Preclinical Studies of Ginger (*Zingiber officinale*) Rhizome and its Clinical Trial as an Add-on Antiepileptic Therapy in Children with Generalized Epilepsies.

By

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Declaration

I declare that; this research has been conducted by my own efforts through an extensive experimental and clinical works under the supervision of senior professors and doctors and no other previous similar researches with similar titles and contents (methodology and results) have been published in any references and citation areas.

Abdel Moniem Abdel Rahman Eisa Ahmed
Dedication

To my Parents, wife and kids
Acknowledgement

Great thanks is due to Allah who owed me with power, patience and health to fulfilled this work. May profound acknowledge and appreciation to my supervisors, Professor El Hadi Mohamed Mohamed Ahmed for his encouragement and supervisory skills that enabled me to accomplished this work. Also I wish to thank Professor Imad Eldin Mohamed Taj Eldin and Professor Hayder El Hadi Babiker for their follow up and guidance during this research. My profound appreciation to Dr Enas Mohammed Awad El Tahir for her support and help in fulfillment of antiepileptic preclinical tests. I would like to express my thanks to my friend Dr. Hauzifa for his technical and laboratory help and continuous support to finish this work. Many thanks to the lab technicians, Faculty of Pharmacy University of Gezira especially Mr. Abdalla Mohamed Osman, Abubaker Ahmed Mohamed and Awad Ali For laboratory assistance. My sincere thanks to my family especially my parents; Abdel Rahman E. Ahmed and Sittana M. Abdalla. Great thanks for my wife Rasha H. Tom for patience. I wish successful life for my children; Abdel Rahman, Amaar, Ludina, Lena and Linda
Preclinical Studies of Ginger (*Zingiber officinale*) Rhizome and its Clinical Trial as an Add-on Antiepileptic Therapy in Children with Generalized Epilepsies.

By

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Abstract

Epilepsy is a disorder of the brain characterized by the generation of epileptic seizures. Botanicals and herbal materials including some parts from plants are prepared and taken in many ways for treatment of children ill with epilepsy. Conventional antiepileptic drugs are associated with adverse effects on long term therapy. This study was conducted to screen ginger for its acute toxicity, gastro protective, antiemetic and antioxidant pharmacological effects. GC-MS analysis was also carried out to identify the phytoconstitutents present in ginger extract. Experimental animal models using maximal electroshock (MES) and pentylentetrazole (PTZ) tests were used to check the anticonvulsive activity of ginger in rats. Moreover a clinical study was conducted on children volunteers with generalized epilepsies to investigate the add-on antiepileptic effect of ginger. Results obtained revealed that ginger possessed gastro-protective activity comparable to that of the H²-receptor antagonist ranitidine and the effect was dose-dependant. Ginger was also found to inhibit emesis to an extent greater than metoclopramide. The remarkable antioxidant activity was produced by ginger extract compared to standard quercetin. The major compounds identified by GC-MS in ginger extract were of vanillyl moiety including 6-gingerol and zingerone and two alkaloids capsaicin and nonivamide. Ginger extract showed 100% protection of seizures induced by MEST and PTZ. Early stage clinical trail in children generalized epilepsies revealed that 87% of participants who administered add-on ginger plus AEDs (sodium valproate / or carbamazepine) were seizure free while all of them (100%) experienced reduction in seizure duration and seizure frequency. Significant reduction in side effects produced by AEDs and/or by seizure were observed. It could be concluded that, ginger could has a high potential in treatment of epilepsy, especially if further studies are conducted to explore its phytoconsitutents possible anticonvulsant effect alone and/or as co-drug in combination with AEDs.
دراسات معملية وأكلينيكية على الزنجبيل (Zingiber Officinale Roscoe.) كمادة مضافة ومضادة للتشنجات وخصائصه العلاجية على الأطفال المصابين باعتلال الصرع العام

عبد المنعم عبد الرحمن عيسى

الخلاصة

الصرع هو اعتلال دماغي يتميز بنوبات تلقائية. بعض المواد النباتية والعشبية يتم تحضيرها وتعاطيها بطرق متعددة لعلاج الأطفال المصابين بهذا المرض. الأدوية التقليدية للمضادة للصرع تكون عادة مصحوبة بآثار جانبية عند العلاج على المدى الطويل. أجريت الدراسة الحالية لتقييم السمية الحادة وآثار الزنجبيل على المعدة، كمضاد للاستفراغ ومضاد للأكسدة. تم استخدام تحليل الكروماتوغرافيا المقارن (GC-MS) لتعريف المركبات الكيميائية في مستخلص الزنجبيل. تم استخدام نموذجي الصدمة الكهربائية القصوى والبنتايلينترترازول لإحداث التشنجات للحيوانات المعملية كالجرذان. بالإضافة إلى ذلك تم أجراء الدراسة الطبية الأكلينيكية على أطفال مرضى الصرع المعمم. أظهرت النتائج المتحصل عليها أن الزنجبيل يمتلك نشاطاً حماياً للمعدة ونشاط مضاد للاستفراغ ونشاط مضاد للأكسدة. المركبات الرئيسية التي تم تعريفها بواسطة تحليل الكروماتوغرافيا المقارن تشمل 6-جنجرول و زنجرون و اثنين من المكونات القلوانية الكابسيسين والفاتاميد. أظهر مستخلص الزنجبيل نشاطاً مضاداً لالعوامل الحيوانية لتشنجات مثل الميتابلاينترترازول بنسبة 100%. الدراسة الطبية الأكلينيكية الأولية للأطفال الذين يعانون من مرض الصرع المعمم أظهرت أن 87% من المتطوعين الذين تناولوا الزنجبيل المضاف للأدوية المعضدية للتشنجات (فالوريدات الصوديوم أو الكاربامازين) تحرروا من النوبات وأظهروا جميعاً انخفاضاً في الفترة الزمنية للنوبات وتزداد النوبات بنسبة 100%. أظهرت الدراسة انخفاضاً واضحاً في الأثار الجانبية المرافقة استخدام الأدوية المعضدية للتشنجات، و النوبات يمكن الاستنتاج بأن الزنجبيل يمكن استخدامه كعلاج للصرع خاصة إذا تم إجراء دراسات أخرى لتوضيح إمكانية الأثر المضاد للتشنجات إذا تم استخدامه منفرداً أو مضافاً للأدوية المعضدية للتشنج التقليدية.
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Chapter One

Introduction
1.1. Introduction:

Epilepsy is a group of neurological disorders characterized by epileptic seizures (Chang and Lowenstein, 2003; Magiorkinis et al., 2010; Fisher et al., 2014). Epilepsy is one of the most common neurological disorders after stroke and affects at least 50 million people worldwide (Stafstrom, 2004). During the past 20 years, major clinical and research efforts have sought to characterize the status of health related quality of life in epilepsy (Sabez et al., 2001). Childhood epilepsies beginning in the first few years of life are frequently characterized by seizures that are resistant to available treatments, including antiepileptic drugs (AEDs), ketogenic diet, high doses of steroids, and surgery (Wheless, 2009). The goal of management of children with epilepsy is to enable the child and his family to lead a life as free as possible of the medical and psychosocial complications of epilepsy. This comprehensive care needs to go beyond simply trying to control seizures with minimal adverse drug reactions. Other factors including social, psychological, behavioral, educational, and cultural dimensions affect children with epilepsy, their families and their close social networks (Testa and Simon, 1996). In 1978 a World Health Organization (WHO) Study Group identified epilepsy to be a disorder whose control should receive top priority, in view of its high prevalence in developing countries and potentially severe consequences (World Health Organization, 1978). Botanicals and herbal materials including any parts from plants are prepared and taken in many ways for treatment of children with epilepsy. Botanical products extracts have been found to block seizures in animal models as well as their centuries old traditions of use in treatment of seizures with actions that include effects on Gama amino butyric acid (GABA) receptors and voltage-gated ion channels, anti-inflammatory and neuroprotective effects (Enas et al., 2012). Published clinical studies of botanicals and epilepsy control are still of inadequate quality concerning safety and efficacy (Steven, 2015).

Many plants were known for their anticonvulsant activity. Among those medicinal plants are found to possess anticonvulsant activity in animal models and/or folk medicine, include: Abelmoschus angulosus, Allium sativum, Artemisia species, Cannabis sativa, Cinchona officinalis, Zingiber officinale, Egletes viscousa, Icacina trichantha,
Magnolia grandifl ora, Plumbago zeylanica and others (Quintans-Júnior et al., 2002). Among those plants tested, a number of them (from different families) are found to possess anticonvulsant activity. While in most cases, the active constituents are yet to be found, for those where the active components are known, they belong to different chemical classes. However, previous studies showed that some natural plant coumarins and triterpenoids exhibit anticonvulsant properties (Chaturvedi et al., 1974; Nsour et al., 2000). In addition, the history of drug discovery showed that plants are highly rich sources in the search for new active compounds and they have become a challenge to modern pharmaceutical industry. Many synthetic drugs owe their origin to plant-based complementary medicine (Howes et al., 2003; Orhan et al., 2004). Various phytochemical and pharmacological studies have been carried out on these anticonvulsant plants (Chauhan et al., 1988 and Nsour et al., 2000). Phytomedicines can potentially play an important role in the development of new antiepileptic drugs to pharmacoresistant patients (Nsour et al., 2000). Using natural product such as spices and herbal agents to treat epilepsy has been going on in communities in developing countries for a long time. Up to our knowledge no proper research on that has been done before, at least in the Sudan. Aware of the great challenge of designing and conducting clinical research studies in the epilepsies. This is a trial to study Ginger as an additive agents to treat epilepsy. Several plants have been investigated for their antiepileptic activity and no evidence that ginger constituents have anti-epileptic activity.

1.2. Rationale:
Ginger is generally considered a safe herbal medicine (Weidner and Sigwart, 2000). A previous studies showed that ginger is a promising source of antiepileptic agents (Enas, et al., 2012). To move forward the concept of botanicals in treatment of epilepsy and other ailments as alternative therapies, investigations was carried out in order to gain insights into the current use of ginger. Hence, this research would be undertaken to investigate the acute toxicity, gastro-protective, anti-emetic, anti-oxidant and anti-epileptic effects of ginger in animals. Clinical study would be undertaken to investigate the anti-epileptic activity in children since no study has been done before addressing this subject in Sudan.
1.3. Objectives:
1.3.1. Main Objective:
Experimental preclinical studies of Ginger (Zingiber officinale) Rhizomes and its clinical Add-on antiepileptic activity on children with generalized epilepsies.
1.3.2. Specific Objectives:
1.3.2.1. Pre-clinical specific objectives:
1- To investigate the acute toxicity of ginger in vivo.
2- To evaluate the gastro-protective effect of ginger by using the Indomethacin induced ulcer method.
3- To investigate the anti-emetic effect of ginger.
4- To evaluate the antioxidant effect of Zingiber Officinale Roscoe Rhizomes.
5- To analyze ginger extract by GC-MS for its active constituents.
6- To verify the anticonvulsant activity of ginger in rats using maximal electroshock-induced seizure test (MST) and pentylentetrazole(PTZ) induced seizures test.
1.3.2.2. Clinical specific objective:
To examine groups of children with idiopathic generalized epilepsy (IGE) in a clinical based sample with control study in patients receiving ginger + AEDs and/or AEDs alone.
Literature Review

2.1. Epilepsy:
The two thousand fourteen definition of the International League Against Epilepsy define epilepsy as disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiologic, cognitive, psychological, and social consequences of this condition (Fisher et al., 2014). The definition of epilepsy requires the occurrence of at least one epileptic seizure (Robert et al., 2005; Panayiotopoulos, 2011). About 39 million people have epilepsy and nearly 80% of cases occur in the developing world, leading to 125,000 deaths and its is more common in older people (Brodie et al., 2009; Holmes et al., 2008). In the developing world, onset of new cases occurs most frequently in babies and the elderly due to differences in the frequency of the underlying causes (Wyllie's, 2016; Newton, 2012).

Generally, information available on epilepsy in developing countries had been derived from hospital-based studies, which made extrapolation to the general population highly conjectural. Since then, new data accumulated from different parts of the developing world give a better understanding of the magnitude of the problem, based on community studies of the prevalence and the possible etiological factors. There has also been a shift of emphasis from hospital-based epilepsy management to community based epilepsy control (Senanayake and Meinardi, 1989). Higher incidences occur in children, age being an important factor in determining the incidence (Mathai, 1968). Most of the prevalence data available for developing countries are based on community surveys of rural populations. Such surveys, in general, have employed a two-phase design, the first phase consisting of screening interviews by field workers and the second phase comprising medical evaluation by neurologists.

Common methodological problems of epidemiological studies of epilepsy include deficiencies in the following: differential diagnosis, case ascertainment methods, definition and classification of epilepsy, and selection bias. (Senanayake, 1987). In some communities, epileptic patients may be expelled from their homes as outcasts, and hence not be available for case ascertainment (Giel, 1970). Prevalence greater than three to nine per 1000 have been found in several African (Gerrits, 1965; Aall-Jilek, 1965) and Latin American countries (Marino, 1987; Gracia, 1990). The rates in some of these studies are
not very different from those in industrialized countries (Marino, 1987). Some of these high prevalence of epilepsy in developing countries probably reflect local or regional characteristics that do not apply to the countries as a whole. It should also be noted that several studies reporting high prevalence used small population samples, some less than 1000 individuals (Osuntokun, 1982; Gracia, 1988). Most studies of epilepsy in industrialized countries report that males are more frequently affected than females, although the difference is seldom statistically significant. Results from developing countries are similar, although, some studies in Nigeria (Osuntokun, 1982) and Latin America (Gomez, 1978; Cruz, 1986) have found higher prevalence for females. Mortality data, based on a single underlying cause of death listed on the death certificate, considerably underestimate the number of deaths among people with epilepsy. Furthermore, the case-fatality ratio for epilepsy is low, so that mortality statistics are not a good indicator of the disease frequency. Also, the accuracy of death certificate diagnosis varies, particularly from one country to another and over long periods of time. Males have a higher age-adjusted mortality rate than females, and developing countries have higher mortality rates than industrialized countries (Massey and Schoenberg, 1985).

The oldest medical records show that epilepsy has been affecting people at least since the beginning of recorded history and throughout ancient history the disease was thought to be a spiritual condition although the world's oldest description of an epileptic seizure comes from a text in Akkadian (a language used in ancient Mesopotamia) and was written around 2000 BC. Person described in the text was diagnosed as being under the influence of a moon god (Magiorkinis, 2010; Saraceno, 2005). Simple febrile seizures do not tend to recur frequently and do not make the development of adult epilepsy. Increased risk of death has been shown for complex febrile seizures, partly related to underlying conditions (Shinnar and Glauser, 2002; Vestergaard et al., 2008). Sudden unexpected death in epilepsy (SUDEP) is a fatal complication of epilepsy and its defined as the sudden and unexpected, non-traumatic and non-drowning death of a person with epilepsy, without a toxicological or anatomical cause of death detected during the post-mortem examination; the number one cause of epilepsy related death in people with pharmaco-resistant epilepsy. Children with epilepsy have a cumulative risk of dying suddenly of 7% within 40 years (Nashef et al., 2012; Surges and Sander, 2012; Rylin et
Surgery is considered in patients whose seizures can not be controlled by adequate trials of two different medications and epilepsy surgery has been performed for more than a century (Spencer et al., 2013; Krucoff et al., 2017). The ketogenic diet is indicated as an adjunctive treatment in children and young people with drug-resistant epilepsy (Vining et al., 1998; Hartman and Vining, 2007). Epileptic seizures are the result of excessive and abnormal neuronal activity in the cortex of the brain (Fisher et al., 2005). The diagnosis involves ruling out other conditions that might cause similar symptoms, such as fainting, and determining if another cause of seizures is present, such as alcohol withdrawal or electrolyte problems (Longo, 2012). An epileptic seizure, also known as an epileptic fit, is a brief episode of signs or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. The outward effect can vary from uncontrolled jerking movement to as subtle as a momentary loss of awareness. Diseases of the brain characterized by an enduring predisposition to generate epileptic seizures are collectively called epilepsy. (Fisher et al., 2005; Fisher et al., 2014). In epilepsy, seizures tend to recur and, as a rule, have no immediate underlying cause (Hughes, 2009). Isolated seizures that are provoked by a specific cause such as poisoning are not deemed to represent epilepsy (Fisher et al., 2005). About 6% of those with epilepsy have seizures that are often triggered by specific events and are known as reflex seizures. Those with reflex epilepsy have seizures that are only triggered by specific stimuli. Common triggers include flashing lights and sudden noises (Xue and Ritaccio, 2006; Steven and Schachter, 2008).

The frequency of use of specific natural products in developed countries appears to parallel the available evidence supporting their use, between 24 and 56% of the adult patients and 12 to 32% of children with epilepsy have used Complementary and alternative medicine therapies at some time (Melchart et al., 2000; Prus and Grant, 2010). The efficacy of natural products for control of seizures cannot be accurately assessed from the available studies because of their significant methodological shortcomings (Easterford, 2005). Because of the risk of pharmacokinetic interactions with AEDs, it is concerning that many patients do not inform their physicians about their use of natural products (Gidal et al., 1999; Peebles et al., 2000; Easterford et al., 2005; Murphy et al., 2008).
2.1.1. Causes of epilepsy:
Epilepsy can have both genetic and acquired causes, with interaction of these factors in many cases. Acquired causes include serious brain trauma, stroke, tumors and problems in the brain as a result of a previous infection; of those with brain tumors, almost 30% have epilepsy, making them the cause of about 4% of cases and the risk is greatest for tumors in the temporal lobe and those that grow slowly. Other mass lesions such as cerebral cavernous malformations and arteriovenous malformations have risks as high as 40–60%. Of those who have had a stroke, 2–4% develop epilepsy (Berkovic et al., 2006).
Seizures may also occur as a consequence of other health problems, if they occur right around a specific cause, such as a head injury, toxic ingestion or metabolic problem, they are known as acute symptomatic seizures and are in the broader classification of seizure-related disorders rather than epilepsy itself (Thurman et al., 2011; Neligan, 2012).
Genetics is believed to be involved in the majority of cases, either directly or indirectly and some epilepsies are due to a single gene defect (1–2%); most are due to the interaction of multiple genes and environmental factors (Pandolfo, 2011). Each of the single gene defects is rare (Dhavendra Kumar, 2008). Most genes involved affect ion channels, either directly or indirectly (Berkovic et al., 2006). These include genes for ion channels themselves, enzymes, GABA, and G protein-coupled receptors (Simon et al., 2012). In identical twins, if one is affected there is a 50–60% chance that the other will also be affected. In non-identical twins the risk is 15% and these risks are greater in those with generalized rather than focal seizures if both twins are affected, most of the time they have the same epileptic syndrome (70–90%) (Pandolfo, 2011). Other close relatives of a person with epilepsy have a risk five times that of the general population. Between 1 and 10% of those with Down syndrome and 90% of those with Angelman syndrome have epilepsy (Bhalla et al., 2011). Benign epilepsy with centrotemporal spikes is thought to be a genetic disorder. An autosomal dominant inheritance with age dependency and variable penetrance has been reported, although not all studies support this theory (Neubauer, 2000; Chahine and Mikati, 2006; Bali et al., 2007). It is not clear why some patients get post traumatic epilepsy while others with very similar injuries do not (Frey, 2003).
2.1.2. Classification of epilepsy:
The earliest classification of seizures can be attributed to Babylonian scholars who inscribed their medical knowledge into stone tablets known as the Sakikku (meaning All Diseases) (Wilson and Reynolds, 1990). These seizures may present in several ways depending on the part of the brain involved and the person's age (Duncan et al., 2006).

The most common type of seizures are convulsive. Of these, one-third begin as generalized seizures from the start, affecting both hemispheres of the brain. Two-thirds begin as focal seizures affecting one hemisphere of the brain, may then progress to generalized seizures. The remaining are non-convulsive. An example of this type is the absence seizure, which presents as a decreased level of consciousness and usually lasts about ten seconds. (Hammer et al., 2010; Hughes, 2009).

Focal seizures are often preceded by certain experiences, known as auras (Shearer and Peter, 2010). They include sensory (visual, hearing, or smell), psychic, autonomic, and motor phenomena (Hammer et al., 2010). Jerking activity may start in a specific muscle group and spread to surrounding muscle groups in which case it is known as a Jacksonian march. Automatisms may occur, which are non-consciously-generated activities and mostly simple repetitive movements like smacking of the lips or more complex activities such as attempts to pick up something (Bradley and Walter, 2012). There are six main types of generalized seizures: tonic-clonic, tonic, clonic, myoclonic, absence and atonic seizures (The National Clinical Guideline Centre, 2012). They all involve loss of consciousness and typically happen without warning. Tonic-clonic seizures occur with a contraction of the limbs followed by their extension along with arching of the back which lasts 10–30 seconds (the tonic phase). A cry may be heard due to contraction of the chest muscles, followed by a shaking of the limbs (clonic phase). Tonic seizures produce constant contractions of the muscles. A person often turns blue as breathing is stopped. In clonic seizures there is shaking of the limbs in unison. After the shaking has stopped it may take 10–30 minutes for the person to return to normal; this period is called the "postictal state" or "postictal phase." Loss of bowel or bladder control may occur during a seizure. The tongue may be bitten at either the tip or on the sides during a seizure. In tonic-clonic seizure, bites to the sides are more common. Tongue bites are also relatively common in psychogenic non-epileptic seizures (Engel, 2008). The ability to categorize a
case of epilepsy into a specific syndrome occurs more often with children since the onset of seizures is commonly early. Less serious examples are benign rolandic epilepsy, childhood absence epilepsy, and juvenile myoclonic epilepsy (Neligan, 2012). Severe syndromes with diffuse brain dysfunction caused, at least partly, by some aspect of epilepsy, are also referred to as epileptic encephalopathies. These are associated with frequent seizures that are resistant to treatment and severe cognitive dysfunction, for instance Lennox–Gastaut syndrome and West syndrome (Nordi, 2012).

2.2. Anti-epileptic drugs (AEDs):

Epilepsy cannot usually be cured, but medication can control seizures effectively in about 70% of cases also supporting people's self-management of their condition may be useful (Michael and O'Connor, 2011; Eadie, 2012). Epilepsy is a tendency to have recurrent seizures. Most patients with epilepsy have a good prognosis and their seizures will be controlled by treatment with a single antiepileptic drug, but up to 30% develop refractory epilepsy that often requires treatment with combinations of antiepileptic drugs (Cockerell et al., 1995). These cases represent a considerable therapeutic problem since up to 2-3% of the population will suffer from epilepsy at some time in their lives (Hauser et al., 1935). Antiepileptic drugs (AEDs) used to treat seizure disorders are today among the most common medications for which clinical laboratories perform therapeutic drug monitoring (Neels et al., 2004; Patsalos et al., 2008). Ideally, any choice made between antiepileptic drugs should be based upon the results of comparative randomized controlled trials. At present there is insufficient evidence to guide a choice between standard treatments such as carbamazepine, phenytoin, and valproate (Marson and Chadwick, 1996). A single medication is recommended initially, if this is not effective switching to a single other medication. In about half, the first agent is effective; a second single agent helps in about 13% and a third or two agents at the same time may help an additional 4% (Robert et al, 2005; Steven et al., 2008; Elaine Wyllie, 2012). About 30% of people continue to have seizures despite anticonvulsant treatment (Edie, 2012). Adverse effects from medications are reported in 10 to 90% of people depending on how and from whom the data is collected also most adverse effects are dose-related. Certain medications have side effects that are not related to dose and up to a quarter of people stop treatment due to adverse effects (Perucca and Gilliam, 2012). the newer antiepileptic
drugs (AEDs) may offer a better adverse events profile in comparison to the older generation antiepileptic drugs and they may still have significant undesired Central Nervous System effects such as decreased cognitive abilities and psychiatric complications (Schmidt, 2009).

2.2.1. Conventional anti-epileptic drugs :

Conventional antiepileptic drugs may block sodium channels or enhance gamma-aminobutyric acid (GABA) function. Several antiepileptic drugs have multiple or uncertain mechanisms of action. Next to the voltage-gated sodium channels and components of the GABA system, their targets include GABA_A receptors, the GABA transporter, and GABA transaminase (Rogawski and Löscher, 2004). Additional targets include voltage-gated calcium channels, SV2A, and alpha-2delta (Rogawski, 2004; Meldrum and Rogawski, 2007). By blocking sodium or calcium channels, antiepileptic drugs reduce the release of excitatory glutamate, whose release is considered to be elevated in epilepsy, but also that of GABA. This is probably a side effect or even the actual mechanism of action for some antiepileptic drugs, since GABA can itself, directly or indirectly, act proconvulsively (Kammer et al., 2011). Another potential target of antiepileptic drugs is the peroxisome proliferator-activated receptor alpha (Maguire and Murthy, 1985; Hall et al., 1990; Lampen and Carlberg, 2001; Frigerio et al., 2006; Porta et al., 2009; Citraro, 2013; Puligheddu et al., 2013). Some anticonvulsants have shown antiepileptogenic effects in animal models of epilepsy (Kaminski et al., 2014). That is, they either prevent the development of epilepsy or can halt or reverse the progression of epilepsy. However, no drug has been shown in human trials to prevent epileptogenesis (the development of epilepsy in an individual at risk, after a head injury) (Khalil, 2007). During pregnancy, the metabolism of several anticonvulsants is affected. There may be an increase in the clearance and resultant decrease in the blood concentration of lamotrigine, phenytoin, and to a lesser extent carbamazepine, and possibly decreases the level of levetiracetam and the active oxcarbazepine metabolite, the monohydroxy derivative. Therefore, these drugs should be monitored during use in pregnancy (Harden et al., 2009). Many of the common used medications, such as valproate, phenytoin, carbamazepine, phenobarbitol, gabapentin have been reported to cause increased risk of birth defects (Weston et al., 2016) and its derivatives such as sodium valproate and
divalproex sodium, causes cognitive deficit in the child, with an increased dose causing decreased intelligence quotient on the other hand, evidence is conflicting for carbamazepine regarding any increased risk of congenital physical anomalies or neurodevelopmental disorders by intrauterine exposure. Similarly, children exposed to lamotrigine or phenytoin in the womb do not seem to differ in their skills compared to those who were exposed to carbamazepine (Bromley et al., 2014). The most effective combination is a drug with a single- plus another with multiple mechanisms of action. In humans, combinations of a blocker of voltage-gated sodium channels plus a drug with multiple mechanisms of action may exert synergistic effects (Giussani and Beghi, 2013) On the other hand, combinations of more than three drugs are not recommended because they rarely result in complete inhibition of epilepsy (Brodie et al., 2012).

2.2.2. Newer anti-epileptic drugs:

Epilepsy is one of the most common neurological diseases; its treatment is the administration of antiepileptic drugs (AEDs). These are divided into first-, second-, and third-generation AEDs (Johannessen Landmark and Patsalos, 2010). The commonly used first-generation AEDs are phenytoin, phenobarbital, carbamazepine, and valproic acid. The third-generation drug includes lacosamide and eslicarbazepine acetate; others recently delivered are included in the second generation. Post-second-generation AEDs are commonly known as new AEDs. gabapentin, topiramate, lamotrigine, levetiracetam, and rufinamide are distributed as oral drugs. Vigabatrin, oxcarbazepine, perampanel, and lacosamide are being considered for approval by the Japanese Ministry of Health, Labour, and Welfare. While therapeutic guidelines have long advocated the administration of carbamazepine and valproic acid as a first drug for focal and generalized seizures (Glauser et al., 2013). In the last 20 years, 14 more so-called newer generation AEDs entered the market: eslicarbazepine acetate, felbamate, gabapentin, lacosamide, lamotrigine, levetiracetam, oxcarbazepine, pregabalin, rufinamide, stiripentol, tiagabine, topiramate, vigabatrin, and zonisamide (LaRoche and Helmers, 2004). the new AEDs are not superior to traditional AEDs in terms of their antiepileptic- and adverse effects (Marson et al., 2007; Trinka et al., 2013). Their prolonged administration elicited fewer adverse effects and milder interactions with other drugs than did traditional AEDs (French et al., 2004; Pastalos, 2013). Most new AEDs involve less
teratogenicity and their effect on the patients’ physical status, including hormone secretion and the bone and lipid metabolism, are milder (Reimers, 2008).

### Table 2.1: Indications for the new antiepileptic drugs

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Focal seizure</th>
<th>Generalized seizure</th>
<th>Induced aggravation of seizure type/epilepsy syndrome</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Focal seizure</td>
<td>Generalized seizure</td>
<td>Induced aggravation of seizure type/epilepsy syndrome</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>+</td>
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<tr>
<td>Topiramate</td>
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<tr>
<td>Lamotrigine</td>
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<td>+</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>+ + (+) + (+)</td>
<td>+</td>
<td>+ + + + +</td>
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<tr>
<td>Rufinamide</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Vigabatrin</td>
<td>+</td>
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<tr>
<td>Oxcarbazepine</td>
<td>+</td>
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<tr>
<td>Perampanel</td>
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<td>Lacosamide</td>
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</table>


### 2.2.3. Anti-epileptic drugs of plant origin:

The 20 century has witnessed considerable progress in anticonvulsant drug development (Loscher and Schmidt, 1988). nature is a rich source of biological and chemical diversity. The unique and complex structures of natural products cannot be obtained easily by chemical synthesis. A number of plants in the world have been used in traditional medicine remedies (Barbosa-Filho et al., 2006; Funke and Melzig,
A number of animal models have demonstrated utility in the search for more efficacious and more tolerable AEDs. In fact, the models employed in the early phase of AED discovery are highly predictive of subsequent efficacy in easy-to-manage generalized and partial epilepsy (Smith et al., 2007). Thus, animal models more employed were leptazole-induced seizure (LIS), maximal electroshock seizure (MES), metrazole-induced seizures (MIS), picrotoxin-induced convulsions (PIC), pilocarpine (PILO), pentyleneetetrazole (PTZ) and strychnine-induced seizures (SIS). However, MES, PIC and PTZ seizure models continue to represent the three most widely used animal seizure models employed in the search for new AEDs (While et al., 2002). In fact, all currently available drugs are anticonvulsant (anti-seizure) rather than antiepileptic. The latter term should only be used for drugs which prevent or treat epilepsy and not solely its symptoms. The goal of therapy with an anticonvulsant drug is to keep the patient free of seizures without interfering with normal brain function (Löscher and Schmidt, 1998). The selection of an anticonvulsant drug is based primarily on its efficacy for specific types of seizures and epilepsy (Mattson, 1995).

2.3. Ginger: The plant of the study

Ginger, the rhizome of Zingiber officinale, was selected for investigation of different pharmacologic actions and anti-epileptic activity. Ginger is one of the most widely used species of the family (Zingiberaceae) and is a common condiment for various foods and beverages. Ginger has a long history of medicinal use dating back 2,500 years in China and India (Grant and Lutz, 2000). Zingiberaceae or the ginger family is a family of flowering plants made up of about 50 genera with a total of about 1600 known species (Christenhusz and Byng, 2016). In Arabian medicine, ginger is considered an aphrodisiac (Qureshi et al., 1989).

2.3.1. Habitat and Distribution:

Ginger originated in the tropical rainforests from the Indian subcontinent to Southern Asia where ginger plants show considerable genetic variation (Thomas, 1982). The plant is native to South-East Asia and is cultivated in the tropical regions in both the eastern and western hemispheres. It is commercially grown Rhizoma Zingiberis in Africa, China,
India, and Jamaica; India is the world’s largest producer. The Synonymous of this plant are Amomum zingiber and Zingiber blancoi Massk. In Arabic ginger is called zanjabil (Benson, 2002). The scientific and common names of ginger are Zingiber officinale, African Ginger, Black Ginger, Cochin Ginger, Gingembre, Ginger root, Jamaica Ginger, Race Ginger, Zingiberis rhizoma, Gingerall, Cayenne Ginger, Ginger Peppermint Combo, Ginger Power, and Ginger Trips (Newall et al., 1996; Fetrow et al., 1999; Jellin et al., 2002).

2.3.2. Plant part used:
Ginger is the dried rhizome of Zingiber officinale (Roscoe) family Zingiberaceae (Fig 2. 1). Ginger occurs in horizontal, laterally flattened, irregularly branching pieces; three to sixteen centimeters long, three to four centimeters wide, up to two centimeters thick; sometimes split longitudinally; pale yellowish buff or light brown externally, longitudinally striated, somewhat fibrous; branches known as “fingers” arise obliquely from the rhizomes, are flattish, obovate, short, about one to three centimeters long; fracture, short and starchy with projecting fibres. Internally, yellowish brown, showing a yellow endodermis separating the narrow cortex from the wide stele, and numerous scattered fibrovascular bundles, abundant scattered oleoresin cells with yellow contents and numerous larger greyish points, vascular bundles, scattered on the whole surface (Benson, 2000).

2.3.3. Description:
A perennial herb with a subterranean, digitately branched rhizome producing stems up to one half meter in height with linear sheathing leaves (5–30cm long and 8–20 mm wide) that are alternate smooth and pale green(Fig 2). Flower stems shorter than leaf stems and bearing a few flowers, each surrounded by a thin bract and situated in axils of large, greenish yellow obtuse bracts, which are closely arranged at end of flower stem forming collectively an ovate-oblong spike. Each flower shows a superior tubular calyx, split part way down one side; an orange yellow corolla composed of a tube divided above into three linear oblong, blunt lobes; six staminodes in to rows, the outer row of three inserted at mouth of corolla; the posterior two, small, horn-like; the anterior petaloid, purple and spotted and divided into three rounded lobes; an inferior, three celled ovary with tufted
stigma. Fruit a capsule with small arillate seeds (Benson, 2002; O'Hanlon and McCauley, 1974). It is a herbaceous perennial, grows annual pseudostems (false stems made of the rolled bases of leaves) about a meter tall bearing narrow leaf blades. The inflorescences bear pale yellow with purple flowers and arise directly from the rhizome on separate shoots (Sutarno et al., 1999).
**Fig 2.1:** Dried ginger (*Zingiber officinale*) Rhizomes

**Fig 2.2:** Ginger plant with flower
2.3.4. Traditional and Medicinal uses:

Ginger (Zingiber officinale Roscoe, Zingiberaceae) is widely used around the world in foods as a spice. For centuries, it has been an important ingredient in Chinese, Ayurvedic and Tibb-Unani herbal medicines for the treatment of catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes (Awang, 1992; Wang and Wang, 2005; Tapsell, 2006). Several reviews have appeared in the literature about this plant, and this may reflect the popularity of the subject and its common use as a spice and a medicinal plant (Afzal et al., 2001; Chrubasik et al., 2005). Medicinal use of ginger dates back to ancient China and India; references to its use are found in Chinese pharmacopeias, the Susruta scriptures of Ayurvedic medicine as well as Sanskrit writings. Only unbleached ginger (scraped or unscraped) is accepted as a medicinal-grade drug, containing one to five percent or more volatile oil (Langner et al., 1998). The ethnomedical and biological effects information's available on ginger (Zingiber officinale) Roscoe revealed that the root is used for:

2.3.4.1. Anti-inflammatory, analgesic, and anti-pyretic actions:

The anti-inflammatory properties of ginger have been known for centuries (Afzal et al., 2001; Grzanna et al., 2005). Several lines of evidence have been provided, mostly in different animal models of inflammation, and to a much lesser extent in humans or human cells, of the effectiveness of either ginger or of compounds isolated there from against inflammation and its mediators. In the early 1980s, it was reported for the first time that ginger has anti-inflammatory actions, as evidenced by its inhibitory effects on prostaglandins synthesis (Kiuchi et al., 1982). Subsequently, it has been demonstrated that ginger contains constituents for example gingerdiones-14 and shogaols (for example 2, 7–10]) that have pharmacological properties mimicking dual-acting non-steroidal antiinflammatory drugs (NSAIDs) in intact human leukocytes in vitro (Flynn et al., 1968). It is known that such inhibitors have fewer side effects and are more effective than conventional NSAIDs (Charlier and Michaux, 2003; Martel-Pelletier et al., 2003). Further, it has been shown that gingerols are very active in inhibiting both prostaglandins and leukotrienes in RBL-1 cells, and that gingerols with long alkyl side chains are more potent inhibitors of leukotrienes synthesis than of prostaglandins synthesis (Kiuchi et al., 1992). More recently, it has been shown that ginger (and some of its constituents) is
effective against cytokines synthesized and secreted at sites of inflammation (Grzanna et al., 2005). Cytokines are small proteins secreted at sites of inflammation by lymphocytes, macrophages, fibroblasts and other cells, and act as chemical messengers between cells involved in immune and inflammatory responses. Ginger was found to modulate some biochemical pathways activated in chronic inflammation. It was found to inhibit the induction of several genes involved in the inflammatory response, and some of these genes encode cytokines, chemokines and the inducible enzyme cyclo-oxygenase-2 (COX-2) to demonstrate that ginger extract has an effect on human monocyte cell activity (Grzanna et al., 2005). Ginger acts as a dual inhibitor of cyclooxygenase (prostaglandins) and lipoxygenase (leukotrienes) pathways (Morelli et al., 2003; Fajardo and Di Cesare, 2005; Wigler et al., 2003; Chrubasik et al., 2005; Chrubasik et al., 2007; White, 2007). Gingerols act as vanilloid receptor (VR1) agonists (Dedov et al., 2002). The VR1 receptor has been shown to integrate chemical and thermal nociceptive stimuli (Ma and Quirion, 2007). Therefore, direct activation or deactivation of the VR1 receptor at the site where pain is generated during inflammation and other painful conditions provides a new strategy for the development of a new class of peripheral analgesics devoid of the well characterized side effects of currently available analgesics and anti-inflammatory drugs. Inducible nitric oxide synthase (iNOS), a pro-inflammatory enzyme responsible for the generation of nitric oxide (NO), has been implicated in the pathogenesis of inflammatory diseases, and as gingerols are known to have anti-inflammatory properties in vitro (Kiuchi et al., 1992; Kim et al., 2005; Grzanna et al., 2005) conducted an experiment with cultured THP-1 monocytes and showed that the extract can inhibit beta-amyloid peptide-induced cytokine and chemokines expression (Grzanna et al., 2005). In an in-vitro study, the same group showed that extract of Zingiber officinale Rhizome suppresses inflammation due to arthritis through suppression of pro-inflammatory cytokines and chemokines produced by synoviocytes, chondrocytes, and leukocytes. Ginger extract was found to be effective in inhibiting chemokines expression (Phan et al., 2005). The anti-inflammatory, analgesic, and anti-pyretic actions of an ethanolic extract of ginger were studied in rats. The extract reduced carrageenan-induced paw swelling and yeast-induced fever, but was ineffective in suppressing the writhing induced by intraperitoneal acetic acid (Mascolo et al., 1989). A dose-dependent inhibition of prostaglandin release
was also observed using rat peritoneal leucocytes. Thomson et al.,(2002) confirmed the inhibitory action of ginger on prostaglandins when they reported that either oral or intraperitoneal administration of a raw aqueous extract of ginger (500 mg/kg) given to rats daily for four weeks was effective in significantly reducing serum prostaglandin-E2.

A study reported reconfirming the anti-inflammatory, analgesic, and anti-pyretic actions of an ethanolic extract of ginger in rats and mice (Ojewole, 2006). Aktan et al.,(2006) examined the effect of a stable [6]-gingerol metabolite, [6]-ihydroparadol [6]-DHP) and a closely related gingerol analog, RAC-2- hydroxy-1-(4-hydroxy-3-methoxyphenyl) dodecan-3-one [a capsaicin/gingerol (capsarol) analog referred to as ZTX42] on nitric oxide(NO) production, inducible nitric oxide synthase (iNOS) activity and protein expression levels in a murine macrophage cell line. It has been found that ZTX42 and [6]-DHP suppress NO production in murine macrophages by partially inhibiting iNOS enzymatic activity and reducing iNOS protein production, via attenuation of NF-kappa ß-mediated iNOS gene expression, providing a possible mechanism of action for the anti-inflammatory activity reported for this class of compounds. Ginger’s anti-inflammatory and analgesic properties are less well supported in human. However, a review of 8 trials (481 participants) indicates a potential anti-inflammatory effect, which may reduce pain in some conditions, such as osteoarthritis (Terry et al.,2011). Ginger has provided mixed results, with the majority of trials showing a trend toward pain relief greater than placebo but less than traditional anti-inflammatory drugs. Ginger used to treat menstrual cramps, heavy menstrual flow, by inhabitation of the pathways that lead to prostaglandin production (Marcus and Snodgrass, 2005).

2.3.4.2. Gastro-protective effects:
The powdered rhizome of ginger has long been used in traditional medicine for alleviating the symptoms of gastro-intestinal tract illnesses (Afzal et al.,2001). An acetone extract of ginger and its constituents have been shown to enhance the gastric emptying of charcoal meal in mice (Yamahara et al.,1990). Most studies in animals have demonstrated that ginger root extracts increase gastric emptying and gastrointestinal transit (Choudhury et al., 2012). It is established that neither ginger nor its constituents produce the gastrointestinal adverse effects that are usually produced by the conventional NSAIDs as a result of prostaglandin inhibition (Goldstein, 2004; Konturek et al.,2005).
In fact, ginger has been shown to protect against ulceration in rats (Yamahara et al., 1988; Wu et al., 1990). Mahady et al., (2003) were the first to provide evidence that the active constituents of ginger (gingerols) are effective in vitro against Helicobacter pylori, the primary etiological factor associated with dyspepsia, peptic ulcer disease and development of gastric and colon cancer. This was further confirmed by Mahady et al., (2004) and Nostro et al., (2006).

2.3.4.3. Cholesterol lowering effect and Hypoglycemic potential:

It has been reported that treatment with a methanolic extract of dried rhizomes of ginger produced a significant reduction in fructose-induced elevation of lipid levels, body weight. Treatment with an ethyl acetate extract of ginger produced a significant reduction in elevated lipid levels and body weight. The concentration of [6]-gingerol was found to be higher in the methanol extract and less in the ethyl acetate extract. The ginger methanolic extract produces greater effects in comparison with the ethyl acetate extract in fructose-induced hyper lipidemia associated with insulin resistance. The extent of activity appears to be dependent on the concentration of [6]-gingerol present in the extracts (Kadnur and Goyal, 2005). In animal studies, ginger oleo-resin, and dried ginger rhizome were found to reduce hypercholesterolemia by disrupting cholesterol absorption from the gastro-intestinal tract (Chen Jaw-Chyun et al., 2007). Ginger might interfere with the diabetic therapy due to its hypoglycemic effects, (Jellin et al., 2002). Aldose reductase inhibitors found in ginger are now considered to have remarkable potential for the treatment of diabetes and its complications without increased risk of hypoglycemia (Giannoukakis, 2006). It has recently been reported that the assay for aldose reductase inhibitors in ginger led to the isolation of five active compounds including 2-(4-hydroxy-3-methoxyphenyl) ethanol and 2-(4-hydroxy-3-methoxyphenyl) ethanoic acid. These two named compounds were good inhibitors of recombinant human aldose reductase, Furthermore, these compounds significantly suppressed not only sorbitol accumulation in human erythrocytes, but also lens galactitol accumulation in 30% of galactose-fed cataract rats, suggesting that protection against or improvement of diabetic complications could be achieved with a dietary supplement of either ginger or its extract containing aldose reductase inhibitors (Kato et al., 2006). However, more research into this is required, not only because of the limited experimental data, but also because of the lack
of information on the effects of the chronic consumption of ginger in humans (Tapsell et al., 2006). Goyal and Kadnur (2006) administered methanol and ethyl acetate extracts of ginger for eight weeks to mice and found that the treatment reduced goldthioglucose-induced obesity in the treated mice, and further reduced the elevated glucose and insulin levels. It was suggested that ginger had significantly improved insulin sensitivity in these animals. Al-Amin et al., (2006) studied the hypoglycemic potentials of ginger in streptozotocin (STZ)-induced diabetic rats given an aqueous extract of raw ginger daily (500 mg/kg, intraperitoneally) for a period of seven weeks. Blood serum from fasting animals was analyzed for glucose, cholesterol and triacylglycerol levels. The STZ injected rats exhibited hyperglycemia accompanied by weight loss. At a dose of 500 mg/kg, raw ginger was significantly effective in lowering serum glucose, cholesterol and triacylglycerol levels in the ginger-treated diabetic rats compared with the control diabetic rats. Ginger treatment also resulted in a significant reduction in urine protein levels. In addition, ginger-treated diabetic rats sustained their initial weights during the treatment period. Moreover, ginger decreased both water intake and urine output in the STZ-induced diabetic rats. These results confirmed the earlier reports that suggested that raw ginger possesses hypoglycemic, hypocholesterolemic and hypolipidemic potential. Additionally, it showed that raw ginger is effective in reversing the diabetic proteinuria and loss of body weight observed in the diabetic rats. Thus, ginger may be of value in managing the effects of diabetic complications in human subjects (Tapsell et al., 2006).

2.3.4.4. Hormonal Effect:
Recent studies performed on rodents suggest that adding ginger to everyday diet could significantly improve testosterone levels and improve sperm quality (Edward, 2012). Enhanced testosterone production was reported by Chrubasik et al., 2005.

2.3.4.5. Antibacterial properties and anti-platelets effect:
Ginger may have antibacterial properties and antiplatelet effects in vitro, but data are inconsistent (Chaiyakunapruk et al., 2006). O’Mahony et al.,(2005) tested the bactericidal and anti-adhesive properties of ginger and several other culinary and medicinal plants against H. pylori and found that ginger was highly effective in killing Helicobacter pylori, but had lesser ability in inhibiting the adhesion of this bacterium to stomach sections. In vitro investigations have repeatedly shown that an aqueous ginger extract
inhibited the formation of thromboxane B2 and platelet aggregation induced by several aggregating agents, an inhibition that has been explained by an inhibitory effect of ginger on platelet COX enzyme (Jiang et al., 2005; Young et al., 2006). The mechanism of action of ginger, gingerol compounds, and their derivatives has been studied by many authors. Gingerols and their derivatives, especially [8]-paradol, have been reported to be more potent anti-platelet and cyclo-oxygenase-1 (COX-1) inhibitors than aspirin, when tested in vitro by the Chrono Log whole blood platelet aggregometer (Nurtjahja-Tjendraputra et al., 2003). These authors proposed that the carbonyl functional group at C3 found in paradol and in the diarylheptanoid series may contribute to their potent anti-platelet activity and inhibition of COX-1. Inhibition of the arachidonic acid (AA) metabolism cascade via the COX-1/thromboxane synthase system by these phenolic compounds may underline the mechanism of their action. Koo et al., (2001) compared the ability of gingerols and related analogs to that of aspirin in inhibiting AA-induced human platelet serotonin release in vitro using the same dose range, it has been found that gingerols and related analogs were approximately two- to three-fold less potent than aspirin against the platelet release reaction initiated by AA, and two- to four-fold less potent than aspirin at inhibiting AA-induced platelet aggregation. Gingerols inhibited COX activity, assessed by measuring PGD2, a product of AA metabolism by COX. These results suggest that inhibition of COX activity by gingerols and related analogs may be the underlying mechanism for their effect on AA-induced platelet activation.

2.3.4.6. Effects on cardiovascular system:

Several pieces of evidence, mainly from rats studies, have suggested that ginger exerts many direct and indirect effects on blood pressure and heart rate (Afzal et al., 2001). In vitro research indicates that gingerols and the related shogaols exhibit cardiodepressant activity at low doses and Cardiotonic properties at higher doses. In animals, gingerol had inotropic and chronotropic effects. (Chrubasik et al., 2005; White, 2007). Ghayur and Gilani (2005) reported that the crude extract of ginger induced a dose-dependent (0.3–3 mg/kg) fall in the arterial blood pressure of anesthetized rats. In Guinea pig paired atria, the crude extract exhibited a cardiodepressant activity on the rate and force of spontaneous contractions. In rabbit thoracic aorta preparation, the crude extract relaxed the phenylephrine induced vascular contraction at a dose 10 times higher than that
required against K-induced contraction. Ca2+ channel-blocking activity was confirmed when the crude extract shifted the Ca2+ dose–response curves to the right, similar to the effect of verapamil. It also inhibited the phenylephrine control peaks in normal Ca2+-plus and Ca2+-free solutions, indicating that it acts at both the membrane-bound and the intracellular Ca2+ channels. When tested in endothelium-intact rat aorta, it again relaxed the K-induced contraction at a dose 14 times less than that required for relaxing the PE-induced contraction. The vasodilator effect of the crude extract was endothelium-independent because it was not blocked by either L-NAME (a non-selective inhibitor of nitric oxide synthase used experimentally to induce hypertension) or atropine and also was reproduced in the endothelium-denuded preparations in the same dose range. These data indicate that the blood pressure-lowering effect of ginger is mediated through blockade of voltage dependent calcium channels. In another paper, the same group (Ghayur et al., 2005) concluded that the blood pressure lowering action of aqueous ginger extract was through a dual inhibitory effect mediated via stimulation of both muscarinic receptors and blockade of Ca2+ channels. Interestingly, they also noted that the different constituents of ginger might have opposing actions on the reactivity of blood vessels. For example, an atropine-resistant and L-NAME-sensitive vasodilator activity was also noted for the ginger phenolic constituents [6]-, [8]-, and [10]-gingerol, while [6]-shogaol showed a mild vasodilator effect.

2.3.4.7. Anti-tumor, antiproliferative and anticancer effects:
Ginger contains active phenolic compounds such as gingerol, paradol and shogoal that have anti-cancer properties. It has also been shown to down-regulate NF-κ B-regulated gene products involved in cellular proliferation and angiogenesis (Grøntved and Pittler, 2000; Nagasawa, 2002; Miyoshi et al., 2003; McGee, 2004; Chrubasik et al., 2005; Marcus and Snodgrass, 2005; Aggarwal and Shishodia, 2006; Shukla and Singh, 2007; Jeong et al., 2009; Oyagbemi et al., 2010).

2.3.4.8. Radioprotective activity:
The radioprotective effect of the hydroalcoholic extract of ginger rhizome(ZOE) was studied in mice given the extract at an intraperitoneal dose of 10 mg/kg, once daily for five consecutive days before exposure to 6–12 Gy of gamma radiation, and were monitored daily up to 30 days post-irradiation for the development of signs of radiation
sickness and mortality (Jagetia et al., 2003). The protection of ginger against radiation lethality was confirmed by the same authors in a subsequent publication (Jagetia et al., 2004). Pretreatment of mice with ZOE reduced the severity of radiation sickness and the mortality, and protected mice from gastrointestinal syndrome, as well as bone marrow syndrome. The dose reduction factor for ZOE was found to be 1.15. The optimum protective dose of 10 mg/kg ZOE was 1/50 of the LD50 (500 mg/kg). Ginger has been reported to increase glutathione, reduce lipid peroxidation in vivo and scavenging of various free radicals in vitro. Ginger crude plant material and single constituents such as [6]-gingerol, [6]-paradol, phenolic 1,3-diketones and zingerone have been shown to protect against lipid peroxidation in various established models (Chung et al., 2001; Patro et al., 2002; Jagetia et al., 2003; Ippoushi et al., 2003; Jagetia et al., 2004).

2.3.4.9. Hepatoprotective activity:
Several extracts and fractions of Zingiber officinale have been shown to protect against chemically-induced tissue damage. For example, it has been shown by Yemitan and Izegbu (2006) that pretreatment of rats with an ethanol extract of the rhizome of Zingiber officinale and oil extracted from the plant were effective in ameliorating carbon tetrachloride and acetaminophen (paracetamol)-induced acute hepatotoxicity.

2.3.4.10. Anti-emetic effect:
Meta-analyses have suggested possible benefits of ginger in the control of postoperative (Chaiyakunapruk et al., 2006) and pregnancy-related (Matthews et al., 2010) nausea and vomiting, but no benefit for chemotherapy-induced nausea and vomiting (Lee, 2013). Some evidence suggests that constituents of ginger have central anti-emetic activity. Another opinion is that the anti-emetic actions of ginger are more likely due to local effects on the gastro-intestinal tract rather than from effects in the central nervous system. The acetone extract of ginger given orally also significantly inhibited serotonin-induced diarrhea. [6]-Shogaol had a more potent anticathartic action than [6]-dehydroygingerdione, [8]- and [10]-gingerol (Ernst and Pittler, 2000; Lien et al., 2003; Manusirivithaya et al., 2004; Chrubasik et al., 2005; Borrelli et al., 2005). As the evidence that ginger helps alleviate chemotherapy-induced nausea and vomiting is inconclusive, it is not recommended for any clinical uses or for nausea (Marx et al., 2013; Ernst and Pittler, 2000). There is no clear evidence of harm from taking ginger during pregnancy, although
its safety has not been established (Ernst and Pittler, 2000). The effectiveness of ginger in emesis due to hyperemesis gravidarum (Fischer-Rasmussen et al., 1990), motion sickness (Stewart et al., 1991) and cancer chemotherapy is clear (Sharma et al., 1997). Ginger has been recorded as being useful in preventing post-operative nausea and vomiting in humans (Phillips et al., 1993b), without a significant effect on gastric emptying (Phillips et al., 1993a). Recently, it has been confirmed that ginger extract, in addition to having a direct cholinergic agonistic effect on the post-synaptic M3 receptors, also has a possible inhibitory effect on pre-synaptic muscarinic auto receptors, similar to standard muscarinic antagonists (Ghayur et al., 2007). In isolated Guinea pig ileum, several compounds in ginger e for example [6]-gingerol, [6]-shogaol, and galanolactone have been shown to have anti-serotonin (5-hydroxytryptamine; 5HT3) effects (Yamahara et al., 1989; Huang et al., 1991). This may possibly suggest that the anti-emetic action of either ginger or some of its constituents may be mediated centrally via 5-HT3 receptors, as these constituents have small molecular weights and could easily cross the blood brain barrier. In Suncus murinus (a house musk shrew), it has been shown that orally administered [6]-gingerol completely prevented vomiting in response to cyclophosphamide, presumably via a central effect (Yamahara et al., 1989). Cisplatin treatment causes nausea and vomiting in man and animals. Acetone and 50% ethanolic extracts of ginger at oral doses of 25, 50, 100 and 200 mg/kg exhibited significant protection, while aqueous extract at these doses was ineffective against cisplatin emesis in dogs (Sharma et al., 1997), and rats (Sharma and Gupta, 1998).

2.3.4.11. Anti-epileptic and anti-seizure effect:

The principle outcome measure is that proposed by Engel (1978). Engel class I outcome basically equates with seizure freedom, class II with rare seizures, class III with worthwhile improvement and class IV with no worthwhile improvement (Engel, 1987). New antiepileptic drugs initially were tested in patients with refractory partial epilepsy as add-on treatment in randomized placebo controlled trials. They are later on compared with standard treatments in monotherapy studies, predominantly in patients with a new diagnosis of epilepsy. As for new AEDs, the usual measure of efficacy is the percentage of patients who achieve at least a 50% reduction in seizures. Ideally, any choice made between antiepileptic drugs should be based upon the results of comparative randomized
controlled trials. At present there is insufficient evidence to guide a choice between standard treatments such as carbamazepine, phenytoin, and valproate (Marson et al., 1996). The place of new treatments is even more uncertain, as there have been few monotherapy studies comparing new antiepileptic drugs with standard treatments and there have been no studies comparing one new drug with another, whether given as monotherapy or add-on treatment. Nevertheless, doctors are faced with an increasing choice of new antiepileptic drugs to prescribe to refractory patients. Enas and her colleagues, (2012) were able to detect the alkaloid constituents, capsaician and nonivamide of ginger, and they were able to detect the anticonvulsant activity of these constituents through Gamma Amin o Butyric Acid (GABA) and vanilloid (VRI) receptors. The latter anticonvulsant effect was also evidently detected by this group. Capsaician, nonivamide, 6 gingerol and zingerone have 100% anti-pentylenetetrazole and a high (66.6%) anti-maximum electroshock activity.

2.3.4.12. Anti-thrombotic effect:
The effect of an aqueous extract of ginger on platelet thromboxane-B2 (TBX2) and prostaglandin-E2 (PGE2) production was examined after giving rats a raw aqueous extract of ginger daily for a period of 4 weeks, either orally or intraperitoneally (IP). A low dose of ginger (50 mg/kg) administered either orally or IP did not produce any significant reduction in the serum TBX2 levels. However, ginger administered orally caused significant changes in the serumPGE2 at this dose. High doses of ginger (500 mg/kg) were significantly effective in lowering serum PGE2 when given either orally or IP. However, TXB2 levels were significantly lower in rats given 500 mg/kg ginger orally, but not IP. These results suggest that ginger could be used as an anti-thrombotic agent (Thomson et al., 2002).

2.3.4.13. Anti-oxidant activity:
Several authors have shown that ginger is endowed with strong in vitro and in vivo anti-oxidant properties. The antioxidant action of ginger has been proposed as one of the major possible mechanisms for the protective actions of the plant against toxicity and lethality of radiation (Jagetia et al., 2003; Haksar et al., 2006) and a number of toxic agents such as carbon tetrachloride and cisplatin (Amin and Hamza, 2006; Yemitan and Izegbu, 2006), and as an anti-ulcer drug (Siddaraju and Dharmesh, 2007). Recently, it has
been shown that [6]-gingerol is endowed with strong anti-oxidant action both in vivo and in vitro, in addition to strong anti-inflammatory and anti-apoptotic actions (Kim et al., 2007). This makes it a very effective agent for prevention of ultra violet B (UVB)-induced reactive oxygen species production and COX-2 expression, and a possible therapeutic agent against UVB-induced skin disorders.

2.3.5. toxicity and teratogenicity of ginger:
Many reviews have been devoted to specific aspects of ginger’s actions. For example, the review of Grzanna et al., (2005) was on the use of ginger as an anti-inflammatory agent, while that of Shukla and Singh (2007) dealt with the cancer prevention properties of the crude drug. The actions of ginger as a post-operative anti-emetic substance were the subject of a review by Chaiyakunapruk et al., (2006). Some minor adverse effects have been associated with the use of ginger in humans. In one clinical trial that involved 12 healthy volunteers who received ginger orally at a dose of 400 mg of ginger (three times per day for two week), one subject in the study reported mild diarrhea during the first two days of ginger pretreatment. Ginger may cause heartburn, and in doses higher than six gram may act as a gastric irritant. Inhalation of dust from ginger may produce IGE-mediated allergy (Chrubasik et al., 2005). In culinary quantities, the root is generally devoid of activity. Larger doses carry the potential for adverse reactions. Adverse reactions reported in trials are uncommon and include mild gastro-intestinal tract effects such as heartburn, diarrhea, and mouth irritation. Case reports of arrhythmia and immunoglobulin E allergic reaction have been documented (White, 2007; Chrubasik et al., 2005). Ginger is generally considered a safe herbal medicine (Weidner and Sigwart, 2000). Toxicologic information regarding use in humans is lacking. The acute oral lethal dose 50 of ginger oil has been estimated to be more than five gram per kilogram of body weight in rats (White, 2007). A patented ginger extract EV.EXT 33 was administered by oral gavage in concentrations of 100, 333, and 1000 mg/kg, to three groups of 22 pregnant female rats from days six to fifteen of gestation. For comparison, a fourth group received the vehicle, sesame oil. Body weight and food and water intake were recorded during the treatment period. The rats were killed on day 21 of gestation and examined for standard parameters of reproduction performance. The fetuses were examined for signs of teratogenic and toxic effects. The ginger preparation was well tolerated. No deaths or
treatment-related adverse effects were observed. Weight gain and food consumption were similar in all groups during gestation Reproductive performance was not affected by the ginger treatment. Examination of fetuses for external, visceral, and skeletal changes showed neither embryotoxic nor teratogenic effects of the ginger preparation. Based on these results, it was concluded that the ginger preparation EV.EXT 33, when administered to pregnant rats during the period of organogenesis, caused neither maternal nor developmental toxicity at daily doses of up to 1000 mg/kg body weight (Weidner and Sigwart, 2001).

2.3.6. Chemical Constituents of ginger:
The major constituents in ginger rhizomes are carbohydrates (50% - 70%) which are present as starch, amino acids, raw fiber, protein, phytosterols, vitamins e.g., nicotinic acid and vitamin A and minerals are among the other constituents. (Langner, et al., 1998; Yang and Chang, 1998; Grzanna et al., 2005; Chrubasik et al., 2005; Young et al., 2006; Shukla and Singh, 2007). The concentration of lipids is three to eight percent and includes free fatty acids (palmitic, oleic, linoleic, capric, lauric, myristic) and triglycerides. The active ingredients in ginger are thought to reside in its volatile oils, which comprise approximately one to five percent of its weight (Newall et al., 1996). The major active ingredients in ginger oil are the sesquiterpenes: bisapolene, zingiberene, and zingiberol (Connell and Sutherland, 1969; Yoshikawa et al., 1993). Recently other major phytoconstituents are reported as a fragrant including vanillin, Zingerone, Gingerols and Shogaols, (Connell et al., 1969; Yoshikawa et al., 1993; Newall et al., 1996). The odor of ginger depends mainly on its volatile oil and over 50 components of the oil have been characterized and these are mainly monoterpenoids [b-phellandrene, (+)-camphene, cineole, geraniol, curcumene, citral, terpineol, borneol] and sesquiterpenoids [a-zingiberene (30-70%), b-sesquiphellandrene (15-20%), b-bisabolene (10-15%), (E-E)-a-farnesene, arcurcumene, zingiberol]. The characteristic odor and flavor of ginger is caused by a mixture of zingerone, shogaols and gingerols (Choudhury et al., 2012). Some of the oil components are converted into less odor-defining compounds on drying (Langner et al., 1998; Evans, 2002). The pungency of fresh ginger is due primarily to the gingerols, which are a homologous series of phenols. The most abundant is [6]-gingerol, although smaller quantities of other gingerols with different chain lengths are also
present. The pungency of dry ginger mainly results from shogaols (for example, [6]-shogaol), which are dehydrated forms of gingerols. Shogaols are formed from the corresponding gingerol during thermal processing (Wohlmuth et al., 2005). Degradation rates of [6]-gingerol to [6]-shogaol were also found to be pH dependent, with greatest stability at pH four, whereas at 100 °C and pH one, the reversible degradation was relatively rapid (Bhattarai et al., 2001). Thermal degradation of gingerols to gingerone, shogaols, and related compounds was demonstrated by Jolad et al., (2004). The concentrations of active ingredients vary with growing conditions (Mascolo et al., 1989; Connell, 1970). Jolad et al., (2004) examined organically-grown fresh ginger and identified 63 compounds, of which 31 had been previously reported as constituents of ginger and 20 were unknown compounds. The identified components included gingerols, shogaols, 3-dihydroshogaols, paradols, dihydroparadols, acetyl derivatives of gingerols, gingerdiols, mono- and di-acetyl derivatives of gingerdiols, 1-dehydrogingerdiones, diarylheptanoids, and methyl ether derivatives of some of these compounds. In addition to [6]-gingerol (1), [4]-, [7]-, [8]-, and [10]-gingerol (3–6) were identified, as well as methyl [4]-gingerol and methyl [8]-gingerol. [4]-, [6]-, [8]-, [10]- and [12]-shogaol were characterized (2, 7–10), as were methyl [4]-, methyl [6]- and methyl [8]-shogaol. Paradols are 5-deoxygingerols. [6]-Paradol (11), along with [7]-, [8]-, [9]-, [10]-, [11]-, and [13]-paradols were detected in the fresh ginger, as was methyl[6]-paradol. Jolad et al., (2005) also examined commercially processed dry ginger using the same techniques that they had utilized in their earlier study Jolad et al., (2004). They identified a total of 115 compounds, of which 88 were reported. Of these, 45 had been recorded previously for fresh ginger (Jolad, et al., 2004) and 31 were new compounds, which included methyl [8]-paradol, methyl [6]-isogingerol (12) and [6]-isoshogaol (13). The remaining 12 constituents had been isolated previously by other workers. [6]- (14), [8]-, [10]- and [12]-gingerdiones were detected, whereas they had not been previously reported in fresh white and yellow gingers. The concentrations of gingerols in the dry ginger were reduced slightly in comparison to fresh ginger, whereas the concentrations of shogaols increased. Diarylheptanoids have been reported as components of both fresh and dry ginger (for example, (Jolad et al., 2004; Jolad et al., 2005; Ma et al., 2004). Ma et al., (2004) reported the isolation of seven previously unknown diarylheptanoids from the ethanol extract of
Chinese ginger, along with 25 known compounds, including 8- diarylheptanoids. An example of one of the novel compounds reported is (3S,5S)-3,5-diacetoxy-1,7-bis(4-hydroxy-3-methoxyphenyl) heptane. Wei et al., (2005) reported significant cytotoxic and apoptotic activities against human promyelocytic leukemia cells of several ginger constituents, including some diarylheptanoids and gingerol-related compounds. They showed that the following structural features contribute significantly to the enhancement of activity; Acetoxyl groups at the 3- and/or 5-positions of the side chain, The appropriate longer alkyl side chain length, the ortho-diphenoxyl functionality on the aromatic ring and the a,b-unsaturated ketone moiety in the side chain.
Chapter Three
Materials and Methods
Materials and Methods

3.1. Materials and chemicals:

Ranitidine                        Glasco and Smithline, England
Indomethacin                     Amipharma Laboratories, Sudan
Copper sulphate                  Sigma Eldritch, USA
Metoclopramide                   L.B.S Laboratories, India
Diphenyle-2 picryl hydrazyl(DPPH) Sigma Eldritch, USA
Methanol                         SD Fine Chem Limited, India.
Pentylenetetrazole(PTZ)           Sigma Aldarich, USA
Sodium valproate                 Sigma Aldarich, USA
Carbamazepine                    Sigma Aldarich, USA
Tween20                          Sigma Aldarich, USA
Ethanol                          SD Fine Chem Limited, India.

3.2. Preclinical Methods:

3.2.1. Plant collection and Preparation of Extract:

Zingiber officinale Roscoe family Zingiberaceae dried rhizomes were purchased from Wad Medani local market, Sudan. The plant species were identified in the Medicinal and Aromatic Plants Research Institute, National Centre for Research, Ministry of Science and Technology, Khartoum, Sudan. The plant material was cleaned, air dried and was milled into a coarse powder. One kilogram of the powdered plant was macerated with pure ethanol for seven days with occasional shaking. The liquid extract obtained was filtered and dried at 60°C using a rotary evaporator. The resulting mass was stored in a refrigerator until use.

3.2.2. Acute toxicity test of ginger:

Male albino rats (Three animals) weighting 280-310 grams and about eight to ten weeks old obtained from the animal house, Faculty of pharmacy, University of Gezira were used for experimental study. They were housed in poly acrylic cage. Animals were acclimatized for the period of seven days under standard laboratory condition, temperature (25 ± 2°C) with dark and light cycle. All the animals were allowed free
access to diet and water. The animals were fasted over night before administration of ginger extract. Acute oral toxicity method was adapted (Organization for Economic Cooperation and Development Guidelines No 423, 2001) for toxicity study using three animals administered dose of 2 g/kg orally daily for three consecutive days. Changes in weights, skin color, urination, sweating, food intake, water intake, motility, and auditory reflexes were observed. Absence or presence of Extract-related morbidity/mortality would determine the toxicity of the extract (Lorke, 1983).

3.2.3. Gastro protective effect of ginger:

Albino rats weighting 150-200 grams of either sex were used for the study. The animals were housed in an air conditioned with sun light and dark cycles and were provided with diet consisting of normal rat food and water. This assay carried out using the method of Urushidani et al., (1979). eighteen adult rats randomly divided into six groups of three rats each. They were deprived of food for 24 hours but given access of water prior to drug administration. The ginger extract and indomethacin used were freshly prepared as a suspension in 10% solution of Tween-20 and administered orally but raniidine was given subcutaneously. The first group administered 10% solution of Tween-20 in dose of 5ml/kg body weight as control and the second group was given indomethacin suspension in dose of 50 mg/kg body weight, the third group treated by ranitidine injection in dose of 100mg/kg, the 4th, 5th and 6th groups administered ginger oily extract in dose of 100mg/kg, 200mg/kg and 400mg/kg body weight as tests respectively. Half an hour later, all animals in all groups except the control were treated with indomethacin suspension 50 mg/kg body weight orally to induce gastric damage. The animals sacrificed after eight hours using chloroform anesthesia. Stomachs were removed and placed on saline soaked paper until inspection. A longitudinal incision along the greater curvature was made with fine cutter. The stomach was inverted over the index finger and the presence or absence of gastric damage is determined by a magnifier. The presence of lesions, erosions, ulcers, perforations and hyperemia indicated gastric damage. Erosions formed on glandular portions of the stomach were counted and the ulcer index were calculated as described by Main and Whittle (1975). The ulcers were counted and scored as: (0: no ulcer. 1: superficial ulcer. 2: deep ulcer. 3: perforation.) and Ulcer index was counted as follow (UI) = UN+US+UP/10) where UN: Average number of ulcers
per animals; US: Average severity scores; UP: Percentage of animals with ulcers. Ulcer index was compared between the treatment and control groups. The data were analyzed by one way analysis of variance (ANOVA) using SPSS 11.0 software. The results of gastro-protective effect were expressed as "total severity score \( \pm SD \)" P values < 0.05 were considered as significant and P < 0.001 as highly significant.

3.2.4. Anti emetic effect of ginger:
Four Days age young male chicks, weighing from 32-52 gram were purchased from the local market. All chicks were kept under laboratory conditions at room temperature allowed free access of food and water and divided into four groups of five chicks each. Group one received copper sulphate 50mg/kg body weight orally as control while group two administered Metoclopramide 50mg/kg intra-peritoneally. Group three, four, five and six Received ginger extract orally in doses of 25,100, 200 and 400 mg/kg respectively. After 10 minutes copper sulphate was administered orally at dose of 50mg/ kg to all test groups. Each chick kept in large beaker at 25 °C for 10 minutes. The anti-emetic effect was determined by using chick emesis model following the protocols of Akita et al.,(1998). The number of retches were observed during the next ten minutes. The anti-emetic effect assessed as a decrease in number of retches in the treated groups in contrast to the group administered metoclopramide.

3.2.5. *In vitro* antioxidant DPPH(1,1-Diphenyl-2-Picrylhydrazyl) assay:
The antioxidant activity of *Zingiber officinale* Rhizomes was estimated according to the procedure described by Bahman et al.,( 2007). One ml of DPPH solution(0.3 mM) in ethanol was mixed with 2.5 ml taken from different concentrations( 50,100 and 250 µg/ml) of extract. After 30 minutes incubated in dark at room temperature, absorbance was measured in a spectrophotometer at 518 nm. The concentrations were prepared in triplicates and the percentage of the radical scavenging activity (RSA) was calculated by the following equation:

\[
RSA = \frac{\text{Control} - (\text{Sample} - \text{Blank})}{\text{Control}} \times 100
\]

Each 2.5 ml taken from the different concentration plus 1 ml of 0.3 mM DPPH solution considered as sample, and 1 ml of ethanol plus 2.5 ml of each extract was used as control. Quercetin was diluted to final concentrations of 50, 100, 250 and 500 µg/ml in ethanol and used as reference standard.
3.2.6. GC-MS analysis of ginger extract for its active constituents:
GC-MS analysis was carried out for sample of ethanolic extract of ginger in a Shimadzu GC-QP 2012(Japan). Capillary column Rtx 50 was used (30m x0.25mmx 0.25 μm) for the analysis carrier gas helium at flow rate 1.2 ml/min. Ginger ethanolic extract one mg was dissolved in 10 ml dichloromethane. One micro liter injection was made in split mode at an injection temperature of 280 °C. The gas oven was initially held at 80°C for one minute then increased at 7°C/ min to 280 °C data was collected from m/z 40 to m/z 350 at 20 spectra per second.

3.2.7. Assessment of anticonvulsant activity of ginger:
The anticonvulsant activity of ginger was tested by using the maximal electroshock test (MEST) introduced by Putnam and Merritt (1973) combine with pentylenetetrazole (PTZ) seizure test. These bioassays were used in the in vitro screening of new anticonvulsant compounds (Raza et al., 2001; Loscher and Schmidt, 1999).

3.2.7.1. Experimental animals:
Albino rats of both sexes weighting 150-200 g were used. The animals were kept and maintained under appropriate laboratory conditions and allowed free access to water and were fasted over night.

3.2.7.2. Maximal electroshock-induces seizure test:
Maximal electroshock seizure was used to evaluate the anticonvulsant activity of ethanolic extract of ginger. Seizures were induced in rats by delivering electroshock of 50 m A for 0.5 second by means of an electro-convulsiometer (Ugobasile ECT unit 57800) through a pair of ear clip electrodes (Kumar, 2008). For ginger extract (400mg/kg) group of rats of both sexes (n=5) was used. Rat group administered the tested material intraperitoneally (Manigauha et al., 2009). The standard group (n=3) was administered sodium valproate(dissolved in water) at 400mg/kg intraperitoneally. Thirty minutes later rats were observed for MES induced seizure response. The experimental group compared to control group treated with oily extract.

3.2.7.3. Pentylenetetrazole-induced seizures test:
The test was carried out as described by Swinyarad and Kupferberg (1985). For ginger extract, group of rats of both sexes (n = 5) were used. Rat group was received the tested
material at 400mg/kg intraperitoneally. The standard group (n=3) was administered sodium valproate (dissolved in water) at 400mg/kg intraperitoneally. Thirty minutes later, rats were injected with pentylentetrazole (90mg/kg in normal saline) subcutaneously, and observed for induced seizure response. The experimental group compared to the control group treated with normal saline.

3.3. Clinical Trials:
A randomized, cross over controlled study was conducted. Only patients with partial epilepsy were recruited. Treatment was continued for twenty four weeks.

3.3.1. Types of Study:
This prospective hospital-based randomized cross over controlled study investigated add-on ginger effect on different types of primary generalized epilepsies in children aged 2-16 years. Adequate methods of concealment as well as randomized trials were considered.

3.3.2. Ginger Rhizomes Supplement:
Ginger was offered to each volunteer in the form of crude powder mixed with teaspoonful of jam.

3.3.3. Types of participants:
The participants of this study were children with diagnosed primary generalized epilepsies aged 2-16 years. Patients identified from a clinical database and Electroencephalogram (EEG) records attended Wad Medani Children Teaching Hospital Referred Clinics. Data recorded prospectively on demographics and clinical information, seizure types, antiepileptic drug treatment details, seizures onset frequency, AEDs side effects and remission rates. Epilepsy diagnosis was made for each patient, based on the clinical and EEG features according to the ILAE classification where possible. Only children with primary generalized seizures including patients with typical absence seizure (petit mal), atypical absence seizure, generalized tonic-clonic seizure, atonic seizure and myoclonic seizure were included.

3.3.4. Types of interventions:
3.3.4.1. Study groups:
Patients with primary generalized epilepsies aged 2-16 years on monotherapeutic drugs of either Sodium valproate (n=15) or Carbamazepine(n=15) drugs were included as
control groups. While another two test groups received either sodium valproate plus ginger (n=15) or carbamazepine plus ginger(n=15) were considered. The whole sample size was sixty children. Ginger in test groups was administered orally in doses of 250 mg for 2-5 years, 500 mg for 6-11 years and 1000 mg for 12-16 years, mixed with teaspoonful of jam extemporaneously for six months. Children in all groups were followed and assessed every month until a final assessment six months later.

3.3.5. Inclusion criteria:
For inclusion into the study the following criteria were included; the clinical criteria for the diagnosis of IGE were fulfilled from review of the case notes even if there was no EEG and Confirmation of generalized spike-wave and electro clinical diagnosis of epilepsy.

3.3.6. Exclusion criteria:
Patients were excluded if these criteria were satisfied, Focal epilepsy, asymptomatic epilepsy, non defined epilepsy syndromes, Age younger than 2 years and more or older than 16 years, lethal or potentially lethal disorders has to be defined or detected, patients with learning disability or history of psychiatric or behavioral disturbance, inability of parents or guardians to give informed signed consent, enrolment in a concurrent trial that either used treatment that might affect the outcome measures, patients whose parents were unable to comply regularly with drug delivery and absence of daily seizure diary.

3.3.7. Procedures:
Treatment was allocated by stratified block randomization using a number of variables as sex, age at randomization (age groups), presence or absence of factors that increase the risk of treatment failure, parents or child careers education and use of daily seizure diary. Treating doctors were allowed to change treatment if considered to be in the child’s best interest. The following data were recorded from the case notes for each patient included basic demographics, family history of epilepsy in first degree relative, history of febrile convulsions, seizure types and dates of onset, EEG results, anti-epileptic drug treatment history and longest seizure-free period on antiepileptic drug regimen.

3.3.8. Outcome measures:
Complete cessation of seizure, reduction in seizure frequency, reduction in seizure duration and presence or absence of side effects induced by AEDs and/or seizure.
3.3.9. Study limitations:
There may be unquantifiable bias in the ways in which treatments were selected for the patients. Patients may be included without typical EEG abnormality of generalized spike–wave pattern if the clinical diagnosis (based on seizure semiology and demographics) is secured. If there was any doubt over the diagnosis on clinical grounds, they were excluded and there may be a minimized inclusion of those without IGE. This approach kept the study relevant to clinical practice.

3.3.10. Ethical approval:
Ethical clearance was obtained from the ethical committee, University of Gezira and Ministry of Health. Verbal and written consent were obtained from each child’s parent accepted to be included in the study.

3.3.11. Statistical analysis:
This was done by using SPSS version 14.0 (SPSS Inc. Chicago, IL, USA). All values were expressed as mean ± SD. Data were analyzed by one-way ANOVA and difference between means was assessed by a two-tailed Student’s T-test. P ≤ 0.05 was considered statistically significant.
Chapter Four
Results and Discussion
4.1. Results and Discussion of pre clinical Tests:

4.1.1. Acute toxicity test of ginger:

Ginger has been widely used at different parts of the globe as spice and it is considered to be safe herbal medicine with few insignificants and adverse/side effects (Badreldin et al., 2007). Acute toxicity test revealed that, no changes were observed concerning: weight, skin color, urination, sweating, food intake, water intake, motility and auditory reflexes that indicates absence of extract-related morbidity/mortality of the animals dosed. Although ginger is safe, it is sensible to avoid using ginger over a long period of time, pending more studies.

4.1.2. Gastro protective effect of ginger:

In this test indomethacin was used to induce gastric damage and the presence of single or multiple lesions were noted. Erosions, ulcers, perforations and hyperemia were considered; the reason being attributed to inhibition of cytoprotective prostaglandins (Goldstein, 2004; Konturek et al., 2005) leading to gastric damage (Table 4.1) with mean total severity scores of (85). The administration of ranitidine 100mg/kg, ginger 200mg/kg and 400mg/kg, along with indomethacin lowered the mean total severity score to 18, 41 and 36 respectively. Furthermore, results demonstrate that the percentage inhibition of stomach induced damage were 49.13%, 54.30% and 75.90% that produced by ginger 200mg/kg, ginger 400mg/kg and ranitidine respectively. Calculation of ulcer indices (Table 4.2) returned values of 5.23 for indomethacin, 2.66, 2.39 and 1.26 for the group of ginger 200mg/kg, 400mg/kg and ranitidine 100mg/kg respectively.

The gastro-protective effect of ginger (Table 4.2; Fig. 4.1) was in agreement with results noted by Yamahara et al., (1990) and Afzal et al., (2001) where as the powder of ginger was used to alleviate symptoms of gastrointestinal tract illness. It was explained that ginger root in animals increase gastric emptying (Choudhury et al., 2010). Ginger has been shown to protect against ulceration in rats and also to provide evidence that the
active constituents of ginger are effective *in-vitro* against *Helicobacter Pylori* bacteria (Mahady *et al.*, 2003; Mahady *et al.*, 2005; Nostro *et al.*, 2006).

**Table 4.1: Effect of Ginger extract on indomethacin-induced gastric damage in rats.**

<table>
<thead>
<tr>
<th>Treatment group (ml/kg or mg/kg)</th>
<th>Total score</th>
<th>Percentage inhibition</th>
<th>Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: (untreated 100mg/kg )</td>
<td>85</td>
<td>--</td>
<td>5.23</td>
</tr>
<tr>
<td>Group II Ranitidine 100mg/kg</td>
<td>18</td>
<td>75.90</td>
<td>1.26</td>
</tr>
<tr>
<td>Group III (Ginger 200mg/kg )</td>
<td>41</td>
<td>49.13</td>
<td>2.66</td>
</tr>
<tr>
<td>Group IV Ginger 400mg/kg</td>
<td>36</td>
<td>54.3</td>
<td>2.39</td>
</tr>
<tr>
<td>P-value</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Control Group   Indomethacin   Ranitidine   Ginger 200mg/kg Ginger 400mg/kg

**Fig 4.1: Photographs of the gastric mucosa in the control (Tween-20) group, indomethacin 100mg/kg group, Ranitidine 50mg/kg group, ginger 200mg/kg with indomethacin group and ginger 400mg/kg with indomethacin group.**

The results explained that ginger rhizomes extract inhibited the gastric damage induced by indomethacin and its efficacy as a gastro-protective agent was comparable to that of the H2- receptor antagonist ranitidine (P ≤ 0.05). The mechanism of ginger protection of the stomach against gastric damage in part may be due to increase in mucosecretion. Although the exact mechanism of action of the anti-ulcer activities of ginger has not been clearly delineated, the plant contains some active constituents whose ulcer protective properties have been identified such as gingerols, capsaicin and nonivamide.
(Yamahara et al., 1988; Enas., et al 2012). The results of the present study shows that ginger extract possess good potential as an antiulcer agent.

4.1.3. Anti-emetic effect of ginger:

As shown in Table 3, ginger extract significantly inhibited emesis induced by Copper Sulphate (CuSO₄) to an extent greater than metoclopramide (P≤0.001), where at dose of 25 mg/kg produced 84% inhibition of retches while at dose of 100 mg/kg no retch was observed.

**Table 4.2: Anti-emetic activity of methanolic extract of Zingiber officinale Rhizomes against copper sulphate induced emesis in checks.**

<table>
<thead>
<tr>
<th>Drug / dose</th>
<th>Mean No of retches ±SEM</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper sulphate (10ml/kg)</td>
<td>44.4±0.570</td>
<td>0.00</td>
</tr>
<tr>
<td>Metoclopramide (50 mg/kg)</td>
<td>17±1.2748</td>
<td>65%</td>
</tr>
<tr>
<td><em>Zingiber officinale</em> (25mg/kg)</td>
<td>7±0.6124</td>
<td>84%</td>
</tr>
<tr>
<td><em>Zingiber officinale</em> (100mg/kg)</td>
<td>0±0.6124</td>
<td>100%</td>
</tr>
</tbody>
</table>

From chemical point of view, rhizomes of *Zingiber officinale* contain alkaloidal constituents capsaicin and nonivamide and terpenes that may play role that provides scientific basis for its use in folk medicine for emesis and management of GI complication.

4.1.4. Antioxidant activity of ginger:

**Table 4.3: DPPH radical scavenging activity of ginger extract (50, 100 and 250 µg/ml):**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Percentage of radical Scavenging activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Zingiber officinale</em> Extract Quercetin</td>
</tr>
<tr>
<td>250 µg/ml</td>
<td>81.5± SD 87.9±SD</td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>78.5±SD 87.7±SD</td>
</tr>
<tr>
<td>50 µg/ml</td>
<td>68.5±SD 84.7±SD</td>
</tr>
</tbody>
</table>
Table 4.3 showed that the level of antioxidant activity of ginger ethanolic extract was comparable with standard quercetin. The plant extract exhibited antioxidant activity dependant on concentration, since the highest scavenging activity was produced at concentration of 250 µg/ml which scavenged 81.5% of DPPH indicating that ginger is rich in known antioxidant compounds represented by phenolic and alkaloidal contents (Yamahara et al. 1988; Enas et al. 2012). This antioxidant property of ginger is worth noted to make it a very effective in fighting against damage of oxidative stress as free radical scavenges and hence acts as anti inflammatory, anti apoptotic, kidney, liver and cardiovascular protective agent (Kim, et al., 2007; Fuhrman, et al., 2000; Yemitan and Izegbu 2006; Afzal et al., 2001; Grzanna et al., 2005).

4.1.5.1. Gas chromatography- mass spectrometric (GC-MS) analysis of ginger:
Gas chromatography- mass spectrometry (GS-MS) has been used in the present study to analyze ginger ethanolic extract. Ethanolic extract showed diverse compounds as reported in the literature.

Table 4.4: Major components isolated and identified by GC-MS in ginger ethanolic extract

<table>
<thead>
<tr>
<th>Retention time/min</th>
<th>Compound names and synonyms</th>
<th>Area%</th>
<th>Diagnostic MS data (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Base peak</td>
</tr>
<tr>
<td>18.248</td>
<td>3-Decanone-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl), 6-gingerol</td>
<td>18.87</td>
<td>137</td>
</tr>
<tr>
<td>26.537</td>
<td>6-Nonenamide-N-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methyl, capsaicin</td>
<td>13.96</td>
<td>137</td>
</tr>
<tr>
<td>27.926</td>
<td>2-butanone,4-(4-hydroxy-3-methoxyphenyl) Vanillyl acetone Zingiberone/Zingerone</td>
<td>5.03</td>
<td>137</td>
</tr>
<tr>
<td>30.933</td>
<td>N-94-hydroxy-3-methoxy benzyl) Nonamide, Nonivamide</td>
<td>3.16</td>
<td>137</td>
</tr>
</tbody>
</table>
The identified compounds includes gingerols, paradols, zingerone, carinol, alkanes, aldehydes and phenols. In this study and as shown in Table 5, two compounds of vanillyl moiety were detected namely, zingerone and 6-gingerol. 6 gingerol was the most abundant component with area% of more than 18. Additionally capsaicin and its analogue nonivamide are detected as constituents of ginger ethanol extract. The mass spectra of the identified compounds (Capsaicin, nonivamide, zingerone and 6-gingerol) showed a base peak at $m/z$137 that is associated with 4-hydroxy-3-methoxybenzyl grouping (Saha et al., 2003). The presence of these natural molecules in ginger are responsible for ginger biological activities reported including its anti convulsant effect. Findings were consistently made in favor of the kitchen herb ginger.

4.1.6. Anti-convulsant activity of ginger:

In the present study ginger was investigated for the potential Anti-convulsant activity using MES and PTZ induced seizure models in experimental rats. Results obtained (Table 6) showed considerable anti-MES and anti- PTZ activities. Ginger ethanolic extract showed 100% seizure protection at 400mg/kg anti-MES and as anti-PTZ.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zingiber officinale Roscoe Rhizomes Screened for anti-convulsant activity using Maximum Electro Shock(MES) and Pentyline Tetrazole (PTZ)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>Dose (mg/kg)</td>
<td>Models</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>
Table 7: Seizure protection

<table>
<thead>
<tr>
<th></th>
<th>Seizure protection%</th>
<th>Seizure protection%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium valproate</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>(standard) 400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (Negative</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>control) 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>400</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.2. Early stage of clinical studies:

Challenges associated with herbal medicines must be overcome before conducting clinical trials necessary for approval by regulatory authority (Steven, 2015). The traditional safety use of kitchen-herb-ginger and the remarkable results obtained from its preclinical laboratory experiments including anticonvulsant activity in rats, all of which encouraged the attempt of conducting this early-stage clinical trail of the antiepileptic effects of ginger used concomitantly (Add-on) with AEDs, sodium valproate and carbamazepine in children generalized epilepsies. Titration of findings made as case by case basis according to the clinical responses and observational data collected from patients and/or parents, compared to the base-line data.

As shown in Table 7, patients (n=30) treated with add-on ginger plus AEDs increasingly experienced seizure free in 87% of participants at the end of six months duration of chronic treatment. While all of them (100%) experienced reduction in seizure duration and frequency with insignificant differences (P≤ 0.244) compared to the control.
Table 4.6: Children with Generalized Epilepsies and became Seizure Free during six month of treatment with Sodium Valproate(250-500mg/kg/day) or Carbamazepine(250-500mg/kg/day) and/or with add-on Ginger(250-1000mg/kg/day).

<table>
<thead>
<tr>
<th>Time of treatment</th>
<th>Group (control/test)</th>
<th>Patients received Carbamazepine (control)/plus ginger (test)</th>
<th>Patients received sodium Valproate (control)/plus ginger (test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>one month of treatment</td>
<td>Test 3/30(10%)</td>
<td>2/15(13%)</td>
<td>1/15(7%)</td>
</tr>
<tr>
<td></td>
<td>Control 0/30(0%)</td>
<td>0/15(0%)</td>
<td>0/15(0%)</td>
</tr>
<tr>
<td>Two months of treatment</td>
<td>Test 8/30(27%)</td>
<td>8/15(27%)</td>
<td>0/15(0%)</td>
</tr>
<tr>
<td></td>
<td>Control 3/30(10%)</td>
<td>1/15(7%)</td>
<td>2/15(13%)</td>
</tr>
<tr>
<td>Three months of treatment</td>
<td>Test 13/30(43%)</td>
<td>11/15(85%)</td>
<td>2/15(13%)</td>
</tr>
<tr>
<td></td>
<td>Control 3/30(10%)</td>
<td>1/15(33%)</td>
<td>2/15(13%)</td>
</tr>
<tr>
<td>Six months of treatment</td>
<td>Test 26/30(87%)</td>
<td>14/15(93%)</td>
<td>12/15(80%)</td>
</tr>
<tr>
<td></td>
<td>Control 24/30(80%)</td>
<td>13/15(87%)</td>
<td>11/15(73%)</td>
</tr>
</tbody>
</table>
Children treated with AEDs plus add-on ginger experienced significant (P≤ 0.05) reduction in side effects produced by the AEDs and/or by seizure on autonomic nervous system, mainly urination (7/7 = 100%), vomiting (15/15 = 100%) and heart burn (6/6 = 100%) and to less extent defecation (1/2 = 50%), salivation (9/13 = 69%) compared to those administered AEDs alone.

It was found that patients and parents reported a high rate of success (100%) in reducing seizure duration and frequency with add-on ginger plus AEDs. While during six months of treatment with this combination 26/30 = 87% of children become seizure free, it is also worth noted that all patients tolerated ginger very well and no signs of toxicity or serious side effects were reported.

Few clinical studies have been conducted using natural traditional nonconventional anti-epileptic drugs in man and to less extent in children. One of which was the use of water extract of *Nigella sativa* in patients with their usual medication where as, three adult patients out of 12 were seizure free by four weeks (Akhondian *et al.*, 2007). Another clinical trail using Cannabidiol-enriched cannabis was conducted by Porter and Jacobson (2013). In this study, survey was presented to parents belonging to a face book group sharing information about the use of Cannabidiol-enriched cannabis to treat their child’s treatment-resistant seizure. Only two (11%) out of 19 reported complete seizure freedom. Sixteen (84%) of parents reported a reduction in seizure frequency and six (32%) reported a 25-60% seizure reduction.

Study of herbal therapies that supported by tradition throughout history and their promising activity in animal epilepsy models provide reasonable basis for their further early-stage clinical studies. Such therapies have the potential to yield new treatments and preventive options of epilepsy. They could also have novel mode of action, leading to find new molecular targets (Porter and Jacobson, 2013).
Conclusions and Recommendation
Conclusions and Recommendation

5.1. Conclusions:

• Screening of the ginger for gastro protective effect revealed that it has a protective effect on the stomach and can be consider as potential treatment of gastric ulcer and other disorder of GIT.

• The anti-emetic effect of ginger has been investigated and results obtained encouraging the use of ginger as potential antiemetic supplement.

• Screening of ginger In-vitro for anticonvulsant activity in rats revealed that it is of high as potential anticonvulsant agent.

• The antioxidant effect of ginger apparently was high due to the presence of constituents that scavenging the free radicals.

• The In-vivo trails of ginger on children with generalized epilepsies proved that it can be used as co-drug in treatment of generalized epilepsies and to counteract the effects produced by AEDs and/or seizure on autonomic nervous system.

Finally we can conclude that: The present investigation of ginger used concomitantly with AEDs, mainly Sodium valproate and Carbamazepine in childhood generalized epilepsies, appears to be of encouraging results and prove that ginger could has a place in epilepsy treatment for many reasons as:

• No side effects were observed as to require ginger intake discontinuation.

• In most of the treated children an improvement of seizure burden was obtained and provide evidence of promising effects.

• Positive satisfactory parents/patients reports.

• The absence of the negative side effects commonly associated with AEDs and/or seizure such as urination, defecation, salivation, sweating Vomiting and heart burn.
5.2. Recommendation:

The overall positive results on seizure control in test groups of patients with generalized childhood epilepsies suggest that further studies of ginger are warranted. Furthermore, to know whether these activity responses are due to a specific bioactive component and/or due to synergistic action of the combined phytoconstituents there in. Bioguided phytochemical investigation is needed to explore the findings.

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