THE EFFECTS OF CRUDE LYOPHILISED AQUEOUS EXTRACT OF Vernonia hochstetteri Schip. Bip. IN RATS WITH PILOCARPINE–INDUCED STATUS EPILEPTICUS

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DEDICATION

This thesis is a special dedication to herbal practice in Kenya.
DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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ABSTRACT

Vernonia hochstetteri (Compositae), a medicinal plant used in traditional medicine to treat epilepsy by East African communities, was screened for anticonvulsant properties in rats with pilocarpine-induced status epilepticus (SE). Pretreatment with crude lyophilised aqueous extract of *V. hochstetteri* (2 g/kg, i.p. - 6 g/kg, i.p.) increased seizure latency in rats. Oral and intraperitoneal pretreatment with lyophilized aqueous extract of *V. hochstetteri* (6 g/kg) prior to pilocarpine (320 mg/kg, i.p.) treatment significantly decreased seizure frequency and duration when compared with controls (P < 0.05). Pretreatment with crude lyophilized aqueous extract of *V. hochstetteri* (6 g/kg, i.p.) decreased pilocarpine-induced mortalities compared to saline pretreated rats (P < 0.05). At a dose of 6 g/kg, i.p, lyophilized aqueous extract of *V. hochstetteri* had minimal acute behavioral neurotoxicity in rats as determined by the position sense test and gait and stance test. Phenobarbital significantly delayed onset of spiking in the EEG of pilocarpine treated rats by 29.5 ± 6.35 min. (P < 0.05). *V. hochstetteri* pretreatment did not significantly delay onset of spiking (15.5 ± 6.35 min) in the pilocarpine treated rats EEG compared to saline pretreated rats (onset of spiking at 14.5 ± 6.35 min) (P > 0.05). These results suggest that *V. hochstetteri* has anticonvulsant principle(s) against pilocarpine-induced seizures in rats.
Chapter 1

Introduction:

Epilepsy is a common chronic brain disorder characterized by recurrent seizures due to excessive discharge of cerebral neurons. Every type of cerebral pathology or insult to the brain could give rise to epileptic seizures. Consequently, every human being is a potential epileptic (1). The prevalence rate for epilepsy in Rochester, Minnesota, was found to be 4-8 cases per 1000 population and the annual incidence rate including patients with recurrent seizures typically varied from between 30 and 50 per 100,000 population per year (2). In Finland, Kera’nen (3) reported an incidence rate of 24/100,000 and a prevalence rate of 6.3/1000 in adult patients with epilepsy. Epilepsy is an important health problem in developing countries where its prevalence can be up to 57 per 1000 population (4). In a study to determine the profile of epilepsy in a developing country among 1000 epileptics, it was found out that partial epilepsy was far more common than generalized epilepsy (80% versus 20%). The higher incidence of partial epilepsy in these patients was attributed to greater frequency of CNS infections and birth injuries (5). In a rural Kenyan population, the prevalence of epilepsy was found to be 0.4% but this figure was reported to be an underestimate of the severity of the situation. Uncontrolled and poorly managed epilepsy inevitably leads to increased risk of premature death among Kenyan epileptics (6). Control of epilepsy has primarily focused on suppressing seizure activity by anti-epileptic drugs (AEDs) after epilepsy has developed. AEDs have greatly improved the lives of people with epilepsy (7). Although beneficial, synthetic psychotropics are usually unavailable to approximately 80% of the world’s population.
Accordingly, the WHO fact sheet N134 reports that a large proportion of the population in a number of developing countries still rely on traditional practitioners such as herbalists and on local medicinal plants to satisfy their primary health care needs (9).

Animal models of epilepsy have been used to screen potential AEDs; indeed several medicinal plants used in folklore medicine to treat epilepsy have been screened for antiepileptic potential using standard rodent models of partial and generalized epilepsy (10, 11, 12, 13, 14). *V. hochstetteri* (Figure 1), is a medicinal plant used to treat epilepsy in East Africa (15). Acute pilocarpine-induced status epilepticus in rats was used to screen crude lyophilized aqueous extract of *V. hochstetteri* for its potential as an anticonvulsant.
Figure 1: Herbarium specimen of *V. hochstetteri*. *V. hochstetteri* is found in mature forests, on edge of swamps. It is a shrub of about 2 meters high and was collected at Kieni forest near Uplands, Nairobi.
Fig 1
1.1: OVERALL OBJECTIVE:

To screen crude lyophilised aqueous extract of *V. hochstetteri* for anticonvulsant properties in rats.

1.1.1: SPECIFIC OBJECTIVES:

1. To test the effect of crude lyophilised aqueous extract of *V. hochstetteri* on seizure latency, duration, and frequency in rats with pilocarpine-induced status epilepticus.

2. To determine the effect of pretreatment with crude lyophilised aqueous extract of *V. hochstetteri* on pilocarpine-induced status epilepticus mortalities in rats.

3. To screen crude lyophilised aqueous extract of *V. hochstetteri* for acute behavioral neurotoxicity in rats.

4. To determine the effect of crude lyophilised aqueous extract of *V. hochstetteri* on the latency of EEG epileptic activity in pilocarpine treated rats.

1.1.2: HYPOTHESIS

Crude lyophilized aqueous extract of *V. hochstetteri*, increases seizure latency, reduces seizure duration and frequency, and delays the appearance of epileptic activity in the EEG of pilocarpine treated rats.
2.0: REVIEW OF THE LITERATURE.

2.1 Epilepsy

2.1.1 Definition

Epileptic seizures can be defined as the manifestation of abnormal, excessive neuronal activity in the gray matter of the cerebral cortex (1). Seizures can be caused by a variety of pathological conditions including acquired brain injuries and genetic abnormalities. Many biochemical and physiological disturbances of brain function can also provoke seizures (16, 17, 18). When unprovoked seizures occur recurrently, characterizing a diverse collection of brain disorders, the condition is referred to as epilepsy (19).

2.1.2: Epidemiology

No large-scale study has been carried out to determine the incidence of epilepsy in Africa, but epidemiological studies have confirmed the importance of postnatal insults as the cause of epilepsy in Africa. Family history is also considered a risk factor for epilepsy in epidemiological terms (20). Epilepsy is an important health problem in developing countries where its prevalence can be up to 57 per 1000 population. The prevalence of epilepsy is particularly high in several African countries, notably Liberia, Nigeria and Tanzania. Parasitic infections such as neurocysticercosis are important etiological factors for epilepsy in these countries. Other reasons for high prevalence include intracranial infections by bacteria, viruses, toxic agents and hereditary factors (4).
In a study to determine the prevalence and incidence of epilepsy in a rural Tanzanian district, it was found that the annual incidence was 73.3 in 100,000 (21).

2.1.3 Classification

The classification of epileptic seizures is based on clinical events (seizure type) and characteristics of the electroencephalogram (EEG) (22). According to this classification, epileptic seizures are divided into partial and generalized seizures. Partial seizures are those which, in general, the first clinical and EEG changes indicate initial activation of a system of neurons limited to a part of one cerebral hemisphere. Partial seizures are classified as simple when consciousness is retained and complex when consciousness is impaired. Simple partial seizures can progress to become complex and they are further classified according to symptoms: motor, sensory, autonomic and psychic. Both simple and complex partial seizures can evolve into secondary generalized seizures with characteristic tonic and clonic manifestations. Generalized seizures are those in which the first clinical manifestation indicates initial involvement of both hemispheres. The initial ictal EEG pattern are bilateral and presumably reflect neuronal discharge, which is widespread in both hemispheres. Generalized seizures are further divided into tonic-clonic, tonic, clonic, absence, myoclonic and atonic seizures. Diverse conditions such as CNS infections, brain tumor, and craniocerebral trauma express themselves by occurrences of recurrent seizures. Classification of epilepsy and epileptic syndromes, which reflect etiology and prognosis, specifies more than 40 distinct types of epileptic syndromes, characterized by signs, symptoms, seizure types, cause, age at onset and EEG pattern occurring together (23).
In spite of this elaborate classification of epilepsy, only a small proportion of patients fit into specific syndromes and a large proportion fall into nonspecific categories, therefore, the classification of epilepsy is still being refined (24).

2.1.4: Epileptic Process

Epileptogenesis refers to a variety of progressive biochemical, anatomical and physiological changes that lead to spontaneous recurrent seizures (25). It is well known from experimental studies with animal models, that there is a latent period between the induction of a localized cerebral insult, such as head trauma or status epilepticus, and the appearance of a chronic epileptic condition. During the latent period, neuronal loss and abnormal synaptic reorganization occurs. This reorganization of the neuronal integration leads to abnormally increased excitability and synchronization, and eventually to the occurrence of spontaneous seizures (26). Once developed, epilepsy should not be viewed as a random succession of seizures but as a dynamic process, which results in both ictal phenomena and interictal functional and structural abnormalities in the brain (27). Patients who develop chronic intractable epilepsy demonstrate progression in both the number of seizures and seizure-related neurological symptoms such as cognitive and behavioural disorders (28). The termination or arrest of seizure activity might depend on a variety of mechanisms. These are, postsynaptic inhibition acting via hyperpolarization of the postsynaptic membrane and presynaptic inhibition, which acts on excitatory fibers and exerts an indirect inhibitory effect on the neuron. Linked to these neural pathways are neural transmitter substances with inhibitory action (29). The inhibitory influences are likely to emanate from the cerebellum (30).
Purkinje cells of the cerebellum have been shown to give rise to inhibitory actions (31). Negative feedback mechanisms have been demonstrated in the hippocampal basket cells acting upon the adjacent pyramidal cells (32), in the ventrobasal thalamus (33), and at the neocortical level (34).

2.1.5 Prognosis

Evidence from population-based studies show that in 50%-70% of patients, seizures will be controlled with an initial anti-epileptic drug, regardless of the specific drug used (35, 36). Up to 30% of all epilepsy patients will develop intractable epilepsy (25). Epilepsy is considered intractable when a patient has epileptic seizures or other symptoms of epileptic syndrome despite optimal treatment, and these symptoms restrict the patient’s ability to lead a full and safe life (1).

2.1.6 Status Epilepticus

Status epilepticus is defined as a condition characterized by an epileptic seizure that is so frequently repeated or so prolonged as to create a fixed and lasting condition (37). An operational definition of status epilepticus is either, continuous seizures lasting at least 5 minutes, or two or more discrete seizures between which there is incomplete recovery of consciousness (38). The morbidity and mortality from status epilepticus are related to three factors: damage to the CNS caused by acute insult precipitating the status epilepticus, systemic stress from repeated generalized tonic-clonic convulsions, and injury from repetitive electrical discharges within the CNS (39).
2.2: Temporal Lobe Epilepsy (TLE)

Temporal lobe epilepsy (TLE) is a localization related epilepsy with typical clinical and EEG characteristics (23). Patients with temporal lobe complex partial seizures constitute the majority of patients referred for surgical consideration (1). In most TLE patients, seizures begin in the mesial temporal structures, specifically in the hippocampus (40). The entorhinal cortex, in particular, serves as a gate for seizure propagation in both experimental (pilocarpine) and in vitro seizure models (41, 42).

2.2.1: Seizure semiology and electroencephalographic findings

Temporal lobe seizures are characterized by simple partial seizures, complex partial seizures and secondarily generalized seizures or a combination of these (23). Most temporal lobe seizures begin with a simple partial seizure such as an abdominal visceral sensation, or an experiential phenomenon (deja vu) (28). Prominent ictal features include arrest of activity, oral-alimentary automatism, motor phenomena and commonly amnesia (43). Interictal and ictal EEG patterns observed in TLE show unilateral or bilateral synchronous, or asynchronous temporal spikes, sharp waves and/or slow waves (44). Systemic administration of the cholinergic agent, pilocarpine, in experimental TLE induces EEG changes in rats, these are, significant theta rhythm, isolated spikes and synchronization of activity in the hippocampus (45). Pilocarpine induces increase in the amplitude of the orthodromic population spike in the hippocampal CA3 area of the urathane-anaesthetised rats (46).
2.2.2: Etiology

Temporal lobe epilepsy may develop after a variety of insults such as head trauma, birth injury or CNS infection (28). When seizures result from specific cerebral pathologic substrate in the temporal lobe, such as traumatic scar, TLE is classified as remote symptomatic. A common factor predisposing some patients to TLE is the occurrence of complex febrile convulsions (47). Cryptogenic TLE refers to an epileptic disorder where no etiology of the condition can be determined. A frequently encountered finding in patients with intractable TLE, both cryptogenic and symptomatic, is hippocampal sclerosis (40).

2.2.3: Histopathological findings in experimental TLE

There is neuronal loss in the CA3 and CA1 subregions of the hippocampus in the pilocarpine murine model of temporal lobe epilepsy (48). In other neuropathological studies in rodents, pilocarpine-induced seizures damage the forebrain, hippocampus, amygdala, thalamus, olfactory cortex, neocortex and substantia nigra (49).

2.2.4: Causes of medial temporal lobe damage

Clinically, TLE often starts as an isolated, prolonged convulsion in early life, followed by a period of remission, after which seizures reemerge and may become intractable. Several histopathological studies have reported a significant correlation between serious childhood illness such as infections, febrile convulsions, head injury, status epilepticus and hippocampal sclerosis in patients with TLE (50, 51).
Patients with an initial precipitating injury such as prolonged seizures, status epilepticus, head trauma or encephalitis before the onset of chronic TLE had severe neuronal loss in the pattern of hippocampal sclerosis (52).

2.2.5: Functional consequences of medial temporal lobe damage

The hippocampus and the adjacent anatomically related cortex, including the entorhinal cortex, perirhinal cortex and parahippocampal cortices, form the neural system for declarative memory (53). A bilateral lesion limited to field CA1 of the hippocampus leads to permanent loss of ability to consolidate short-term memory to long-term memory in adults (54). In humans, damage involving the parahippocampal gyrus produces a syndrome of topographical disorientation (55). Studies in animal models show that the amygdala is crucial for the normal regulation of emotions (56). It is also involved in learning and storage of information about emotional significance of events.

Selective damage in the emotional memory system, results in inability to identify emotional facial expressions, which has been observed in some epilepsy patients after amygdalectomy (57). Global hyposxuality is a very common finding in temporal lobe epilepsy and occurs in a vast majority of these patients (58, 59). Personality changes attributed to epilepsy are mostly confined to TLE (1, 27).
Seizures produced by the systemic administration of pilocarpine hydrochloride, a cholinergic muscarinic agonist, are a useful model of epilepsy. Pilocarpine–induced seizures in rats and mice are characterized by sequential development of behavioral and electroencephalographic signs, which are followed by widespread damage to the forebrain, hippocampus, amygdala, thalamus, olfactory cortex, neocortex and substantia nigra. In experiments designed to examine the neuronal networks engaged in the generation and spread of pilocarpine-induced convulsions, a marked role of the basal ganglia was demonstrated. The caudate–putamen, the substantia nigra and the entopenducular nucleus were found to govern the propagation of seizures produced by pilocarpine (49). Pilocarpine-induced chronic seizures replicate several features of human temporal lobe epilepsy (hippocampal cell loss, supra- and intragranular mossy fiber sprouting, dentate granule cell dispersion, and spontaneous recurrent seizures), and are a useful model in studying the human condition (26). Following 380 mg/kg, i.p., pilocarpine treatment in rats, three distinct phases are observed: 1) an acute period of 1-2 days which corresponds to a pattern of repetitive seizures and status epilepticus, 2) a silent period (4-44 days) characterized by progressive return to normal EEG and behavior, 3) a period of recurrent seizures, which starts 5-45 days after pilocarpine treatment and lasts for 120 days. The seizures last for 50-60 seconds, recur 2-3 times per week, and are more frequent during the light period of the light–dark cycle (60, 61). The pilocarpine model of epilepsy is a useful model for screening anti-epilepsy drugs (AEDs). Local or systemic administration of pilocarpine in rodents leads to a pattern of repetitive limbic seizures and status epilepticus, which can last for several hours.

2.2.6: Animal experimental model for temporal lobe epilepsy
A latent period follows status epilepticus and precedes a chronic phase, which is characterized by the occurrence of spontaneous limbic seizures. These distinct features have allowed anti-epileptic drugs (AEDs) studies with different purposes: (a) in the acute phase, identification of compounds with efficacy against refractory status epilepticus or neuroprotection against damage induced by sustained seizures, (b) in the latent period, identification of agents with a potential for preventing epileptogenesis or against seizure-induced long term behavioral deficits or both (c) in the chronic phase, testing drugs effective against partial and secondary generalized seizures (62). A comparison of the pharmacology of chronic models with models of acute (reactive or provoked) seizures in previously healthy (nonepileptic) animals, demonstrates that drug testing in chronic models of epilepsy yields data which is more predictive of clinical efficacy and adverse effects than reactive models, and therefore chronic models should be more frequently used for routine preclinical screening of potential AEDs than the reactive models. A problem for clinical validation of the TLE models is the lack of any AED, which effectively prevents epilepsy in humans. Indeed clinically utilized drugs such as carbamazepine and diphenylhydantoin block neither convulsions nor brain damage induced by pilocarpine, and ethosuximide and acetazolamide increase the susceptibility of rats to the convulsant action of pilocarpine (7). The resistance of seizures produced by pilocarpine in rats to anti-epileptic drugs reaffirms the clinically obvious lack of effective treatments for limbic convulsions (63). There are inter animal variations in incidence and severity of pilocarpine-induced convulsions. Variations in seizure onset time (SOT) are due to food deprivation, repeated nociceptive stimulation, continuous lighting, handling during the previous 7 to 14 days and blood corticosterone levels.
These suggest that neurological mechanisms affecting the range in SOT could involve the adrenocorticotropic-corticosterone hormone system. Previous studies have shown that the susceptibility to pilocarpine induced status epilepticus (SE) in female rats changes according to estrous cycle phases, suggesting that female sexual hormones could have protective effects against pilocarpine induced SE (64). Finally, the susceptibility of rats to pilocarpine-induced seizures is age-dependent. During the third week of life, rats show increased susceptibility to the convulsant action of pilocarpine relative to younger and older rats. The susceptibility to seizures induced by pilocarpine increases in rats aged over 4-months (65). Behavioral characteristics are used to quantify pilocarpine-induced convulsions, however most of the current tools used to quantify seizure events need to be coupled to electrophysiology and more sophisticated systems for recording and analyzing behavior (62).

2.3: Herbal Medicines in Epilepsy Treatment
The use of alternative medicine by epileptic patients who have used these forms of treatment before seeking hospital treatment has been evaluated. Among the 265 epileptic patients studied, 47.6% used African traditional medicine alone, 24.1% combined traditional medicine with spiritual healing, 20.4% used spiritual healing alone, and 7.5% used other forms of alternative medicine. Patients used the alternative treatment for < 1 year to > 5 years before seeking hospital treatment, presumably when alternative medicine failed to control seizures. After initiation of hospital treatment, only 14.6% of patients who had earlier used African traditional medicine continued with such treatment.
This observation suggests that the practice of alternative medicine has widespread use in the management of epilepsy in Africa. Further investigations are required to determine the efficacy, supportive role, and limitations of alternative medicine in the management of epilepsy in developing countries (66). The use of herbal products to treat a wide range of conditions is rising rapidly, leading to increased intake of phytochemicals. However, recent studies have revealed potentially fatal interaction between herbal remedies used in the treatment of epilepsy and conventional drugs, for instance, in transplant patients self-medication with St. John’s Wort (Hypericum perforatum) has led to a drop in plasma levels of immunosuppressant drug, cyclosporine, causing tissue rejection. Herbal remedies with potential to modulate cytochrome P450 activity and thus participate in interaction with conventional drugs include Milk thistle, Angelica dahurica, ginseng, danshen and liquorice. If potential drug interactions are to be predicted, it is essential that the ability of herbal products to interfere with drug metabolizing enzyme systems be fully established (67). Toxicity related to traditional medicines is becoming more widely recognized as these remedies become popular in developed countries. Accidental herbal toxicity occurs not only as a result of lack of pharmaceutical quality control in harvesting and preparation but also because herbal remedies are believed to be harmless (68). Ginkgo biloba extract, advertised as improving cognitive function and treating epilepsy, has been reported to cause spontaneous bleeding, and may interact with anticoagulant and antiplatelet agents. St. John’s Wort, promoted for treatment of depression may have monoamine oxidase-inhibiting effect and thus cause increased levels of serotonin, dopamine and norepinephrine. Ephedrine containing herbal products have been associated with adverse cardiovascular events, seizures and even death (69).
Although beneficial, synthetic psychotropics are usually unavailable to approximately 80% of the world’s population, and therefore improved understanding of appropriate and safe use of naturally occurring substances as psychotropic agents will greatly contribute to global mental healthcare (8). Several plant extracts have been screened for antiepileptic potential. The ethyl ether and hydroalcoholic extract of Magnolia grandiflora L. (Magnoliaceae) seeds, a popular plant used in the Mexican traditional medicine were used in studies with adult Wistar rats. Oral administration of 250 mg/kg abolished extensor reflex of maximal electric induced-seizures in 50% of experimental animals. The results suggest that the chemical constituents of this plant could have utility in the control of epileptic seizures (10). In another study, the effect of the essential oil of Eugenia caryophyllata (Myrtaceae), an anti-epileptic remedy in Iranian folk medicine, against seizures induced by maximal electroshock (MES) and pentylenetetrazole (PTZ) in male mice was studied. The essential oil exhibited anticonvulsant activity against tonic seizures induced by MES and increased the seizure threshold of PTZ, determined by an increase in the dose of intravenously infused PTZ required to induce clonus. In addition, at some anticonvulsant doses, the essential oil produced motor impairment of the rototord (13). The lyophilized root decoction of Afrormosia laxiflora (Leguminosae) claimed to be beneficial in epilepsy and psychoses was screened for depressant and anticonvulsant activities using in vivo models. The root decoction, 150-300 mg/kg, showed significant inhibition of motor activity in mice, indicating depressant actions. Similarly, doses of 150-300 mg/kg of this extract significantly diminished the duration of convulsive symptom and increased the seizure latency, in both picrotoxin and electroshock-induced seizures when compared with the controls.
The results suggest possible beneficial effects of the plants' roots, and offer a rational explanation for its folklore usage in epilepsy and related disorders (12). *Delphinium denudatum* Wall. (Ranunculaceae) is a medicinal herb used in the treatment of epilepsy in India. An aqueous fraction (FS-1) isolated from the roots of *D. denudatum* exhibits very potent anticonvulsant activity that is comparable to the effects of a well-known antiepileptic drug, phenytoin, in CF1 mice (14). The anticonvulsant effect of Chai-Hu-Long-Ku-Mu-Li-Tan (TW-001), a Chinese herbal medicine, and its mechanism of action in several rodent models of generalized seizures has been evaluated. TW-001 (4 g/kg, orally) significantly increased the threshold for tonic electroconvulsion, and the threshold for tonic seizures in response to i.v infusion of pentylenetetrazol (PTZ) in the s.c. PTZ seizure test. Both the incidence and severity of seizures was decreased by TW-001 (70). Japanese herbal medicine (Kampo) is used in the treatment of intractable epilepsy (71). A herbal mixture, “Saikokeishito-Ka-Shakuyaku” (SK or TJ-960), is used to treat epilepsy (72). TJ-960/SK shows normalizing effects on intracellular calcium-related and protein-related pathological changes induced by pentylenetetrazol (PTZ) application in snail neurons and shows marked protective effects against neuron damage induced by the cobalt focus epilepsy model, cytochalasin B and severe stress (73). TJ-960 shows complete preventive effects on the abnormal expression of one of the seizure related gene (SEZ), PTZ – 17, induced by PTZ in *Xenopus* oocytes injected with PTZ-17 RNA (74). Herbal prescriptions are promising candidates for new AEDs development (72). *Vernonia* spp. (Asteraceae) is of pharmacological significance.
Methanolic extracts of *Vernonia* analyzed with High Pressure Liquid Chromatography (HPLC) and Centrifugal Partition Chromatography (CPC) show the main phytochemicals to be triterpene glycosides, sesquiterpene lactones, flavones, flavonic glycosides and dicaffeoylquinic acids (75). Several species of this genus used in folklore medicine have been screened for biological activity (76, 77, 78, 79, 80, 81, 82, 83, 84). *V. hochstetteri* is a tall, often single stemmed shrub with ovate to oblong minutely toothed leaves. In Kenya, it is found in Elgon Highlands, Cherangani Highlands, Loita Highlands, Aberdare Highlands, Mumias, Machakos, Nairobi, and Kajiado (Figure 2), (85). In East Africa, *V. hochstetteri* decoction of whole plant is drunk to treat epilepsy (15).
Figure 2: (•) Distribution of *V. hochstetteri* in Kenya. In Kenya *V. hochstetteri* is found in Elgon Highlands, Cherangani Highlands, Loita Highlands, Aberdare Highlands, Mumias, Machakos, Nairobi and Kajiado.
Fig 2
3.0: MATERIALS AND METHODS

3.1: Plant material:

_Vernonia hochstetteri_ was collected from Kieni forest (Uplands, Nairobi) and authenticated by the University of Nairobi herbarium. The leaves, bark, flowers and roots from a single plant were macerated, homogenized and boiled in 5 liters of water for 1-hour, to obtain an aqueous fraction / decoction* (15). The aqueous fraction was filtered to yield 3.2 liters of dark brown liquid, which was then lyophilized to yield 192 grams of white powder. The lyophilized extract was stored at 4 °C. The lyophilised aqueous extract was then redisolved in saline at a concentration of 1g _V. hochstetteri_ extract / 1ml of saline. The term _V. hochstetteri_, unless otherwise indicated will mean crude lyophilised aqueous extract of _V. hochstetteri_.

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*Note: The term "decoction" refers to the traditional process of steeping or boiling plant materials in water to extract their properties for medicinal or culinary purposes.
The scheme for preparation of lyophilized aqueous extract of *V. hochstetteri* is shown (Figure 3).

* Protocol for preparation of plant material was based on how it is traditionally prepared, which is not reproducible.

3.2: Animal and animal welfare:

Adult male Sprague-Dawley rats (*n* = 83, 300 - 400g) were purchased from the Biochemistry Department, University of Nairobi. The rats were kept according to guidelines for the care and use of mammals in neuroscience and behavioral research (86). The rats were kept in groups of five in aluminum-bottomed cages with wire tops (65cm x 45cm x 30cm).
The rats had access to water and rat chow (Unga feeds, Nairobi) *ad-libitum* and were subjected to standard light/dark cycles (lights on from 6.00am to 6.00pm) and average room temperatures of 23 ± 5.9 °C. Their beddings, which consisted of sawdust, were changed weekly. The rats were weighed one week before experiments commenced.

### 3.3: (Experiment 1)

**Effects of different doses of *V. hochstetteri* on seizure latency in pilocarpine treated rats:** 35 rats were used in this experiment: 5 controls and the rest randomly divided into 6 groups of five rats and given the following doses of *V. hochstetteri*, i.p. (i) group A – 0.2 g/kg (ii) group B – 1 g/kg (iii) group C – 2 g/kg (iv) group D – 3 g/kg (v) group E – 4 g/kg (vi) group F – 6 g/kg. The rats were injected with *V. hochstetteri*, 1-hour before pilocarpine injection (pretreatment at this time was found to significantly increase seizure latency, defined as onset of forelimb clonus with lordosis in pilocarpine treated rats, in a pilot study). The rats were injected with atropine (1 mg/1kg, i.p. Rotexmedica, Germany), a muscarinic receptor antagonist, to prevent the peripheral effects of pilocarpine. Pilocarpine hydrochloride (320 mg/kg, i.p. Aldrich, USA) was administered 30 minutes after atropine injection. The controls (n = 5) were given 3 ml of saline (as this was the highest volume used to redisolve *V. hochstetteri*) 1-hour prior to pilocarpine treatment as described above. All the pilocarpine treated rats were observed for seizure latency (time taken to observe forelimb clonus with lordosis after pilocarpine (320 mg/kg, i.p.) treatment). A technician who was blind to the experimental treatments administered the drugs in all the experiments to minimize observation bias.
3.4: (Experiment 2)

**Effects of *V. hochstetteri* and phenobarbital on seizure latency, frequency and duration in pilocarpine-treated rats:** 20 rats were used in this experiment. 5 rats chosen randomly were pretreated with *V. hochstetteri* (6 g/kg, i.p.), 5 other randomly chosen rats were orally pretreated with *V. hochstetteri* (6 g/kg) and 5 other rats were pretreated with phenobarbital (10 mg/kg, i.p.) as positive control. The remaining 5 rats were pretreated with 3 ml of saline (as this volume was used to dissolve *V. hochstetteri* and phenobarbital). All the 20 rats were pretreated with *V. hochstetteri*, phenobarbital or saline 1-hour prior to pilocarpine (320 mg/kg, i.p.) treatment as in experiment 1. The protocol for drug treatments is shown (Figure 4)

![Figure 4](image-url)

- n = 5 phenobarbital (10 mg /kg, i.p.)
- n = 5 *V. hochstetteri* (6 g /kg, i.p.)
- n = 5 *V. hochstetteri* (6 g /kg, orally)
- n = 5 Saline (3 ml, i.p.)

Fig 4
The rats were monitored by a JVC digital video recorder, (model DVL 100) for 7 days (3 rats per video monitoring session (Rat #1 - phenobarbital treated, Rat #2 - saline treated, Rat #3 - *V. hochstetteri* intraperitoneally treated) except on the sixth and seventh day where only rats orally pretreated with *V. hochstetteri* (6 g/kg) were monitored. All rats were video monitored for 4-hours after pilocarpine treatment. Seizures were scored according to a modification of the scale of Racine (87). Only motor seizures (Figure 5, 6, 7) were considered (i.e. class I and II seizures were not scored, because they involved mouth and facial movement and head nodding, which are not clearly discernible under video monitoring conditions. (88). Seizures were graded as shown (Table I) during observation of the videotapes.

**Table I.** Classification of behavioral motor seizures according to Racine (87)

<table>
<thead>
<tr>
<th>SEIZURE CLASS</th>
<th>BEHAVIORAL SYMPTOMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLASS III</td>
<td>Rats have fore limb clonus with lordotic posture</td>
</tr>
<tr>
<td>CLASS IV</td>
<td>Rats rear with concomitant fore limb clonus</td>
</tr>
<tr>
<td>CLASS V</td>
<td>Rats have class IV seizure and fall</td>
</tr>
</tbody>
</table>
Figure 5: Rats showing class III seizures (forelimb clonus with a lordotic posture) 1-hour after pilocarpine (320 mg/kg, i.p.) treatment.
Figure 6: Rats showing class IV seizures (forelimb clonus with rearing) 1-hour after pilocarpine (320 mg/kg, i.p.) treatment.
Figure 7: Rat showing class V seizure (rat has class IV seizure and falls), 1-hour after pilocarpine (320 mg/kg, i.p.) treatment.
3.5: (Experiment 3)

**Effects of *V. hochstetteri* and phenobarbital on pilocarpine–induced status epilepticus mortalities in rats:** 40 rats were used in this experiment. 20 rats were from experiment 1, that is, group A, B, E and F pretreated intraperitoneally with 0.2 g/kg, 1 g/kg, 4 g/kg, and 6 g/kg, of *V hochstetteri*, 1-hour prior to pilocarpine (320 mg/kg, i.p.) treatment. 5 rats pretreated with phenobarbital (10 mg/kg, i.p.) and 5 other rats pretreated with saline prior to pilocarpine (320 mg/kg, i.p.) treatment from experiment 2, and finally 5 rats pretreated with (1mg/kg, i.p.) phenobarbital and another 5 rats pretreated with (5 mg/kg, i.p.) phenobarbital prior to pilocarpine treatment, were monitored for 16-weeks post-treatment with pilocarpine (320 mg/kg, i.p). The rats were kept in cages, in groups of five; according to dose of *V. hochstetteri* or phenobarbital they received. Mortalities were scored daily for 16-weeks (duration of study), after pilocarpine treatment.

3.6: (Experiment 4)

**Determination of Acute Neurotoxicity of *V. hochstetteri* in rats:** 10 rats were used in this experiment, 5 were treated with 3 ml of saline intraperitoneally as control and the other 5 were treated with *V. hochstetteri* (6 g/kg, i.p.) 24-hours prior to neurological evaluation. The rats were examined for behavioral toxicity by the positional sense test and gait and stance test. In the positional sense test, one hind limb is lowered over the edge of the table, whereupon the rat, experiencing neurological deficit, fails to lift its leg quickly (> 5 sec) back to a normal position.
In the gait and stance test, neurotoxicity is indicated by a circular or zigzag gait, ataxia, abnormal spread of legs, abnormal posture, tremor and hyperactivity, lack of exploratory behavior, somnolence, stupor or catalepsy (89).

3.7: (Experiment 5)

**Effects of *V. hochstetteri* and phenobarbital on epileptic activity of pilocarpine treated rat EEG:** EEG was done on six rats. 5 Stainless steel screw electrodes were secured on the skull using dental acrylic. Two of the screws were fixed on the parietal bones and another two on the occipital bone and an indifferent screw electrode was fixed at a point in the skull anterior to the two parietal screw electrodes. The screws were soldered to a 5-way female connector. The female connector was connected to a 5-way male connector with leads to the EEG machine (NIHON – KOHDEN, model 4113). EEG was done on two saline treated rats (3 ml, i.p.). The same rats were then treated with pilocarpine (320 mg/kg, i.p) 1 – hour later and then EEG recorded. 2 other rats were pretreated with *V. hochstetteri* (6 g/kg, i.p.) and two other rats were pretreated with phenobarbital (10 mg/kg, i.p) 1- hour prior to pilocarpine (320 mg/kg, i.p) treatment, EEG was done immediately after pilocarpine treatment. Time to the onset of spiking in the EEG of all the rats was recorded.
3.8: Statistical analysis:

Seizure frequencies and duration for the different treatments were expressed as \( \text{mean } \pm \text{SEM} \) and were compared using one-way ANOVA with the Student-Newman-Keuls (SNK) test as the post-hoc test. Seizure latencies were compared using the Student t-test and expressed as \( \text{mean } \pm \text{SEM} \). Pilocarpine-induced status epilepticus mortalities were compared using Chi-square test. Significant differences were considered at 5% level and expressed as \( P < 0.05 \).
Chapter 4

4.0: Results:

4.1: Effect of *V. hochstetteri* on motor seizure latency in pilocarpine treated rats.

The effects of *V. hochstetteri* on onset of pilocarpine-induced seizures was determined in 30 rats pretreated with *V. hochstetteri* at different dose levels, 1- hour prior to pilocarpine (320 mg/kg, i.p.) treatment. Rats pretreated with *V. hochstetteri* (0.2 g/kg, i.p.) had seizure latency (defined as forelimb clonus with lordosis in pilocarpine treated rats) of $28.4 \pm 0.2$ min. Rats pretreated with *V. hochstetteri* (1 g/kg, i.p.) had seizure latency of $29.8 \pm 0.4$ min. Both these doses of *V. hochstetteri* did not significantly delay the onset of seizures when compared to saline ($28.8 \pm 1.2$ min, $P > 0.05$). At a dose of 2 g/kg, i.p., *V. hochstetteri*, the onset of seizures was $38 \pm 0.6$ min. and with 3 g/kg, i.p. the onset of seizures was $39.6 \pm 0.7$ min. Pretreatment with *V. hochstetteri* (4 g/kg, i.p.) the onset of seizures was $41 \pm 0.4$ min. and *V. hochstetteri* (6 g/kg, i.p) the onset of seizure was $42 \pm 0.6$ min. Rats given orally *V. hochstetteri* (6 g/kg) had onset of seizures $34.4 \pm 0.5$ min after pilocarpine treatment. There was a significant delay in time to seizure onset of rats pretreated with *V. hochstetteri* at doses of (2 g/kg, 3 g/kg, 4 g/kg and 6 g/kg) compared to controls. ($P < 0.05$). There was also a significant difference in seizure onset time between rats given orally *V. hochstetteri* (6g/kg.) and rats given intraperitoneally *V. hochstetteri* (6 g/kg). ($P < 0.05$). Pretreatment with *V. hochstetteri* increased seizure latency in a dose-dependent manner (Figure 8). Pretreatment with phenobarbital (10 mg/kg, i.p.) prevented motor seizure activity in all the rats during the 4-hour observation period.
Figure 8: Effect of *V. hochstetteri* on onset of motor seizures (latency, mean ± SEM) in pilocarpine (320 mg/kg, i.p.) treated rats. Student t-test, n = 5. P < 0.05.
Dose response curve for *V. hochstetteri*

![Graph showing the dose response curve for V. hochstetteri. The x-axis represents the dose (g/Kg) ranging from 0.2 to 6, and the y-axis represents latency (Min) ranging from 0 to 45. The graph shows a trend of increased latency with increasing dose.]
4.2: Effects of *V. hochstetteri* and phenobarbital on seizure latency, frequency and duration in pilocarpine treated rats.

5 rats were orally pretreated with *V. hochstetteri* (6 g/kg,) and 5 other rats with *V. hochstetteri* (6 g/kg, i.p.), 1- hour prior to treatment with pilocarpine (320 mg/kg, i.p.) to determine the effects of pretreatment with *V. hochstetteri* on onset and severity of pilocarpine-induced seizures. 5 other rats were pretreated with phenobarbital (10 mg/kg, i.p.), 1- hour prior to pilocarpine (320 mg/kg, i.p.) treatment and 5 other rats were pretreated with saline (3 ml, i.p.), 1 – hour prior to pilocarpine (320 mg/kg, i.p.) treatment. Pretreatment with phenobarbital (10 mg/kg, i.p.) (as this dose was found to be effective in controlling motor seizure activity in a pilot study), prevented motor seizure activity in all the pilocarpine (320 mg/kg, i.p.) treated rats, (n = 5). Rats pretreated with *V. hochstetteri* (6g/kg, i.p) (which was the highest dose tested and found to maximally increase seizure latency in experiment 1) (n = 5), had seizure latency of 42 ± 0.6 min. Rats orally pretreated with *V. hochstetteri* (6g/kg,) (n = 5) had onset of seizures 34.4 ± 0.5 min after pilocarpine (320 mg/kg, i.p.) treatment. Oral and intraperitoneal pretreatment with *V. hochstetteri* significantly delayed onset of motor seizures when compared to saline pretreated rats, which had seizure latency of 28.8 ± 1.2 min. (P < 0.05). (Figure 9). Orally and intraperitoneally administered *V. hochstetteri* (6g/kg) significantly reduced average seizure frequency and duration over the 4-hour observation period when compared to saline pretreated rats. There was a significant difference in class III, class IV, and class V motor seizure frequency and duration between saline treated rats and rats orally and intraperitoneally pretreated with *V. hochstetteri* (Table II, Figure10 and Figure11) (P < 0.05).
Intraperitoneally administered *V. hochstetteri* had a greater effect in reduction of seizure frequency and duration than orally administered *V. hochstetteri*. Significant differences were determined by one-way ANOVA. *V. hochstetteri* (6g/kg), orally and intraperitoneally administered had preferential effects against class V and class IV seizures when compared to class III. (V > III, V > IV) (P < 0.05). There was also a significant difference in the effect of *V. hochstetteri* against class III and class IV seizures. IV > III (P < 0.05). Post-hoc test used was the Student-Newman-Keuls test. The effects of phenobarbital (10 mg/kg, i.p.) and *V. hochstetteri* (6 g/kg, i.p.) in rats after seizure onset were determined in pilot studies. Treatment with phenobarbital (10 mg/kg, i.p.) in pilocarpine (320 mg/kg, i.p.) treated rats having class III motor seizures resulted in death of the rats (n = 3), immediately after phenobarbital treatment. Other rats (n = 3) having class III motor seizures after pilocarpine (320 mg/kg, i.p.) treatment died when they were treated with *V. hochstetteri* (6 g/kg, i.p.). Phenobarbital treatment might have depressed the cardiorespiratory system, further complicating the anoxic brain damage during the ictal events, leading to death of the rats. The mechanism of action of *V. hochstetteri* has not been determined, and therefore the cause of death in the *V. hochstetteri* treated rats remains unknown.
Figure 9: Seizure latencies in pilocarpine-treated rats (mean ± SEM) with pretreatment with saline and *V. hochstetteri* pretreatment. Student t-test, n = 5. P < 0.05.
Seizure Latencies

Saline

V hoch., p.o.

V hoch., i.p.

Time (minutes)

Fig 9
Table II: Average 4-hour seizure frequencies and duration in rats given saline or *V. hochstetteri* (6 g/kg) orally and intraperitoneally before pilocarpine (320 mg/kg, i.p.). One-way ANOVA with SNK *post hoc* test, n = 5. P < 0.05.
Table II: Average 4-hour seizure frequencies and duration (mean ± SEM) in rats given saline or *V. hochstetteri* (6 g/kg, i.p., or orally) before pilocarpine (320 mg/kg, i.p).

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>AVERAGE SEIZURE FREQUENCY AFTER 4- HOURS</th>
<th>AVERAGE SEIZURE DURATION (SEC) AFTER 4-HOURS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>III</td>
<td>IV</td>
</tr>
<tr>
<td>Saline 3 ml, i.p</td>
<td>19.4 ± 0.5</td>
<td>16.6 ± 0.5</td>
</tr>
<tr>
<td><em>V. hoch</em>, 6 g/kg, i.p</td>
<td>11.8 ± 0.5</td>
<td>5.4 ± 0.7</td>
</tr>
<tr>
<td><em>V. hoch</em>, 6 g/kg, orally</td>
<td>13 ± 0.6</td>
<td>7.4 ± 0.8</td>
</tr>
</tbody>
</table>

Value of mean ± SEM *(P < 0.05)
Figure 10: Seizure frequencies (mean ± SEM) in pilocarpine-treated rats with pretreatment with saline (3 ml, i.p) and *V. hochstetteri* (6 g/kg, i.p., and orally).

One-way ANOVA with SNK *post hoc* test, n = 5. P < 0.05.
Seizure Frequencies

Average Seizure/hr

9 AM-10 AM 10 AM-11 AM 11 AM-12 AM 12 PM -1PM

Duration

Fig 10
Figure 11: Seizure duration in pilocarpine-treated rats (mean ± SEM) with pretreatment with saline (3 ml, i.p.) or *V. hochstetteri* (6 g/kg, i.p., and orally). One-way ANOVA with SNK post hoc test. n = 5. P < 0.05.
Seizure Duration

Average Seizure Duration (seconds)

9 AM - 10 AM
10 AM - 11 AM
11 AM - 12 AM
12 PM - 1 PM

Fig 11
4.3: Effects of *V. hochstetteri* and phenobarbital on pilocarpine-induced status epilepticus mortalities in rats.

Intraperitoneal pretreatment with phenobarbital (1 mg/kg, 5 mg/kg, 10 mg/kg) protected all the rats, (n = 15), from pilocarpine-induced status epilepticus mortalities, 16-weeks post-treatment with pilocarpine (320 mg/kg, i.p.). Rats pretreated with *V. hochstetteri* (0.2 g/kg, i.p.) had 100% mortality (n = 5), 16-weeks post-treatment with pilocarpine (320 mg/kg, i.p.). In rats pretreated with (1 g/kg, i.p.) *V. hochstetteri* there was 80% mortality (n = 5), 16-weeks post-treatment with pilocarpine (320 mg/kg, i.p.). Rats pretreated with *V. hochstetteri* (4 g/kg, i.p.) (n = 5), had 60% mortality 16-weeks post-treatment with pilocarpine (320 mg/kg, i.p.). Rats pretreated with *V. hochstetteri* (6 g/kg, i.p.) (n = 5), had 40% mortality 16-weeks post-treatment with pilocarpine (320 mg/kg, i.p.). Saline (3 ml, i.p.) pretreated rats (n = 5) had 80% mortality 16-weeks post-treatment with pilocarpine (320 mg/kg, i.p.). There was a significant difference in mortalities between saline pretreated rats and rats pretreated with phenobarbital (1 mg/kg, 5 mg/kg, 10 mg/kg.) (P < 0.05). Pretreatment with *V. hochstetteri* (1 mg/kg, i.p.) did not significantly reduce pilocarpine-induced status-epilepticus mortalities when compared to saline pretreated rats (P > 0.05). *V. hochstetteri* (6 g/kg, i.p.) significantly reduced pilocarpine-induced status epilepticus mortalities when compared to saline pretreated rats (P < 0.05) (Table III). Chi-square test.
Table III: Pilocarpine-induced mortalities in rats (N = 40) randomly pretreated with saline (3 ml, i.p.), or different doses of *V. hochstetteri* or phenobarbital, 1-hour prior to pilocarpine (320 mg/kg, i.p) treatment. Chi-square test, n = 5. P < 0.05.
<table>
<thead>
<tr>
<th>Pre-treatment dose given to group of 5 rats</th>
<th>% Mortality 16 weeks after pilocarpine (320 mg/kg i.p.) treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 g/kg, i.p. <em>V. hochstetteri</em></td>
<td>100</td>
</tr>
<tr>
<td>1 g/kg i.p. <em>V. hochstetteri</em></td>
<td>80</td>
</tr>
<tr>
<td>4 g/kg, i.p. <em>V. hochstetteri</em></td>
<td>60</td>
</tr>
<tr>
<td>6 g/kg, i.p. <em>V. hochstetteri</em></td>
<td>40*</td>
</tr>
<tr>
<td>Saline 3 ml, i.p.</td>
<td>80*</td>
</tr>
<tr>
<td>1 mg/kg, i.p. Phenobarbital</td>
<td>0</td>
</tr>
<tr>
<td>5 mg/kg, i.p. Phenobarbital</td>
<td>0</td>
</tr>
<tr>
<td>10 mg/kg, i.p. Phenobarbital</td>
<td>0</td>
</tr>
</tbody>
</table>

Value % of n = 5 * P < 0.05
The potential harm, which an anticonvulsant drug can cause, must be weighed against the potential benefits, which it can produce. Rats (n = 5), were treated with *V. hochstetteri* (6 g/kg, i.p.) (Dose found to significantly delay the onset and severity of pilocarpine-induced seizures in rats, in experiment 2) to determine its acute behavioral neurotoxicity in rats. Rats pretreated with *V. hochstetteri* (6 g/kg, i.p.) (n = 5) showed acute minimal behavioral neurotoxicity. All the 5 rats when placed in an open field for neurological evaluation, showed inhibited exploratory behavior (Rats did not move at all during the 1-hour observation period), somnolence, stupor and an abnormal position sense (Rats took 38 ± 14.2 sec to retract displaced leg). Saline treated rats (n = 5) had all examined neurological parameters normal (Rats took 3.4 ± 2.1 sec to retract displaced leg in the position sense test, and circled the open field 7 ± 3.4 times, in the gait and stance tests). (Table IV).
Table IV: 5 rats treated with *V. hochstetteri* (6 g/kg, i.p.,) and 5 other rats treated with saline (3 ml, i.p.) were evaluated 24-hours later for symptoms of acute behavioral neurotoxicity.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rat Number</th>
<th>Lack of Position sense</th>
<th>Circular or zigzag gait</th>
<th>Ataxia abnormal spread of legs</th>
<th>Abnormal posture</th>
<th>Lack of exploratory behavior</th>
<th>Somnolence</th>
<th>Stupor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
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Legend: 0 = normal; + = Present/Abnormal, - = Absent
4.5: Effects of *V. hochstetteri* and phenobarbital on latency of spiking in the EEG of pilocarpine treated rats:

Rats (n = 2) were pretreated with saline (3 ml, i.p.) and their EEG done immediately. The same rats were then treated with pilocarpine (320 mg/kg, i.p.) 1-hour later and EEG done immediately after pilocarpine treatment. 2 other rats were pretreated with phenobarbital (10 mg/kg, i.p.) 1-hour prior to pilocarpine (320 mg/kg, i.p.) treatment. EEG was done immediately after pilocarpine treatment. Other rats (n = 2) were pretreated with *V. hochstetteri* (6 g/kg, i.p.), 1-hour prior to pilocarpine (320 mg/kg, i.p.) treatment and EEG done immediately after pilocarpine treatment. Rats pretreated with saline showed 4 - 8 Hz theta rhythm (Figure 12). When the same rats were treated with pilocarpine (320 mg/kg, i.p.), the EEG showed spiking at 14.5 ± 6.35 min. (n = 2) (Figure 13, 14, 15). Rats pretreated with *V. hochstetteri* (6 g/kg, i.p.) (n = 2), prior to pilocarpine (320 mg/kg, i.p.) treatment showed spike activity at 15.5 ± 6.35 min (Figure 16). Rats pretreated with phenobarbital (10 mg/kg, i.p.) (n = 2) had onset of spiking at 29.5 ± 6.35 min after pilocarpine (320 mg/kg, i.p.) treatment (Figure 17). Pretreatment with *V. hochstetteri* (6 g/kg, i.p.) did not significantly delay the onset of spike activity in the pilocarpine treated rats EEG when compared to saline treated rats (P > 0.05). Pretreatment with phenobarbital (10 mg/kg, i.p.) significantly delayed onset of spiking in the pilocarpine treated rat EEG when compared to saline and *V hochstetteri* treated rats. (P < 0.05).

Saline and *V. hochstetteri* pretreated rats showed behavioral motor seizures, which were correlated with irregular spike activity in the EEG after pilocarpine treatment. The EEG of rats pretreated with phenobarbital prior to pilocarpine treatment, displayed irregular spike activity, which was not correlated with behavioral motor seizures.
Figure 12: EEG tracing of rat after saline (3 ml, i.p.)
Figure 13: EEG tracing of rat during class III motor seizure. The rat was given saline (3 ml, i.p.) 1-hour prior to pilocarpine (320 mg/kg, i.p.)
Figure 14: EEG tracing during class IV seizure in rat given saline (3 ml, i.p.) prior to pilocarpine (320 mg/kg, i.p.) treatment.
Figure 15: EEG tracing of rat given saline (3 ml, i.p.) prior to pilocarpine (320 mg/kg, i.p.) treatment displaying class V seizure.
Figure 16: EEG tracing of rat given *V. hochstetteri* (6 g/kg, i.p.), before pilocarpine (32 mg/kg, i.p.).
Figure 17: EEG tracing of rat given phenobarbital (10 mg/kg, i.p.) before pilocarpine (320 mg/kg, i.p.).
Chapter 5:

5.0: Discussion:

*Vernonia hochstetteri* is a medicinal plant used in traditional medicine to treat epilepsy by East African communities (15). To date there has been no study on the efficacy of *V. hochstetteri* or its anti-epileptic properties, if any. Phytochemical investigation of *V. hochstetteri* has shown that a flavone and flavonic glycosides are among the main phytochemicals (75). Flavonoids have CNS activity (90). Indeed flavones such as chrysin have anticonvulsant activity (91). The present study screened crude lyophilised aqueous extract of *V. hochstetteri* for anticonvulsant activity in rats with pilocarpine-induced status epilepticus. The results of these experiments suggest that *V. hochstetteri* has anticonvulsant principle(s) against seizures induced by pilocarpine. Pretreatment with *V. hochstetteri* (6 g/kg, i.p.) prior to pilocarpine treatment significantly increased motor seizure latency, decreased motor seizure frequency and duration when compared to saline treated rats. Both orally and intraperitoneally administered *V. hochstetteri* significantly delayed onset and severity of pilocarpine-induced seizures. Intraperitoneally administered *V. hochstetteri* had greater anticonvulsant activity when compared to orally administered *V. hochstetteri*. A possible explanation for the difference in anticonvulsant activity of *V. hochstetteri*, orally and intraperitoneally administered, is that orally administered *V. hochstetteri* might not have reached plasma levels for maximum anticonvulsant activity 1-hour prior to pilocarpine treatment or *V. hochstetteri* is poorly absorbed in the gastrointestinal tract.
Pretreatment with *V. hochstetteri* (6 g/kg, i.p.) protected 60% of the rats from pilocarpine-induced status epilepticus mortalities, when compared with saline treated rats (20% survived). *V. hochstetteri* reduced the incidence and severity of pilocarpine-induced seizures. Surface EEG recordings of pilocarpine treated rats showed that pretreatment with *V. hochstetteri* (6 g/kg, i.p.) does not significantly delay onset of epileptic spike discharge when compared to saline. Phenobarbital (10 mg/kg, i.p.) on the other hand significantly delayed onset of epileptic spike discharge when compared to both saline and *V. hochstetteri* treated rats. Acute pilocarpine-induced seizures are used to screen compounds with efficacy against refractory status epilepticus or neuroprotection against damage induced by sustained seizures or both (62). A problem with clinical validation of TLE models is the lack of any AED that effectively prevents epilepsy in humans. Indeed clinically utilised drugs such as carbamazepine and diphenylhydantoin block neither convulsions nor brain damage induced by pilocarpine, and ethosuximide and acetazolamide increase the susceptibility of rats to the convulsant action of pilocarpine (7). *V. hochstetteri* should be screened with a battery of other rodent models of epilepsy to establish its anticonvulsant activity in rats, but even if it has anticonvulsant activity in rats, it still has to undergo secondary evaluation to determine its clinical utility as an anticonvulsant. Behavioral parameters such as seizure latency, frequency and duration of pilocarpine treated rats show various sources of variation. It is important to appreciate that different animals show different susceptibility to pilocarpine-induced convulsions. Other sources of variation in seizure onset and severity in pilocarpine treated rats are lighting, previous handling in the past days, nociceptive stimulation, age, and the hormonal environment (64, 65, 92).
It is important to appreciate these sources of variability in designing experiments to study therapy-induced changes in behavioral seizure parameters. Our study design had inherent limitations, since a different set of animals served as the control for another set of animals, together with the small sample sizes, inter animal variability might introduce false positive or negative results in the observed seizure parameters between the treatment and the control group. Another serious handicap was the subjective nature of scoring seizures. More sophisticated systems of analyzing behavior such as chronic electroencephalography should be used in such studies. The protocol for the preparation of aqueous extract of *V. hochstetteri* is not reproducible. In future, standard phytochemical extraction protocols such as column chromatography, to obtain specific phytochemicals, should be used in such studies. The specific phytochemicals from *V. hochstetteri* should then be assayed on behavioral seizure parameters rather than the crude extracts of *V. hochstetteri*. 
6.0: Conclusion

In conclusion, the present study has found that crude lyophilized aqueous extract of *Vernonia hochstetteri* significantly delayed the onset of motor seizures in pilocarpine treated rats. Oral and intraperitoneal pretreatment with lyophilized aqueous extract of *V. hochstetteri* significantly reduced seizure frequency and duration in pilocarpine treated rats. *V. hochstetteri* 6 g/kg, i.p. treated rats showed symptoms of acute behavioral neurotoxicity. Finally, *V. hochstetteri* significantly reduced pilocarpine induced status epilepticus mortalities in rats. *V. hochstetteri* has anticonvulsant effects in rats with pilocarpine-induced status epilepticus. However, it is recommended that further studies should be done to:

I. Screen of *V. hochstetteri* against other experimental models of epilepsy such as PTZ, MES, picrotoxin and bicuculine for anticonvulsant activity.

II. Bioassay the flowers, shoot, bark and roots of *V. hochstetteri* to determine which plant part has greatest anticonvulsant principle(s).

III. Screen of *V. hochstetteri* against chronic spontaneous seizures induced by pilocarpine, which replicates key features of human temporal lobe epilepsy.

IV. Examine the long-term use of *V. hochstetteri* for teratogenicity, hepatotoxicity, and neurotoxicity in rats.

V. Investigate the anticonvulsant activity of flavonic glycosides and the flavone from *V. hochstetteri*. 
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*Indian J. Pediatr*; 67 (1 Suppl); S 4 –11).


Deiters neurons I, monosynaptic inhibition of the inhibitory postsynaptic potential.

hippocampal neurons II. After potentials and repetitive firing.

the thalamus, types of cells, their responses, and their functional organization.


36. Collaborative Group for the study of epilepsy prognosis in newly referred
patients; a multicentre prospective study of the effects of monotherapy on the


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