.p) and phenobarbitone (12 mg/kg, i.p). Phenytoin had no effect on tonic convulsions induced by NMDLA, Strychnine, picrotoxin, PTZ or bicuculline. Diazepam also had no effect on tonic convulsions induced by NMDLA and strychnine. Tonic convulsions induced by NMDLA were not significantly antagonized by phenobarbitone.

Oral doses of 100-4000 mg/kg of *E. rhinocerotis* were found to be non-toxic in experimental mice. No signs of acute toxicity or death were observed. The LD_{50} value obtained for *E. rhinocerotis* may be greater than 4000 mg/kg.

In conclusion, the results obtained from this study showed that the methanol leaf extract of *E. rhinocerotis* possess anticonvulsant activities. The mechanism of the anticonvulsant effect of *E.rhinocerotis* may be through involvement in the gamma aminobutyric acid (GABA), glutamate and glycine systems. Since *E. rhinocerotis* significantly delayed the onset of tonic convulsions induced by NMDLA and strychnine, the mechanism of the anticonvulsant activity of the plant may not be specific. The phytochemical analysis showed the leaves of *E. rhinocerotis* contain some chemical compounds such as; alkaloids, saponins, flavonoids, tannins, cardiac glycosides and triterpene steroids. The HPLC fingerprint of the leaf methanol extract of *Elytropappus rhinocerotis* showed distinct peaks at the following retention times, 4.227, 5.030, 7.698, 12.810, 13.306, 15.333 and 18.563 minutes.

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CHAPTER 1

INTRODUCTION

Epilepsy is a worldwide neurological disorder which is characterized by recurrent seizures and it is also prevalent in South Africa. It is associated with episodes of altered behavior and/or consciousness, which can take many forms, but occurs from an abnormal electrical paroxysmal discharge in cerebral neurons (Cull and Goldstein, 1997). The type of epileptic seizures such as toni-clonic seizures also known as grand mal seizures, typical absence seizures, and myoclonic seizure depend on the part of the brain affected. Epilepsy is extremely common, and has been presumed to be as a result of the massive interconnected organization the brain has. The prevalence of epilepsy worldwide reaches 1% and it is said that 10% of the population will have an epileptic seizure at some time but only 5% of these are febrile seizures of childhood (Zeman *et al*., 2012). It often starts in childhood and old age, less often in early/mid-adulthood. Its prognosis depends upon its cause, but about two-thirds of people who develop epilepsy stop having seizures either spontaneously or treatment. Causes of epilepsy varies from genetic predisposition as shown by recent studies that some single gene mutations have epilepsy as their main manifestation, abnormalities of brain development, brain infections such as meningitis and encephalitis, head trauma, stroke, dementia (Zeman *et al*., 2012). Another known cause of epilepsy is as a result of imbalance in the GABAergic system which is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS). Gamma-aminobutyric acid (GABA) exerts its physiological effects by binding to three different receptor types in the neuronal membrane; $GABA_A$, $GABA_B$ and $GABA_C$ receptors. The $GABA_A$ receptor is mainly involved in epilepsy. It has a binding sites for compounds such as benzodiazepines that allosterically modify the chloride channel gating of GABA (Jäger *et al*., 2005).

In adult onset epilepsy, the cause remains unknown in about 50% of the cases. Epilepsy is not easily diagnosed. It depends on a vivid description of the events occurring in seizures, both from the victim's perspective and by an eye witness. Several tests can be helpful in making a diagnosis, but it may remain unclear despite these. Standard investigations include EEG (Zeman *et al*., 2012).

Medicinal plants and their derived products have been a key source of traditional medicine (TM) from time immemorial worldwide prior to the synthetic era. Around 80% of all medicines were obtained from natural products mainly from medicinal plants and play an important role in the healthcare systems of developing countries (Singh et al., 2014)

According to the World Health Organization (WHO), more than 80% of the world population relies on traditional healthcare practices to cater for their healthcare needs. TM comprises of the knowledge, beliefs, skills and lifestyle practices including the use of plants, animals, spiritual therapies, exercises, mineral based medicines and experiences that are practised by different cultures to improve and maintain the healthcare needs of various communities. Although, as important as TM is, some countries still underestimate its importance (WHO, 2005; WHO, 2015).

Sometimes, the terms complementary and alternative medicine are used interchangeably with TM in some countries. Different countries have their various ways of practicing TM as the practices depend on the cultures, long history and tradition passed on from generation to generation, perceptions, personal belief and attitudes which have all in one way or another proven the safety, efficacy and reliability of TM. The use of TM is rapidly spreading in most industrialized countries because its popularity has been maintained in developing countries and

the market growth rate for TM worldwide is increasing and steady (WHO, 2005). It has been observed that there has been a continued demand for TM around the world since WHO developed its first global strategy, Many countries now use TM to treat chronic diseases and other ailments such as cancer, diarrhoea, malaria, pain and fever, arthritis, anxiety, physical and mental disorders. It is also used in public health promotion and prevention of most diseases and the reasons why most countries prefer the use of TM is because of its cost-effectiveness, easy to use and reliability (WHO, 2005; Kabatende, 2005).

Although, TM is recognized in so many countries, it is still not officially recognized in most countries and it is still lacking enough scientific evidence from research to prove its safety and efficacy. Therefore, more and improved research is needed but it is vital that when conducting these researches, the long history, tradition, practices, experience and knowledge of TM should be taken into considerations. This then leads to the support of training and education needed for the research of TM. Even though, there have been some evidence so far from research that has proven the safety and efficacy of some of the TMs, the results are still not sufficient to meet the criteria that enables a world wide support because of the health care policies of some countries and also some research methodologies used for evaluating and assessing TM are lacking. Most countries have been able to provide some evidence which have been published and not published so this requires an improvement in the quality of research and more scientific evidence to prove the safety and efficacy of TM (WHO, 2015).

In order, to improve the quality of research in TM and to provide more proof for safety and efficacy, some guidelines and strategies were created by the World Health Organization (WHO) to assist with the research process, methodologies used during research to have enough assurance and guarantee the safety and efficacy of herbal medicines and traditional procedure-based

therapies and avoidance of obstacles that might arise when developing and during the application of TM. However, this has been difficult for research organizations and scientists to adhere to. The purpose of the guidelines outlined by WHO were basically to help improve and promote the proper use and development of TM as previously mentioned, so it is very crucial for researchers, healthcare providers and TM practitioners to always refer to these guidelines. The guidelines also suggest botanical verification (i. e. Identification of the plant species as a first stage in quality assurance, the safety and efficacy of herbal medicine which should be practiced in every country (WHO, 2015).

Safety and efficacy issues remain a huge concern when it comes to the use and research of TM as the inappropriate, unregulated use and practices of TM can be detrimental and pose a big threat to the world. Some evidence have been brought to light concerning the safety issues of some herbs; for instance, the case of the Chinese herb "*Ma Huang"* also known as *Ephedra* which was known to be safe for the treatment of respiratory congestion and was later found out to be marketed as a product to aid diet and had fatal effects such as death, stroke and cardiac arrest in the US. Another guideline was then created by WHO to assist countries to develop adequate patent laws under approved international standards to protect the knowledge and biodiversity of TM, to create stronger evidence of safety and efficacy, to enable a more cost-effective and affordable TM and the documentation of remedies used (WHO, 2015).

South Africa is a diverse nation with many tribes and traditional practices which are sometimes connected to the practice of traditional medicine. The Zulu tribe is most commonly associated with traditional healers which they refer to as, "inyangas", or herbalists and "isangomas", or diviners (Kabatende, 2005). The knowledge of herbs is mostly acquired by the elderly folks in rural areas mostly because of their passion for plants and herbs and the knowledge gained from

experience that has been passed on from generation to generation. There are also the spiritual healers who treat CNS disorders and other related problems. For most people in the rural areas, the use of plants have been known to them as the only source of different remedies and the only cure and treatment that they trust to be effective to treat all kinds of ailments from generation to generation (Van wyk *et al*., 2000; Kabatende, 2005).

In most African countries including South Africa, TM is a significant part of the culture of the people that indulge in its practice. Hence, it is associated with their beliefs and attitudes. The quality of the plants use in TM can be affected by various factors such as; environmental conditions, species variation, the time of harvesting, storage and processing. These factors can make the plant's medicinal use less potent and unsafe. Therefore, it is very essential for the quality control (QC) of plant extracts of any research to involve; safety, efficacy and effective therapy. Although, the QC of medicinal plants is a very complex task because medicinal plants comprise of various compounds that are not easily identified (Kabatende, 2005). Some scientific researches have shown that some active compounds including flavonoids, terpenes and some derivatives of caffeic acid are presents in medicinal plants (Mauri *et al*., 2000). Some studies have also shown that the basis of any classification of medicinal plant from data interpretation such as potential therapeutic use of the plant extract is from a complete study of the pharmacological profile of the plant extracts (Atta and Alkofahi, 1998; De Sarro *et al*., 2000; Rabbani *et al.*, 2003).

People with epilepsy have used different types of plants and herbs now known as *herbal therapies* over thousands of years although no clinical benefit is implied by this term (Schachter, 2009). Moreover, the use of TM to treat CNS disorders such as epilepsy in South Africa still lacks a lot of scientific data although a study that was conducted in the Western Cape province of

South Africa, revealed that the people in the Bredasdorp community use *Elytropappus rhinocerotis* to treat convulsions. Hence, the main objective of this current study was to evaluate the anticonvulsant activity of *Elytropappus rhinocerotis* (Thring and Weitz, 2005).

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

2.1.1. Overview on traditional medicines.

The interest in the knowledge of traditional medicines is rapidly increasing and gaining a significant amount of recognition worldwide and is included in the development of policies and scientific literatures. TM plays a significant role in the health of majority of the people in Africa. Medicinal plants are quickly regaining their famous position that they were known for in past eras in modern medicines and has always held in most part of the world which has made the use of the plants become an important part of the lives of the people in Africa as well as in the western world, Asia and other undeveloped parts of the world despite the fact that modern medicine and pharmaceutical research have gained a lot of advancement. In the Venda area of South Africa, for instance, there are about 200-700 people attended to a traditional medicine practitioner, while a university trained medical doctor has about 1639 people assigned to him/her. African traditional medicine has its own methodology, the fact that certain aspects of it do not follow/ are not on the same level of knowledge in science should not cause much limitations. Basically, it has been known to treat and cure many ailments which were all not as a result of chance, but based on years of experience, teaching and observations passed on from generation to generation. The Pharmacopoeia of the African medicinal healer comprises of treatment and cures for various ailment and diseases that cause affliction to people such as; dysentery, snake bite, skin conditions, whooping cough, diabetes, pain, inflammation and so on. Modern medical practitioners have indicated that medicinal plants can cure some difficult

diseases that affect humans such as cancers and various immune deficiency disorders. The World Health Organization (WHO) has described TM as one of the surest means to gain total healthcare coverage of the world's population. The attention currently received by TM from various governments to allow for a widespread healthcare application, has lead to an increase in research, funding and development of programmes in several developing countries despite the hindrance it has received over the past years (Iwu, 1993; Cunningham, 1997; Li, 2002; Kabatende, 2005).

In the past, plants were discovered to have medicinal values based on traditional practices and accidental discoveries without scientific proofs. Recently, researches conducted on medicinal plants have generated some proof and a significant amount of information about the biologically active chemical components that are responsible for the medicinal claims. Almost 50% of medicines on the market are made from natural fundamental materials. A lot of the active ingredients in medicinal plants cannot be prepared synthetically yet, which will likely increase the market demand for medicinal plants which have potenial in healthcare improvement. High cost of modern medicines and pharmaceutical services in the developing world has created room for medicinal plants to be used continuously in medical practice (Iwu, 1993; Li, 2002).

Until recently, the people who have mainly used medicinal plants in Africa have been the local people in the communities. The field has now attracted a number of different researchers who have discovered the value of traditional healing and this has resulted in more interests in different areas of research of TM. Some of the aspects of medicinal plants that researches were first conducted on were; ethno-botany which gave researchers the insight for new drug discoveries based on medicinal plants uses; phytochemistry and chemistry of natural products, organic synthesis and the usefulness of medicinal plants. Some literatures have considered African medicinal plants from two points of view; firstly, as sources of biologically active

compounds and secondly, as elements in the complex equation of healing. One might think that the two aspects are different but a closer inquiry will show a similarity in the two modes of medicinal plant utilization in African traditional medicine (Iwu, 1993; Kabatende, 2005).

The first consideration of treatment that comes to the mind of many Africans when they are ill/sick, especially the under-privileged in both rural and urban areas, is usually to use medicinal herbs. Even up to date, there are still many rural communities in Africa that still rely on traditional herbal medicines as their major and first point of healthcare source (Kabatende, 2005). Hence, it is difficult not to accept and doubt the efficacy of herbal remedy within the African society (Sofowora, 1982). The importance of using TM and the use of medicinal plants as a strategy for healthcare delivery have long been recognized by community workers in poor urban and rural areas which has allowed the communities to accept the safety and efficacy of TM but also to recognize the fact that the use of TM needs to be scientifically validated. The use of herbal medicines in communities has resulted not only in economic benefits and accessibility to much needed remedies. The use of TM has somehow, partially freed people from dependence on commercial drugs and has shown that the community has the ability to use and develop local plant resources to solve their healthcare problems which has led to their trust in the efficacy of herbal medicines. In many areas, especially the rural areas, the accessibility and availability of medicinal herbs have been able to offer people the use of safe and effective products to prevent and treat illness through self-medication. Some of such plants have been shown to be useful in modern medicine as well (Tomlinson and Akerele, 1998).

However, some literature has suggested that it is very important for the traditional medical system to remain distinct and should not be consumed by modern medicine. The system should be allowed to complement the modern medical system by accepting its complexity furthermore,

the promotion of the herbal medicines that have been proven to be safe and effective (i.e. scientifically) should be encouraged at all times and in every sector of the community (Tomlinson and Akerele, 1998).

South Africa has a rich cultural diversity which is reflected in the various ways in which medicine is practised presently in different parts of the nation (Kabatende, 2005). The use of traditional medicine is been referred to as *phytotherapy* by Biomedical literature. Traditional medicines and traditional healers form part of a broader field of study classified by medical anthropologist as *ethnomedicine.* Traditional medicine is usually associated with herbal remedies and sort advice from the *sangomas,* which makes the role of the traditional healers important and considerable in developing the healthcare system in South Africa (Campaign and Richter, 2003). Traditional medicine is a comprehensive term that does not just refer to traditional medicine systems such as traditional Chinese medicine, but also Indian ayurveda and Arabic unani medicine, and also to other various forms of indigenous medicine. TM has been described by WHO as a system of medicine that is based on past experience and cultural beliefs and practices passed on from generation to generation, verbally or in writing, and traditional medicine includes diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied alone or in combination to maintain well-being, as well as to treat, diagnose or prevent illness. A deeper look into the concept shows that TM also include mystical and magical rituals, that is other kinds of treatment which may not be explained by modern medicine (Sofowora, 1982; Van Wyk *et al*., 2000; Bienvenu *et al*., 2002; Tabuti *et al*., 2003; Kabatende, 2005; WHO, 2015).

A traditional healer is referred to as a person who is known by the community to which he or she belongs to be competent to provide healthcare. They are involved in diagnosing usually through spiritual means and choosing and applying relevant remedies for the diagnosed condition. In most cases, the roots, leaves, barks or flowers of the plant are used as the compositions of the treatments (Richter, 2003; Kabatende, 2005; Reid *et al*., 2005).

Although, a lot of research has been carried out about the constituents and biological activity of African medicinal plants and the scientific proof documented and published about these plants, some societies are still neglecting and have not fully accepted the development of therapeutic agents from African medicinal plants. Some studies have shown that the African continent is experiencing a rapid loss of the natural habitat of some of the plants due to anthropogenic activities which have helped to increase the research and documentation of the medicinal uses, the constituents and pharmacological activity of African plants (Iwu, 1993). Over the years, nature has been a source of medicinal agents, and an impressive number of modern day drugs have been isolated from natural sources, many based on their use in traditional medicine. These plant-based traditional medicine systems play a significant role in healthcare, with a huge amount of the world's population relying mainly on traditional medicine for their primary health care need. People in the local rural areas and some parts of the urban community use a wide range of plants therapeutically to maintain their health. Hence, there is a great promise for new drug discoveries based on traditional plant uses (Lewis and Elvin-Lewis, 2003).

2.1.2. Advantages and Disadvantages of Traditional Medicine

Some reports have revealed that the Western Cape Province of South Africa is enriched with a huge diversity of plants species which are used for treatment of different ailments due to their medicinal properties. These plants include *Elytropappus rhinocerotis* that is used for the treatment of epilepsy and other conditions such as fever, bladder and kidney infections in some Western Cape communities (Van wyk *et al*., 2000; Thring and Weitz, 2006).

Although, there have been a lot of reports and researches that have revealed the possible uses of traditional medicines and there is a continuous increase in traditional medicine awareness, many of the herbs are still untested. Their uses are not regulated and monitored which may lead to a high risk of harm as a result of lack of knowledge about their possible side effects. This limits the promotion of their rational use and makes it more difficult to identify the safest and most effective therapies (WHO, 2001; Kabatende, 2005). Medicinal plants/traditional medicines thus have some disadvantages which include;

- The lack of scientific proof of their efficacy and toxicological profile (Sofowora, 1982).
- Limited information in literatures regarding the proper usage of herbal medicines such as correct dosage, route of administration, frequency and when to use the herbs, possible side effects, drug and disease interaction (Li, 2002).
- Some medicinal plants are infested by some pests and pathogenic bacteria which can cause some diseases to the individual taking them if not properly cultivated before administration for treatment thus causing harmful effects.

- Some soil contains toxic chemicals such as selenium and arsenic which can contaminate the plants growing in that particular area (Muhizi, 2002). The contaminated plants can cause harmful effects when used as a medicine.
- The symptoms of the diseases are mostly treated rather than the disease itself because majority of the traditional healers are not equipped with the knowledge of the pathology of some diseases. They can also diagnose and treat the wrong ailment due to the knowledge they have of a different disease which present with same symptoms and was treated with the same herb (Sofowora, 1982; Muhizi, 2002).
- The growing high demand for medicinal herbs has threatened the existence of some plants which can put these plants in the endangered species list thereby leading to prohibition of further harvesting by law (Li, 2002).
- The lack of suitable technology has affected the cultivation of medicinal plants which leads to low yield and product of poor quality and can cause possible harm when used as medicinal product (Li, 2002).
- Some of the herbal products on the market are a mixture of two or more herbs and there is limited research on the effect of combinations of herbs in humans which can lead to possible toxicity when consumed by humans (Li, 2002).
- Lack of mechanisms to control and regulate TM advertising and claims (WHO, 2015).
- Lack of financial support for TM research.

Some literature has implied that plant medicines can be safer and less damaging to the human body than synthetic drugs (Williamson *et al*., 1996). The market demand for medicinal plants is growing at a high and steady rate and is likely to remain high because a lot of the active ingredients in the medicinal plant cannot yet be prepared synthetically (Li, 2002). The increase

in medicinal plants research is due to the fact a lot of the commercially important drugs in use today were isolated from plants or starting molecules of plant origin such as: digoxin/digitoxin, the vinca alkaloids, reserpine and tubocurarine which have all been shown to yield extremely valuable molecules that can be used as tools in the characterization of enzymes and classification of receptor systems. The efficacy and popularity of medicinal plants have encouraged pharmacists and pharmaceutical sciences researchers to carry out thorough analysis on the herbs to understand, establish and correlate the relationship between the chemical composition and therapeutic activities (Vickery *et al*., 1979; Bienvenu *et al*., 2002; Kabatende, 2005).

2.1.3. Basic comparison of Traditional and Modern Medicine.

Plants were once the only main source of medicine worldwide. New remedies are still been obtained from plants on a regular basis. 50% of the drugs in use today are obtained from natural products and their derivatives. Almost 80% of the African population still rely on traditional medicines as their primary source of healthcare and in developed countries traditional medicine is rapidly gaining recognition. Almost 80% of the population has tried some traditional therapy such as homeopathy or acupuncture (Li, 2002; Shetty, 2010). The South African traditional medical system has not yet been systematised, and is passed on by word of mouth and learning experience from generation to generation. The formal system of medicine, which is well documented and systematised, was introduced to South Africa by the Europeans and other settlers over three centuries ago which is now practiced and referenced by today's modern western medicine (Van wyk *et al*., 2000).

Each system of medicine is an art of and science of diagnosing the cause of disease, treating the disease, and maintain health in the sense of physical, spiritual, social and psychological well-being. Each system has found solutions to the preventive, promotive and curative aspects of healthcare that agrees with the world wide view. For instance, modern medicine may diagnose a disease caused by a bacterial infection and treat such disease with an antibiotic while an African traditional healer will seek to understand why the patient became ill and presented such symptoms in the first place, then treat the cause of the illness, and usually add other therapies to alleviate the signs and symptoms of the condition (Van wyk *et al*., 2000).

Studies have shown that some modern medicine practitioners believed that it would be beneficial to modern medicine if some of traditional medical practices are integrated into their systems. Scientists and pharmaceutical companies are urgently searching for new drug sources and increasingly turning their eyes to traditional medicines because; it takes years for a new drug to be developed and the cost is very high. Furthermore, drug resistance is on the increase which is partly due to misuse of medications and has rendered most antibiotics and other drugs used in the treatment and management of life threatening disease useless. Foods such as red yeast and oyster mushrooms have been used to synthesize compounds such as mevastatin and pravastatin. Modern medicine practitioners and pharmaceutical researchers have been able to achieve a few successes from the interest in traditional medicine such as the antimalarial drug Artemisinin which is derived from the plant, *Artemisia annua*. In many studies, researchers have pointed out the use of traditional medicine as first line of treatment before application of modern drugs (Shetty, 2010).

Modern medicine explains the cause of disease to be due to unwanted pathophysiological agents in the body causing the infection, whereas traditional medicines believes that diseases are due to

supernatural or spiritual causes or imbalance between forces of nature causing harm to the body. New scientific techniques are now being used in the application of traditional medicine during the process of new drug discovery from medicinal plants. An example of this new approach is reverse pharmacology. The end product is usually the starting point of the research, researchers work backwards to find out what a clinically useful compound contains and how it works, since most traditional medicine practitioners do not know how most of their remedies work. This new approach helps to offer clues about how a particular medicine works and where they exhibit their functions in the body (Shetty, 2010). The use of new scientific techniques in plant analysis should always take the cultural beliefs and practices into consideration and respect the way people use and practice their medicines.

Some initiatives have been created in Africa such as African network for drug and diagnostics. This is an innovation which encourages the mining of Traditional medicines. Global health has a lot of benefits to gain from traditional medicine, especially as the need for new drug discovery is continuously on the increase. If both modern medicine and traditional medicine can work together by collaborating and conributing research capacities equally; there could be a ground breaking discovery and revival in health research and development worldwide (Shetty, 2010).

2.1.4. The need for safety and efficacy data

The use of traditional medicines needs to be properly regulated in order to avoid negative effects which could be harmful. The lack of safety and efficacy data on TM has brought several challenges to the usage of herbal medicines such as; lack of adequate or accepted research methodology for evaluating TM, unpublished data on research in TM, lack of proper training and

education about the knowledge of TM practitioners as it relates to its rational use and practice. Some scientific evidence which was obtained from randomized clinical trials is only enough for the use of few herbal medicines that involve manual therapies and acupuncture (WHO, 2002). The world health organization (WHO) has developed strategies to provide support for clinical research projects on TM safety and effectiveness including methodologies for research and evaluation of TM. The strategies outlined by WHO will help guarantee the safety and efficacy of TM and the procedures involved in these therapies. Since the practice of TM relies on a holistic approach, the evaluation of the efficacy of TM has to be done in an integrated manner taking the fact that traditional medicine comprises of herbal medicines and traditional procedure-based therapies (which can be combined for successful results) into consideration (WHO, 2005; WHO, 2015).

Recently, the quality of research has improved and better safety and efficacy data have been obtained which have lead to the tremendous increase in the number of patients and medical practitioners in developing countries using herbal medicines as an adjunct to or substitute to prescription drugs (Li, 2002). WHO provided statistics which shows the increasing use and popularity of traditional medicine in developing countries. The global market for herbal medicines currently stands at over 60 billion US dollars annually and is growing steadily (WHO, 2015). These figures prove that even with the lack of credible scientific safety and efficacy data, the use of herbal medicines will continue to grow so there is a greater need for further research to ascertain credible safety and efficacy of traditional medicines and its practice thereof.

The use of medicinal plants are rapidly regaining their prominent position that they possessed a long time ago due to their perceived safety and long history of usage of folk medicines compared to drugs manufactured synthetically Also the fact that plant medicines are natural makes people

to trust in their safety. These claims can be detrimental as they are not based on sufficient quality clinical data (Amabeoku and Kinyua, 2010).

A lot of randomized clinical trials are been carried out to authenticate their therapeutic success scientifically which will eventually help to speed up the acceptance of medicinal plants into modern medicine. Scientific validation increases popularity and approval by practitioners of modern medicine in developed and industrialized world such as Europe and the United States of America (Amabeoku and Kinyua, 2010). The world health organization has developed strategies designed to assists countries develop national policies on the evaluation and regulation of traditional medicine practices. These policies help to control and license the use of herbal medicines with regards to proper preparation procedure, regulated clinical trials to ensure quality and credible safety and efficacy and monitoring of treatment as compared with modern medicine (Amabeoku and Kinyua, 2010; WHO, 2015).

2.2 Technologies for herbal medicine discovery and analysis

2.2.1 Chemical fingerprinting

Chromatography is a method in drug discovery which is applied to isolate the active ingredients from the main plant component and also to shorten the time needed for the isolation. The chromatographic methods commonly in use are liquid chromatography, gas chromatography and column chromatography. These methods include, combining both natural product chemistry and organic synthesis of lead compounds from herbs in drug design and discovery and production of samples that are easy to use in screening by purification or fractionation of samples from the crude extract. With this done, the final resolution of the active compound is less complex, and

additional purification is required. High-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) and mass spectrometry are used to simplify purification and classification of active components from herbal products (Kayprime, 1992; Amabeoku and Kinyua, 2010). High-performance liquid chromatography was employed in this study to obtain the fingerprint of *E. Rhinocerotis* for characterization of the plant species.

2.3 Toxicity and Evaluation of Toxicity

The scientific study of the harmful effects of chemical compounds on biological components is known as toxicology. The harmful effects of the chemicals either occur from the natural part of a molecule, or chemical interaction with a particular biological system (Loomis and Hayes, 1996; Timbrell, 2001). There is a correlation between the main pharmacological effect of a drug and its toxic effects, for example, toxic effects of non steroidal anti-inflammatory drugs is gastric bleeding. Some toxic effects that occur in therapeutic doses are unpredictable, severe and rare while other effects unrelated to the major action of drug are often caused by reactive metabolites and/or immunological reactions (Loomis and Hayes, 1996; Rang *et al*., 2012).

Before new drugs or food containing chemical components are administered to humans, broad toxicological tests are required in order to discover possible toxic substances and the safety limits associated with the use of the compounds (Loomis and Hayes, 1996; Amabeoku and Kinyua, 2010; Rang *et al* 2012). There are three main methods used to evaluate the toxicity of a compound: a) Epidemiology- the process whereby the human, animal or plant populations exposed to a chemical compound are been observed. b) *in vivo*- *in vivo* administration of test compound to the plants or animals under well controlled conditions. c) *in vitro*- these

experiments are carried out by exposing the test compound to cells, sub-cellular fractions or single celled organisms and observing them (Timbrell, 2001). Most of the data on the toxicity of chemical compounds are obtained from the experimental studies carried out in animals. Hence, obtaining the biological information responsible for toxicity that is dose-related, dependable and reproducible, and which can be interpreted, extended and/or improved for the assessment of health risks to humans should be the main aim of toxicological studies (Ecobichon, 1997; Timbrell, 2001). Modern toxicological studies require series of toxicological tests to determine the different types of toxicity. The needed tests are reliant on four major principles: species selection, group sizes, dose selection and route of administration. There are other points to consider apart from the ones previously mentioned, which are, knowledge of the physio-chemical characteristics of the test compound which is helpful to determine if the compound will be toxic or not; the biological considerations, for example, sex of the animals to be used, breed of the animals (in or out bred strains), health status of the animal, diet, metabolic similarity to man; and finally the duration of the toxicity study (Poole and Leslie, 1989; Timbrell, 2001).

2.4 Species Selection and Group sizes

Species selection is based on the type of toxicity test carried out. Existing data on the particular species, financial and ethical aspects are also considered (Loomis and Hayes, 1996; Rang *et al*., 2012).

When carrying out toxicological studies, there should be enough number of animals to allow statistical significance to be calculated. There are three major *in vitro* safety profiles commonly referred to as the 3R's governing the research involving the use of live laboratory animals. They

are, the principle of replacement, reduction and refinement. This entails replacing the live animals with other alternatives, reducing the number of test animals and finally, refining the existing test methods to decrease unwarrantable suffering to animals. Some literature suggests using about six to ten animals per treatment group and a minimal amount of five dosages tested in toxicity studies (Poole and Leslie, 1989; Ecobichon, 1997; Timbrell, 2001; Amabeoku and Kinyua 2010).

2.5 Route of administration

The dosing in experimental animals should be performed in the same manner as it would be performed in humans according to the guidelines (Rang *et al*., 2012). The route of administration commonly used for most compounds is the oral route. In order to measure the toxicity of compounds affected by metabolism or absorption, it is suggested to use the parenteral routes. The parenteral route of drug administration involves: injection into the skin (intra-dermal), injection beneath the skin (subcutaneous), injection into the muscle (intramuscular), injection into the spine (intra-thecal), injection into the veins (intra-venous) and injection into the arteries (intra-arterial). Injection into the abdominal fluid (intra-peritoneal) is the most common route of administering test compounds to laboratory animals. The volume of the test compound to be administered to test animals requires a stipulated dose and is restricted by the size of the animal, hence, there should be maintenance and uniformity of the dose within and between groups of animals (Loomis and Hayes, 1996; Timbrell, 2001). The volume of solution administered to test animals should always be consistence (expressed as millilitre per kilogram of body weight) and must always be maintained as discrepancies in the absorption rates and subsequent biological effects could result. Large volumes should be avoided as they are liable to hinder efficient

absorption of the toxicant. Volumes ranging between 2.0 to 5.0 ml/kg may be administered conveniently to Rodents (Ecobichon, 1992; Amabeoku and Kinyua, 2010).

It is suggested by literature that the test compound in a toxicological test should be administered to the test subjects via a route that the species is prone to get exposed to. In toxicity tests involving animals, it is vital to come up with a appropriate means of administering the test compound to the animals. The method of intubation or incorporation of the compound in the animal feed or drinking water is the simplest way of administering test compound orally to animals. However, there is the disadvantages of: the test compound changing the taste of the animal feed thereby making it unpleasant and changing the colour which will result in the animal rejecting the feed which will give a false toxicological response. Another disadvantage is that mixing the test compound with the animal feed will increase the volume of the feed to be ingested by the test animal which will result in imprecise dosing and thus, give false results. Practically, the intravenous route is the most convenient way to administer compound to test animals as the chemical compounds go directly to the tissue. However, the intravenous route is not commonly used in toxicity testing of animals especially rodents because of the rapid systemic distribution of the compound in animals resulting in false responses. Therefore, the most ideal route of administration is the intraperitoneal route as dose inconsistencies observed with oral route, rapid systemic distribution with the intravenous route and substance accumulation at the site of injection observed in other parenteral routes are all avoided (Poole and Leslie, 1989; Ecobichon, 1992; Loomis and Hayes, 1996; Amabeoku and Kinyua, 2010).

2.6 Dose selection

In toxicological studies, there are three doses (high, medium and low) that are the accepted dosing schedule designed with a negative control group. This is important in order to characterise the toxic response over an experimental dose range, compare the relative toxicity of the test compound in different species and also to determine the 'no-observed-adverse-effect-level' (NOAEL). A NOAEL is referred to as the maximum amount of dose administered to an experimental animal without producing any toxic effects. It is always associated with the route of administration and species of animals used in experiments. It is important to always determine the NOAEL so as to ensure that the dose which produces the desired pharmacological effect is lower than the dose causing toxic effects (Poole and Leslie, 1989; Loomis and Hayes, 1996; Amabeoku and Kinyua, 2010).

2.7 Therapeutic index

The margin of safety between the dose producing a lethal effect and the dose producing the desired effect is referred to as the therapeutic index (TI) (Rang *et al*., 2012). This is obtained experimentally from two dose-response curves obtained on appropriate biological systems of animals such as mice or rats. The data represented on one curve is that from the drug's therapeutic effect and the data represented on the second curve is that of the lethal effect of the drug. This curve is mainly constructed in order to establish a maximum no-observed-adverse-effect-level (NOAEL), and also as a proof of potency and safety about the test compound to the researcher and regulatory authorities (Loomis and Hayes, 1996; Amabeoku and Kinyua, 2010). Comparison of four main statistically obtained values from the dose-response curve which are,

LD_{50}, ED_{50}, LD_1 and ED_{99} are used to measure the safety parameters of drugs. The dose which will cause mortality in 50% of the animals in a test population is the LD_{50} and the ED_{50} is the dose which produces the desired response in 50% of test animals. The therapeutic index can be obtained in two ways: one is by dividing the LD_{50} by the ED_{50} that is representing therapeutic index with ratio LD_{50}/ED_{50}. The dose-response relationship is determined by experimental administration of the test compound via a particular route of route of administration to groups of homogenous species such as mice. It involves dose variation; the initial dose is usually a small dose which has no effect on the test animal while subsequent doses are increased in multiples of two on a logarithmic basis in preceding group of animals till death of the test animal result from the highest dose (Poole and Leslie, 1989; Loomis and Hayes, 1996). There are two major limitations to the determination of the therapeutic index of a drug: one is that it does not show signs of toxic reactions and secondly, it is based on the data from animal toxicity, which does not always reflect forms of toxicity that are clinically important (Rang *et al*., 2012).

2.8 Toxicity tests

Toxicity testing entails a firm protocol that vividly outlines the procedures for a specific test. The principles of the guidelines require the type and number of test animals, the particular route of compound administration, dose schedules, duration of administration of the test compound and thorough pathological and functional procedures should be adhered to (; Loomis and Hayes, 1996; Amabeoku and Kinyua, 2010). There have been enhancement and modification of toxicity testing methods over the years to make them appropriate for different types of toxicity. There are three broad categories of toxicity testing: acute toxicity test, sub-chronic toxicity test and the

chronic toxicity test (Timbrell, 2001). The acute toxicity test which was carried out in this study involves the administration of the test compound on only one occasion.

2.8.1 Acute toxicity tests

Acute toxicity test is carried out preceding other toxicity tests on chemical compounds relevant to any biological system (Amabeoku and Kinyua, 2010). The aim of this test is to establish the symptomatology consequent to administration of the test compound (i.e. the dose response relationship) and the order of how lethal the compound is (LD_{50} value) (Ecobichon, 1992).

A series of dose-range finding experiments is involved in the establishment of the sequence to determine the acute toxicity of a new compound. Subsequent experiments are then performed in order to narrow the range of effective doses to measure how lethal the compound is, and finally, a perfect experiment for establishing the dose-response curve for lethality is carried out (Loomis and Hayes, 1996). In cases where no dose range-finding have been done, four dosages are used which may be in logarithmic progression. The data obtained from acute toxicity tests shows a dose-response relationship and also reveals toxic effects and time of death (if any) of the test animals. There should be a wide range of doses to allow for manifestation of toxic effects associated with the highest dosages used unless the required dose are improbable in relation to the expected dose (Ecobichon, 1992; Timbrell, 2001 Amabeoku and Kinyua, 2010).

2.8.2 Plant toxicity

If proper quality control measures are enforced in traditional medicines, a lot of indirect adverse effects of herbal medicines could be avoided (Angell and Kassirer, 1998). Some of the toxic effects which arise from traditional medicines are due to, misdiagnosis by the traditional medicine practitioner or use of wrong herbs, mixing the medicinal plant extract with other toxic plant extract or non-organic contaminants such as heavy metals and inclusion of synthetic drugs into the natural plant extract; which could have side effects on their own hence aggravating the condition (Vickers and Zollman, 1999; Ernst, 2002). Climatic and soil conditions where plants are grown, lack of dose-safety studies and variability in chemical components that make up the herbs can also cause toxic effects of medicinal plants (Tyagi and Delanty, 2003). Medicinal plants should not be viewed as risk-free for they can be linked with toxic effects as a result of the plant exposure to the intrinsic ingredients in them. There are various harmful effects of medicinal plants. Many plants have the potential to be toxic, carcinogenic, and mutagenic as observed through extensive screening program of some South African medicinal plants such as: *Tulbaghia violacea, Sclerocarya birrea, Prunus africana, Hypoxis colchicifolia* and *Chaetacme aristata*. All these plants have the potential to cause long term damage to a patient's genetic makeup (genetoxic) (Fennell *et al*., 2004). Some other examples of toxic effects of medicinal plants include, liver damage resulting from Kava extracts (Ernst, 2002) and brain hemorrhage from *Ginkgo biloba* due to interaction with other platelet inhibitors (Izzo and Ernst, 2001). The same thorough and vigorous test applied to synthetic drugs before human consumption should also be applied to medicinal plants to ensure optimal safety.

2.9 Pathophysiology of epilepsy and antiepileptic drugs

Epilepsy is a central nervous system disorder characterized by spontaneous, recurring seizures associated with loss of consciousness usually but not always with convulsions. The form of the seizure depends on the part of the brain affected. Epilepsy is known to affect approximately 0.5-1% of the worldwide population. The distinguishing sign of epilepsy is the seizure that is mostly associated with it. Infants, young children and the elderly are mostly found with epilepsy. Females have a higher rate of the disease than males as stated by some literature (Brown *et al*. 1993; Cull and Goldstein, 1997; Rang *et al*., 2015). Causes of seizures include, brain abnormalities, brain infections such as meningitis and encephalitis, neurocysticerosis, stroke, head trauma, dementia, and psychotropic drugs known to induce seizures; although the cause in adult on-set epilepsy remains unknown (Zeman *et al*., 2012; Rang *et al*. 2015).

The nature of epilepsy is the characteristic seizure mostly associated with epilepsy and high episodes of frequently discharged impulses by a group of brain neurons. The abnormal discharge can spread to other parts of the brain. The part of the brain where the discharge was first initiated and the extent to which the discharge spreads would determines the symptoms produced which include the brief lapse of attention to a full convulsing episode alongside other abnormal behaviour like the characteristic body movement observed in patients experiencing epileptic episodes (Rang *et al*. 2015).

Epilepsy is treated mainly with antiepileptic drugs (AEDs). There are a lot of new AEDs available today which were developed in the past two decades. They are effective enough in controlling about 70% of cases of seizures but their use is limited because of the adverse effects they cause. Furthermore, a significant amount of epileptic patients still experience seizures

continuously despite being on AEDs. As a result, research is now focusing more on ways to improve their efficacy and adverse effect profiles. The research has been steady but epilepsy still remains a very difficult problem to overcome. It has been suggested that the problem might be easier to solve if the parts of the brain responsible for emotions, mood and cognitive abilities can be controlled (Kabatende 2005; Rang *et al*., 2015). Low levels of gamma aminobutyric acid (GABA), the inhibitory neurotransmitter are often found in the post mortem parts of the brain in dead epileptic patients and high levels of glutamate, the excitatory neurotransmitter, is found in the post mortem parts of the brain. This can trigger a seizure. Increased intracellular sodium may also underlie epilepsy. Therefore, the development of GABA receptor agonist, glutamate receptor antagonist and sodium channel inhibitor has been put into consideration as an important potential therapeutic strategy when developing drugs for many neurological disorders including epilepsy (Barton *et al*., 2003; Ulbricht and Seamon 2010).

The spreading of the neuronal excitation occurring during epileptic episodes is prevented by anticonvulsants although the mechanisms of how they act are not fully understood. Therefore most AEDs are designed to target neuronal mechanisms although a deeper understanding of the mechanisms is needed to develop adequate therapies (Devinsky *et al*., 2013)

In South Africa, epilepsy is speculated to affect 1 in every 100 person, but more accurate statistics is unknown. This is due to the fact that epilepsy statistics are combined with other neurological and neurodegenerative illnesses such as schizophrenia, bipolar affective disorder and dementia (Keikelame *et al.,* 2012).

2.9.1 Neuropathology of epilepsy

The effects of seizures are intricate and have to be separated from the effects of the major, principal neurological disease process that has led to increased susceptibility of seizures. Although, there has been proof to support detrimental effects of seizures on brain histology, neuropathology is not predictable. Apoptotic (active) or necrotic pathways, gliosis and microglial activation may lead to prolonged seizures which may eventually cause neuronal death (Thom *et al*., 2008).

There are three types of pathology found in the brains of epileptic patients, which are:

1.) Focal lesions which are the main cause of secondary epilepsy. They include congenital malformations, cysts, parasitic infections, abscesses, traumatic lesions and infarcts (Webster and Jordan, 1989). There is no distinctive differentiation between focal lesions, whether they occur in patients with or without epilepsy.

2.) Degenerative disease (i.e. diffuse infective): This is not specifically related to epilepsy but may be associated with myoclonic and focal seizures. Pathologies such as cerebral malaria, leucodystrophies, Huntington's chorea, Alzheimer's disease and HIV-associated seizures are all included in this group (Webster and Jordan, 1989).

3.) Epileptic brain damage: a pathological syndrome which occurs differently in idiopathic primary epileptic patients or patients whose epilepsy is as a result of generalized or partial focal disorder. This type of neuropathology is characterised by extremely selective neuronal loss and glial proliferation, which is partially selective and partially diffuse (Webster and Jordan, 1989).

Some neuropathological alterations may be adaptive and reversible while others are permanent (Thom *et al*., 2008).

2.9.2 Types of epilepsy

There are two main categories based on the clinical classification of epilepsy, they include; partial and generalized seizures. Although, each has many varieties which are either complex (lost of consciousness) or simple (no loss of consciousness) (Rang *et al*., 2015).

The particular symptoms produced which determine the type of seizure depend on the part of the brain affected, thus convulsions result from involvement of the motor cortex while the peripheral autonomic discharge is triggered by the hypothalamus. The loss of consciousness often experienced by some epileptics is as a result of the reticular formation in the upper brain stem. The type of seizure can be detected by using an electroencephalography (EEG) which involves placing electrodes at various points over the scalp to figure out the abnormal electrical activity occurring during and after a seizure. Inquiring from a third party about the symptoms displayed by the patient during the seizure can also be of help to diagnose epilepsy (Haslett *et al*., 2002; Zeman *et al*, 2012; Rang *et al*., 2015).

2.9.2.1 Partial seizures

In this type of seizure, the discharge starts locally and regularly remains localized. Symptoms such as involuntary muscle contractions, autonomic discharge or effects on mood and behaviour which are displayed with partial seizures depend on the part of the brain affected. These symptoms are referred to as psychomotor epilepsy and patients who experienced them usually

have no recollection of the event. The EEG discharge in partial seizures is limited to one brain hemisphere. The incidence of partial seizures in epileptic patients increases with age. Partial seizures are subdivided into simple partial seizures and complex partial seizures (Rang *et al*., 2015).

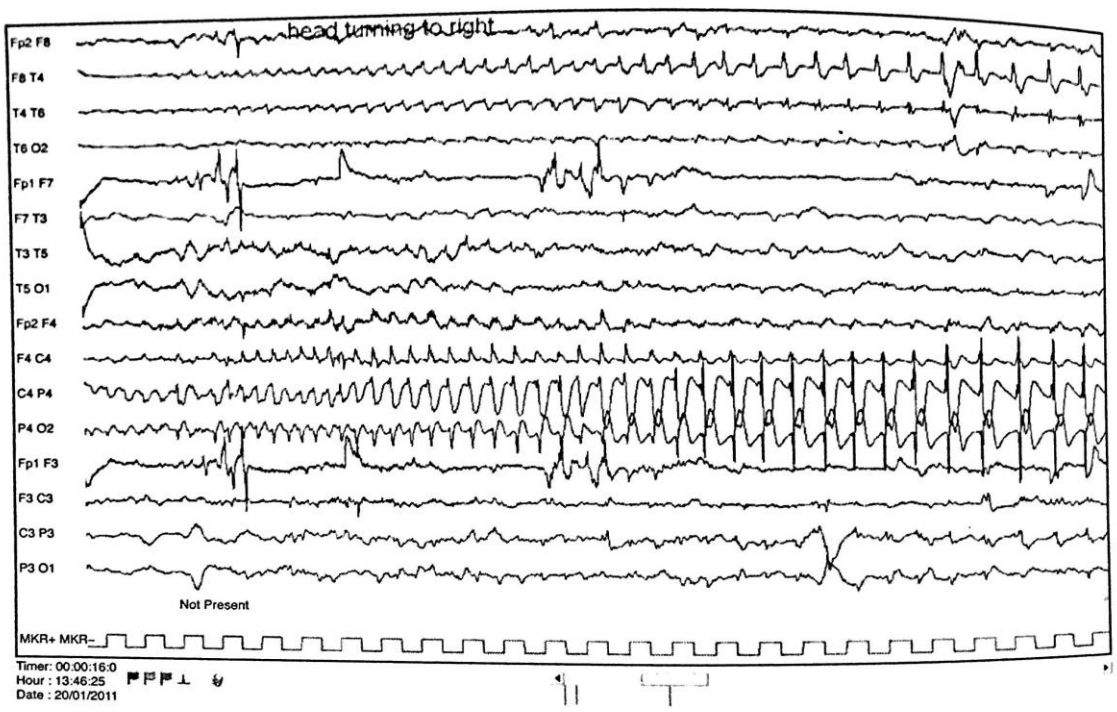


Fig 2.1: an example of an electroencephalogram (EEG- brain wave recording) showing a focal seizure recorded from the scalp. The outstanding 'spike and wave' activity observed particularly in 'C4 P4' and 'P4 O2' reflects epileptic activity originating over the right side of the brain (Zeman *et al*., 2012)

Simple partial seizures- Jacksonian epilepsy is a manifestation of partial seizures. This results from epileptic focus in the motor cortex which leads to attacks consisting of continuous jerking of a specific muscle group. It begins from one side of the body (usually the thumb, big toe or

angle of mouth) and then spreads to the rest of the body within two minutes before ending. Patients do not experience loss of consciousness (Rang *et al*., 2015).

Complex partial seizures- these are the most common types of epilepsy, patients having this type of epilepsy usually experience loss of consciousness mostly at the beginning of epileptic attack or later when the discharge has spread from its site of origin (brain) to other parts of the brainstem and reticular formation (Rang *et al*., 2015).

2.9.2.2 Generalized seizures

The whole brain including the reticular system is involved in this type of seizures. Abnormal electrical activity is produced throughout both brain hemispheres. There is immediate loss of consciousness experienced in generalized seizures. Generalized seizures are categorized under two main types, which are tonic-clonic seizures (grand mal) and absence seizures (petit mal) (Rang *et al*., 2015).

Tonic-clonic seizures- This seizure usually begins with contraction of the entire body muscles. It causes a rigid extensor spasm and the patient cries involuntarily. There is cessation of respiration, patient often defecates, micturition and salivation occur. The duration of the tonic phase is usually a minute, during which the face turns blue (not syncope), and is followed by a series of violent, synchronous jerks that last for about 2-4 minutes. The patient remains unconscious for a few minutes after the incidence, followed by a gradual process in recovering and also with possible injuries incurred on the patient during the convulsive episode. The generalized continuous high-frequency activity which takes place during the tonic phase and the

intermittent discharge which occurs in the clonic phase are observed from the EEG readings (Rang *et al*., 2015).

Absence seizures- This type of seizures mostly occur in children. They are not as dramatic as the tonic-clonic seizures but they occur more frequently (lots of seizures in a day) than the tonic-clonic seizures. During incidence of convulsion, the patient suddenly stops doing anything that he/she was doing before, stops speaking in mid-sentence and stares impassively for a few seconds with little or no motor disturbance. The patient is not entirely aware of his/her environment and recovers at a speedy rate with no attacks afterwards (Rang *et al*., 2015). A characteristic rhythm as seen in the figure below is observed from the EEG during an absence seizure attack.

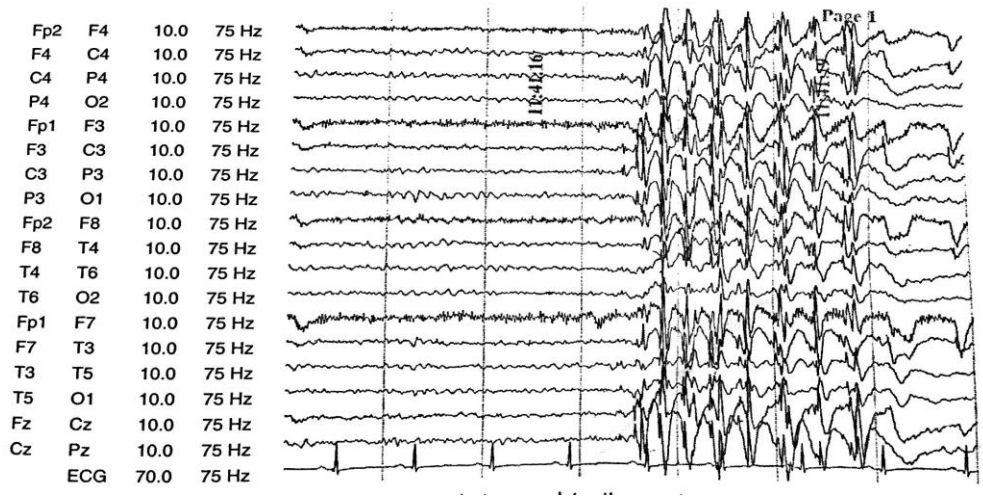


Figure 2.2: An example of an EEG showing a brief burst of 3 cycle/seconf 'spike and wave' activity across the entire scalp from a patient experiencing generalized absence seizure (Zeman *et a*l ., 2012)

Some literature has stated that the rhythmic movement is likely caused by oscillatory responses which link the cortex and the thalamus to where calcium channels are found expressed by the thalamic neurones. This clearly differentiates generalized seizure and partial seizure where the

point of origin and the high-frequency asynchronous discharges spread from a local focus. Because of this, modern treatment of epilepsy involves the use of drugs that are able to block the calcium channels for treatment of absence seizures, drugs blocking sodium channels to treat generalised and partial tonic-clonic seizures (Ure and Perassolo, 2000; Eadie and Vajda, 2012) and drugs which can enhance the inhibition mediated gamma-aminobutyric acid (GABA) to treat generalized and partial tonic-clonic seizures. (Czuczwar and Patsalos, 2001).

2.10 Neurotransmitters in the central nervous system implicated in Epilepsy

There is synergic relationship between inhibitory and excitatory amino acids which control the central nervous system (CNS) (Jansen and Dannhardt, 2003). There are two main categories of the amino acids: a) glycine and gamma-aminobutyric acid which are the inhibitory neutral amino acids. b) Glutamate which is the excitatory acidic amino acid (Katzung, 2001). Epilepsy is thought to arise as a result of imbalance between low levels of gamma-aminobutyric acid (GABA) and high levels of glutamic acid activities as shown in the post mortem brains of epileptic patients (Rang *et al*., 2015).

2.10.1 Glutamic acid (glutamate)

The excitatory amino acid (EAAs) includes glutamate, aspartate and homocysteate. These are the major fast excitatory neurotransmitters in the central nervous system (CNS). Glutamate is also referred to as L-glutamic acid (Trist, 2000) which mediates the synaptic excitation at major synapses in mammalian CNS (Ure *et al*., 2006). It is common and fairly consistently distributed

in the CNS. Glutamate is more in the CNS than other tissues. The formation of glutamate in the CNS is mainly from glucose through the tricarboxylic acid cycle (Krebs cycle) where the glial cells synthesis it. It is then taken up by neurons with the aid of some specific transporter proteins together with Na^+ (sodium ions) and stored in the synaptic vessels which is the main storage site for neurotransmitters (Rang *et al*., 2015).

Glutamate binds to specific receptors in the CNS; these receptors are widely divided into two classes: **1) the ionotropic glutamate receptors (iGluRs)** which allow channelling of small citations such as Na^+ and Ca^{2+}, and **2) the metabotropic glutamate receptors (mGluRs)** that are responsible for the modulation of second messenger systems (Trist, 2000). **The ionotropic receptors** are subdivided into three main types: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl Isoxazole-4-proprionic acid (AMPA), and Kainate (Trist, 2000; Ure *et al*., 2006;). There is a distinctive difference between the ionotropic receptor and the metabotropic receptor in the sense that the ionotropic receptors have a slow rise time, a glycine co-agonist is required, the permeability of Ca^{2+} is high, have voltage-dependent Mg^{2+} blockade and are modulated by Zn^{2+} and polyamines. While the metabotropic receptors are fast mediators of synaptic neurotransmission, permeability of kinetics and Ca^{2+} are low (Barnard, 1997; Ure *et al*., 2006;). The metabotropic receptors are subdivided into eight types ($mGlu_1$-$mGlu_8$), using agonist specificity, selective antagonism and structural heterogeneity which are also used to classify ionotropic receptors (Rang *et al*., 2015). Neuronal differentiation, migration of neuron, synapse formation and the shaping of axons during early CNS development are all linked to NMDA receptors (Sucher, 2006).

Over-activation of glutamate receptors may be the cause of many neurodegenerative conditions associated with most chronic and acute diseases such as epilepsy, Parkinson's disease, stroke, cerebral ischaemia, Huntington's disease and AIDs-linked dementia (O'Shea, 2002; Sucher, 2006).

Most importantly, glutamate plays a vital role in epileptogenesis and that over stimulation of NMDA receptor by glutamate and its glycine co-agonist causes convulsion. The presence of glycine at the NMDA receptor level increases the receptor's affinity to glutamate (Ure and Perassolo, 2000). Hypothetically, the suppression of these seizures would require blockade of the action of glutamate on the NMDA receptors. However, studies carried out to determine the effectiveness of NMDA antagonist in chemically-induced seizures have been unsuccessful in humans, but only successful in animal models. This could be related to the adverse effects experienced with classical NMDA antagonists (Dansysz and Parsons, 1998).

2.10.2 Gamma-aminobutyric acid (GABA)

Amino acids gamma-aminobutyric acid (GABA) and glycine, are the main inhibitory neurotransmitters in mammalian central nervous system (CNS). GABA is the major inhibitory neurotransmitter in the brain (Rang *et al*., 2015) with roughly 40% of all cerebral synapses being gabaergic (Laube *et al*., 2002; Amabeoku and Kinyua, 2010). GABA is present in brain tissues but not in other mammalian tissues. It is present in lower concentrations but abundant in the nigrostriatal system. GABA originates from glutamate by the glutamic acid decarboxylase (GAD) enzyme in GABA-synthesizing neurons present in the brain (Rang *et al*., 2015). The alteration of GABA levels in the brain as well as regulation of GABAergic brain function can

occur by physiologically and pharmacologically manipulating the synthesis and catabolism (Katzung, 2001; Amabeoku and Kinyua, 2010).

There are two main types of receptors in GABA mediating activities: the ionotropic ($GABA_A$ and $GABA_C$) receptors linked to a chloride channel and a G-protein-couple metabotropic ($GABA_B$) receptor. $GABA_A$ are found in the post-synaptic part of the brain where there is fast mediation of inhibitory neurotransmission with a high selective permeability for chloride ions (Cl^-). $GABA_A$ receptors are the major targets for some anticonvulsants drugs. The $GABA_A$ receptor chloride ionophore complex consists of several binding sites for different compounds which include gamma aminobutyric acid (GABA) itself, benzodiazepines and barbiturates, some convulsant such as picrotoxin (a chloride channel blocker) and bicuculline (a Selective $GABA_A$ receptor competitive antagonist), neurosteroids which affect inhibitory neurotransmission and ethanol (Ure and Perassolo, 2000; Czuczwar and Patsalos, 2001; Amabeoku and Kinyua, 2010).

$GABA_B$ receptors are located pre-and post-synaptically and directly look like the glutamate receptors. They mediate post-and pre-synaptic effects, inhibiting the postsynaptic excitability and also causing decrease in inhibitory and excitatory neurotransmission at both synapses, respectively, by enhancing K^+ efflux and decreasing Ca^{2+} efflux (Ure and Perassolo, 2000; Czuczwar and Patsalos, 2001; Rang *et al*., 2015).

$GABA_C$ receptors are found in the retina and they mediate responses through the Cl^- channels (Czuczwar and Patsalos, 2001).

2.10.3 Glycine

Just like gamma-aminobutyric acid, glycine increases membrane permeability to Cl^- subsequent to binding of an agonist to glycine receptors, thus, inhibiting depolarization and firing of neuron induced by excitatory neurotransmitters (Barry *et al*., 1999; Katzung, 2001).

Glycine's activity is mediated via the ionotropic glycine receptors (GlyRs) which are, strychnine-sensitive $glycine_A$ receptor and strychnine-insensitive $glycine_B$ receptor (Jansen and Dannhardt, 2003; Amabeoku and Kinyua, 2010). The major mediator of fast inhibitory synaptic neurotransmission in the mammalian spinal cord and brain stem is the strychnine-sensitive $gycine_A$ receptor (Breitinger and Becker, 2002). Glycinergic transmission is important in controlling motor functions, coordination of reflex responses and processing of sensory signals, especially for glycine receptors in the dorsal horn of the spinal cord (Laube *et al*., 2002; Kirsch, 2006).

Compounds such as picrotoxin block the Cl^- at the GlyRs, while some anticonvulsants modulate GlyRs activity. Furthermore, the GlyR has a high affinity for strychnine, which is a very poisonous white odourless alkaloid commonly used as an adulterant for common drugs of abuse like cocaine (Katz *et al*.,1996). Strychnine is a competitive glycine antagonist at the strychnine-sensitive $glycine_A$ receptor.

2.11 Epilepsy management

Administration of antiepileptic drugs (AEDs) is currently the first approach in the clinical management of people with epilepsy with the main aim of keeping the patient seizure-free and maintaining minimal side effects of the drugs without interference of normal brain function (Löscher, 2002a ; Zeman *et al*., 2012). AED selection is particularly based on its efficacy against specific types of seizures, patient's tolerability and safety. There has been a massive growth in AEDs discovery and development in the last two decades, with over twenty various drugs to choose from, which includes, lamotrigine, gabapentin, topiramate and tiagabine discovered in the 20th century, levetiracetam, oxcarbazepine and pregabalin discovered in 21st century. These are the newer 'second generation drugs'. The first generation drugs include the old ones such as: phenytoin, carbamazepine, phenobarbital and valproate. AEDs have been known to effectively control the seizures in 70-80% of patients. The remaining 20-30% with severe treatment-refractory epilepsy show no response with conventional AEDs and 5% of these patients are eligible for epilepsy neurosurgery (Czuczwar and Patsalos, 2001; Zeman *et al*., 2012). There has been steady progress recently in the pharmacotherapy of epilepsy. In addition to the newly discovered drugs, there has been an improvement in the efficacies of the older anticonvulsants (McCabe, 2000).

Despite the advancements in drug discovery, resistance to conventional AEDs has been observed in a third of the patients been treated with the drugs. AEDs do not appear to affect the main natural history of epilepsy nor prevent the progression of the disease in patients who show positive response to current pharmacotherapies (Löscher, 2002a) for example after injury to the head (Temkin, 2001). Additionally, most of the AEDs are associated with severe adverse effects

occurring in approximately 60-70% in epileptic patients on AEDs therapy. Central nervous system (CNS) side effects are more common and include symptoms like, fatigue, headache, blurred vision, dizziness and some cognitive impairment (Zeman *et al*., 2012), other adverse effects commonly seen in relation to AEDs include, hypersensitivity reactions, teratogenenicity, behavioural and mood changes (Rang *et al*., 2015). Management of epilepsy still lacks complete efficiency possibly due to the fact that most AEDs mainly control reverberative neuronal discharges whereas, the bigger picture would be to control the areas of the brain functions responsible for emotions, mood and cognitive function (Rang *et al*., 2015). Therefore, another area of focus to consider when developing new AEDs would be to develop drugs that prevent either the development or progression of epilepsy in addition to developing drugs used to treat patients resistant to the current AEDs (Löscher, 2002a).

2.12 Mechanism of action of antiepileptic drugs

Antiepileptic drugs act through three main mechanisms:

- Enhancement of GABA action
- Inhibition of calcium channel function
- Inhibition of Sodium channel function

Other mechanisms include blockade of glutamate receptors to inhibit the effect of glutamic acid (Rang *et al*., 2015).

2.12.1 Enhancement of GABA action

The most commonly used and prescribed AEDs such as: phenobarbital, valproic acid, benzodiazepines and some the newer AEDs (vigabatrin and tiagabine) act through this mechanism. Most of these AEDs act through GABA-mediated synaptic transmission attained by either the enhancement of postsynaptic action of GABA or inhibition of GABA-transaminase enzyme (main enzyme responsible for GABA inhibition) (Rang *et al*., 2015). Some research conducted suggested that the enhancement of GABAergic neurotransmission aggravated the occurrence of absence seizure using the WAG/Rij rat (a model designed for absence seizure) tested with vigabatrin, a GABA transaminase enzyme inhibitor (Marescaux *et al.,* 1992). However this research did not help to determine the development of this class of drug, which then lead to development of newer AEDs released into the market over the last two decades. Although, there is still no improvement in prognosis of refractory epilepsy in epileptic patients been treated with these drugs. The lack of sufficient evidence for a rational choice of drug in terms of efficacy and/or patient's tolerability and the many adverse effects associated with these AEDs can be the cause of the poor improvement in prognosis of the disease. Other factors beside the fact that GABA is a vital factor in epileptogenesis are also considered in the process of AEDs research and development (Czuczwar and Patsalos, 2001; Amabeoku and Kinyua, 2010).

2.12.2 Inhibition of calcium channel function

Many of the AEDs have very little effects on the calcium channels. The main drug that acts through this mechanism is ethosuximide. It acts by specific blockage of the T-type calcium

channels, consequently controlling the rhythmic discharges associated with generalised absence seizures (Rang *et al*., 2015).

2.12.3 Inhibition of sodium channel function

A lot of the most important and generally prescribed AEDs such as phenytoin, carbamazepine, valproate and lamotrigine acts through this mechanism by affecting the excitability of the membrane and acting on the voltage-dependent sodium channels thus blocks the influx of Na^+ (Rang *et al*., 2015). This then reduces the number of functional channels capable of generating action potentials. There is a high frequency discharge from the neurones during an epileptic seizure episode. These AEDs specifically block the channels discriminately to a point whereby they are no longer active thus depolarising the neuron (Rang *et al*., 2015)

2.12.4 Other mechanisms of action of AEDs

The mechanisms through which many AEDs act are still poorly understood. The barbiturate; phenobarbital, for example has more antiepileptic effect and less sedative action compared to other barbiturates, but has same GABA-potentiating action. Phenobarbital has same effect on convulsions induced electrically and convulsions induced by PTZ in rats or mice, while benzodiazepines, whose actions are also on GABA-mediated transmission have no effect on convulsions induced electrically. Benzodiazepines such as diazepam prevent the electrical activity of neurons within a chemically induced epileptic focus from spreading out of the cortex, while phenobarbital decreases the electrical focal activity. Therefore, the action of phenobarbital

cannot be only as a result of its interaction with GABA, it is possible that it also acts through inhibition of excitatory synaptic responses, but not so much is known about this mechanism of action (Rang *et al*., 2015).

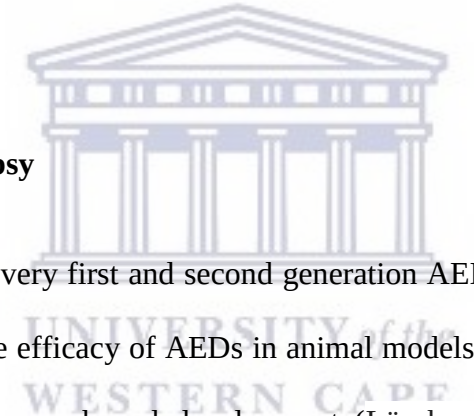
Many studies have been carried on phenytoin. It has not only been known to cause use-dependent block of sodium channels but can also affect other areas of membrane function, calcium channels included, which could also disturb membrane excitability and synaptic function (Rang *et al*., 2015).

Levetiracetam and zonisamide, some of the newer AEDs act through poorly understood mechanisms but they have been known to have very little or/weak effects on several receptors and channels involved in epilepsy (Rang *et al*., 2015).

2.13 Alternatives to conventional pharmacotherapy of Epilepsy

Besides the common systemic (oral) administration of AEDs use in treating epilepsy, several alternatives or adjunct treatment procedures are being developed for patients experiencing resistant and adverse effects from AEDs (Aiken and Brown, 2000). Neurostimulation of the vagus nerve by using a neurocybernetic prosthesis implant is one of the most common non-pharmacological adjunctive therapies for pharmacologically resistant partial complex epilepsy (Morrow *et al*., 2000). The others include transcranial magnetic stimulation or direct current stimulation through the scalp (Ziemann *et a*l., 1998) and electrical stimulation of brain regions through depth electrodes (Löscher, 2002a). Placing the epileptic patients on a ketogenic diet is another way to reduce the episodes of neuronal discharge in severe cases of epilepsy in children

(Lefevre and Aronso, 2000). Immune therapy (Aarli, 2000), gene therapy (Scheffer and Berkovic, 2003), resective surgery for the treatment of drug resistant epilepsy (Beghi and Tonini, 2006), radio surgery for mesial-temporal and extra-temporal epilepsy management (Romanelli and Anschel, 2006), and use of focus-targeted drug treatment, which uses a biosensor device that detects the beginning of seizures and then applies treatment to the specific area through a minipump to prevent occurrence of seizures (Le Van quyen *et al*., 2001) have all been used in epilepsy. These unconventional methods look promising, therefore, phytomedicines could serve as lead compounds in developing the therapeutics that can target $GABA_A$ receptors minimal side effects and refined therapeutic spectrum (Tsang and Xue, 2004).



2.14 Animal models of Epilepsy

The anticonvulsant effect of every first and second generation AEDs was originally determined in animal models, therefore the efficacy of AEDs in animal models is still an important factor to be considered in new AED research and development (Löscher, 2002b). Since detailed and extensive studies are difficult to carry out on epileptic patients, a lot of various animal models of epilepsy have been developed (Rang *et al*., 2015).

Three main objectives should be of high importance and put into consideration when embarking on epilepsy research and new drug development: 1) the Pathophysiology of epilepsy; the basic neuronal abnormality in epilepsy should be clearly understood so as to develop adequate therapies intended to avoid epilepsy in patients at risk, 2) the biological mechanisms associated with pharmacoresistance and development of drugs capable of reversing or preventing resistance

should be clearly understood as well and 3) development of disease-modifying therapies that are capable to slow down epilepsy progression (Löscher, 2002b; Rang *et al*., 2015).

Two types of animal models are used in epilepsy. A) **the acute (reactive or provoked) model:** this model involves the chemical or electrical induction of seizures in healthy, non-epileptic animals (Klitgaard, 2005; Löscher, 2002b) This model uses two major preclinical tools in AEDs development, which are the maximal electroshock seizure (MES) and the pentylenetetrazole (PTZ)-induced seizure tests. These two acute model tests predict clinical activity and efficacy in animal models particularly in mice and rats and are the most important factors to consider in new AED research. The MES test is carried out by inducing tonic hind limb seizures electrically in animals (Löscher, 2002a; Löscher, 2002b; Klitgaard, 2005) and is used in the early phase of AED discovery and it identifies agents with action against generalized tonic-clonic and partial seizures. While PTZ-induced seizure test is the most common model. It is mostly used in early AED discovery particularly against non-convulsive (generalized) absence or myoclonic seizures. PTZ is administered parenterally (Amabeoku and Kinyua, 2010). B) **The chronic model** involves inducing experimental animals electrically or chemically or using animals born with epilepsy. The chronic model has helped to improve the testing and development of AEDs. Results obtained with the chronic model show that it yields more predictive data and allows for more accurate preclinical testing of the efficacy of AEDs (Löscher, 2002a; Lö scher, 2002b; Klitgaard, 2005).

2.15 Epilepsy management in South Africa: a primary health care concern

In South Africa, the report of the awareness of the burden of non-communicable disease such as epilepsy is growing and yet the burden is unknown, but the incidence is likely to be as huge as that typically found in other developing countries around the world (Eastman, 2005). About one in every hundred South African suffers from epilepsy, but the figures known are not always accurate (Eastman, 2005; Spangenberg and Lalkhen, 2006; Ackerman and Van Toorn, 2011). The reason could be that the statistics for epilepsy are combined with those of other neurological diseases such as schizophrenia, bipolar disorder and dementia which account for a 6% of the total burden of non-communicable diseases (Mayosi *et a*l., 2009). Studies have shown that Neurocysticerosis which is one of the causes of epilepsy has a 20% rate of prevalence mainly in the rural areas of Eastern Cape Province in South Africa (Mafojane *et al*., 2003; Foyaca-Sibat *et al*., 2004). The management of epilepsy is regularly accessed through means of clinical governance process and in 2011, the Western Cape government, Provincial Department of Health reported epilepsy as *"a relatively new area for audit"* (De Vries, 2011).

A pilot study carried out by (Keikelame *et al*., 2012) revealed that the management of epilepsy in primary care settings in Cape Town region of South Africa was superficial and poorly taken care of. Factors such as inaccessibility of epilepsy management guidelines and lack of access to different range of AEDs, wrong seizure type diagnosis by doctors, poor seizure control (due to lack of compliance and drug interactions with alcohol dagga and so on), epileptic patients experiencing adverse reactions to AEDs, illiteracy, poverty, lack of continuity of care and poor doctor-patient relationship perpetuate the poor management of epilepsy. These reports are consistent with reports from other studies regarding the factors related to treatment-gap in sub-

Saharan Africa (Birbeck, 2010) The fact that there is a wide range of old and new AEDs available makes the report about lack of access to different range of AEDs to be of a great concern and a disturbing factor. This can lead to patients and health care providers tending to accept or ignore side effects due to limited access to various range of AEDs (Belhocine *et al*., 2004). Hence, there is an urgent need for integrated and combined approaches to improve the management of epilepsy putting the factors responsible for the poor outcomes into consideration (Keikelame *et al*., 2012).

2.16 Description of the plant *Elytropappus rhinocerotis*

2.16.1 Introduction

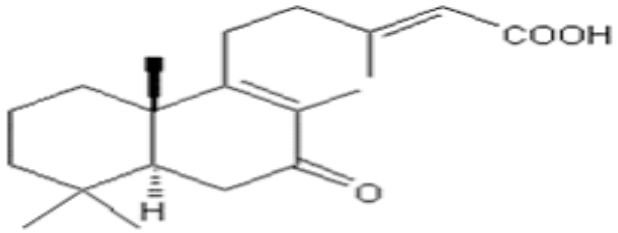
Elytropappus rhinocerotis belongs to the daisy plant family of Asteracea. *E. rhinocerotis* is common in the Cape region of the Western Cape Province in South Africa. It is among the medicinal plants used for the treatment of various ailments by South African traditional medicine practitioners (van Wyk *et al*., 1997).

This common cape plant is an erect bushy shrub which grows up to 1 to 2 m in height; with tiny, greyish-green leaves which are tightly grouped on the thin stems. The flower heads are tiny and are not easily seen with a single floret in each (van Wyk *et al*., 1997). It produces many seeds which are borne on each twig. The seeds are tiny and are dispersed by wind by a means of feathery pappus. The plants are fast growing and have a long life span. *E. rhinocerotis* is a dominant member of the unique Renosterveld vegetation type in some type of Western and Eastern Cape provinces. The species is widely distributed in the Western, Northern and Eastern

Cape (van Wyk *et al.,* 1997). It is common and abundant on road verges and can grow in disturbed or overgrazed lands. It is locally known as "renosterbos" in Afrikaans (van Wyk *et al.,* 1997).

The tips of the young branches are the parts of the plants used. It is used traditionally to treat indigestion, dyspepsia, ulcers and stomach cancer by making infusions of the young branches in alcohol. A tonic can also be made from the plant to improve lack of appetite (van Wyk *et al.,* 1997). The powdered twigs are used to treat diarrhoea in children. The preparations are said to induce sweating and can be used in the treatment of influenza and fevers. It was used as a remedy during an influenza epidemic in 1918 and used in the Bredasdorp community in the Western Cape to treat epilepsy (Thring and Weitz, 2005). Some of the activity of the medicine is due to the chemical Rhinocerotinoic acid which has been isolated from *E.rhinocerotis*. Rhinocerotinoic acid has significant anti-inflammatory activity (van Wyk *et al.,* 1997).

Fig 2.3. Elytropappus rhinocerotis image sourced from https://commons.wikimedia.org/wiki/File:Elytropappus_rhinocerotis_-_Renosterbos_-_Cape_Town_2.jpg



Rhinocerotinoic Acid

Fig 2.4 structure of Rhinocerotinoic acid sourced from http://www.medicinenet.com/seizure/article.

2.16.2 Study Objective

The use of traditional medicines in particular medicinal plants, in Africa and South Africa particularly has long been considered as an important part of the daily lives of individuals and their socio-cultural heritage (Van Wyk *et al*., 2000). Plants were once the only and major source of medicine in the world and up till present time, they still continue to provide mankind with new remedies. However, the claims made by traditional medicine practitioners regarding the therapeutic activity of medicinal plants have not been subjected to enough well and detailed scientific evaluation. Therefore, plants with medicinal properties are selected on the basis of their perceived ability to treat the particular ailment intended for and frequently used in a manner which does not allow pharmacological interpretation of their efficacy (Van Wyk *et al*., 2000; Kabatende, 2005). The validation of the claims made by the traditional healers is required for three reasons: firstly, the claims need to be justified so as to ensure the public is exposed to safe and efficacious medicines (Chan, 2003; Govender *et al*., 2006). Secondly, the Medical Research

Council of South Africa highly demands proof of efficacy and safety of herbal medicines for the purpose of registration and regulation of the products with the main aim of integrating the products into the primary health care framework (Govender *et al*., 2006). Thirdly, despite the years of researching epilepsy, neuroscience and pharmacotherapy, positive results in rational AED designs was only achieved in the last two decades. Discovery and development of AEDs have been especially difficult mainly because of the strict regulatory policies. Clinical trials especially, those involving human subjects, have been extremely difficult and challenging due to the fact that seizures are erratic in nature due to the complexity of epilepsy (Aiken and Brown, 2000). *Elytropappus rhinocerotis* was studied due to the claim of its potential anticonvulsant activity by the people in the Bredasdorp community in the Western Cape where it is used for convulsions (Thring and Weitz, 2005). This method of practice is not safe because little is known about the mechanism of its action and its potential level of toxicity. No scientific data exist about *E. Rhinocerotis* pertaining to its anti-epileptic properties.

This study, hence, investigated the anticonvulsant activity of *E. rhinocerotis* by studying the effects of the leaf methanol plant extract induced against seizures induced chemically by pentylenetetrazole PTZ, picrotoxin, bicuculline, N-methyl-DL-aspartic acid (NMDLA) and strychnine in experimental mice.

CHAPTER 3

MATERIALS AND METHODS

3.1 plant materials

3.1.1. Selection, collection and identification of plant material

Elytropappus rhinocerotis was selected based on documented reported claims by traditional healers and a study that was conducted in the Western Cape province of South Africa which revealed that the people in the Bredasdorp community use *Elytropappus rhinocerotis* to treat convulsions (Thring and Weitz, 2005). The plant materials, fresh leaves were collected from Kirstenbosch National Botanical garden, Cape Town, Republic of South Africa in March 2014. A sample of the collected leaves was verified by the curator of the Garden and also authenticated by a taxonomist, Mr. Frans Weitz, in the Department of Biodiversity and Conservative Biology at the University of the Western Cape and voucher specimen (Amabeoku 008) was deposited in the University's Herbarium.

3.1.2. Preparation of crude methanol extract of *E.rhinocerotis*

The freshly collected plant leaves were weighed (3124.3g) and separated from the thin stems. The leaves were washed with distilled water, air dried for an hour and dried in a ventilated oven at 35^{o}C for 5 days. The dried leaves (3104.7g) were milled into fine powder using Waring (885g) commercial laboratory blender. The fine powder was then stored in amber bottles for further use. The dried powder (20g) was extracted in a Soxhlet extractor then in 500ml of

methanol for 2 hours. The resultant filtrate was evaporated to a semi-solid extract using a Buchi RE11 rotavopour and Buchi 461 water bath. The semi-solid extract was then freeze-dried under vacuum at 44ºC for 7 days using a freeze-drier to obtain a dried leaf methanol extract (68g) which was then stored in an amber bottle in the refrigerator for further use.

Fresh solutions of *E.rhinocerotis* were prepared on each day of the experiment by reconstituting weighed quantities of the crude leaf methanol extract in a minimum amount of dimethylsulfoxide (DMSO) and then made up to appropriate volumes with physiological saline. The plant extract was injected intraperitoneally (i.p) into mice in a volume of 1 ml/100g of body weight.

3.2. Experimental animals

Male albino mice bred in the Animal House of the discipline of Pharmacology, School of pharmacy, University of the Western Cape, South Africa, weighing between 18 and 30g were used in this study. The mice were housed in groups of eight per cage and had access to food (standard mouse pellet diet) and water *ad libitum* except for the short fasting period prior to the commencement of the experiments when they had access only to water. Laboratory conditions of temperature (25 ± 1ºC), humidity and alternate 12 hour-light and 12 hour-dark cycle were maintained at all times during the experiments. Each mouse was used for one experiment only.

3.3 Drugs and chemicals

Pentylenetetrazole (PTZ, sigma Chemical Co.), picrotoxin (Sigma Chemical Co.), N-methyl-DL-aspartic acid (NMDLA) (Sigma Chemical Co.), strychnine (Sigma Chemical Co.), muscimol (Sigma chemical Co.), LY233053 (Sigma Chemical Co.), phenobarbitone (Gardenyl®, Rhone-poulene Rorer, South Africa) and 5,5 diphenylhydrantoin sodium salt (Phenytoin, Sigma

Chemical Co.) were all dissolved in physiological saline to appropriate volumes. Bicuculline (Sigma Chemical Co.) was suspended in 0.5 ml of Tween 80 and adjusted to an appropriate volume with physiological saline. Diazepam (Valium®, Roche, South Africa) was suspended in a volume of polyethylene glycol 400 (Fluka AG, Buchs) and adjusted to an appropriate volume with physiological saline. All drugs were injected intraperitoneally (i.p) in a volume of 1ml/100 g body weight of animals. Control animals received equal volume injections of the appropriate vehicle. Fresh drug solutions were prepared on each day of the experiment. The doses and pre-treatment times of leaf methanol extract, the standard antiepileptic drugs and DMSO used were obtained from previous similar studies (Amabeoku *et al*., 215). The pre-treatment times following the administration of pentylenetetrazole, bicuculline, picrotoxin, strychnine, or NMDLA were; plant extract (15 min), phenobarbitone (10 min), diazepam (20 min), phenytoin (20 min), muscimol (1 h), LY233053 (30 min) and DMSO (15 min).

3.4. Phytochemical anaylsis of *Elytropappus rhinocerotis*

The methods as described by Harborne (1984) and Ikhiri *et al*. (1992) were used for the phytochemical analysis to detect some of the chemical compounds present in the leaves of *E.rhinocerotis.*

3.4.1 Alkaloids

The powdered leaf of *E.rhinocerotis* (0.5 g) was boiled with 10 ml of dilute hydrochloric acid (alcoholic) in test tube for 5 minutes. The boiled mixture was cooled and the debris was allowed to settle. The supernatant liquid was filtered into another test tube and 1ml of the filtrate was taken into another test tube to which three drops of dragendorffs' reagent (potassium bismuth

iodide solution) was added. The solution was shaken and observed for the appearance of an orange-red spot and the formation of a precipitate which would indicate the presence of alkaloids.

3.4.2 Saponins

The powdered leaf of *E.rhinocerotis* (0.2 g) was shaken in clean water in a test tube and observed for the presence of a persistent froth (foam) which would indicate the presence of saponins.

3.4.3 Flavonoids

The powdered leaf of *E.rhinocerotis* (10 g) was boiled for 2 to 3 minutes in 100ml of water in a water bath. 3ml of the filtrate was taken into a test tube and 3ml of acid-alcohol (Ethanol: water: concentrated hydrochloric acid in a ratio of 1:1:1), solid magnesium (1cm) and 1ml of t-amyl-alcohol were added. The mixture was then observed for the appearance of a rose-orange or violet colour change which would indicate the presence of flavonoids.

3.4.4 Tannins

The powdered leaf of *E.rhinocerotis* (0.2g) was boiled in 5ml of water. The mixture was cooled and filtered. 2-3 drops of 5% ferric chloride solution were added to the filtrate and observed for the formation of a blue-black precipitate which would indicate the presence of tannins.

3.4.5 Cardiac glycosides

The powdered leaf of *E.rhinocerotis* (0.5g) was boiled in 5ml of 70% ethyl alcohol for 2 minutes. The mixture was filtered and 10ml of water and 5ml of chloroform were added to the filtrate and shaken. The lower chloroform layer was taken off and evaporated to dryness in a water bath. The cooled chloroform residue was dissolved in 3ml glacial acetic acid containing 0.1ml of ferric chloride. The solution was carefully transferred to the surface of 2ml sulphuric acid and observed for a reddish-brown layer that was formed at the interface and if the upper layer gradually acquired a bluish-green colour that would indicate the presence of cardiac glycosides.

3.4.6 Quinones

The powdered leaf of *E.rhinocerotis* (10g) was moistened with a 10% hydrochloric acid (HCL) solution and allowed to stand in ether: chloroform mixture (3:1, 40ml). The mixture was filtered and 1ml of the resultant extract was treated with 1ml of 10% sodium hydroxide (NaOH) solution. The solution was observed for the appearance of a red discolouration which would indicate the presence of quinones.

3.4.7 Triterpene steroids

The powdered leaf of *E.rhinocerotis* (1g) was extracted for 24 hours in ether. The resultant solution was filtered and 1ml of the filtrate evaporated to dryness and the residue was re-dissolved in several drops of acetic anhydride, several drops of sulphuric acid were then added to

the solution which was observed for the appearance of a green colour change which would indicate the presence of triterpene steroids.

3.4.8 Reducing sugars

The powdered leaf of *E.rhinocerotis* (0.2g) was boiled in 5ml of water; the mixture was cooled and filtered. An equal amount of Fehling's solutions A and B in a ratio 1:1 was added to the filtrate and the solution was heated in a water bath. It was then observed for the appearance of a red-brown precipitate which would indicate the presence of reducing sugars.

3.5 Acute toxicity testing

The lethal dose (LD_{50}) of the plant extract was determined using the method of Lorke (1983) as modified by Ojewole (2006), and El Hilaly *et al*. (2004). Mice were fasted for 16 hours. The animals were then randomly divided into groups of eight mice per cage. Graded doses of the plant extract (100, 200, 400, 800, 1200, 1600, 2000, 2400, 2800, 3200, 3600 and 4000 mg/kg) were separately administered orally by means of a bulbed steel needle to the mice in each test group. The control group was administered with 0.25ml (p.o) of normal saline by means of a bulbed steel needle. The mice in both the control and test groups were then allowed free access to food and water, and observed for over 48 hours for signs of acute toxicity such as piloerection, salivation, immobility, respiratory distress and death. Log dose-response curves would then be constructed for the plant extract from which the lethal dose would be calculated.

3.6 PHARMACOLOGICAL SCREENING

3.6.1 Anticonvulsant activity assessment

The method described by Vellucci and Webster (1984) and modified by Amabeoku and Chikumi (1993), was used to assess the anticonvulsant activity of the leaf methanol extract of *E. rhinocerotis*. The mice were housed singly in transparent perspex mice cages half an hour prior to the commencement of the experiment to adapt to their new environment. Control mice were pretreated for 15 min with physiological saline (0.25 ml, i.p.) and then standard convulsant agents, such as, PTZ (100 mg/kg, i.p.), bicuculline (30 mg/kg, i.p.), picrotoxin (20 mg/kg, i.p.), strychnine (2 mg/kg, i.p.) or NMDLA (500 mg/kg, i.p.) was administered to induce convulsion in the mice. The animals were observed for 30 min for tonic convulsion. Convulsions were manifested as tonic hind- limb extensions. The time of the onset of tonic convulsions and the number or proportion of animals convulsing or not convulsing were obtained during the 30 min observation period. Test animals, eight per group, were pre-treated with either the leaf methanol extract of *E. rhinocerotis* (100-400 mg/kg, i.p.), phenobarbitone (12 mg/kg, i.p.), diazepam (0.5 mg/kg, i.p.), phenytoin (30 mg/kg, i.p.), muscimol (0.2-2 mg/kg, i.p) or LY233053 (1-5 mg/kg, i.p.) prior to the administration of either PTZ, bicuculline, picrotoxin, strychnine or NMDLA. The animals were also observed for 30 min for tonic convulsion. The time of the onset of tonic convulsions and the number of animals convulsing or not convulsing were obtained during the 30 min period of observation. The experiment was repeated with another group of eight mice pretreated for 15 min with DMSO (0.25 ml, i.p.) prior to the administration of any of the convulsant agents. The ability of the plant extract to prevent or delay onset of the tonic hind limb extensions was taken as an indication of anticonvulsant activity (Amabeoku *et al*., 1998, Amabeoku and Kinyua, 2010).

3.6.2 HPLC analysis

The HPLC finger print of the plant species was obtained using standard HPLC chromatographic methods Chromatographic system: Agilent 1200 system consisting of degassing system, quaternary pump, auto loading sampler, thermostatted column compartment, diode array detector, fluorescence detector, analyte fraction collector and Agilent ChemStation software ; column: Phenomenex Luna (C18) 5µm and dimensions (250 cm x 4.6 mm).

Chromatographic conditions: Mobile phase degassed with helium, solvent A: water containing 0.1% formic acid; solvent B: Acetonitrile containing 0.1% formic acid, Mode: flow rate, 0.8 ml/min; injection volume, 50 µl, detector, UV at 370 nm. The eluent was monitored at several wavelengths ranging from 210 to 370nm, the specific wavelength of interest being 350 nm. The HPLC operating conditions were programmed to give the following: 0 min, solvent B: 18%; 15 min, solvent B: 25%; 20 min, solvent B: 35%, 30 min, solvent B: 90%. The run rate was 30 min.

3.7 statistical analysis

The one way of variance (ANOVA) followed by Dunnette's multiple comparison test (GraphPad Prism, version 5.0, GraphPad software, Inc., San Diego Cap 2130, USA), was used to analyze the data on the onset of tonic convulsions. The data on the proportion of animals convulsing was analyzed using the chi- squared test (Amabeoku and Kinyua, 2010). Data obtained were expressed as mean (±SEM). P values of less than 5% ($p<0.05$) were considered statistically significant.

3.8 Ethical considerations

The experimental protocol used in this study was approved (14/7/46) by the University of the Western Cape Ethics Committee, Bellville 7535, South Africa and conforms to the University's Regulations Act concerning animal experiments.

CHAPTER 4

RESULTS

4.1 Phytochemical Analysis

The phytochemical tests used to detect the chemical compounds present in the dried powdered leaf of *E. rhinocerotis* leaves indicated the presence of the following chemical compounds; alkaloids, saponins, flavonoids, tannins, cardiac glycosides and triterpene steroids (Table 1).

Table 1. Chemical compounds present in the dried powdered leaf of *E. rhinocerotis*

Chemical compound	**Result**
Alkaloids	+
Saponins	+
Flavonoids	+
Tannins	+
Cardiac glycosides	+
Quinones	_
Triterpene steroids	+
Reducing sugars	_

Key

+ (positive) indicates present

_ (negative) indicates absent

4.2 Acute toxicity test

Doses of 100-4000 mg/kg of *E. rhinocerotis* administered orally to mice did not cause any signs of acute toxicity or deaths. Therefore, the no-adverse-effect-level (NOAEL) of the plant extract was 4000 mg/kg, this being the highest dose tested without any adverse consequence. The LD_{50} value obtained for *E. rhinocerotis* may be greater than 4000 mg/kg (p.o.) (Table 2).

4.3 ANTICONVULSANT ASSESSMENT

4.3.1 Effect of leaf methanol extract of *E. rhinocerotis* (ER) on pentylenetetrazole (PTZ) - induced seizures

The dose of 100 mg/kg (i.p.) of pentylenetetrazole (PTZ) manifested tonic convulsions in 100% of the experimental animals used. Leaf methanol extract of *E. rhinocerotis* (100 mg/kg, i.p.) did not significantly affect the onset or incidence of the tonic convulsions produced by PTZ (100mg/kg, i.p.). The doses of 200 mg/kg (i.p.) and 400 mg/kg (i.p.) of *E. rhinocerotis* significantly delayed the onset but did not significantly alter the incidence of PTZ (100 mg/kg, i.p.)-elicited tonic convulsions. *E. rhinocerotis* (400 mg/kg, i.p.) protected only 12.5% of mice against the tonic convulsion produced by PTZ (100mg/kg, i.p.). Phenobarbitone (12 mg/kg, i.p.) and diazepam (0.5 mg/kg, i.p.) completely protected 100% of the experimental animals used against PTZ (100 mg/kg, i.p)-induced tonic convulsion. Muscimol (2 mg/kg, i.p.) significantly delayed the onset of tonic convulsion produced by PTZ (100 mg/kg, i.p.) And also significantly decrease the proportion of animals convulsing. Muscimol (2 mg/kg, i.p.) protected 62.5% of mice against the tonic convulsion. Muscimol (0.2 mg/kg, i.p.) did not significantly affect the onset or incidence of the tonic convulsion induced by PTZ (100mg/kg, i.p.). It protected 25% of

mice against the tonic convulsion. However, combined therapy of the subeffective doses of leaf methanol extract of *E.rhinocerotis* (100 mg/kg, i.p.) and muscimol (0.2 mg/kg, i.p,) significantly delayed the onset of tonic convulsion produced by PTZ (100 mg/kg, i.p.) but only protected 25% of the number of animals convulsing. The onset or incidence of the tonic convulsion produced by PTZ (100mg/kg, i.p) was not significantly affected by either phenytoin (30 mg/kg, i.p) or DMSO (0.25 ml, i.p.) (Table 3).

Table 2. Acute toxicity of leaf methanol extract of *E. rhinocerotis* (ER) in mice

Dose (mg/kg)	Number of animals dead/Number of animals treated	Toxic symptoms
PS (0.25 ml)	0/8	None
100	0/8	none
200	0/8	none
400	0/8	none
800	0/8	none
1200	0/8	none
1600	0/8	none
2000	0/8	none
2400	0/8	none
2800	0/8	none
3200	0/8	none
3600	0/8	none
4000	0/8	none

None= no sign of any toxic symptoms observed

PS: Physiological saline

4.3.2. Effect of leaf methanol extract of *E.rhinocerotis* (ER) on bicuculline-induced seizures

All the eight experimental animals used experienced tonic convulsions when administered 30 mg/kg (i.p.) of bicuculline. Leaf methanol extract of *E. rhinocerotis* (200 and 400 mg/kg, i.p.) significantly delayed the onset of bicuculline (30mg/kg, i.p.)-induced tonic convulsion but did not significantly affect the proportion of mice convulsing. *E. rhinocerotis* (100mg/kg, i.p.) did not significantly affect the incidence nor the onset of bicuculline (30 mg/kg, i.p.)-induced tonic convulsion. Phenobarbitone (12 mg/kg, i.p.), diazepam (0.5 mg/kg, i.p.) and muscimol (2 mg/kg, i.p.) significantly delayed the onset of tonic convulsion produced by bicuculline (30 mg/kg, i.p.) and also significantly reduced the number of experimental animals convulsing. Phenobarbitone (12 mg/kg, i.p.) and muscimol (2 mg/kg, i.p.) protected 75% and 87.5% of the mice against bicuculline (30 mg/kg, i.p.)-induced tonic convulsion respectively. Diazepam (0.5 mg/kg, i.p.) protected all the mice against the tonic convulsion. The onset or incidence of bicuculline (30 mg/kg, i.p.)-induced tonic convulsion was not significantly altered by Phenytoin (30 mg/kg, i.p.), muscimol (0.2 mg/kg, i.p.) or DMSO (0.25 ml, i.p.). The combined therapy of the subeffective doses of leaf methanol extract of *E. rhinocerotis* (100 mg/kg, i.p.) and muscimol (0.2mg/kg, i.p.) did not significantly reduce the number of experimental animals convulsing but significantly delayed the onset of bicuculline (30 mg/kg, i.p.)-induced tonic convulsion. The combined therapy only protected 50% of the test animals against the tonic convulsion (Table 4).

Table 3: Effect of leaf methanol extract of *E. rhinocerotis* (ER) on pentylenetetrazole (PTZ) - induced seizures in mice

Dose (mg/kg)									
PTZ	ER	PB	DZ	PHY	MS	DMSO (ml)	No. Convulsed/ No. Used	Percentage protection (%)	Onset of Tonic Convulsions(min) Mean ± S.E.M
100	—	—	—	—	—	—	8/8	0	2.13 ± 0.23
100	100	—	—	—	—	—	8/8	0	6.38 ± 1.56
100	200	—	—	—	—	—	8/8	0	16.1 ±1.20*
100	400	—	—	—	—	—	7/8	12.5	16.3 ±2.39*
100	—	12	—	—	—	—	0/8	100	0*
100	—	—	0.5	—	—	—	0/8	100	0*
100	—	—	—	30	—	—	8/8	0	2.09±0.50
100	—	—	—	—	2	—	3/8*	62	21.0±4.42*
100	—	—	—	—	0.2	—	8/8	0	2.75±0.45
100	100	—	—	—	0.2	—	6/8	25	17.0±2.94*
100	—	—	—	—	—	0.25	8/8	0	2.88±0.48

*p < 0.001 compared to PTZ (100mg/kg, i.p) control, ANOVA (n=8).

$^{+}p<0.05$, $^{++}p<0.001$ compared to PTZ (100mg/kg, i.p) control, Chi-squared test (n=8)

PB: Phenobarbitone

DZ: Diazepam

PHY: Phenytoin

MS: Muscimol

DMSO: Dimethylsulfoxide

Table 4: Effect of leaf methanol extract of *E.rhinocerotis* (ER) on bicuculline (BIC)-induced seizures in mice

Dose (mg/kg)									
BIC	ER	PB	DZ	PHY	MS	DMSO (ml)	No. Convulsed/ No. Used	Percentage protection (%)	Onset of Tonic Convulsions(min) Mean ± S.E.M
30	—	—	—	—	—	—	8/8	0	2.38 ± 0.42
30	100	—	—	—	—	—	8/8	0	4.76 ± 0.67
30	200	—	—	—	—	—	8/8	0	13.38*±1.57
30	400	—	—	—	—	—	8/8	0	15.75*±0.49
30	—	12	—	—	—	—	2/8+	75	17.93*±1.41
30	—	—	0.5	—	—	—	0/8+++	100	0*
30	—	—	—	30	—	—	8/8	0	2.44 ± 0.75
30	—	—	—	—	2	—	1/8++	87.5	16.98* ±0.87
30	—	—	—	—	0.2	—	8/8	0	2.31 ± 0.35
30	100	—	—	—	0.2	—	4/8	50	15.29*±0.62*
30	—	—	—	—	—	0.25	8/8	0	2.41 ± 0.53

*p < 0.001 compared to bicuculline (30 mg/kg, i.p) control, ANOVA (n=8).

+p <0.01, ++p<0.005, +++p<0.001 compared to bicuculline (30 mg/kg, i.p) control, Chi-squared test (n=8)

PB: Phenobarbitone

DZ: Diazepam

PHY: Phenytoin

MS: Muscimol

DMSO: Dimethylsulfoxide

4.3.3 Effect of leaf methanol extract of *E.rhinocerotis* (ER) on picrotoxin induced seizures

All the eight experimental animals used experienced tonic convulsions when administered with picrotoxin (20 mg/kg, i.p.). Leaf methanol extract of *E.rhinocerotis* (200 and 400 mg/kg, i.p.) significantly delayed the onset of tonic convulsion produced by picrotoxin (20 mg/kg, i.p.) but did not significantly affect the proportion of animals convulsing. *E. rhinocerotis* (100 mg/kg, i.p.) did not significant affect the onset or incidence of picrotoxin (20 mg/kg, i.p.)-induced tonic convulsion. Phenobarbitone (12 mg/kg, i.p.), diazepam (0.5 mg/kg, i.p.) or muscimol (2 mg/kg, i.p.) significantly delayed the onset of tonic convulsion induced by picrotoxin (20 mg/kg, i.p.) and also significantly reduced the number of experimental animals convulsing. They protected 87.5% of mice against the tonic convulsions. Muscimol (0.2 mg/kg, i.p.) did not significantly affect the onset or the incidence of picrotoxin (20 mg/kg, i.p.)-induced tonic convulsion. However, combined therapy of subeffective doses of leaf methanol extract of *E. rhinocerotis* (100 mg/kg, i.p.) and muscimol (0.2 mg/kg, i.p.) significantly delayed the onset of tonic convulsion induced by picrotoxin (20 mg/kg, i.p.) and also significantly reduced the number of experimental animals convulsing. The combined therapy protected 62.5% of mice against the tonic convulsion. The onset or incidence of picrotoxin (20 mg/kg, i.p.)-induced tonic convulsion was not significantly altered by either phenytoin (30 mg/kg, i.p.) or DMSO (0.25 ml, i.p.) (Table 5).

Table 5. Effect of leaf methanol extract of *E.rhinocerotis* (ER) on picrotoxin (PIC)- induced seizures in mice

Dose (mg/kg)									
PIC	ER	PB	DZ	PHY	MS	DMSO (ml)	No. Convulsed/ No. Used	Percentage protection (%)	Onset of Tonic Convulsions(min) Mean ± S.E.M
20	—	—	—	—	—	—	8/8	0	8.50 ± 0.42
20	100	—	—	—	—	—	8/8	0	9.50 ± 0.57
20	200	—	—	—	—	—	8/8	0	16.00* ± 1.22
20	400	—	—	—	—	—	8/8	0	19.13* ± 1.18
20	—	12	—	—	—	—	1/8^{++}	87.5	20.04**±1.01
20	—	—	0.5	—	—	—	1/8^{++}	87.5	23.16***±0.84
20	—	—	—	30	—	—	8/8	0	9.17±0.33
20	—	—	—	—	2	—	1/8^{++}	87.5	28.71***±1.12
20	—	—	—	—	0.2	—	8/8	0	10.00±1.08
20	100	—	—	—	0.2	—	3/8^{+}	62.5	18.29*±1.24
20	—	—	—	—	—	0.25	8/8	0	9.17±0.92

*p < 0.02, **P<0.001 compared to picrotoxin (20 mg/kg, i.p) control, ANOVA (n=8).

$^{+}$p<0.05, $^{++}$p<0.005 compared to picrotoxin (20 mg/kg, i.p) control, Chi-squared test (n=8).

PB: Phenobarbitone

DZ: Diazepam

PHY: Phenytoin

MS: Muscimol

DMSO: Dimethylsulfoxide

4.3.4 Effect of leaf methanol extract of *E.rhinocerotis* (E.R) on Strychnine induced seizures

Strychnine (2 mg/kg, i.p.) produced tonic convulsions in all the eight animals used. Leaf methanol extract of *E. rhinocerotis* (100 and 200 mg/kg, i.p.) did not significantly affect the onset or incidence of strychnine (2 mg/kg, i.p.)-induced tonic convulsion. Leaf methanol extract of *E. rhinocerotis* (400 mg/kg, i.p.) significantly delayed the onset of tonic convulsion produced by strychnine (2 mg/kg, i.p.) but did not affect the number of animals convulsing. Phenobarbitone (12 mg/kg, i.p.) significantly delayed the onset of strychnine (2 mg/kg, i.p.)-induced tonic convulsion and also significantly reduced the number of experimental animals convulsing. It protected 62.5% of the animals against strychnine (2 mg/kg, i.p.)-induced tonic convulsion. Diazepam (0.5 mg/kg, i.p.), phenytoin (30 mg/kg, i.p.) or DMSO (0.25 ml, i.p.) did not significantly alter the onset or incidence of strychnine (2 mg/kg, i.p.)-induced tonic convulsion (Table 6).

Table 6. Effect of leaf methanol extract of *E.rhinocerotis* (E.R) on strychnine (STN)-induced seizures in mice

Dose (mg/kg)								
STN	ER	PB	DZ	PHY	DMSO (ml)	No.Convulsed/ No.Used	Percentage protection (%)	Onset of Tonic Convulsions(min) Mean ± S.E.M
2	—	—	—	—	—	8/8	0	2.63±0.26
2	100	—	—	—	—	8/8	0	3.63±0.18
2	200	—	—	—	—	8/8	0	3.75±0.16
2	400	—	—	—	—	8/8	0	11.25*±1.77
2	—	12	—	—	—	3/8+	62.5	19.34*±0.65
2	—	—	0.5	—	—	8/8	0	2.81±0.44
2	—	—	—	30	—	8/8	0	2.59±0.25
2	—	—	—	—	0.25	8/8	0	2.76±0.37

*$p < 0.001$ compared to strychnine (2 mg/kg, i.p.) control, ANOVA (n=8).

+$p<0.05$ compared to strychnine (2 mg/kg, i.p.) control, Chi-squared test (n=8)

PB: Phenobarbitone

DZ: Diazepam

PHY: Phenytoin

DMSO: Dimethylsulfoxide

4.3.5. Effect of leaf methanol extract of *E. rhinocerotis* (ER) on N-methyl-DL aspartic acid (NMDLA)-induced seizures

NMDLA (500 mg/kg, i.p.) produced tonic convulsions in the eight experimental animals used. Leaf methanol extract of *E. rhinocerotis* (400 mg/kg, i.p.) significantly delayed the onset but did not significantly affect the incidence of NMDLA (500 mg/kg, i.p.)-induced tonic convulsion. *E. rhinocerotis* (400 mg/kg, i.p.) protected only 12.5% of the test animals from convulsing. The doses of leaf methanol extract of *E. rhinocerotis* (100 and 200 mg/kg, i.p.) had no significant effect on the onset and incidence of NMDLA (500 mg/kg, i.p.)-induced tonic convulsion. The onset or incidence of NMDLA (500mg/kg, i.p) induced tonic convulsion was not significantly altered by phenobarbitone (12 mg/kg, i.p.), diazepam (0.5mg/kg, i.p.), phenytoin (30 mg/kg, i.p.) or DMSO (0.25 ml, i.p.). LY233053 (5 mg/kg, i.p.) significantly delayed the onset of tonic convulsion induced by NMDLA (500 mg/kg, i.p.) and also significantly reduced the number of experimental animals convulsing. This dose protected 87.5% of mice against the tonic convulsion. LY233053 (1mg/kg, i.p) had no significant effect on the onset and incidence of the tonic convulsion produced by NMDLA (500 mg/kg, i.p.). However, the combined therapy of the subeffective doses of LY233053 (1 mg/kg, i.p.) and leaf methanol extract of *E. rhinocerotis* (100 mg/kg, i.p.) significantly delayed the onset of NMDLA (500mg/kg, i.p.)-induced tonic convulsion but did not significantly affect the number of the experimental animals convulsing (Table 7).

Table 7: Effect of leaf methanol extract of *E. rhinocerotis* (ER) on N-methyl-DL aspartic acid (NMDLA) induced seizures in mice

Dose (mg/kg)									
NMDLA	ER	PB	DZ	PHY	LY	DMSO (ml)	No. Convulsed/ No. Used	Percentage protection (%)	Onset of Tonic Convulsions(min) Mean ± S.E.M
500	–	–	–	–	–	–	8/8	0	4.38±0.32
500	100	–	–	–	–	–	8/8	0	6.38±0.53
500	200	–	–	–	–	–	8/8	0	7.25±0.25
500	400	–	–	–	–	–	7/8	12.5	12.38**±2.54
500	–	12	–	–	–	–	8/8	0	4.19±0.12
500	–	–	0.5	–	–	–	8/8	0	5.03±0.84
500	–	–	–	30	–	–	8/8	0	4.46±0.38
500	–	–	–	–	5	–	1/8+	87.5	22.13***±0.98
500	–	–	–	–	1	–	8/8	0	7.20±0.65
500	100	–	–	–	1	–	8/8	0	10.88*±0.71
500	–	–	–	–	–	0.25	8/8	0	4.29±0.55

*P < 0.01, **P <0.005, ***P<0.001 compared to NMDLA (500mg/kg, i.p) control, ANOVA (n=8). +P<0.005 compared to NMDLA (500mg/kg, i.p) control, Chi-squared test (n=8).

PB: phenobarbitone

DZ: Diazepam

PHY: phenytoin

LY: LY233053

DMSO: Dimethylsulfoxide.

4.4 HPLC ANALYSIS

The HPLC fingerprint of the leaf methanol extract of *Elytropappus rhinocerotis* showed distinct peaks at the following retention times, 4.227, 5.030, 7.698, 12.810, 13.306, 15.333 and 18.563 min

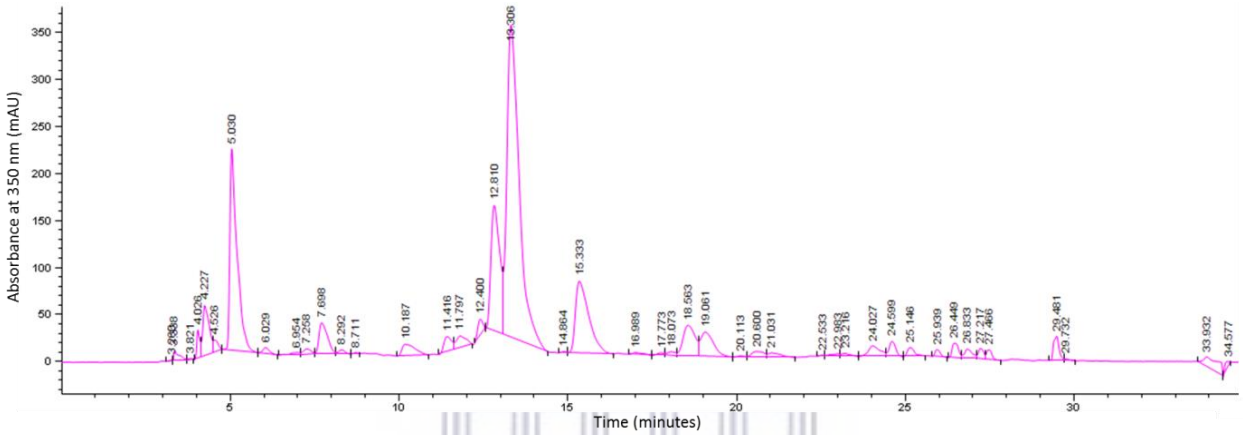


Fig.4.1. HPLC fingerprint of leaf methanol extract of *Elytropappus rhinocerotis*

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The study investigated the anticonvulsant activity of the leaf methanol extract of *Elytropappus rhinocerotis* by studying the effect against tonic convulsions produced by pentylenetetrazole (PTZ), bicuculline, picrotoxin, strychnine or N-methyl-DL-aspartic acid (NMDLA). The leaf methanol extract of *E. rhinocerotis* (200-400 mg/kg, i.p.) was shown in this study to antagonize tonic convulsions produced by PTZ (100 mg/kg, i.p.), bicuculline (30 mg/kg, i.p.), picrotoxin (20 mg/kg, i.p.), strychnine (2 mg/kg, i.p.) or NMDLA (500 mg/kg, i.p.). Phenobarbital (12 mg/kg, i.p.), diazepam (0.5 mg/kg, i.p.), muscimol (2 mg/kg, i.p.) or muscimol (0.2 mg/kg, i.p.) + leaf methanol extract of *E. rhinocerotis* (100 mg/kg, i.p.) antagonized PTZ (100 mg/kg, i.p.), bicuculline (30 mg/kg, i.p.) or picrotoxin (20mg/kg, i.p.)-elicited tonic convulsions. Phenobarbital (12 mg/kg, i.p.) or leaf methanol extract of *E. rhinocerotis* (400 mg/kg, i.p.) and not diazepam (0.5 mg/kg,i.p.) antagonised strychnine (2 mg/kg, i.p.)-elicited tonic convulsion. LY233053 (5 mg/kg, i.p.), leaf methanol extract of *E. rhinocerotis* (400 mg/kg, i.p.) or LY233053 (1 mg/kg, i.p.) + leaf methanol extract of *E. rhinocerotis* (100 mg/kg, i.p.) But not phenobarbital (12 mg/kg, i.p.) or diazepam (0.5 mg/kg, i.p.) antagonized the tonic convulsion produced by NMDLA (500 mg/kg, i.p.). Phenytoin (30 mg/kg, i.p.) or DMSO (0.25 ml, i.p.) did not affect tonic convulsions produced by PTZ (100 mg/kg, i.p.), bicuculline (30 mg/kg, i.p.), picrotoxin (20mg/kg, i.p.), strychnine (2 mg/kg, i.p.) or NMDLA (500 mg/kg, i.p.).

According to Meldrum (1975), Olsen (1981), Czuczwar and Patsalos (2001) and Rang et al. (2015), gamma aminobutyric acid, a major inhibitory neurotransmitter in the CNS, facilitates

increased chloride ion conductance into the brain cells by stimulating $GABA_A$ receptors to open the $GABA_A$-linked chloride channels. This enhances GABA neurotransmission and antagonises convulsions while inhibition of GABA mediated neurotransmission at $GABA_A$ receptors causes convulsion. On the other hand, stimulation of NMDA receptors by glutamic acid in the CNS enhances glutamic acid neurotransmission and causes convulsions while inhibition of the neurotransmission antagonises the convulsions. Pentylenetetrazole (PTZ) was shown in the study to induce tonic convulsion in the experimental animals. According to Kalant and Roschlau (1998), De Sarro et al.(1999) and Rang et al. (2015), PTZ produces convulsion by inhibiting GABA Neurotransmission at the $GABA_A$ receptors in the brain. Phenobarbitone and diazepam, standard antiepileptic drugs, exhibit their anticonvulsant effects by increasing GABA mediated inhibition in the brain through the $GABA_A$ receptor ionophore complex (Kalant and Roschlau 1998; Rang et al. 2015). The data obtained in this study show that phenobarbitone and diazepam significantly antagonized PTZ- induced seizures which may probably be due to their activating GABA mechanism. On the other hand, phenytoin, a standard antiepileptic drug, did not affect PTZ-induced tonic convulsion because it exerts its anticonvulsant effect by inhibiting sodium ion entry into the brain cells resulting in the inhibition of the generation of repetitive action potential (Kalant and Roschlau 1998; Rang et al. 2015). Muscimol was shown in the study to significantly antagonise PTZ- induced tonic convulsions. This may be due to muscimol being a specific $GABA_A$ receptor agonist and produces similar effect to that of GABA by stimulating $GABA_A$ receptor in the brain (Lanca, 1998; Rang et al., 2015). Leaf methanol extract of *E. rhinocerotis* (200 and 400 mg/kg, i.p.) and a combined therapy of the subeffective doses of muscimol (0.2 mg/kg, i.p.) and leaf methanol extract of *E. rhinocerotis* (100 mg/kg, i.p.) significantly

antagonised PTZ- induced tonic convulsions which may probably be due to enhancement of GABA neurotransmission.

Bicuculline, a known potent and specific $GABA_A$ receptor antagonist in the brain (Lanca, 1998; Rang et al., 2015), was shown to produce tonic convulsions in the present study. Phenobarbitone and diazepam known to antagonize convulsions by

Increasing GABA neurotransmission through $GABA_A$ receptor ionophore complex

(Kalant and Roschlau 1998; Rang et al. 2015), were shown to antagonize bicuculline-induced tonic convulsion. In addition, muscimol, a specific $GABA_A$

Receptor agonist, known to have anticonvulsant property (Lanca, 1998; Rang et al.,2015), significantly antagonized bicuculline-induced tonic convulsion. However, phenytoin known to have anticonvulsant effect by blocking sodium channel and hence blocking sodium ion entry into brain cells (Rang et al., 2016) did not affect bicuculline-induced tonic convulsion. Leaf methanol extract of *E. rhinocerotis* (200 and 400 mg/kg, i.p.) and a combined therapy of the subeffective doses of muscimol (0.2 mg/kg, i.p.) and leaf methanol extract of *E. rhinocerotis* (100 mg/kg, i.p.) significantly antagonised bicuculline- induced tonic convulsions. This therefore, supports the assertion that GABA mechanism may be involved in the anticonvulsant activity of the leaf methanol extract of *E. rhinocerotis*.

According to Kalant and Roschlau (1998) and Rang et al. (2015), picrotoxin, a $GABA_A$ receptor antagonist, blocks GABA neurotransmission and causes convulsion by binding to its binding site inside the $GABA_A$ receptor-linked chloride channel. This blocks the chloride channel and inhibits the entry of chloride ions into the brain. Picrotoxin produced tonic convulsion in experimental mice in the present study. It is not surprising therefore, that, in this study, phenobarbitone and diazepam, or muscimol known to enhance GABA neurotransmission or

mimic the effect GABA at $GABA_A$ receptor respectively (Lanca 1989, Rang et al., 2016), antagonised the tonic convulsion produced by picrotoxin. Phenytoin known to prevent convulsion by blocking sodium channel and hence blocking sodium ion entry into brain cells (Rang et al., 2016), did not affect picrotoxin-induced tonic convulsion. Similar to phenobarbitone and diazepam, leaf methanol extract of *E. rhinocerotis* (200 and 400 mg/kg, i.p.) and a combined therapy of the subeffective doses of muscimol (0.2 mg/kg, i.p.) and leaf methanol extract of *E. rhinocerotis* (100 mg/kg, i.p.) significantly antagonised picrotoxin-induced tonic convulsions. This therefore, further supports the assertion that GABA mechanism may be involved in the anticonvulsant activity of the leaf methanol extract of *E. rhinocerotis*.

In the present study, strychnine produced tonic convulsion in experimental animals. Strychnine, a selective glycine receptor antagonist, exerts its convulsant effect by blocking glycine receptor which is also linked to chloride channel in the brain (Lanca, 1998,; Rang et al., 2016). Phenobarbitone and leaf methanol extract of *E. rhinocerotis* (400 mg/kg, i.p.) were shown in the study, to significantly antagonised strychnine-induced tonic convulsions. However, diazepam and phenytoin did not affect the tonic convulsion produced by strychnine. The above data indicate that the anticonvulsant activity of leaf methanol extract of *E.rhinocerotis* may be underpinned by the enhancement of glycine neurotransmission. Report by Rang et al. (2015) showed that besides enhancing

GABA neurotransmission to prevent convulsion, phenobarbitone may also have other mechanisms involved in its anticonvulsant activity. Since it was shown in

the study to antagonize strychnine-induced tonic convulsion, it is probable that glycine mechanism may be involved in the anticonvulsant activity of phenobarbitone in the brain. Rang et al. (2015) have also reported that benzodiazepines do not to affect convulsion produced by

strychnine in experimental animals. It is not surprising therefore, that diazepam, a benzodiazepine, did not alter strychnine-induced tonic convulsion in the study.

However, it is important to mention that according to Lambert et al. (1981) and Boyd et al. (1983) , moderate to high doses of IV phenobarbitone, IV diazepam and IV phenytoin have been effective in the prevention of strychnine convulsion in people suffering from strychnine poisoning.

The present study shows that N-methyl-DL-aspartic acid (NMDLA) produced tonic convulsion in experimental animals. The reports by Chapman and Meldrum (1991), Besancon et al. (2008) and Rang et al. (2015) show that NMDLA produces convulsion by stimulating NMDA receptors in the brain to produce effects similar to that of glutamate, the excitatory amino acid implicated in epilepsy.

The data obtained in the study show that, phenobarbitone, and diazepam, known enhancers of GABA neurotransmission, and phenytoin, known blocker of sodium ion entry into the brain, did not affect NMDLA-induced tonic convulsion. LY233053, a competitive NMDA receptor antagonist, known to prevent glutamic acid-induced convulsion by blocking glutamic acid effect at NMDA receptors

(Madden et al., 1992; Borowicz et al., 1996), was shown to block NMDLA-induced tonic convulsion in the study. Similar to LY233053 (5 mg/kg, i.p.), leaf methanol extract of *E. rhinocerotis* (400 mg/kg, i.p.) and a combined therapy of the subeffective doses of LY233053 (1 mg/kg, i.p.) and leaf methanol extract of *E.rhinocerotis* (100 mg/kg, i.p.) significantly antagonised NMDLA-induced tonic convulsions indicating the possibility of the involvement of

glutaminergic mechanism in the anticonvulsant activity of leaf methanol extract of *E. rhinocerotis*.

The phytochemical analysis, in this study, reveals the presence of alkaloids, saponins, flavonoids, tannins, cardiac glycosides and triterpene steroids in the leaves of *Elytropappus rhinocerotis*. This is in addition to the labdane diterpene, rhinocerotinoic acid, which is responsible for the anti-inflammatory activity (Dwarka, 2012). Triterpene steroids have been shown to possess strong anticonvulsant activity in various medicinal plants (ihumans, 2001; Chauhan *et al*.,1998; Amabeoku and Kinyua, 2010). Terpenoids includes saponins and triterpene steroids. It is therefore, possible that the saponins and triterpene steroids present in the plant species may be contributing to its anticonvulsant activity. The LD_{50} value proposed for the leaf methanol extract of *E. rhinocerotis* administered orally should be over 4000 mg/kg since no death of the animals was observed at this dose in the study. It is pertinent to mention that the leaves of *E. rhinocerotis* are given orally as an infusion by traditional medicine practitioner. Therefore, the LD_{50} value obtained shows that the plant material is non toxic to the mice used. The HPLC fingerprint obtained becomes very important because of the characteristic peaks shown by the fingerprint which could be useful in the verification of this species of plant.

5.2 CONCLUSION

The results of this study confirm that *Elytropappus rhinocerotis* possesses strong anticonvulsant activity; thus affirming its use by the traditional medicine practitioners in Western Cape region of South Africa in treating convulsions. Other findings from this study suggest the involvement of gabaergic, glutaminergic and

Glycinergic systems to control chemically-induced seizures by the leaf methanol extract of *Elytropappus rhinocerotis*. It is therefore possible to suggest that more than one mechanism may be involved in the plant's anticonvulsant activity. The oral acute toxicity study shows that *Elytropappus rhinocerotis* is conveniently safe at all oral doses tested as neither lethality nor unpleasant behaviour of animals were observed.

5.3 FURTHER WORK

Further work should include an elaborate toxicological studies and clarification of the mechanism (s) by which the plant species produces its anticonvulsant activity.

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