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ORIGINAL ARTICLE

Beneficial Effect of *Moringa stenopetala* (Bak.f) Cuf. on Lithium–Pilocarpine-Induced Temporal Lobe Epilepsy in Experimental Animals

Etsay Weldekidan Tsegay[1,2], Rajkapoor Balasubramanian[1,3], Kald Beshir Tuem[1], Abadi Kahsu Gebre[1]

[1]Department of Pharmacology and Toxicology, School of Pharmacy, Mekelle University, Mekelle, [2]Department of Pharmacy, College of Medicine and Health Sciences, Adigrat University, Adigrat, Ethiopia, [3]Department of Pharmacology, JKK Nattraja College of Pharmacy, Namakkal, Tamil Nadu, India

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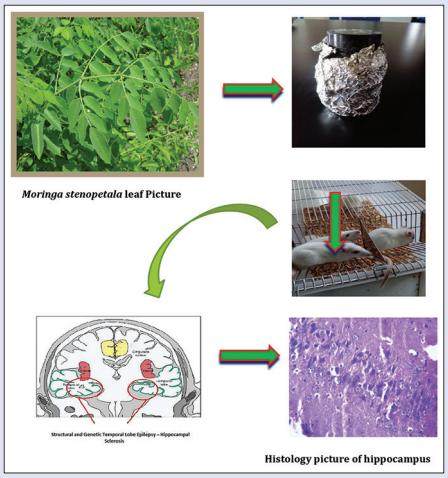
ABSTRACT

Background: *Moringa stenopetala* is traditionally used for the treatment of epilepsy in southern Ethiopia. Additionally, the plant material is used for the treatment of various ailments such as pain, diabetes, hypertension, and hyperlipemia. **Aim of the Study:** The aim of the study was to investigate the effect of *M. stenopetala* crude extract on the development of lithium–pilocarpine-induced temporal lobe epilepsy model in rats. **Materials and Methods:** Male Sprague-Dawley rats weighing 200–225 g were randomly divided into nine groups. Groups I, V, and VI served as normal control, positive control, and negative control, respectively. While Groups II–IV were treated with three different doses (400, 600, and 800 mg/kg) levels of crude extract in pre- and postinduction of status epilepticus (SE). On the other hand, Groups VII–IX were treated with the same three dose levels but after induction of SE only. All the rats, except those in normal control, were subjected to lithium (3 meq/kg) and pilocarpine (35 mg/kg) for the induction of SE. In addition, the elevated plusmaze and infrared actimeter models were used to evaluate anxiolytic and antidepressant efficacy in SE animals. **Results:** The rats treated with the crude extract of *M. stenopetala* significantly increased the latency period to the first motor seizure (58.1 min) and SE (80.4 min) compared to the control group; those developed seizure and SE at 13.3 and 35.4 min, respectively. The chronic spontaneous seizure was less severity just below spontaneous recurrent seizure at Pinel and Rovner scale 6 in rats treated with extract pre- and postinduction. The crude extract also showed significant dose-dependent anxiolytic activity ($P < 0.001$) and improved locomotor activity ($P < 0.001$). The histopathological studies of hippocampus formation revealed that the extract prevented damages compared to control animals. **Conclusion:** The current findings suggest that a crude extract of *M. stenopetala* has anticonvulsant activity in a lithium–pilocarpine-induced epilepsy model *M. stenopetala* also reduced depression and anxiety associated with epilepsy that indicates that it is having antidepressant and anxiolytic activity.

Key words: Antidepressant, anxiolytic, lithium–pilocarpine, *Moringa stenopetala*, temporal lobe epilepsy

SUMMARY

- The *Moringa stenopetala* is traditionally used for epilepsy treatment among Konso people, Ethiopia. However, there is no scientific study conducted so far that verified the ethnobotanical claim of the plant for epilepsy treatment. Hence, the aim of the study was to investigate the effect of *M. stenopetala* crude extract on the development of lithium–pilocarpine-induced temporal lobe epilepsy (TLE) model in rats. From experimental studies showed that *M. stenopetala* extract decreased convulsion and behavioral epilepsy parameters including histopathology changes in lithium–pilocarpine-induced TLE model. It also possesses antidepressant and anxiolytic activities.



Abbreviations used: AED: Antiepileptic drug; CA: Cornu Ammonis; CNS: Central nervous system; GABA: Gamma-aminobutyric acid; Lit-Pilo: Lithium–pilocarpine; NMDA: N-methyl-D-aspartate; TLE: Temporal lobe epilepsy; SE: Status epilepticus; SRS: Spontaneous recurrent seizure.

Correspondence:

Mr. Etsay Weldekidan Tsegay,
Department of Pharmacy, College of Medicine and Health Sciences, Adigrat University, Adigrat, Ethiopia.
E-mail: rajkapoorb@gmail.com
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INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by recurrent and unprovoked seizures arising from the excessive synchronized and sustained discharge of a group of neurons.[1] Fifty million people have epilepsy globally, of which approximately 80% are from low- and middle-income countries.[2] In Ethiopia, there is no comprehensive study conducted so far, but it varies from 5.2% to 29.5%.[3,4]

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The current antiepileptic drugs provides only symptomatic relief.[5] This is further complicated by the high adverse effect and cost of the medications. In addition, a high incidence of psychiatric comorbidities such as anxiety and depression are also reported in patients with epilepsy. The prevalence of comorbid depression is as high as 37% in a community-based study of people with epilepsy.[6,7] The increased prevalence of depression and anxiety with epilepsy increases morbidity and disability of patients.[8] Therefore, this warrants the need for a new drug or lead compound for improving epilepsy treatment.

Herbal medicine has been used for centuries for the treatment of various ailments, and they have been a major source of modern medicines. Ethiopia is reaching in terms of biodiversity, and the societies have been using traditional medicine for millennia. *Moringa stenopetala* is known with vernacular name *Aleko* or *Shiferaw*. *M. stenopetala* is a highly nutritious edible plant that mostly grows in southern Ethiopia.[9] This plant is also used for the treatment of various ailments such as pain,[10] diabetes,[11] hypertension, and hyperlipemia.[12] The *M. stenopetala* is traditionally used for epilepsy treatment among Konso people.[9] However, there is no study conducted so far that verified the ethnobotanical claim of the plant for epilepsy treatment. Whereas, pilocarpine-induced model is the best chronic model and is widely used to study the pathophysiology of seizures.[13-15] The aim of this study is to evaluate the antiepileptic properties of *M. stenopetala* and to rationalize its traditional use based on scientific findings.

MATERIALS AND METHODS

Drugs and chemicals

Pilocarpine hydrochloride (Himedia Laboratories Pvt. Ltd., India), lithium chloride (Indenta Chemicals Pvt. Ltd., India), scopolamine methyl nitrate (Sigma-Aldrich, USA), hematoxylin monohydrate (Sisco Research Laboratories Pvt. Ltd., India), formaldehyde (Abron Chemicals. Pvt. Ltd., India), and ethanol absolute (Fine Chemical G.T.PLC, Ethiopia) were procured from the respective companies. All other chemicals were used of analytical grade.

Plant material collection and extraction

Fresh leaves of *M. stenopetala* were collected from Alamata city, Tigray, Ethiopia. The plant was also authenticated by Dr. Getinet Masresha Kassa, and a voucher specimen (MM0028/2010) was deposited in the herbarium of Faculty of Biology, Gondar University, Ethiopia. Extraction was performed, in briefly the leaves were shade dried, ground into coarse powder, and extracted with ethanol (70%) using a Soxhlet apparatus. The extract was dried and kept in desiccator throughout the study. The percentage of extract yield was 17%.

Experimental animals

The experiment was performed on adult male Sprague-Dawley rats weighing 200–225 g. They were all acclimatized to the laboratory setting for 2 weeks prior to use. The rats were kept in cages under controlled room temperature at 27°C ± 2°C and equal light/dark cycle of 12 h. They were fed standard pellet diet and water *ad libitum*. Experiments mentioned in this study were conducted in accordance with guidelines of local animal ethical committee and approval was obtained from the Ethics Review Committee, College of Health Sciences (HRERC, 1210/2018).

Oral acute toxicity testing

Oral acute toxicity of *M. stenopetala* was evaluated according to the Organization for Economic Co-operation and Development 425 guidelines for the testing of chemicals.[16] Prior to dosing, the animals were fasted for the night. Following the fasting period, the animals were weighed and the test substance was given to them. Each stage was completed with three animals, and the beginning dose was 100 mg/kg. This pattern maintained until the maximum dose of 2000 mg/kg was reached. Any sign of toxicity (diarrhea, decrease of appetite, hair erection and loss, lacrimation convulsion, salivation, lethargy, paralysis, and mortality) was observed individually after dosing for the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h and daily thereafter, for a total of 14 days.

Experimental design

Induction of status epilepticus

To induce status epilepticus (SE), animals were randomly assigned into nine groups (n = 7). Based on the previous study, the rats received lithium chloride (LiCl, 3 meq/kg i.p) and, 20 h later, scopolamine butylbromide (1 mg/kg s.c) to limit the peripheral effects of pilocarpine. SE was induced by injecting pilocarpine hydrochloride (35 mg/kg, s.c) 30 min later.[17] Protocols for Lit-Pilo-induced seizure model are tabulated in Table 1.

The occurrence of seizure was assessed based on the manifestation of seizure symptoms but not necessarily of clonic movements of the four paws plus rearing and falling. SE onset was determined by behavioral observations and was considered started when three Racine's scale Stage V seizures (clonic movements of the four paws plus rearing and falling) occurred and the time of the onset after pilocarpine injection were recorded.[18]

The overall follow-up was continued for 30 days for any parameters such as the manifestation of spontaneous recurrent seizures (SRSs), mean behavioral score, seizure frequency, seizure duration, and mortality rate was recorded, score were categorized based on the eight-scale established by Racine and modified by Pinel and Rovner[19] which is simply extension of Racine scale.

Affective behavior test

Elevated plus-maze model

Elevated plus-maze is validated to use in rats and mice for anxiety experiments.[20] An arm entry was defined as the entry of all four feet into one arm.[21] In brief, animals (n = 5) were placed individually in the center of the maze, facing toward open arm. Number of entries into open and closed arm, average time spent in the open and closed arms, and time spent in the neutral zone were recorded in 5 min.[22]

Infrared actimeter model

Animals (n = 5) were placed individually in the infrared actimeter and allowed to explore freely for 10 min, and locomotor activity was recorded in terms of the total number of interruptions of the photo beams in X, Y direction and T (total).

Histological studies

Animals were anesthetized with ketamine 160 mg/kg ketamine[23] and subsequently perfused transcardially with 10% of paraformaldehyde in sodium phosphate buffer 0.1 M, pH 7.4. Then, the brains were extracted and postfixed by immersion in the same fixative solution at 4°C till the next process. The hippocampus was separated, processed, and dehydrated with different grades of alcohol, xylene, and paraffin. The brain was sectioned and stained with hematoxylin and eosin coloration. Histopathological changes were examined with a microscopic magnification of × 4–×40.[24]

Statistical analysis

Data were analyzed by SPSS version 20.0 (IBM, New York, USA) and expressed as means ± standard error of mean. The mean difference

among different groups was analyzed by one-way ANOVA, followed by Tukey's *post hoc* test. The mortality rate was evaluated using Kaplan–Meier survival analysis. The statistical significance was set as $P < 0.05$.

RESULTS

Oral acute toxicity study

In acute toxicity studies, there was no mortality or any signs of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma up to the dose of 2000 mg/kg. This finding suggests that 70% of ethanol extract of *M. stenopetala* is non-toxic up to 2000 mg/kg. Additionally, the plant is edible in southern Ethiopia, but there is no report of undesirable outcomes related to the consumption of the plant.

Effect of *Moringa stenopetala* extract on seizure and latency to status epilepticus

Pretreatment of the crude extract of the plant material has significantly ($P < 0.01$; 0.001) delayed the mean latency to the first episode of limbic seizure in a dose-dependent manner. Duration, frequency, and mortality associated with SE were significantly lower in animals treated with 800 mg/kg among the extract-treated groups [Table 2]. Similarly, there were disparities in the rats' long-term survival, as seen in Figure 1's mortality graph.

Effect of *Moringa stenopetala* crude extract on seizure behavioral score

The eight-class scale modified by Pinel and Rovner[19] is used to summarize the development of elicited motor seizures [Table 3]. This is useful in characterizing seizures that occurred spontaneously. The severity of epilepsy increased as the scale advanced from 1 to 8, and only rats with three Pinel and Rovner scale V (facial movement, head nodding, and forelimb clonus, rearing and falling) are regarded to have acquired SRS.

Scale 1 motor seizures were very common to all groups, whereas scale 8 motor seizures were never observed. Even though the treatment of *M. stenopetala* ethanol extract (MSEE) could not prevent totally the occurrence seizure, the pre- and post-treatment of MSEE significantly ($P < 0.001$) reduces the development of seizures with scales 1–4 and prevents the progress of those seizures into SRS. This is shown by total absence of scales 6, 7, and 8 in both MSEE pre- and posttreated groups. Data from Table 4 show the behavior score of rats from MSEE pretreated group that reached SE is dose dependent and similarly their progress to SRS is also dose-dependent manner [Table 4].

Effect of *Moringa stenopetala* on the occurrence of spontaneous recurrent seizures

When a subject reached the criterion of three spontaneous motor seizures of scale V or greater, the rat was considered as it developed SRS [Table 5]. During the 30-day follow-up period, pretreatment of the crude extract at a dose of 600 mg/kg and 800 mg/kg prevented the occurrences of SRS and there was no SRS which shows that the latency period for SRS is beyond the 30 days of follow-up period. Whereas, rats pretreated by 400 mg/kg of MSEE showed the lowest SRS scale 5 with latency period of 2.0 ± 0.2 days. Post-treatment of MSEE crude extract after the induction

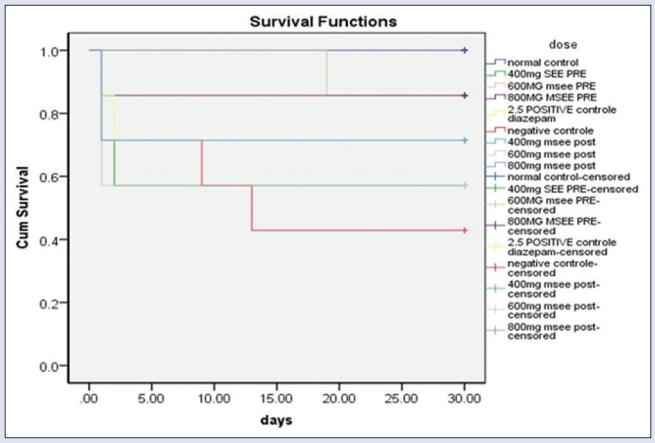

Figure 1: Mortality graph

Table 1: Protocol for lithium-pilocarpine-induced seizure model

Group	Day 1-14	Day 15	Day 16-30
Group I	Vehicle	NS	Vehicle
Group II	*M. stenopetala* (400 mg/kg)	Lit-Pilo	*M. stenopetala* (400 mg/kg)
Group III	*M. stenopetala* (600 mg/kg)	Lit-Pilo	*M. stenopetala* (600 mg/kg)
Group IV	*M. stenopetala* (800 mg/kg)	Lit-Pilo	*M. stenopetala* (800 mg/kg)
Group V	Vehicle	Lit-Pilo	Diazepam (3 mg/kg)
Group VI	Vehicle	Lit-Pilo	vehicle
Group VII	Vehicle	Lit-Pilo	*M. stenopetala* (400 mg/kg)
Group VIII	Vehicle	Lit-Pilo	*M. stenopetala* (600 mg/kg)
Group IX	Vehicle	Lit-Pilo	*M. stenopetala* (800 mg/kg)

Lit-Pilo: Lithium-pilocarpine; *M. stenopetala: Moringa stenopetala*

Table 2: Effect of *Moringa stenopetala* on lithium-pilocarpine-induced seizures latency and lethality

Dose	Latency to first seizure	Onset of SE (min)	Frequency of SE	Duration of SE (min)	Percent of animals reaching SE (%)	Percent of mortality in 24 h (%)	Percentage of long-term survival (30 days)
10 ml/kg normal saline	0.0±0.0	0.0±0.0	0.0±-0.0	0.0±0.0	0	0.00	100
400 mg/kg MSEE + S + Li-Pilo	20.1±0.7**	41.4±1.2	3.2±0.3**,##	35.4±2.6##	100	28.6	71.4
600 mg/kg MSEE + S + Lit-Pilo	42.0±1.3**,###	63.4±1.8***,###	2.2±0.5***,##	30.9±5.4**,###	85.7	14.3	85.7
800 mg/kg MSEE + S + Lit-Pilo	58.1±1.9***,###	80.4±2.5***,###	1.3±0.4***,###	18.4±3.4***,###	71.4	14.3	85.7
S + Lit-Pilo + diazepam	22.7±0.8***	43.3±1.4	0.36±0.2***	12.6±4.5***	100	28.6	71.4
S + Lit-Pilo	13.3±0.7	35.4±2.6	4.8±0.4	57.4±2.2	100	14.3	42.9

**P<0.01, ***P<0.001 respectively when compared to negative control, ##P<0.01, ###P<0.001 when compared to positive control (diazepam). Values are expressed as mean±SEM (n=7). Lit-Pilo: Lithium-pilocarpine; SEM: Standard error of mean, MSEE: *Moringa stenopetala* ethanol extract; S: Scopolamine; SE: Standard error

of SE significantly reduces the severity of SRS and significantly decreases latency period to scale 5 SRS.

Affective behavior tests

Elevated plus-maze test

In anxiolytic activity, the highest dose (800 mg/kg) of crude extract has significantly increased the number of entries and time spent in open arm as compared to negative control group animals [Table 6].

Infrared actimeter model

All doses of crude extract which were administered pre- and postinduction of seizures produced significant ($P < 0.01$ and $P < 0.001$, respectively) improvement in locomotor activity in the infrared actimeter model when compared to negative control group animals [Table 7]. The doses of 600 and 800 mg/kg in posttreatment group showed more pronounced locomotor activity improvements.

Histological studies

Histological examination of hematoxylin- and eosin-stained sections revealed the areas of hippocampus formation [Figure 2a and b] with difference in histopathological features. The section of cornius ammonium 3 (CA3 and CA1) shows dispersed free neuron area, neuronal shrinkage, and dark nuclei in negative control group animals. However, the extract-treated animals reverse the changes occurred in negative control group animals.

DISCUSSION

Epilepsy is a chronic central nervous system (CNS) disorder characterized by recurrent unprovoked epileptic seizures and cognitive, social, and psychological consequences of this condition. Temporal lobe epilepsy (TLE) is cryptogenic epilepsy having presumptive lesion due to brain insult because of various factors including SE. Despite many progresses being made in epilepsy treatment, still people at risk can identify, but there is no prophylactic drug therapy to give for those patients to prevent the disease.[25] Therefore, prevention, interruption, or reversing the epileptogenesis can decrease the disease development in the risk group or it can increase good long-term outcomes.[26] The present study was to investigate the potential disease prevention and disease modification effect of *M. stenopetala* in lithium–pilocarpine-induced animal model of epilepsy.

TLE is the common brain insult associated with epilepsy characterized by having long latency period and being refractory for medical therapy. It is originated from the medial and lateral temporal lope, most often hippocampus and amygdala. Patients with TLE are suffering not only by the recurrent seizure but also by behavioral alterations such as depression and anxiety due to morphological and functional alterations of temporal lobe.[27]

Lithium–pilocarpine model of TLE is the most popular and widely used rodent model of this common and difficult to treat the type of epilepsy. The cholinomimetic convulsant pilocarpine is used to induce a SE, which is followed by hippocampal damage and development of SRSs. In rats, administered lithium with pilocarpine allows a reduction of the pilocarpine dose required to induce SE and increases the percentage of animals developing SE.[28]

The long latency period is indicated by a very short mean latency period (2.0 ± 0.2 days) to SRS (scale V and above) developing for 400 mg/kg pretreatment of MSEE and being completely free of SRS symptoms for at least the 30 day follow up period for 600 mg/kg and 800 mg/kg. This result shows the insult modification effect of MSEE at higher doses. MSEE treatment increased the mean latency period time to develop SRS scale V. Even though rats in those groups are not free from development of SRS (scale V), they did not develop SRS more than scale V and their mean value for seizure-free period is longer than the traditionally described period.

The latency period after brain insult offers opportunity window, in which the best drug therapy can modify or stop the epileptogenesis due to brain insult.[28] The prevention of SRS is one of the ultimate goals of prophylactic medication therapy.[26] As a result, highdose pretreatment of crude *M. stenopetala* extract meets this primary goal (prevents SRS; only motor seizures were detected), indicating that it has real antiepileptic properties. While therapy with a crude extract of *M. stenopetala* after a seizure did not prevent SRS, it did change the normal course of the disease by making it less severe, less frequent, and with a lower number of rats developing SRS. This shows the disease modification effect of the crude extract of *M. stenopetala*.

In Lit-Pilo-induced SE, lipid peroxidation is increased,[29,30] indicating the involvement of free radicals in Lit-Pilo-induced neuronal damage that is why antioxidants like ascorbic acid are displaying anticonvulsant activity in this model.[31] Antioxidants are compounds that prevent or prolong the oxidation process that damages the neuronal cells. The presence of some antioxidants such as flavonoids and phenols in MSEE may contribute to prevent the lipid peroxidation-mediated free radical formation after SE and hence contribute to MSEE anticonvulsant effect

Table 3: Limbic seizure severity indexes (Pinel and Rovner, 1978)

Scale	Behavior
1	Facial movements only
2	Facial movements and head nodding
3	Facial movements, head nodding, and forelimb clonus
4	Facial movements, head nodding, forelimb clonus, and rearing
5	Facial movements, head nodding and forelimb clonus, rearing and falling
6	Facial movements, head nodding, forelimb clonus, rearing, falling, terminated with multiple rearing and falling
7	Running fit
8	Running fit with periods of tonus

Table 4: Effects of *Moringa stenopetala* on lithium-pilocarpine-induced seizures behavioral scores

Dose	Scale 1	Scale 2	Scale 3	Scale 4	Scale 5	Scale 6	Scale 7	Scale 8
10 ml/kg NS	0.0±0.0	0.0±0.0	0.0±0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
400 mg/kg MSEE + S + Lit-Pilo	770.0±10.7***,###	636.6±24.3*	461.2±13.5***,###	405.8±10.9***,###	405.8±7.0***	0.0±0.0	0.0±0.0	0.0±0.0
600 mg/kg MSEE + S + Lit-Pilo	756.6±4.8***,###	545.2±14.1***,###	423.6±6.7***,###	355.4±12.1***,###	355.4±2.2***	0.0±0.0	0.0±0.0	0.0±0.0
800 mg/kg MSEE + S + Lit-Pilo	689.8±7.9***,###	489.4±18.5***,###	374.0±8.3***,##	145.4±7.1***,###	145.4±4.6***	0.0±0.0	0.0±0.0	0.0±0.0
S + Lit-Pilo + diazepam	467.0±6.8	287.0±4.2***	176.0±10.1***	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
S + Lit-Pilo	893.8±33.5	722.8±26.1	599.8±24.3	502.0±25.9	502.0±3.7	11.8±2.1	48.0±1.8	0.0±0.0
Lit-Pilo + S + 400 mg/kg MSEE	745.0±7.8***	604.2±15.1**,#	467.4±7.4***,###	353.4±5.8***,###	353.4±3.9***	0.0±0.0	0.0±0.0	0.0±0.0
Lit-Pilo + S + 600 mg/kg MSEE	69.4±6.0***	512.6±27.2***,###	358.0±9.4***,###	163.2±4.5***,###	163.2±2.1***	0.0±0.0	0.0±0.0	0.0±0.0
Lit-Pilo + S + 800 mg/kg MSEE	487.4±25.5***	316.0±9.9***,###	160.6±14.2***,###	54.8±4.7***,#	54.8±0.0***	0.0±0.0	0.0±0.0	0.0±0.0

*P<0.05, **P<0.01, ***P<0.001 when compared to negative control, #P<0.05, ##P<0.01, ###P<0.001 when compared to positive control (diazepam). Values are expressed as mean±SEM (n=7). Lit-Pilo: Lithium-pilocarpine; SEM: Standard error of mean, MSEE: *Moringa stenopetala* ethanol extract; S: Scopolamine; NS: Normal saline

by preventing SE-induced neuronal damage.[32] Additionally, muscarinic activation by pilocarpine is responsible for seizure initiation, but glutamate acting on N-methyl-D-aspartate (NMDA) receptor sustains the seizure activity and excitotoxic injury mediated neuronal cell death in Lit-Pilo model.[33] Therefore, the alteration of seizure induced by Lit-Pilo due to MSEE might be attributed to one or more antioxidant effect, increasing GABAergic effect, decreasing glutamate, or blocking of glutamate receptor or activating potassium channel as increasing potassium conductance involves in inhibition of glutamate release.[34]

The above possible mechanism action for crude extract of *M. stenopetala* is supported by preclinical and clinical studies conducted on currently available antiepileptic drugs tested for their disease modification and insult modification effect in post-SE lithium–pilocarpine model. For example, carisbamate is a neuroprotective and antiepileptogenic drug that acts by inhibiting voltage-sensitive sodium channel, but the data were inconsistent in two clinical trials.[35] Preclinical results on rats showed that modulation of sodium channel, GABAA receptors, and AMPA/kainate-type glutamate receptors were contributed for the disease-modifying effect of topiramate. Epilepsy secondary to SE is associated with histone deacetylase, and this development of

Table 5: Effects of *Moringa stenopetala* on latency period in days to spontaneous recurrent seizures

Group	Scale 5	Scale 6	Scale 7	Scale 8
10 ml/kg NS	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
400 mg/kg MSEE + S + Lit-Pilo	2.0±0.2**,##	0.0±0.0	0.0±0.0	0.0±0.0
600 mg/kg MSEE + S + Lit-Pilo	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
800 mg/kg MSEE + S + Lit-Pilo	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
S + Lit-Pilo + diazepam	10.0±1.3	12.4±0.5	0.0±0.0	0.0±0.0
S + Lit-Pilo	28.8±4.3	8.6±1.9	6.2±0.6	0.0±0.0
Lit-Pilo + S + 400 mg/kg MSEE	19.8±2.1	0.0±0.0	0.0±0.0	0.0±0.0
Lit-Pilo + S + 600 mg/kg MSEE	13.6±1.5	0.0±0.0	0.0±0.0	0.0±0.0
Lit-Pilo + S + 800 mg/kg MSEE	9.0±0.6*,#	0.0±0.0	0.0±0.0	0.0±0.0

*P<0.05, **P<0.001when compared to negative control, #P<0.05, ##P<0.001 when compared to positive control (diazepam). Values are expressed as mean±SEM (n=5). Lit-Pilo: Lithium-pilocarpine; SEM: Standard error of mean, MSEE: *Moringa stenopetala* ethanol extract; S: Scopolamine; NS: Normal saline

Table 6: Effect of *Moringa stenopetala* ethanol extract on anxiolytic activity in lithium-pilocarpine-induced seizure animals

Dose	Number of closed arm entries	Number of open arm entries	Time spent in closed arm (s)	Time spent in open arm time (s)	Time spent in neutral zone (s)
10 ml/kg control (NS)	6.0±0.7	3.4±0.5	187.0±10.7	45.8±3.1	27.2±7.6
400 mg/kg MSEE + S + Lit-Pilo	4.8±0.4	2.2±0.4	193.4±12.1	65.4±8.2	41.2±8.3
600 mg/kg MSEE + S + Lit-Pilo	5.2±0.9	2.8±0.4	181.4±15.0	74.4±12.2	44.2±13.7
800 mg/kg MSEE + S + Lit-Pilo	5.8±0.8*	4.6±0.5***	169.2±13.3	88.2±2.9**	42.6±15.6
S + Lit-Pilo + diazepam	4.8±0.4	4.2±0.5	151.6±8.9	97.6±8.9	50.8±9.7
S + Lit-Pilo	2.6±0.6	1.4±0.4	211.2±15.6	33.6±9.0	55.2±6.5
S + Lit-Pilo + 400 mg/kg MSEE	4.0±0.9	2.8±0.6	199.8±19.2	54.4±13.6	45.8±13.2
S + Lit-Pilo + 600 mg/kg MSEE	4.6±0.6	4.4±0.4**	174.8±19.4	64.6±5.5	44.6±6.8
S + Lit-Pilo + 800 mg/kg MSEE	6.0±0.3*	4.8±0.6***	139.8±8.9*	119.0±6.0***	41.8±5.4

*P<0.05, **P<0.01, ***P<0.001 when compared to negative control (S + Lit-Pilo). Values are expressed as mean±SEM (n=5). Lit-Pilo: Lithium-pilocarpine; SEM: Standard error of mean, MSEE: *Moringa stenopetala* ethanol extract; S: Scopolamine; NS: Normal saline

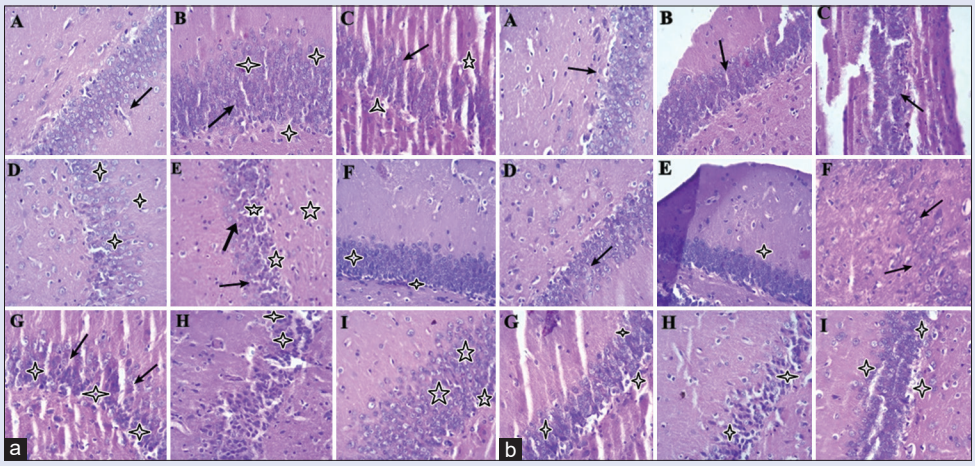

Figure 2: (a) Histological pictures of cornius ammonium 3 (CA3) ×40. (A) Normal control groups, (B) *Moringa stenopetala* ethanol extract 400 mg/kg pretreated group, organized, few dark nuclei (star) and neuron-free area (arrow) with shrinkage in size, (C) *Moringa stenopetala* ethanol extract 600 mg/kg pretreated group, organized, few dark nuclei (star) and neuron-free area (arrow) with shrinkage in size, (D) *Moringa stenopetala* ethanol extract 800 mg/kg pretreated group, organized, dark nuclei (star), (E) Positive control, diazepam-treated group, very organized, dark nuclei (star), (F) Negative control group, disorganized, many dark nuclei (star), shrinkage of neuronal size, (G) *Moringa stenopetala* ethanol extract 400 mg/kg post-treated group, organized, dark nuclei (star) and neuron-free area (arrow), (H) *Moringa stenopetala* ethanol extract 600 mg/kg post-treated group, few dark nuclei (star), (I) *Moringa stenopetala* ethanol extract 800 mg/kg posttreated group, very organized, few dark nuclei (star). (b) Histological pictures cornius ammonium 1 × 40, (A) normal control groups, (B) 400 mg/kg pretreated group, very dense, few neuron area and few enlarged neurons (double head arrow), (C) *Moringa stenopetala* ethanol extract 600 mg/kg pretreated group, few neuron-free areas, (D) *Moringa stenopetala* ethanol extract 800 mg/kg pretreated group, few neuron areas, and some dark nuclei, (E) Positive control, diazepam-treated group, organized, but many dark nuclei (star), (F) Negative control group, dispersed, free neuron area, but normal nuclei (G) *Moringa stenopetala* ethanol extract 400 mg/kg post-treated group, organized with free dark nuclei (star), (H) *Moringa stenopetala* ethanol extract 600 mg/kg post-treated group, organized with free dark nuclei (star), (I) *Moringa stenopetala* ethanol extract 800 mg/kg post-treated group, organized with free dark nuclei (star)

Table 7: Effect of *Moringa stenopetala* ethanol extract on locomotor activity in lithium-pilocarpine-induced seizure animals

Dose	Locomotor activity in 10 min		
	X	Y	T
10 ml/kg control (NS)	31.2±2.2	27.6±2.0	58.8±2.5
400 mg/kg MSEE + S + Lit-Pilo	30.6±0.5	28.0±1.0**	58.6±1.0**
600 mg/kg MSEE + S + Lit-Pilo	28.6±1.9	29.4±1.3**	58.0±3.0**
800 mg/kg MSEE + S + Lit-Pilo	32.0±1.0	25.4±1.5	57.4±2.1**
S + Lit-Pilo + diazepam	36.2±2.6	25.8±2.1	64.0±3.9
S + Lit-Pilo	21.0±2.0	16.2±1.5	37.2±1.5
S + Lit-Pilo + 400 mg/kg MSEE	33.6±4.8*	24.6±4.7	60.4±6.5***
S + Lit-Pilo + 600 mg/kg MSEE	37.4±3.6*	27.2±3.8	66.6±5.1***
S + Lit-Pilo + 800 mg/kg MSEE	35.4±2.0**	32.2±1.7***	67.6±1.8***

*P<0.05, **P<0.01, ***P<0.001 when compared to negative control (S + Lit-Pilo). Values are expressed as mean±SEM (n=5). Lit-Pilo: Lithium-pilocarpine; SEM: Standard error of mean, MSEE: *Moringa stenopetala* ethanol extract; S: Scopolamine; NS: Normal saline

epilepsy in rats delays by the administration of valproate because of its inhibitory effect on histone deacetylase.[36] Additionally, valproate acts on GABAA and inhibits NMDA ion channels that contribute for its antiepileptogenesis effect.[37]

Anxiety and depression are the most common psychiatry comorbidities in patients with epilepsy.[38] Therefore, we have also examined the possible antianxiety and antidepressant activity of the extract. Anxiolytic activity of the crude extract was assessed by the elevated plus-maze apparatus. A high dose of pre- and postseizure treatment of the extract increased the number of entries and time spent on the open arm but decreased the number of closed-arm entries. An increase in the activity of rats in open arms refers to a decrease in anxiety[39,40] and a decrease in these behavioral parameters in closed arms reflects a reduction in stress.[41,42] The anxiolytic activity is also positive in other species of *Moringa* such as *Moringa oleifera*.[43] Therefore, those observations support the anxiolytic activity of the crude extract.

In antidepressant activity, the number of movements within the infrared actophotometer is a manifestation of locomotor activity in rats.[44] Increment in number of locomotor activities indicates the CNS stimulant effect of the test.[45] Therefore, the improvement in locomotor activity after pre- and post-treatment with crude extract of *M. stenopetala* indicates the CNS stimulant effect of the extract and it could be beneficial in epilepsy patients coexisted with depression.

Free radicals from oxidative reactions and inflammatory reactions are associated with some minor and major depressive disorders. It was reported that *M. stenopetala* has a strong antioxidant and anti-inflammatory activity[10] thereby it could be useful in treating depression caused by oxidative reactions or inflammation-induced neurochemical imbalance in the brain. *M. stenopetala* contains many constituents such as flavonoids and phenols, which are vital antioxidants. It has been reported that flavonoids act through their antioxidant mechanism as well as through enhancing neurogenesis.[46,47] In addition to the above antidepressant-like activity of *M. stenopetala* may be due to increase in the serotonin[48,49] by enhancement of norepinephrine neurotransmission effect as reported in other species of *M. oleifera*.[50] Overall, the antidepressant-like potential of the *M. stenopetala* crude extract might be due to various phytochemicals that are known to exist in the plant extract.

Furthermore, the elevated plus maze and infrared actimeter demonstrated that M. stenopetala improved cognitive deficits. Given the fact that TLE epileptogenesis is linked to cognitive deterioration.[51] Those findings further suggest antiepileptogenesis effect of *M. stenopetala* crude extract.

Hippocampus is a major brain part that is highly sensitive for epilepsy and has a prominent role in learning, memories, and behaviors related to food and appetite.[52] Although causes of TLE are different, hippocampus sclerosis is a common histopathological finding for all patients. The presence of mossy fiber sprouting, neuronal damage, and gliosis is characteristic of classical hippocampus sclerosis, but a selective neuronal loss from CA1, CA3, and dentate gyrus is the most common future of TLE patients.[53] Histological result shows marked disorganization of neurons, neuronal loss in some areas, neurons with dark nuclei, and some shrinkage of neuronal size in different layers of pyramidal cells and granular cells of CA1 and CA2. This finding is also consistent with the previous report. The presence of neuron-free area is significant in large pyramidal cells of CA3, whereas cells with dark nuclei are dominant in small pyramidal cells of CA1. Pretreatment of *M. stenopetala* crude extract showed better prevention of neuronal shrinkage, cell disorganization, dark nuclei formation, and neuronal loss as compared to postseizure treatment of crude extract. This supports the insult factor modification and disease modification effect of MSEE observed in the above behavioral parameters.

Preliminary phytochemical study showed the presence of alkaloids, polyphenols, flavonoids, coumarins, terpenoids, anthraquinones, tannins, proteins, and phytosterol in the crude extract. Although it is difficult to state the anticonvulsant activity of the extract in relation to the reported chemicals, some phytochemicals, such as flavonoids[54] and alkaloids,[55] as well as triterpenic steroids and triterpenoidal saponins, have been found to have anticonvulsant effect.[56-58] It is also found that flavonoids can act as benzodiazepine-like molecules that modulate GABA-generated chloride ion currents in animal models of convulsion.[54,59] Therefore, the presence of those phytochemicals in the crude extract of *M. stenopetala* supports the antiepileptogenesis effect of the extract.

The present study's findings demonstrated clearly *M. stenopetala's* antiepileptic action against Lit-Pilo induced seizure, as stated by there was a much lower frequency of epileptic episodes and a significantly longer latency to SE. Furthermore, our study consistently demonstrated that pharmacological intervention using *M. stenopetala* significantly and dose dependently improves alertness and anxiety like symptoms.

CONCLUSION

The present experimental studies showed that *M. stenopetala* extract decreased convulsion and behavioral epilepsy parameters including histopathology changes in lithium–pilocarpine-induced TLE model. It also possesses antidepressant and anxiolytic activities. This may indicate that the anticonvulsant activity of *M. stenopetala* and support the traditional claim in the use of plants for epilepsy in the south nation and nationality people of Ethiopia.

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Conflicts of interest

There are no conflicts of interest.

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