Department of Pharmacology and Clinical Pharmacy

School of Pharmacy

College of Health Sciences

Evaluation of anticonvulsant activity of aqueous and 80% methanol extract of *Pterolobium stellatum (Forssk)* in Swiss albino mice

By: Yimer Hussien (BPharm)

A Thesis Submitted to the Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University in partial fulfillment of the requirements for the Master of Science Degree in Pharmacology

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Advisor: - Dr. TeshomeNedi

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School of Graduate Studies

This is to certify that the thesis prepared by Yimer Hussien, entitled "Evaluation of anticonvulsant activity of aqueous and 80% methanol extract of *Pterolobium stellatum (Forssk)* in Swiss albino mice" submitted in partial fulfillment of the requirements for the Master of Science degree in pharmacology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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_____

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Abstract

Evaluation of anticonvulsant activity of aqueous and 80% methanol extract of *Pterolobium stellatum (Forssk)* in Swiss albino mice

Yimer Hussien

Addis Ababa University, 2020

Pterolobium stellatum is used for the treatment of various illnesses including tuberculosis, pneumonia,and epilepsy in Ethiopian traditional medicine. The present study was conducted to evaluate anticonvulsant activity of aqueous and 80% methanol extract of *Pterolobium stellatum (Forssk)* in Swiss albino mice using pentylenetetrazole (PTZ) induced seizure test. In addition, anticonvulsant activity of the study plant was evaluated using maximal electric shock (MES) induced seizure tests. The effect of both aqueous and 80% methanol extract on motor coordination was evaluated using a rota rod test. The 80% methanol extract has shown a dose dependent increase in latency to onset of seizure against PTZ induced seizure tests. In addition, both aqueous and 80% methanol extract have shown dose-dependent reduction in duration of hind limb tonic extension against MES induced seizure test. Furthermore, the aqueous extract at dose 400mg/kg and methanol extract at doses 200mg/kg and 400mg/kg possess a significant reduction in duration of myoclonic seizure in the PTZ test ($P<0.05$, $P<0.05$and $P<0.01$) respectively. Similarly,administration of 80 % methanol extract at all tested dose levels (100mg/kg, 200mg/kg and 400mg/kg) and aqueous extractat the dose level of 200mg/kg and 400 mg/kg have shown significant reduction on the duration of hind limb tonic extension (HLTE) in MES induced convulsion tests. All animals received both aqueous and 80% methanol extract at the dose level of 100mg/kg, 200mg/kg and 400mg/kg did not show motor deficit inrota road test. Phytochemical screening tests on both aqueous and 80% methanol extract revealed the presence of alkaloids,tannins, saponins,terpenoids, and steroids. The present study revealed that the aqueous and 80% methanol leaf extracts of *Pterolobium stellatum* possess anticonvulsant activity in PTZ and MES induced seizure tests. Results of the present study validated the traditional use of the study plant in the treatment of epilepsy, as its hydroalcoholic extract pretreatment prevents MES and PTZ-induced convulsions in mice.

Keywords: Seizurepentylenetetrazole maximal electric shock rota rod Laboratory animals *Pterolobium stellatum*

Contents

Abbreviations and acronyms

AAU	Addis Ababa University
AEDs	Antiepileptic Drugs
ANOVA	Analysis of Variance
EEG	Electroencephalography
ILAE	International League Against Epilepsy
HLTE	Hind Limb Tonic Extension
MES	Maximum Electric Shock
MRP	Multi-drudResistent associated Protein
OECD	Organization for Economic Co-operation and Development
PTZ	Pentylenetetarazole
SC	Subcutaneous
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization

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1. Introduction

1.1. Definition and classification

Epilepsy is a heterogeneous group of neurological disorders characterized by enduring predisposition of the brain to generate seizures with neuronal hyperexcitability and hypersynchronous neuronal firing presented with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness (Kumar, Singh, Singh, Jain, Roy and Mill-, 2014).

According to the 2017 International League against Epilepsy official report (ILAE), depending on the clinical presentationseizures are fundamentally divided into two major groups: partial and generalized seizures. Partial (focal, local) seizures are those in which clinical or electrographic evidence exists to suggest that the attacks have a localized onset in the brain, usually in a portion of one hemisphere. Partial seizures further subdivided into simple partial, complex partial and partial seizures evolving to secondarily generalized seizures. Generalized seizures are those in which evidence for a localized onset is lacking. Moreover, generalized seizures are categorized into absence (no convulsive), myoclonic, clonic, tonic, tonic-clonic, and atonic seizures (Aaberg, Surén, Søraas, Bakken, Lossius, Stoltenberg, *et al.*, 2017).

Epilepsy can be considered to be present after one unprovoked seizure in individuals who have other factors that are associated with a high likelihood of a persistently lowered seizure threshold and therefore a high recurrence risk. Such risk should be equivalent to the recurrence risk of a third seizure in those with two unprovoked seizures, approximately at least 60%. The latter risk level occurs with remote structural lesions, such as stroke, CNS infection, certain types of traumatic brain injury, diagnosis of a specific epilepsy syndrome, or in some circumstances with the presence of other risk factors (Fisher, Acevedo, Arzimanoglou, Bogacz, Cross, Elger, *et al.*, 2014).

1.2.Epidemiology

Epilepsy isa chronic neurological disorder characterized by recurrent or multiple seizures which accounts for 1% of the overall burden of disease and affects over 65 million people worldwide. The global burden of epilepsy which is measured by death, prevalence and disability-adjusted

life years (DALY) is highly increased in recent times with substantial physical and psychological impacts especially in low-income countries (Bill and Foundation, 2019).

Even though, epilepsy is a treatable condition with high rates of therapeutic response the incidence and prevalence of epilepsy are thought to be higher in low- or middle-income countries than in high-income countries (Seid, Demilew, Yimer and Mihretu, 2018). The rate of mortality is high in women of childbearing age (Christensen, Vestergaard and Hammer Bech, 2018), high childhood which decreases in adulthood and can rise again in older age (Hussain, 2017). Around three-fold of the population in those areas are affected by epilepsy due to treatment gaps associated with cultural beliefs, inequity in the distribution of public health services, poor health system infrastructures, and inadequate supplies of antiepileptic drugs. Furthermore, less opportunities for education and employment, CNS infections, and head trauma, in poor regions of the world raise the prevalence and incidence of epilepsy (Guekht, Zharkinbekova, Shpak and Hauser, 2017).

Studies done in sub-Saharan countries revealed that there is high prevalence of epilepsy in those areas which accounts 63–158 per100 000 people. Epilepsy becomes one of the most common neurological disorder in Ethiopia with the incidence of 64 per 100,000 peoples which usually affects young children's less than nine years of old and people over the age of 65 years; however, it can occur at any time (Dargiea, Alemnewa, and Admasu, 2019).

1.3.Etiology

In most developing countries people associate epilepsy with evil spirit and supernatural origin. Hence, those people with epilepsy are prone to be stigmatized, discriminated, and develop psychosocial mental problems (Fanta, 2015). Etiology of epilepsy is associated with comorbid conditions like behavioral and psychiatric disorders, intellectual disabilities, tumors, infections like meningitis, traumatic brain injury, metabolic syndrome like hyperinsulinemia, chromosomal anomalies (Sokka, Olsen, Kirjavainen, Harju, Keski-Nisula, Räisänen, *et al.*, 2017). Patients with cardiovascular disorders like dyslipidemia and hypertension are also at high risk of developing epilepsy (Vivanco-Hidalgo, Gomez, Moreira, Díez, Elosua and Roquer, 2017). Protein deficiency disease like kwashiorkor or protein-calorie malnutrition, in which both protein and calories are deficient increase risk of epileptic seizures (Gietzen, Lindström, Sharp, Teh and

Donovan, 2018). The risk of epileptic seizure is high in elderly patients as elderly patients had more frequent brain trauma and stroke (Geller, Skarpaas, Gross, Goodman, Barkley, Bazil, *et al.*, 2017).

Furthermore, the risk of epilepsy increases in alcoholic individuals and drivers who might have head injuries due to accidents. Individualsworking at different industries who might have exposure with chemicals solvents and cleaning agents are also at high risk of developing epileptic seizures (Vozikis, Goulionis and Nikolakis, 2012). It was reported that overuse of some dietary supplements obtained from natural products and herbal remedies marketed for management of inflammatory disorders, weight control, bodybuilding, and for management of different behavioral disorders increase the risk of epileptic seizures (Tyagi and Delanty, 2003). Plants like artist Vincent van Gogh, Evening primrose oil, grapefruits, St. Johns wart, Ginkgo Biloba and ephedra alkaloids increase the risk of seizure. Their potential risk might be associated with the presence of neurotoxic compounds and contamination with heavy metals. They alter the pharmacokinetic profile of antiepileptic drugs or they might have complex mechanisms which trigger seizure propagation (Samuels, Finkelstein, Singer and Oberbaum, 2008).

1.4. Pathophysiology

Despite extensive mechanistic experimental research and several advances of in *vivo* and *in vitro* models proposed for finding the compounds used forthe treatment of epilepsy, the cellular basis of epilepsy is still not clearly understood. Around one in three patients with epilepsy are pharmacoresistant and it becomes one of the most common serious neurological disorders, responsible for substantial morbidity and mortality due to the seizures and the existing medications (Wahab, 2010).

Even though it is not clear which mechanisms are required or necessary for the genesis of epilepsy, progress in the field revealed that epileptic syndromes share common ictogenesis-related characteristics such as increased neuronal excitability and synchronicity. It involves various biological pathways orprocesses, structural and functional changes within brain cells (Yin,Ahmad , and Makmor-Bakry,2013). An epileptic seizure results from transient abnormal synchronization of neurons in the brain that disrupts normal patterns of neuronal communication which results in neocortical hyperexcitability and subsequent alterations of ion membrane permeability or ion exchange activity leading to recurrent seizures (Kim and Lee, 2017).

Alteration at many levels of brain function, from genes and subcellular signaling cascades to wide spread neuronal circuits, might resultin increased excitatory activity by hyperactivity of glutamatergic transmission and functional disturbances of the ligand or voltage-gated sodium and calcium channels and the inhibitory neuronal activity. This might be due to loss ordecrease activity of gamma-aminobutyric acid (GABA) mediated neurotransmission and extracellular potassium currents within brain cells (Stafstrom and Carmant, 2015).

According to studies done on different experimental models on the mechanisms of seizure generation and propagation AMPA receptors which have an important role in the spread of seizures and seizure-induced damage have been identified as new targets for the development of potential AEDs and several AMPA receptor antagonists were active in animal models of seizures and epilepsy (Perucca, French and Bialer, 2007). Furthermore, numerous proteins and processes are involved in the regulation of the neuronal micro-environment and in maintaining the delicate balance between excitation and inhibition in the brain. Those proteins and processes might be used as additional or secondary targets for AED action. For instance, experimental studies done in animal models revealed that overexpression of inflammatory mediators like cyclo-oxygenase-2 (Cox-2) enzyme, neuropeptides like vasoactive intestinal peptide (VIP),proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukins (IL-1β and IL-6) in astrocytes are involved in the pathogenesis of epilepsy possibly by inducing neuroinflammation which results excessive neuronal discharge in brain cells (Kilinc, 2019).

It was reported that the incidence of drug resistance epilepsy was increased in recent times in patients who were on AEDs for the last decades. However, no promising therapeutic remedies available to manage drug resistance epilepsy in the present clinical scenario (Pan, Wang, Wang, Li and Liu, 2016). Among the common pathogenesis mechanism which leads to emergency of pharmacoresistant epilepsy,decrease in alpha-1 subunit of GABA receptors were the most common probable pathways. Abnormality in those receptors leads to alteration of GABA receptor activity, increased expression of T-type calcium channels which result in alteration of voltage-gated calcium channel activity, down-regulation of accessary sodium channels which result in alteration of voltage-gated sodium channel activity and overexpression of multidrug-resistant associated protein(MRP1 and MRP2) gene which result in overactivity of P-glycoproteins that increase efflux of AEDs from neurons (Sharma *et al.*, 2015).

1.5.Management

1.5.1. Non-pharmacologic treatments

Lifestyle modifications such as optimizing sleep, improving medication compliance, reducing stress and causes of symptomatic epilepsies, such as head trauma, perinatal injury, and brain infections like neurocysticercosis are among the non-pharmacologic treatment approaches in management of epilepsy. In addition, non-pharmacological treatment approaches like dietary therapy (ketogenic diet), surgery, and immune therapy are used for patients living with epilepsy (Stafstrom and Carmant, 2015).

Non-pharmacological treatment such as cognitive therapy,psychological and educational approaches including digital technologies like internet-based software and mobile applications have shown a reduction in the development of epileptic seizures. Accordingly, study done on the use of digital health technologies for the treatment of epilepsy suggests that possible combinations of non-pharmacologic interventions (ketogenicdiet, low glycemic diet, and neurostimulation) and pharmacologic treatments with mobile medical application will reduce the occurrence of seizure. This might be associated with stable physiological response as individuals become relaxed when they are engaged with activities like puzzles, games, and music since those activities did not require mental creativity (Afra, Bruggers, Sweney, Fagatele, Alavi, Greenwald, *et al.*, 2018).

Dietary supplements containing vitamin E, folic acid, and pyridoxal phosphate are known to have anticonvulsant activity in neonatal seizures which are associated with inherited metabolic defects. They serve as anticonvulsant agents probably as replacement therapy and through resetting the inhibitory gamma-amino butyric acid (GABA) and excitatory (glutaminergic) systems (Kneen and Appleton, 2006).

1.5.2. Pharmacologic treatments

Pharmacological treatments are the mainstay treatments of epilepsy for many centuries. The majority of antiepileptic drugs protect against seizures through interactions with a variety of cellular targets and suppress abnormal hyper synchronous activity in brain circuits, leading to protection against seizures. Based on their action on these targets currently, we have a number of AEDs approved for the treatment of epilepsy which exerts their anti-seizure activity:-(1) by increasing GABA-ergic action mechanisms; (2) by depressing excitatory glutamate

neurotransmission; (3) by depressing voltage-dependent Ca^{2+} mobilizing mechanisms; (4) by blocking voltage-dependent Na^{+} conductance (Lasoñ, Dudra-jastrzêbska, Rejdak and Czuczwar, 2011).

Drugs that block voltage-dependent sodium or calcium channels are effective against generalized tonic-clonic and partial seizures via blockage of sustained repetitive firing in individual neurons. At the same time drugs like ethosuximide are active against absence seizure via blockage of T-type calcium channels and drugs which exert their anti-epileptic activity by enhancing inhibitory events mediated by gamma-aminobutyricc acid (GABA) show better activity in all seizure types (absence, generalized tonic-clonic, and partial seizures) (Czapin, Blaszczyk and Czuczwar, 2005). In addition, drugs like levetiracetam enter in to synaptic vesicles andprevent seizures, as well as other neurological diseases caused by disordered neurotransmission via selective inhibition of neurotransmitter release in the later impulse of long and high frequency brust while they interact with synaptic vesicular proteins (Meehan, Yang, Mcadams, Yuan, and Rothman, 2019).

It is advantageous to start AEDs therapy at low dose as possible in order to prevent their harmful effect. However, the selection, initiation of AED therapy, type and number of AEDs to be indicated for epileptic patients depends on seizure type, patient age, other comorbid conditions, spectrum of activity, drug interactions, and potential side effects (Stafstrom and Carmant, 2015). In addition to this, it is better to consider many important factors, including efficacy, adverse events, spectrum, pharmacokinetic drug interactions, pharmacodynamics interactions, titration speed, and onset of AEDs. However, combination therapy is preferred for patients with symptomatic and cryptogenic epilepsy who are refractory to antiepileptic drugs (Ajmera, 2018). Furthermore, when a drug is added or removed including non-AEDs it will be better to recommend therapeutic drug monitoring (Elger and Schmidt, 2008).

Antiepileptogenesis or disease modifying treatment drugs are given prior to epilepsy onset to prevent or to delay the development of epilepsy, and after the diagnosis of epilepsy to alleviate seizure severity, to prevent or reduce the progression of epilepsy, or change the seizures from drug-resistant to drug-sensitive and to get a complete and permanent reversal of epilepsy such that no seizures occur after treatment withdrawal (Galanopoulou, Pitkänen, Buckmaster and Moshé, 2017). In recent times, as the role of inflammation in the pathophysiology of epilepsy

have been evidenced by different clinical and experimental models, ongoing clinical researches are focusing on the development of novel anti-epileptogenesis or disease modifying drugs by targeting those inflammatory mediators and cellular receptors (Aronica, Bauer, Bozzi, Caleo, Dingledine, Gorter, *et al.*, 2017).

1.5.3. Herbal treatments

Traditional medicine, despite its limitations, is addressing the health care needs of millions of people world wide both in developing and industrialized countries. It was reported that more than half of the population in industrialized countries and around 80% of the rural population relies on traditional medicines or folk remedies for their primary health care need (Ekor, 2014). In african traditional medicine, herbal medicine plays a substantial role in the treatment of epilepsy. As a result they may contribute to the discovery of modern drugs and can be an alternative source for antiepileptic drugs with new structures and better safety and efficacy profiles (Rahman and Parvin, 2016).

Starting from the time immemorial to-date people living in urban and rural areas of Ethiopia use herbal remedies to combat different ailments and human sufferings. This is due to the fact that traditional medicines are accessible, acceptable and have biomedical benefits which are reflected in various medico-religious manuscripts by different languages. Furthermore, people with epilepsy take herbal remedies because of economic factors, cultural attitudes toward orthodox Western scientific medicine, and substitute for artificial medicine that fails to control seizure. Therefore, herbal medicine may provide high possibility for researchers to find new molecular mechanism of epilepsy and new antiepileptic drugs (Amenu, 2007). In addition, peoples with epilepsy in low and middle income countries are often not aware that their disease can be potentially treated with AEDs. Because of their cultural beliefs and socio-economic situations, they search for help through alternative and complementary medicine practitioners (Espinosa-jovel, Toledano and Aledo-serrano, 2018).

The reseason behind the research on traditional medinal plant is to identify and confirm traditional herbal medinial plants with a potential component of novelbioacitive principles with different mechanisms of actions and lower toxicity in epilepsy. Several medicinal plants which are believed to be an important source of new chemical substances with potential therapeutic effects used for the treatment of epilepsy in different systems of traditional medicine have shown

activity when tested in modern bioassays for the detection of anticonvulsant activity. However, many such plants are yet to be scientifically investigated further (Liow, Ablah, Nguyen, Sadler, Wolfe, Tran, *et al.*, 2007). For instance aqueous root extract of *Glycerrhiza glabra, Saussurea lappa*, aqueous leaf extract of *Berberis vulgeris* and *Clerodendrum infortunatum*, ethanolic leaf extract of *Oscimum sanctum*, and *Pongami apinnata* as well as hydro alcoholic leaf extract of *Cissussicyoides, Drosera burmannii and Passion flowe r*have shown better anticonvulsant activity against acute seizure models done on experimental mice's (Malvi Reetesh, Bigoniya Papiya, 2017).

1.5.4.Over view of experimental plant

The genus, Pterolobium consists of more than 10 species of perennial flowering plants in the family Fabaceae. They are sometimes called redwing flowersand are native to the tropical to subtropical climates of Africa and Asia, including Indonesia and the Philippines. They are large scrambling or climbing shrubs that grow in riverside, rocky slopes or at forest margins (Jansen PCM, 2005). In Ethiopia, the genus Pterolobium is represented by *Pterolobium stellatum*. It is locally known as Kentefa (Amharic) ,Mucarba (Afanoromo) ,Qontaftafo (Tigrigna), Mutanda (swuhli) (Ragunathan and Abay, 2009) (Enyew, Asfaw, Kelbessa and Nagappan, 2014). *P.stellatum* is a scrambling or climbing, rarely semi-erect, multi-stemmed shrub with recurved prickles, growing from 2 to 15 m tall. This plant is widespread in Africa, where it is found from Sudan and Eritrea south wards throughout Central, East and South Africa. It is most frequently found with plant communities grown along the river edges known as the riparian vegetation (Meragiaw, Woldu, Martinsen and Singh, 2018). Phytochemical studies on the root methanol extracts of *P.stellatum* revealed the presence of various secondary metabolites, which include terpenoids, saponins and tannins (Andualem, Umar, Getnet, Tekewe, Alemayehu and Kebede, 2014).

Ethno botanical surveys have revealed that the roots, leaves, and flowers of *P.stellatum* were used for treatment of various illness including tuberculosis, pneumonia, goiter and epilepsy in African traditional medicine (Sahranavard, Ghafari and Mosaddegh, 2014, Andualem, Umar, Getnet, Tekewe, Alemayehu and Kebede, 2014) (Omwoyo, Maingi and Kebira, 2017). In Kenya its root decoction is used to treat stomach-ache and pneumonia (Nyang'au, Maingi and Kebira, 2017). Furthermore, a root infusion is drunk by women against infertility as well as juice of the

roots is swallowed to treat snake bites. Because of the presence of sharp, recurved prickles, and branches leaves it is used to make rat traps in Tanzanian traditional medicine (Shangali, Zilihona, Mwang'ingo and Nummelin, 2008).

According to ethinobotanical study done in Amhara regional state, Ethiopia; the dried powder of its root was mixed with half cup of local spirit (tella) and taken orally to treat epilepsy (Aschale,Wubetu and Reta, 2018). Another study which was done in Amhara regional state, Ethiopia; Indicates whole plant juice were given orally for treatment of epilepsy (Ragunathan and Abay, 2009).

Figure 1: Photograph of *Pterolobium stellatum*

1.6. Rationale for the study

Even though epilepsy is a treatable disease, most of currently available AED fail to prevent epileptogenesis. It was reported that only about half of newly diagnosed patients with epilepsy achieve seizure control with their first AED and an additional one out of ten patients may switch to a second or third AED to achieve adequate seizure control, and a minority of patients require adjunct therapy with multiple AEDs. Above one fourth of patients do not achieve seizure control at all (Ajmera, 2018). In addition to that, most of commercially available drugs fail to stop the effect of epileptogenic agents that can prevent the development of epilepsy and its co-morbidities (Ventola, 2014). Frequent medications and the subsequent effects of seizures had an impact on children's learning especially epileptic seizures due to extensive brain abnormality resulted in specific learning difficulties and developmental delays (Shoukat, Ilyas, Azam and Ch, 2013).

Moreover, a substantial treatment gap is evident specially in low income countries because of adverse effects associated with currently available AEDS,misconceptions and stigma surround the disorderand limited human and financial resources for diagnosis and treatment (Wahab, 2010). In addition, there is an increased treatment burden associated with AEDs due to high number of pills, dosing frequency and drug-related costs. Reduction of treatment burden via selection of AED therapy with lower pill numbers and dosing frequency is important in order to improve treatment adherence and both health and economic outcomes (Ajmera, 2018).

Despite their limitations herbal remedies were used for treatment of epilepsy, most of which are experimental tests in laboratory animals. There is still a lack of critical analysis on efficacy and adverse effects for their clinical use. Yet, no experimental studies have been conducted in Ethiopia so far to confirm medicinal value of claimed anti-epileptic plant *P. stellatum*. The present study was done to provide scientific evidence for the anticonvulsant activity of the study plant used by traditional healers. Furthermore,it might initiate further research to be conducted on *P.stellatum* as well as to confirm anticonvulsant activity of other herbs found in Ethiopia.

2. Objectives

2.1. General objective

To evaluate anticonvulsant activity of aqueous and 80% methanol extract of *Pterolobium stellatum* (forssk).

2.2. Specific objectives

- To evaluate anticonvulsant activity of aqueous and 80% methanol extract of *Pterolobium stellatum* (forssk) in pentylenetetrazol induced seizure test.
- To evaluate anticonvulsant activity of aqueous and 80% methanol extract of *Pterolobium stellatum* (forssk) in maximal electric shock-induced seizures test.
- To evaluate the effect of aqueous and 80% methanol extract of *Pterolobium stellatum* (forssk) on motor co-ordination in Rota rod test.
- To perform preliminary phytochemical screening on the aqueous and 80% methanol extract of *Pterolobium stellatum* (forssk).

3. Materials and methods

3.1. Drugs and chemicals

The main chemicals and drugs that were used includes: Distilled water (Ethiopian Pharmaceutical Manufacturer, Ethiopia), Methanol (Carlo Erba Reagents, France), Sodium Valproate (Remedica, Cyprus), Diazepam (Remedica, Cyprus), Phenytoin (Brawn Laboratory, India), Normal Saline (Acu Life Health Care, India),Pentylenetetrazol (Sigma Aldrich, Germany,Hydrochloric acid (BDH, England), Sodium hydroxide (Merck,Germany), Dragendrof's reagent (Fisher Scientific, UK), Glacial acetic acid (Fisher Scientific, UK), Sulfuric acid (Fisher Scientific, UK), Ferric chloride (Qualikems, India), Potassium ferrocyanide (Fisher Scientific, UK) and Acetic Anhydride (Park Scientific, UK). All chemicals used in this experiment were of analytical grade reagents.

3.2. Instruments, apparatus and supplies

Instruments, apparatus and supplies used in the study include: Rotary evaporator (Heidolph, Germany), Lyophilizer (operon, opr-fdu-5012, Korea), electronic balance (kern-alj 220-4, Germany), mini orbital shaker (ssm1-stuart), Separatory funnel, Flasks, Cotton pellets, Syringes with needles,Guavage, Whatman filter paper (Number-42), Electro-convulsometer (Rolex Ambala, India), Rota rod (Biocraft Scientific System Pvt. Ltd., Agra, India).

3.3. Animals

Healthy Swiss male albino mice weighing 25-32 gram were used for the experiment. The mice were obtained from the animal house of School of Pharmacy of Addis Ababa University, Addis Ababa, Ethiopia.

The animals were randomly assigned in to five groups. For each extract six mice are used as one group for the test. The first group was assigned as negative controls and treated with the vehicles used for reconstitution. The second group was assigned as positive control and treated with standard drug (Sodium valproate 200mg/kg p.o) for PTZ induced seizure test, phenytoin 25 mg/kg p.o. for MES -induced seizures test and diazepam 5mg/kg p.o. for Rota rod test). The rest of three groups were treated with various doses. The animals were maintained under standard environmental condition (12h each light/ dark cycle) on a standard animal diet with water *ad libitum,*but fasted prior to dosing (food but not water was withheld for 3-4 hours).

Dose selection was made based on the acute toxicity test in such a way that one tenth of the toxic dose was taken as middle dose and the other two doses were half and twice of the medium dose. All procedures and techniques used in this study were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (Guideline, Testing and Chemicals, 2001).

3.4. Plantmaterial

Fresh leaves of plant *P.stellatum* were collected from its natural habitat around Bahirdar district, which is located 565km from Addis Ababa in Amhara regional state. Identification & authentication of the plant specimens was done by botanist Mr.Melaku Wondafrash at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University, where a voucher specimen can be deposited for future reference with voucher No. of W001.

3.5. Extraction

The leaf of the plant was thoroughly washed with tap water to remove dirt and soil. After that, it was cut into pieces manually, dried under shade, and then, pulverized using a mortar and pestle to get a coarse powder used for the extraction. A weighed quantity (200gm) of air-dried and powdered plant materials divided into two equal portions. The dried powder were exhaustively extracted with water and 80% methanol using maceration technique. Maceration was carried out using one liter of the respective solvent for 72 hours, with regular shaking. The marc was macerated again in the same solvent two times and filtered.

The resulting liquid extract was filtered with Whatman filter paper (Whatman No.42, England) and the filtrate was kept at $+4^{o}$c. The 80 % methanol extract filtrates were combined evaporated under reduced pressure on a rotary evaporator (Heidolph, Germany) at a temperature not exceeding 50ºC. The resultant liquid extract obtained from both aqueous and 80 % methanol were dried as well as concentrated using lyophilizer (operon, opr-fdu-5012, Korea) and reduced pressure.

A percentage yield of (29.2% W/W and 43.2% W/W) was obtained for aqueous and 80% methanol extract,respectively. The dried and concentrated extracts were transferred into vials and kept refrigerated and away from light until use for the proposed experiment.

3.5. Anticonvulsant activity tests

3.5.1. Pentylenetetrazole induced seizure test

PTZ at the dose of 85 mg/kg was injected subcutaneously to induce tonic-clonic convulsions in nearly all mice tested during the observation period usually 30 min (Giardina, Gasior, Laboratories, Park and Chester, 2009).

The mice received 100mg/kg, 200mg/kg, and 400mg/kg of both aqueous and 80% methanol extracts, sodium valproate (200mg/kg) and distilled water (10ml/kg) through the oral route. One hour after administration of standard drug,vehicle and test extracts, PTZ at 85 mg/kg in normal saline solution was injected through subcutaneous route for each mouse. Each mouse was placed into a transparent cage and observed for convulsive behavior for 30 min by using a video recorder.

Latency to the onset time of myoclonic seizure,duration of various phases of ensuing convulsions and percentage of protection were recorded during the first 30 min after PTZ administration and one hour after different doses of aqueous and 80% methanol extract *P.stellatum* administration. The suppression ofclonic seizure was taken as an indicator of anticonvulsant action (Khoshnoud, Tanideh and Namdarian, 2015) (Manasa and Sachin, 2016).

The percentage reduction of clonic convulsion was calculated as:

$$\frac{\text{Duration of clonus in Control-Duration of clonus in Test/Standard}}{\text{Duration of clonus in Control}} * 100$$

- Mice that did not convulse 30 min after PTZ administration was considered as protected (Randrianarivo, 2016).

Figure 2: Clonic seizure exhibited after subcutaneous injection of PTZ.

3.5.2. Maximalelectricshockinducedseizuretest

Anticonvulsant activity of aqueous and 80% methanol extract *P.stellatum* were evaluated using healthy Swiss albino mice (weighing 25-32 g),each group received 100mg/kg, 200mg/kg and 400mg/kg of both aqueous and 80% methanol extracts, standard drug (phenytoin 25 mg/kg), and distilled water (10ml/kg)one hour before application of maximal electric shocks. One hour after administration of standard drug and test extract, animals received maximal electroshocks of 50 mA for 0.2 seconds through ear-clip electrodes by using electro convulsometer and observed closely for 5 min.

Characteristic maximal electroshock convulsions like duration of hind limb tonic extension and the protection against mortality were recorded by using video recorder (Giardina, Gasior, Laboratories, Park and Chester, 2009).

Then the percentage protection was calculated as:

Duration of HLTE in Control-Duration of HLTE in Test/Standard *100

Duration of HLTE in Control

- Prevention or decrease in hind limb tonic extension was considered as protective action.

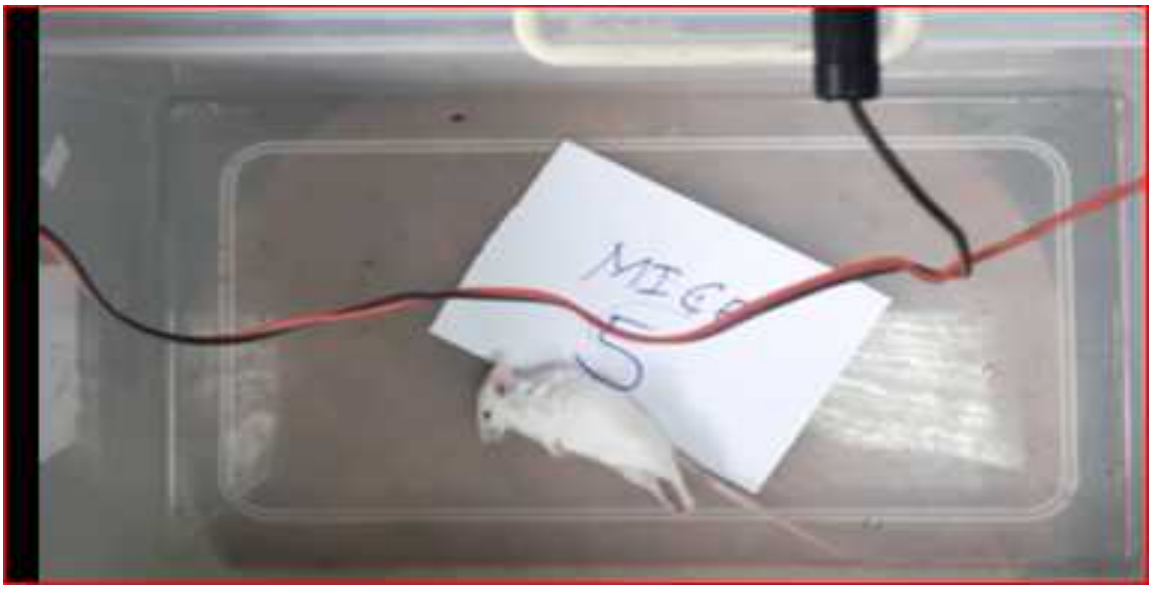

Figure 3: Hind limb tonic extension after application of MES

3.5.3.Rotarodtest

Rota rod test was carried out in order to evaluate any motor incoordination effect the extract might possess. The mice were screened on a rota rod apparatus with slowly revolving rods of 3 cm diameter at 10 rpm for 180 seconds. Mice that remained on the rod for 180 seconds or longer were selected and allotted to five groups with six mice per group. The time each animal spent on the rod was noted and recorded. Accordingly before the training sessions, the mice were habituated to stay on the stationary drum for 3minutes. Habituation was repeated every day for 1minute just before the session.

Acceleration of the rotation was abandoned and the rotation was set at a relatively slow speed (10rpm, 2.8m/min on the surface), to make the task easier for learned animals. The animal was

placed back on the drum immediately after falling, up to 5 times in one session. A fall was over looked when the animal remained on the drum for 180 seconds (Shiotsuki, Yoshimi, Shimo, Funayama and Takamatsu, 2010).

The effect of aqueous and 80% methanol extract of *P. stellatum* on motor co-ordination was evaluated in an accelerating rota rod test. The animals were evaluated for motor coordination one hour after administration of 100mg/kg, 200mg/kg and 400mg/kg of both aqueous and 80% methanol extract, diazepam (5mg/kg) and distilled water (10ml/kg). The time when each animal falls off from the rod for the first time wasnoted during the 3 minute test period (Kudagi, 2012). Motor impairment was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 minute in each of the trials (Kumar, Singh, Singh, Jain, Roy and Mill-, 2014).

3.6. Preliminary phytochemical screening tests

The preliminary phytochemical screening of secondary metabolites of aqueous and 80 % methalonic leaf extract of *P.stellatum* were carried out using phytochemical analysis methods which involve different chemical reactions based on liguid-liquid partitions with solvents. All experimental processes were carried out according to standard procedures.

Test for saponins

To 0.25 g of each aqueous and 80% methanol extract, 5 ml of distilled water was added. Then, the solution was shaken vigorously and observed for a stable persistent froth. Formation of a stable froth that persists for about half an hour indicated the presence of saponins (Tradit, Altern, Sasidharan, Chen, Saravanan, Sundram, *et al.*, 2007) (Ahmed, Ji, Qin, Gu, Liu, Sikandar, *et al.*, 2019,).

Test for terpenoids

To 0.25 g of each aqueous and 80% methanol extract, 2 ml of chloroform was added. Then, 3ml concentrated sulfuric acid was carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids (Tradit, Altern, Sasidharan, Chen, Saravanan, Sundram, *et al.*, 2007) (Ahmed, Ji, Qin, Gu, Liu, Sikandar, *et al.*, 2019,).

Test for tannins

About 0.25 g of each aqueous and 80% methanol extract were boiled in 10 ml of water in a test tube and then filtered with filter paper (Whatman No.42). A few drops of 0.1% ferric chloride were added to the filtrate. A brownish green or a blue-black precipitate indicated the presence of tannins (Tradit, Altern, Sasidharan, Chen, Saravanan, Sundram, *et al.*, 2007) (Ahmed, Ji, Qin, Gu, Liu, Sikandar, *et al.*, 2019,).

Test for flavonoids

About 10 ml of ethyl acetate was added to 0.2 g of each aqueous and 80% methanol extract, and heated on a water bath for 3 minutes. The mixture was cooled and filtered. Then, about 4 ml of the filtrate was taken and shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and the yellow color in the ammonia layer indicated the presence of flavonoids (Tradit, Altern, Sasidharan, Chen, Saravanan, Sundram, *et al.*, 2007) (Ahmed, Ji, Qin, Gu, Liu, Sikandar, *et al.*, 2019,).

Test for cardiac glycosides

To 0.25 g of each aqueous and 80% methanol extract were diluted with 5 ml of water, 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underlayed with 1 ml of concentrated sulfuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides (Tradit, Altern, Sasidharan, Chen, Saravanan, Sundram, *et al.*, 2007) (Ahmed, Ji, Qin, Gu, Liu, Sikandar, *et al.*, 2019,).

Test for steroids

Two ml of acetic anhydride was added to 0.25 g of each aqueous and 80% methanol extract with 2 ml sulfuric acid. The color change from violet to blue or green in some samples indicated the presence of steroids (Tradit, Altern, Sasidharan, Chen, Saravanan, Sundram, *et al.*, 2007) (Ahmed, Ji, Qin, Gu, Liu, Sikandar, *et al.*, 2019,).

3.7. Data analysis

The dose of the compound required for inducing anticonvulsant effect in nearly all animals and its associated 95% confidence limit were calculated by SPSS software version 25. Data obtained from delay convulsion behavior were expressed as Mean ± SEM and were analyzed by One-way ANOVA along with Tukey multiple comparison test.

4. Results

4.1. Anticonvulsant effect

4.1.1. PTZ induced seizure test

Oral administration of aqueous extract of *P.stellatum* was shown to possess anticonvulsant effects on PTZ-induced clonic seizures at a wide range of different doses. The aqueous extract at dose 400mg/kg shown significant increase in latency to the onset of myoclonic seizure compared with the control group ($p<0.05$). The aqueous extract also showed significant reduction on the duration of seizure at doses 200 and 400mg/kg compared to the control group *(*$p<0.01$ and$p<0.001$) respectively (Table 1).

Table 1: Anticonvulsant activity of aqueous extract of *Pterolobium stellatum* against PTZ induced seizure in mice

Groups	Treatment	Onset of Seizure (sec) Mean ± SEM	Duration of Seizure (sec) Mean ± SEM	% of protection
I	DW 10ml/kg	148.67 ± 12.34	92.67 ± 13.13	-
II	SVP 200mg/kg	1574.17 ± 225.83***	3.33 ± 3.33***	83.33
III	PSA100mg/kg	611.00 ± 245.15	52.67 ± 14.10	16.67
IV	PSA 200mg/kg	868.00 ± 301.39	27.83 ± 12.44**	33.33
V	PSA 400mg/kg	1125.83 ± 304.70*	18.83 ± 9.80***	50

Note: Data was analyzed using one way ANOVA followed by Tukey multiple comparison test. Values are expressed as mean ± SEM. n=6, *** $p< 0.001$ is considered as highly significant.

DW= Distilled water, PSA=Pterolobium stellatum Aqueous, SVP=Sodium valproate,n=number of animals

As shown in (Table2) from a total of six animals in each group, 5(83.3%) of animals which received standard drug sodium valproate, 4(66.67%) of animals which received 400mg/kg of methanol extract and 2(33.3%) of animals which received 100mg/kg and 200mg/kg of 80% methanol extract of *P.stellatum* were protected from development of seizures. Animals which received 400 mg/kg of 80% methanol extract of *P.stellatum* exhibited significant delay in the onset of seizures ($p<0.01$) and animals which received 100,200 and 400mg/kg of 80% methanol extract of the study plant showed significant reduction in the duration of seizure ($p< 0.001$) when compared with control (Table 2). The 80% methanol extract of study plant has shown dose dependent increase in latency to onset of seizure ($R^2=0.91$).

Table 2: Anticonvulsant activity of 80% methanol extract of *Pterolobium stellatum* against PTZ induced seizure in mice

Groups	Treatment	Onset of Seizure (sec) Mean ± SEM	Duration of Seizure (sec) Mean ± SEM	% of protection
I	DW 10ml/kg	148.67 ± 12.34	92.67 ± 13.13	-
II	SVP 200mg/kg	1574.17 ± 225.83***	3.33 ± 3.33***	83.33
III	PSM 100mg/kg	918.33 ± 282.40	31.50 ± 11.15***	33.33
IV	PSM200mg/kg	1021.67 ± 261.62	22.00 ± 8.43***	33.33
V	PSM 400mg/kg	1366.67 ± 285.88**	7.17 ± 4.62***	66.67

Note: Data was analyzed using one way ANOVA followed by Tukey multiple comparison test. Values are expressed as mean ± SEM. n=6, *** $p < 0.001$ is considered as highly significant.
DW= Distilled water, PSM= Pterolobium stellatum Methanol, SVP=Sodium valproate, n=number of animals

4.1.2. MES induced seizure test

The mean duration of HLTE in the control group (Group I) was 20.50±1.46 seconds. The mean duration of HLTE were 13.67± 0.88, 10.50± 2.17 and 8.83± 0.91 seconds for groups received 100mg/kg, 200mg/kg and 400mg/kg of aqueous extract respectively (Table3). The reduction in the HLTE were statistically significant at dose levels of 200mg/kg and 400mg/kg ($p<0.01$ and $p<0.001$) respectively when compared with the control group. The aqueous extracts of *P.stellatum* showed dose dependent reduction in the duration of HLTE ($R^2=0.97$).

Table 3: Anticonvulsant activity of aqueous extract of *Pterolobium stellatum* against MES induced seizure in mice

Groups	Treatment	Duration of HLTE (sec) Mean ± SEM	% of reduction to mortality
I	DW 10ml/kg	20.50 ± 1.46	-
II	Phenytoin 25mg/kg	3.17 ± 3.17***	83.33
III	PSA 100mg/kg	13.67 ± 0.88	33.33
IV	PSA 200mg/kg	10.50 ± 2.17**	50
V	PSA400mg/kg	8.83 ± 0.91***	83.33

Note: Data was analyzed using one way ANOVA followed by Tukey multiple comparison test. Values are expressed as mean ± SEM. n=6, *** $p < 0.001$ is considered as highly significant.
DW= Distilled water, PSA= Pterolobium stellatum Aqueous, n=number of animals

As shown in (Table 4) 80% methanol extract of *P.stellatum* at dose of 100mg/kg, 200mg/kg and 400 mg/kg has shown significant duration of HLTE ($p<0.05$, $p<0.01$ and $p<0.001$) respectively. The study plant has shown dose dependent reduction in duration of HLTE ($R^2=0.88$). Rate of mortality were reduced by 83.3% in animals which received standard drug phenytoin at dose of 25mg/kg and 50% in animals which received 400mg/kg of 80% methanol extract of study plant respectively. Animals which received 100mg/kg and 200mg/kg of 80% methanol extract were shown only 33.3% reduction in mortality.

Table 4: Anticonvulsant activity of 80% methanol extract of *Pterolobium stellatum* against MES induced seizure in mice

Groups	Treatment	Duration of HLTE (sec) Mean± SEM	% of reduction to mortality
I	DW 10ml/kg	20.50 ± 1.46	-
II	Phenytoin 25mg/kg	3.17 ± 3.17***	83.33
III	PSM 100mg/kg	11.67 ± 1.15*	33.33
IV	PSM 200mg/kg	7.17 ± 2.44***	33.33
V	PSM 400mg/kg	6.17 ± 1.35***	50

Note: Data was analyzed using one way ANOVA followed by Tukey multiple comparison test. Values are expressed as mean ± SEM. n=6, *** $p < 0.001$ is considered as highly significant.
DW= Distilled water, PSM= Pterolobium stellatum Methanol, n=number of animals

4.1.3. Rota rod test

In rota rod test the aqueous extract of the study plant did not show motor defect at all doses administered (100mg/kg, 200mg/kg and 400mg/kg). However, diazepam at dose of 5mg/kg has shown significant reduction of retention time on rotating road when compared with control groups ($p< 0.01$).

Table 5: Effect of aqueous extract of *Pterolobium stellatum* on motor coordination against Rota rod test

Groups	Treatment	Mean latency of fall (Sec)
I	DW 10ml/kg	236.67 ± 42.09
II	DZP 5mg/kg	56.67 ± 12.09**
III	PSA 100mg/kg	224.17 ± 42.63
IV	PSA 200mg/kg	212.50 ± 41.99
V	PSA 400mg/kg	206.67 ± 23.05

Note: Data was analyzed using one way ANOVA followed by Tukey multiple comparison test. Values are expressed as mean ± SEM. n=6, *** $p < 0.001$ is considered as highly significant.
DW= Distilled water, PSA= Pterolobium stellatum Aqueous, n=number of animals,
DZP= Diazepam

As shown in (Table 6) any dose of the 80% methanol extract administered (100mg/kg,200mg/kg and 400mg/kg) did not show motor deficit, as all treated mice were maintained in their equilibrium on the rotating road for more than 180seconds, which was also comparable to control group.

Table 6: Effect of 80% methanol extract of *Pterolobium stellatum* on motor coordination against Rota rod test

Groups	Treatment	Mean latency of fall (Sec)
I	DW 10ml/kg	236.67 ± 42.09
II	DZP 5mg/kg	56.67 ± 12.09*
III	PSM 100mg/kg	228.67 ± 45.13
IV	PSM 200mg/kg	222.17 ± 37.27
V	PSM400mg/kg	219.17 ± 51.13

Note: Data was analyzed using one way ANOVA followed by Tukey multiple comparison test. Values are expressed as mean ± SEM. n=6, *** $p < 0.001$ is considered as highly significant.
DW= Distilled water, PSM= Pterolobium stellatum Methanol, n=number of animals
, DZP= Diazepam

4.2. Preliminary Phytochemical screening tests

As shown in (Table7) phytochemical screening study revealed the presence of alkaloids, terpenoids, steroids, saponins and tannins in both aqueous and 80% methanol extract. The aqueous extract contains glycosides whereas the 80% methanol extract contains flavonoids.

Table 7: Phytochemical components of aqueous and 80% leaf extract of *Pterolobium stellatum*

Phytochemical components	Aqueous extract	80% methanol extract
Alkaloids	+	+
Glycosides	+	_
Steroids	+	+
Flavonoids	_	+
Terpenoids	+	+
Sapponins	+	+
Tannins	+	+

-, Absent; +, Present.

5. Discussion

Substantial treatment gaps, increased treatment burdens, drug-resistance and adverse effects of currently available AEDs are the main challengesin the treatment of epilepsy (Ajmera, 2018). Therefore,it is worthwhile to investigate fornew types of anticonvulsants with broad spectrum and lower toxicity. One of the approaches to search for new antiepileptic drugs is the consideration of naturally occurring compounds from traditional medicinal plants.

In the present study, the effect of aqueous and 80% methanol extract of *P.stellatum* on seizure induced by MES and PTZ in mice were studied. Both aqueous and 80% methanol extracts were orally administered asthe hydro alcoholic extract of claimed plant have been traditionally recognized to produce beneficial effect in management of epilepsy (Ragunathan and Abay, 2009).

Anticonvulsant activity of the study plant was evaluated using the acute seizure tests, the MES and PTZ tests. Those tests are assumed to identify anticonvulsant drugs effective against grand mal type and petit mal type respectively as well as they are used to predict the clinical spectrum of activity of experimental compounds (Giardina, Gasior, Laboratories, Park and Chester, 2009). The acute seizure models were used in the present study becausethey were remained as standards for more than six decades in early stages of many AED screening programs (Giardina, Gasior, Laboratories, Park and Chester, 2009). Epileptic seizures have multiple pathological processes (Grone and Baraban, 2015), and are suitable for studying seizures and memory impairment associated with epilepsy (Khoshnoud, Tanideh and Namdarian, 2015).

The present study provide evidence for the anticonvulsant activity of both aqueous and 80% methanol extract of *P.stellatum* against PTZ-induced generalized tonic-clonic seizures in a dose-dependent manner at a wide range of different doses. The aqueous extract of the study plant at doses 200mg/kg and 400mg/kg decreases duration of myoclonic seizure. Furthermore, the aqueous extract at dose 400mg/kg possess significant increase in onset of myoclonic seizure compared to control. This indicates the aqueous extract at this dose have better anticonvulsant activity than other lower doses which might be due to the presence of higher concentration of phytochemical constituents.

In addition, the 80% methanol extract at dose of 400mg/kg have shown better protection against PTZ-induced myoclonic seizure, increase in onset of myoclonic seizure and its protection against PTZ-induced myoclonic seizure at doses 100mg/kg and 200mg/kg were comparable with control groups. Furthermore, the 80% methanol extract have shown significant reduction in duration of myoclonic seizure against PTZ-induced seizure tests. This indicates 80% methanol extract showed better effect in tonic-clonic seizure than absence seizures.

Both aqueous and 80% methanol extract of the study plant exhibited anticonvulsant activity as a result of increased time taken for onset of convulsion and tonic convulsion induced by PTZ. The anticonvulsant effect of the extract against PTZ-induced convulsion might be due to GABA agonist like effect. PTZ is a non-competitive antagonist at GABA receptors and it blocks the GABA activated chloride ionophore (Löscher, 2011). Since, the extract was counteracting the action of PTZ, that effect might be through modification of the function of GABA receptor mediated chloride channel.

Indeed, several studies have shown that generalized tonic-clonic seizures in animals which are induced by a non-competitive antagonist of γ-aminobutyric acid type A (GABA-A) receptors, pentylenetetrazol (PTZ) can be prevented by different classes of drugs. Those drugs include drugs that reduce T-type Ca^{2+} currents, such as ethosuximide, enhance gamma amino butyric acid type A (GABA-A) receptor mediated inhibitory neurotransmission such as benzodiazepines and phenobarbital and perhaps valproate and felbamate (Löscher, 2011). Hence,our observations from PTZ-induced generalized tonic-clonic seizure tests suggests that the study plant potential anticonvulsant effects were probably attributed by elevating the seizure threshold in the brain secondary to inhibition of T-type calcium currents or enhancement of GABAergic neurotransmission and inhibition of initiation and generalization of PTZ- induced convulsion by N-methyl D-aspartate (NMDA) receptors.

The potential anticonvulsant activity of both aqueous and 80% methanol extract of *P.stellatum* were evaluated using MES-induced seizure test. This test was used because of the fact that it is most likely the best validated preclinical test that predicts drugs effective against generalized seizures of the tonic–clonic (grand mal) type), induces seizure particularly due to the spread of stimulus throughout the body. Oral administration ofaqueous extract of *P.stellatum* at the dose level of 200mg/kg and 400 mg/kg have shown significant reduction on the duration of HLTE in

MES induced convulsion tests compared to control.In addition to that the study plant at the dose level of 400mg/kg decrease death due to MES-induced seizure by 83.33%. This indicate that the higher dose could be taken as the maximum effective dose relative to lower and middle dose and the presence of good concentrations of active constituents.

Moreover, the 80% methanol extract has significant ability to slowdown the spread of seizure by decreasing duration of HLTE at all tested dose levels. Observations from MES test suggested that the study plant have the ability to inhibit the HLTE in MES test as compared to phenytoin which indicates its anticonvulsant activity for the management of generalized seizures.

It was interesting to note that both aqueous and 80% methanol extract of the study plant were found to be effective in reduction of HLTE against MES-induced seizure test. It has been shown that characteristic maximal electroshock convulsions like hind limb tonic extension (HLTE) can be prevented using anticonvulsant drugs which exert their effect by blocking the seizure spread via inhibition of voltage-dependent Na^{+}channels or by drugs that block glutamatergic receptor (Lopes-Aguiar, Ruggiero, Umeoka, Kandratavicius, Alves Balista, Lopes-Aguiar, *et al.*, 2014). Therefore, the anticonvulsant activity of *P.stellatum* might be attributed to one or more of the above mechanisms. Though, the exact mechanisms underlying the anticonvulsant activity of the study plant is remain to be elucidated.

The present study provides evidence that both the aqueous and 80% methanol extract of *P.stellatum* are effective against both MES and PTZ-induced seizure types and may be acting through synergistic effects in ionic movements across the seizure foci. The multiple mechanisms of the study plant might be due to the presence of different active components. However,compared to the aqueous extract relatively the 80% methanol extract has shown better anticonvulsant activity in both PTZ and MES-induced seizure tests. The fact that the extracts of the plants are showing different efficacy against different animal models presumably because of extraction ability of active ingredients responsible for anticonvulsant activity by 80% methanol and presence of different secondary metabolites present in aqueous and 80% methanolextracts of the study plant. It was reported that natural products containing flavonoids have better anticonvulsant activity. This is because flavonoids are structurally similar with benzodiazepines, and hence they act on GABA receptors and potentiate its effect. Flavonoids have also antioxidant activity so that they decrease oxidative stress in brain cells (Diniz, Silva, Lima-

Saraiva, Ribeiro, Pacheco, De Freitas, *et al.*, 2015). Therefore, from the present study it is important to note that flavonoids might play an important role in prevention of both grand-mal and petit-mal type of seizures. In addition to this the presence of different type and concentration of active constituents in each extract might result discrepancy in effectiveness of the study plant against acute animal models of epilepsy.

The effect of both aqueous and 80% methanol extract on motor coordination were evaluated using rota rod tests. This test was carried out in order to rule out any motor incoordination effect the extract might possess (Shiotsuki, Yoshimi, Shimo, Funayama and Takamatsu, 2010). Our findings showed that, mice which received different doses of both aqueous and 80% methanol extract did not show motor deficit. In the present study, we found that all mice which received 100mg/kg, 200mg/kg and 400mg/kg of both aqueous and 80% methanol extracts were easily able to maintain balance on the rota rod for more than 3 minutes, which was also comparable to control group. However, diazepam at dose of 5mg/kg has shown significant reduction of retention time on rotating road when compared with control groups.

Since, both the aqueous and 80%methanol extracts of the study plant at their effective doses allowed the animals to maintain their equilibrium on the rotating rod, indicating them to be devoid of neurotoxic effects. Observations from the rota rod test suggested that the anticonvulsant activity of the study plant was not due to muscle relaxation associated with blockage of neuromuscular junctions. Therefore, it is possible to say that *P.stellatum* might serve as a potential candidate or additional source of AEDs in the treatment of epilepsy.

Phytochemical analysis of *P.stellatum* leaf extracts showed the presence of saponins, terpenoids, alkaloids, steroids and tannins in both aqueous and 80% methanol extract whereas absence of glycosides in 80% methanol extract and flavonoids in aqueous extract. Our result is supported by (Omwoyo, Maingi and Kebira, 2017), who reported the presence of flavonoids,terpenoids and tannins in methanolic root extracts of study plant. However, in the present study the leaf extract contains additional active constituents. This might be attributed due to difference in plant morphology, area of collection or solvents used for extraction of such phytochemicals.

Alkaloids, terpinoids, saponins and flavonoids have been variously reported to possess anticonvulsant activities and since similar phytoconstituents are also present in the tested extract of the study plant which may be responsible singly or in combination for the observed activity. It was reported that the presence of such phytochemical constituents in natural products prevent occurrence of seizure by decreasing oxidative damages, inhibition of glutamate-induced over-excitation and reduces synaptic release of glutamate or NMDA, calcium channel, sodium channels and via inhibition of calcium overload they prevent an epileptic episode induced by Mg^{2+} deficiency (Liu, Ge, Pan, Leng, Lv and Li, 2017).

Astudy done on medicinal value of terpenoids revealed promising antiepileptic activity of those plant secondary metabolites via regulation of GABA and glutaminergic neurons in brain cells (Gao, Yan, Jin and Lei, 2016). Another study which was done on different solvent fractions of herbal remedies used for treatment of epilepsy have shown that saponin rich fractions of plant extracts possess better anticonvulsant activities over other solvent fractions which lacks saponins possibly due to inhibition of GABA up take and NMDA receptors in brain cells (Singh, Singh and Goel, 2012).

Likewise, a study done on experimental mice using MES and PTZ models revealed that glycosides and terpenoids abolish or tend to reduce PTZ-induced seizure and reduce MES-induced convulsions possibly through the stimulation of the CNS inhibitory pathway or through the reduction of muscle tone, sedation and induction of sleep by antagonizing the GABA receptor/ chloride channel complex (Ilodigwe, Akah, Okoye and Omeje, 2010). Therefore,it is thus noteworthy that the anticonvulsant activity of *P.stellatum* might be attributed due to the presence of those phytoconstituents which exert their effect via multiple mechanisms of action andpossess their anticonvulsant activity because of synergetic effect of different phytochemical components.

6. Conclusion

From the present study it's evident that the hydro alcoholic extract of *Pterolobium stellatum* possess anticonvulsant activity. Both aqueous and 80% methanol extracts have shown significant anticonvulsant activity against PTZ and MES-induced seizure tests.

Moreover, results from rota rod test indicates the anticonvulsant activity of the study plant is not caused by muscle relaxation associated with blockage of neuromuscular junctions. The results of present study validates the traditional use of *P.stellatum* leaves in treatment of epilepsy, as its hydroalcoholic extract pretreatment prevented MES and PTZ-induced convulsions in mice.

7. Recommendations

The study plant possess anticonvulsant activity; accordingly, the following activities are recommended to be done in future;

- Additional anticonvulsant activity studies on different solvent fractions should be done to clearly describe the potential anticonvulsant activity of the study plant.
- Further studies should be done to isolate, purify and identify pharmacologically active principle (s) responsible for anticonvulsant activity displayed by the plant.
- The potential anticonvulsant activity of the study plant should be investigated using chronic animal models of epilepsy
- Further in-vitro experimental studies should be done in order to investigate the basic mechanisms of the study plant.

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